

## *Lactobacillus* GG Improves Recovery in Infants with Blood in the Stools and Presumptive Allergic Colitis Compared with Extensively Hydrolyzed Formula Alone

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**Objectives** To determine the benefits of *Lactobacillus rhamnosus* GG (LGG) in an extensively hydrolyzed casein formula (EHCF) in improving hematochezia and fecal calprotectin over EHCF alone.

**Study design** Fecal calprotectin was compared in 30 infants with hematochezia and 4 weeks after milk elimination with that of a healthy group. We also compared fecal calprotectin and hematochezia on 26 formula-fed infants randomly assigned to EHCF with LGG (Nutramigen LGG) (EHCF + LGG) or without (Nutramigen) (EHCF – LGG) and on 4 breastfed infants whose mothers eliminated dairy.

**Results** Fecal calprotectin in those with hematochezia was significantly higher than in comparisons (mean  $\pm$  SD  $325.89 \pm 152.31$  vs  $131.97 \pm 37.98$   $\mu\text{g/g}$  stool,  $t = 6.79$ ,  $P < .0001$ ). At 4 weeks, fecal calprotectin decreased to 50% of baseline but was still significantly higher than in comparisons ( $157.5 \pm 149.13$  vs  $93.72 \pm 36.65$   $\mu\text{g/g}$ ,  $P = .03$ ). Fecal calprotectin mean decrease was significantly larger among EHCF + LGG compared with EHCF – LGG ( $-214.5 \pm 107.93$  vs  $-112.7 \pm 105.27$   $\mu\text{g/g}$ ,  $t = 2.43$ ,  $P = .02$ ). At 4 weeks, none of the EHCF + LGG had blood in stools, and 5/14 on EHCF – LGG did ( $P = .002$ ).

**Conclusion** Fecal calprotectin is elevated in infants with hematochezia and possible allergic colitis. EHCF + LGG resulted in significant improvement of hematochezia and fecal calprotectin compared with the EHCF alone. (*J Pediatr* 2010;156:397-401).

Cow's milk allergic colitis (CMAC), a benign condition that affects term infants and children younger than 3 years of age, is suspected in otherwise healthy infants with blood in the stools, mucus, with or without diarrhea.<sup>1</sup> The condition occurs in breast- and formula-fed infants.<sup>1-3</sup> Rectal biopsy specimens show an abundance of eosinophils,<sup>4</sup> suggesting an allergic cause, which is supported by resolution of signs after dietary antigen elimination.<sup>5,6</sup> Because biopsy specimens are not obtained routinely, diagnosis of CMAC remains presumptive in most cases and is assumed when 1 or more of the above-mentioned signs are present (suspected CMAC [s-CMAC]).

Fecal calprotectin, a neutrophil-derived protein, is a reliable marker of intestinal inflammation.<sup>7-9</sup> We are not aware of data on levels of fecal calprotectin in infants with CMAC.

Probiotics are live microorganisms that, when ingested in adequate amounts, may affect the host by supplying beneficial intestinal bacteria and may provide health benefits.<sup>10</sup> *Lactobacillus rhamnosus* GG (LGG) is a bacterial strain isolated from human feces, capable of transiently colonizing the human intestine.<sup>11,12</sup> Preventive and therapeutic properties of LGG related to atopic disease, particularly in infants with atopic dermatitis sensitized to cow's milk protein, have been described.<sup>13-16</sup> However, there are only limited published data on the clinical effects of LGG supplementation of infant formulas.

The aims of this study were to determine: (1) whether fecal calprotectin can be used as a marker of intestinal inflammation in infants suspected of having CMAC by comparing its concentration at diagnosis to that of healthy, age matched infants; (2) whether fecal calprotectin can be used to monitor clinical improvement in s-CMAC by comparing its baseline values to those after 4 weeks of dietary antigen elimination and resolution of signs; and (3) whether the addition of LGG to an extensively hydrolyzed casein formula (EHCF) (EHCF + LGG) has therapeutic advantages in the recovery of infants with s-CMAC compared with that of an EHCF without LGG (EHCF – LGG).

CFU	Colony-forming units
CMAC	Cow's milk allergic colitis
s-CMAC	Suspected cow's milk allergic colitis
EHCF	Extensively hydrolyzed casein formula
LGG	<i>Lactobacillus</i> GG

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## Methods

Infants from 0 to 12 months of age, fed a casein-based routine formula or breast milk, with presumptive diagnosis of CMAC (see below) were enrolled in group A; group B (comparison group) included healthy infants of the same sex and age, matched for the type of feeding (formula or breast milk), recruited from the general population of a local pediatric practice. Exclusion criteria were as follows: infants with Hirschsprung's disease, any kind of malformation, history of intestinal intussusception or surgical intervention, or intercurrent illness at the time of enrollment. Several local pediatricians had agreed to refer potential patients for the study. Each time any pediatrician identified a qualifiable infant, they contacted the principal investigator within 48 hours of the initial consultation and referred the baby for further evaluation. CMAC was suspected in infants with the following: (1) presence of macroscopic or occult blood in stool, (2) with or without mucus, (3) with or without diarrhea or increased number of stools, or loose stools in the absence of any systemic symptoms. Occult fecal blood was determined by use of OC-Hemocard cards (Alfa-Wassermann Diagnostics, West Caldwell, New Jersey). Fecal calprotectin was quantified by means of a commercial kit on the basis of an enzyme-linked immunosorbent assay (Calprest; Eurospital, Trieste, Italy) carried out at the laboratory of the Pediatrics Department of the Hospital in Bari. Sigmoidoscopy was not performed in any of the infants. The study was presented to the parents, and those who agreed to participate signed a written informed consent. The study was approved by the ethics committee of the Bari Hospital.

Sample size calculation was directed at having a 90% power of detecting, with a significance level of 1%, a mean reduction in fecal calprotectin of 30  $\mu\text{g/g}$  between the EHCF + LGG and the EHCF – LGG groups. That number was not less than 26 infants with blood in the stools to be randomized between the 2 study groups. Assuming an expected dropout rate of 10%, we decided to recruit not fewer than 30 infants with possible diagnosis of CMAC.

### Design for Infants in Group A

All infants in group A underwent at enrollment history taking and physical examination, anthropometric measurements, prick by prick test for whole cow's milk, patch test for cow's milk proteins, determination of occult blood in 3 different stool samples, quantitation of fecal calprotectin, complete blood count, serum iron, transferrin, ferritin, C-reactive protein, serum albumin, stool culture for bacteria, and determination of parasites. Infants had repeated anthropometric measurements and determination of fecal calprotectin and fecal occult blood at the conclusion of the study 4 weeks after enrollment. Occult blood was documented once at the doctor's office and in 2 additional tests with guaiac cards that were given to the parents to take home and subsequently sent back to the hospital for analysis. Mothers of breastfed infants with s-CMAC were advised to follow a dairy-free diet.

For formula-fed infants, this was a prospective, randomized, double-blind, placebo-controlled study. A randomization sequence had been generated by computer, and formula assignment was written in sequentially numbered sealed envelopes. Infants received 1 of 2 commercially available EHCF, either containing LGG (Nutramigen LGG; Mead Johnson Nutritionals, Evansville, Indiana) or not. The LGG concentration per manufacturer's specification at release was  $2.50 \times 10^7$  to  $5 \times 10^8$  colony-forming units (CFU)/g and the guaranteed level of LGG is  $1.46 \times 10^7$  CFU/100 mL ( $\sim 1 \times 10^6$  CFU/g). The 2 types of formulas were provided by the manufacturer to the principal investigator in powder form, in identical packaging. Physical and organoleptic properties of the 2 formulas were identical. The principal investigator was not aware of formula assignment. To ensure the viability of the probiotic, mothers were told to prepare the formula with water no hotter than 40°C (104° F), which was verified by the application of a temperature-sensitive tape provided by the investigators, applied to the exterior of the formula bottle. Parents were given enough formula for 15 days, and then they returned to the hospital to obtain another similar supply. At the conclusion of the study, all infants in group A were fed commercially-available EHCF + LGG until their doctors decided to discontinue it.

### Design for Infants in Group B

Infants in group B continued with the same feeding that they were receiving at enrollment, whether formula or breast milk. Infants in this group only had fecal calprotectin determination at enrollment and 4 weeks later.

### Data Analysis

Characteristics of the infants in groups A and B were compared by means of the Student *t* test for independent samples. Further analysis was carried out among 5 dietary groups as follows: (1) EHCF + LGG with s-CMAC, (2) EHCF – LGG with s-CMAC, (3) healthy comparison, formula-fed infants, (4) breastfed with s-CMAC, and (5) healthy comparison, breastfed infants. Differences in fecal calprotectin at baseline among the above 5 groups were analyzed with 1-way analysis of variance. To evaluate the effect of 4 weeks diet on fecal calprotectin values and body weight among the above groups, a 2-way analysis of variance test for repeated measures was performed, followed by a post-hoc test. Among the group of infants with s-CMAC, the effect of diet on presence of occult blood stool after a 4-week diet was evaluated with exact contingency tables methods. Statistical analysis was performed with the SAS statistical packages (SAS Institute, Cary, North Carolina). A *P* value of  $<.05$  was considered to be statistically significant.

## Results

Thirty infants in group A and 32 in group B were enrolled. Both groups were similar with respect to gestational age

**Table I.** Characteristics of patients with cow's milk allergic colitis (group A) and comparisons (group B) at enrollment in the study

	Group A Mean (95% CI)	Group B Mean (95% CI)	t	P
Age (mo)	4.03 (3.16; 4.90)	4.41 (3.75; 5.06)	-0.71	.48
Weight (g)	6145.0 (5403.1; 6886.9)	6311.6 (5796.6; 6826.5)	-0.38	.70
Length (cm)	60.70 (57.28; 64.12)	61.87 (59.97; 63.78)	-0.62	.54
Head circumference (cm)	40.18 (38.84; 41.52)	39.90 (38.97; 40.82)	0.36	.72
Fecal calprotectin ( $\mu\text{g/g}$ stool)	325.89 (269.01; 387.76)	131.97 (118.56; 145.38)	6.79	<.0001

and anthropometric data at birth (not shown) and at the time of enrollment (Table I). Age ranged from 1 to 10 months. At enrollment, 26 infants in group A were being fed a casein-based formula, and 4 were exclusively breastfed. Eighteen infants in group B were fed a casein-based formula, and 14 were breastfed. As anthropometric data were not significantly different between formula and breastfed infants, this information is presented together.

At the time of diagnosis, 18 patients in group A had mucus in the stool, and 8 also had loose or soft stools. Absolute peripheral eosinophil count was above the highest accepted normal levels ( $0.3 \times 10^3/\mu\text{L}$ ) in 7 patients. Serum albumin, iron, transferrin, and ferritin, C-reactive protein and stool culture for bacteria and parasites were either normal or negative in all patients. Occult blood in stool was negative in all group B infants. The skin prick test result was positive in 3/30 patients (10%). Patch test for casein was positive in 1/30 (3.33%).

After randomization, 12 patients in group A received EHCF + LGG and 14 EHCF - LGG. Mean fecal calprotectin values at enrollment with respect to group and type of milk feeding are listed in Table II. At the time of enrollment, mean ( $\pm$  SD) fecal calprotectin values were significantly higher in group A ( $325.89 \pm 150.29 \mu\text{g/g}$  of stools) than in group B ( $131.97 \pm 37.3 \mu\text{g/g}$  of stools) ( $t = 6.79$ ,  $P < .0001$ ) (Table I). After 4 weeks of dietary treatment or continuation of their original feedings in the comparison group, fecal calprotectin values decreased significantly with

respect to baseline in infants with and without S-CMAC (Table III and Figure). In fact, the interaction between effect and type of feeding was statistically significant ( $F = 12.45$ ;  $P < .0001$ ). In particular, after 4 weeks of dietary antigen elimination, mean decrease in fecal calprotectin values were significantly larger in infants who received EHCF + LGG compared with the infants who received EHCF - LGG who showed a mean decrease in fecal calprotectin values similar to the comparison group (Table III).

After the 4-week dietary period, occult blood in the stool was not detectable in 19/30 (63.33%) of group A infants, as follows: in 12/12 patients who received EHCF + LGG, in 5/14 who received EHCF - LGG ( $\chi^2 = 11.85$ ;  $P = .027$ ) (Table IV). Two of the 4 breastfed infants continued to have blood in the stools, and their fecal calprotectin values were close to those of the EHCF + LGG. Mean weight increase during the dietary treatment period was 621.4 g for the EHCF - LGG group and 457.5 g for the EHCF + LGG group ( $t = 1.16$ ;  $P = .26$ ).

## Discussion

In allergic enteritis/colitis the mucosal inflammatory process is most likely result of a reaction to dietary antigens, typically, cow's milk proteins.<sup>1,2,4,17</sup> The condition rapidly resolves after antigen exclusion, and a normal diet can usually be achieved by 1 year of age.<sup>18</sup>

Calprotectin is a calcium and zinc-binding protein that accounts for 60% of the cytosol proteins in neutrophils and is elevated in the presence of ongoing inflammation.<sup>19</sup> There is an age-dependent variation in fecal calprotectin<sup>20-22</sup> where upper reference values for newborns are higher than those in children.<sup>23-25</sup> Our results indicate that fecal calprotectin was significantly higher in infants suspected of having CMAC than in a comparison group of healthy infants. There was a significant decrease in fecal calprotectin in infants with s-CMAC after 4 weeks of dietary antigen elimination, although levels remained higher than in age- and diet-matched comparisons. However, a significant decrease in fecal calprotectin was also observed in the comparison group. It could be argued that without a rectal mucosal biopsy, up to 64% of infants could be misdiagnosed<sup>26</sup>; in our study, we did not confirm the diagnosis with a biopsy. However, as a group, all infants with blood in the stools had significantly higher fecal calprotectin than the comparisons matched for age and type of feeding, which makes us believe that indeed the infants

**Table II.** Fecal calprotectin values in patients with cow's milk allergic colitis (group A) and comparisons (group B) at enrollment with respect to type of milk

	Fecal calprotectin mean (95% CI)
Group A (n = 30)	
EHCF + LGG	283.49 <sup>a</sup> (195.68; 371.30)
EHCF - LGG	368.76 <sup>a</sup> (268.37; 469.15)
Breastfed	303 <sup>a</sup> (172.38; 433.62)
Group B (n = 32)	
Breastfed	137.64 <sup>b</sup> (108.06; 167.22)
Formula-fed	127.56 <sup>b</sup> (116.80; 138.30)

<sup>a,b</sup>Same letter indicates no significant difference in post-hoc analysis, 1-way ANOVA results:  $F = 13.55$ ;  $P < .0001$ .

**Table III.** Mean decrease in fecal calprotectin values in patients, with cow's milk allergic colitis (Group A) and comparisons (Group B) 4 weeks after enrollment for type of milk

	Fecal calprotectin mean (95% CI)
Group A (n = 30)	
EHPF + LGG	-214.47 <sup>a</sup> (-283.04; - 145.90)
EHPF - LGG	-112.70 <sup>b</sup> (-173.48; - 51.91)
Breastfed	-225.00 <sup>a</sup> (-423.74; - 26.25)
Group B (n = 32)	
Breastfed	-39.36 <sup>b</sup> (-72.93; - 5.80)
Formula-fed	-37.39 <sup>b</sup> (-58.54; - 16.23)

Infants in group B remained on the same diet.  
 Results from repeated measures analysis of variance:  
 4-week diet effect  $F = 107.7$ ;  $P < .0001$ .  
 Type of milk effect  $F = 12.35$ ;  $P < .0001$ .  
 Interaction: 4-week diet effect for type of milk  $F = 12.45$   $P < .0001$ .  
<sup>a,b</sup>Same letter indicates no significant difference in post-hoc analysis.

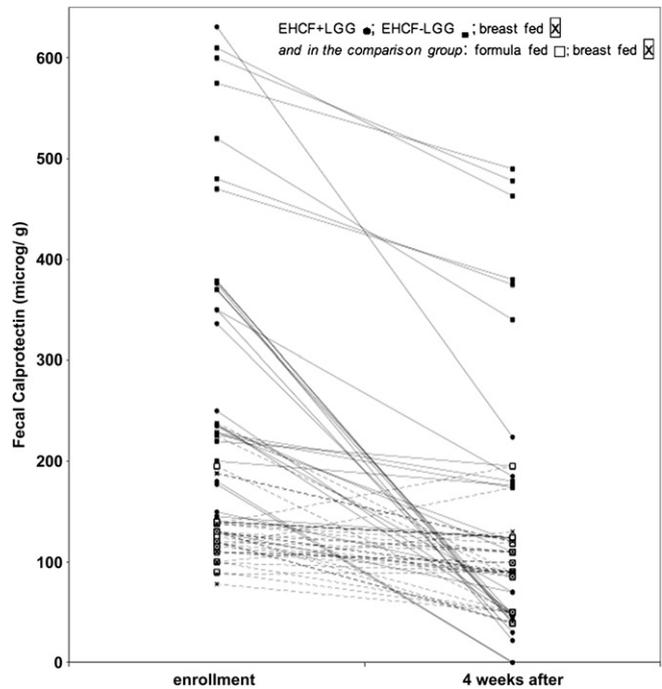
studied had colitis. The low incidence of positive skin prick test results in the infants with s-CMAC is not surprising because this entity is caused mainly by non-immunoglobulin E-mediated mechanisms.

LGG is a prototypic strain of probiotic lactobacilli and has been used in food products in both Europe and North America for more than 10 years. LGG has been evaluated in numerous clinical studies involving preterm and term infants at dose levels ranging from  $10^8$  to  $10^{10}$  CFU per day. A study designed to assess the effectiveness of LGG administered to breastfed infants with rectal bleeding as an adjunct to cow's milk restriction in the mother's diet failed to demonstrate any benefit compared with those who received placebo.<sup>27</sup> In our study, 2 of the 4 breastfed infants that were enrolled continued to have occult blood in the stools 1 month after their mothers eliminated cow's milk products from their diet. It could be hypothesized that other antigens may also be playing a role in causing persistence of blood in the stools among breastfed infants. In the study by Szajewska,<sup>27</sup> one could speculate that LGG was not able to overcome the antigenic effect of other food items that mothers continued to ingest or that mothers did not completely adhere to the dairy-free diet. A more complete elimination of noxious dietary antigens may be achieved by use of an extensively hydrolyzed formula, and in that case, LGG may have a potentiating beneficial effect as seen in our study. Arvola et al<sup>28</sup> demonstrated that the number of days with persistent rectal bleeding was identical in infants randomly assigned to

**Table IV.** Occult blood stool after 4 weeks of diet in patients with s-CMAC with respect to the type of milk feeding

	EHCF + LGG	EHCF - LGG	Breast milk	Total
OBS-	12	5	2	19
OBS+	0	9	2	11
Total	12	14	4	30

OBS, Occult blood stool.  
 $\chi^2 = 11.85$ ;  $P = .027$ .



**Figure.** Fecal calprotectin at enrollment and 4 weeks after.

an elimination diet or to continue their previous diet. In that study, however, 68% of the infants were exclusively breastfed, and only 12% were exclusively formula fed and presence of fecal blood was only assessed macroscopically.

In this study, we demonstrated that the addition of LGG to an EHCF significantly improved the recovery of the inflamed colonic mucosa as indicated indirectly by greater decreases in fecal calprotectin and in the number of infants with persistence of occult blood in stools after 1 month. The mechanisms of this beneficial effect are not apparent from this study but may be related to the effects that LGG has on enhancing the intestinal mucosa's barrier function, participating in degradation of protein antigens, competing with pathogenic bacteria, and promoting early immune system maturation toward nonallergy, as well as alleviating symptoms of eczema attributed to CMA.<sup>14,16,29,30</sup>

Fecal calprotectin seems to be a useful marker of intestinal inflammation in infants with s-CMAC, which can be used to monitor response to antigen elimination. The addition of LGG to an EHPH resulted in a statistically significant greater decrease in values of fecal calprotectin and presence of intestinal blood loss at one month of dietary therapy. Additional long-term studies with fecal calprotectin may be helpful in assessing complete recovery of the intestinal mucosa and subsequent response to reintroduction of cow's milk products to the patients' diet. ■

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