

Enhancement of the Circulating Antibody Secreting Cell Response in Human Diarrhea by a Human *Lactobacillus* Strain

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ABSTRACT. Human *Lactobacillus* sp strain GG (*Lactobacillus* GG) administered during acute rotavirus diarrhea has been shown to promote clinical recovery. To elucidate the immune mechanisms behind such a favorable outcome, the ELISPOT (solid phase enzyme-linked immunospot) assay of Ig- and specific antibody-secreting cells among circulating lymphocytes was used, giving indirect evidence of the immunologic events in the gut. After rehydration, 39 children with acute rotavirus diarrhea, mean age 16 (SD 6) mo, randomly received either a *Lactobacillus* GG fermented milk product (study group) or a pasteurized yogurt (placebo group). The duration of diarrhea was significantly shorter in the study group than in the placebo group [mean 1.1 (SD 0.6) versus 2.5 (SD 1.4) d, $p = 0.001$]. *Lactobacillus* GG therapy was associated with a significantly enhanced nonspecific humoral response during the acute phase of the infection, reflected in the IgG, IgA, and IgM Ig-secreting cell numbers. At convalescence, 90% of the study group versus 46% of the placebo group had developed an IgA specific antibody-secreting cell response to rotavirus ($p = 0.006$). The results indicate that *Lactobacillus* GG promotes recovery from rotavirus diarrhea via augmentation of the local immune defense. Furthermore, specific IgA response to rotavirus is endorsed, which is possibly relevant in protection against reinfections. (*Pediatr Res* 32: 141-144, 1992)

Abbreviations

Lactobacillus GG, *Lactobacillus* sp strain GG
BLG, β -lactoglobulin
CI, confidence interval
ISC, Ig-secreting cell
sASC, specific antibody-secreting cell
ELISPOT, solid phase enzyme-linked immunospot

Rotaviruses have emerged as a major cause of acute diarrhea in infants and children worldwide (1). The infection is characterized by vomiting, watery diarrhea, and fever. The main goal of treatment, correction of the dehydration, is achieved by oral rehydration. The issue of nutritional therapy in acute diarrhea is still an open question.

The nutritional state of the patient has a strong impact on the

Received September 6, 1991; accepted March 17, 1992.

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Supported by the Foundation for Nutrition Research and the E. Aaltonen Foundation.

course of diarrhea. Malnutrition has been reported to increase the absorption of potentially harmful antigens (2) and to impair immune responses (3). A poor nutritional state may thus increase the risk of protracted diarrhea as well as enhance susceptibility to other infections and to gastrointestinal allergy. The nutritional state of even well-nourished infants deteriorates rapidly during diarrhea, either because of loss of appetite, deliberate withholding of oral feeds, or partial malabsorption caused by viral invasion of enterocytes (4).

Rapid reintroduction of oral feedings after rehydration has been advocated to counteract the potential hazards related to fasting during diarrhea (5). We have previously shown that rapid refeeding results in earlier cessation of diarrhea in well-nourished children; also, cow milk products are tolerated (6). A further shortening of diarrhea resulted from administration of human *Lactobacillus* strain (*Lactobacillus* GG, Gefilac, Valio Finnish Co-operative Dairies' Association, Helsinki, Finland) together with the rapid refeeding schedule (7). The mechanisms behind such a favorable outcome remain poorly understood. The effect of nutritional therapy may be immunologically mediated and may prove important in eradicating enteric infections in the immunocompromised host.

The objective of the present study was to evaluate the effect of *Lactobacillus* GG on the intestinal immune response triggered by rotavirus infection in well-nourished children. For this purpose, we used the ELISPOT assay, which measures ISC and sASC among circulating lymphocytes. These cells are arrested during their maturation cycle in peripheral blood, giving indirect evidence of gut local immune response (8-10).

MATERIALS AND METHODS

Patients. Forty-four well-nourished children (33.4% female), between 7 and 37 mo of age, were enrolled in the present study. They were admitted for acute gastroenteritis of less than 7 d duration at the Department of Pediatrics, Tampere University Hospital, during a rotavirus epidemic.

Informed consent was obtained from the patients' parents, and the protocol was approved by the hospital's Committee on Ethical Practice.

Management and samples. Upon admission, the children were weighed and clinically examined. The severity of dehydration (%) was estimated. The children were treated according to standard practice: oral rehydration was accomplished in 6 h with a solution containing Na^+ 60 mmol/L and glucose 144 mmol/L (Osmosal Novum, Leiras, Turku, Finland) given at twice the estimated fluid loss with a minimum of 30 mL/kg body weight. The patients were weighed daily. The attending nurses followed the quality (characterized as watery, loose, or solid) and number of stools and vomitus. The duration of diarrhea was counted

from the last appearance of watery stools. The patients were discharged according to the clinical judgment of the attending physician. They were seen 3 wk later or sooner if diarrhea recurred.

Blood was drawn on admission for determination of acid-base balance and serum sodium concentration. For the ELISPOT assay, blood in heparin was drawn 1 d after admission and 3 wk later. At convalescence, blood was also drawn for measurement of serum cow milk and rotavirus antibodies.

Feces were tested for rotavirus antigen with an enzyme immunoassay (Rotazyme, Abbott Laboratories, North Chicago, IL) and cultured for *Salmonella*, *Shigella*, *Campylobacter*, and *Yersinia*.

Dietary therapy. After rehydration, alimention appropriate for age was resumed, excluding only fermented milk products. Infants still totally breast-fed were not included in the study.

The patients randomly received one of two dietary regimens for 5 d in addition to the rapid refeeding schedule. The study group received 10^{10} – 10^{11} colony-forming units *Lactobacillus* GG, as a fermented milk product, 125 g twice daily. The placebo group received a fermented-then-pasteurized yogurt, with $<10^3$ colony-forming units lactic acid bacteria, 125 g twice daily. Both *Lactobacillus* GG and the placebo yogurt were supplied by the Research and Development Centre of Valio Finnish Co-operative Dairies' Association, Helsinki, Finland.

Determination of the immune response. Mononuclear cells containing mainly lymphocytes were obtained by FicollPaque (Pharmacia, Uppsala, Sweden) centrifugation of the heparinized blood. The total number of ISC (antigen-nonspecific immune response) and sASC (antigen-specific immune response) were determined by the ELISPOT method, as described previously (8–10). We and others have shown that the number of these lymphocytes in peripheral blood reaches a maximum 6–8 d after oral administration of antigen (8–12).

In brief, cells are incubated in antigen-coated, flat-bottomed, 96-well microtiter plates (Immunoplate R I, a/s Nunc, Roskilde, Denmark) to allow the antibodies secreted by them to react with the antigen near them. The antibodies from each cell are visualized by application of enzyme-labeled antisera followed by a substrate-agarose overlay and by observing the colored spots.

For determination of ISC, coating of the plates was done with rabbit Ig to human IgA (α -chain) and IgM (μ -chain) (Dakopatts a/s, Roskilde, Denmark), goat anti-human IgG (γ -chain specific) F(ab')₂ fragment of antibody, goat anti-human IgG (γ -chain specific), IgA (α -chain specific), and IgM (μ -chain specific) alkaline phosphatase conjugate (Sigma Chemical Co., St. Louis, MO). For determination of sASC to rotavirus, the coating was done in two stages. First, the wells were coated for 2 h at 37°C or overnight at 4°C with rabbit antirotavirus (human) (Dakopatts a/s, Glostrup, Denmark). After washing the wells with PBS-0.05% Tween 20, rotavirus antigen (for the complement fixation test, Behringwerke AB, Marburg, Germany) was applied for 2 h at 37°C. This two-stage coating was found superior to mere rotavirus antigen coating in preliminary ELISA tests on rotavirus-positive and -negative sera. To determine sASC against BLG, wells were coated with BLG as described in elsewhere (13). 5-bromo-4-chloro-3-indolylphosphate *p*-toluidine salt was used as substrate for alkaline phosphatase. It is considered an immune response if >0.5 sASC/ 10^6 cells are detected (13).

Serum antibodies against cow milk antigens, BLG A and B, α -casein, α -lactalbumin, BSA, and total milk protein, reflecting the systemic immune response, were determined by ELISA (14). In brief, the antigens were diluted to appropriate concentrations. Microtiter plate wells (Nunc-Immuno plate MaxiSorp, Nunc) were coated with the antigen solution. Test sera were added and, after incubation and washing, antihuman antisera conjugated with alkaline phosphatase were added. Absorbances were determined. Serum rotavirus antibodies were measured using a complement fixation test.

Statistical analyses. Because of skewed distributions of ISC

and sASC numbers, logarithmic transformation was used and the numbers are thus given as geometric mean (95% CI). Two-tailed independent *t* test was applied for comparison of differences. Paired *t* test was used for comparing repeated measurements. The χ^2 test was applied to determine differences in proportions. Spearman rank correlation coefficient was applied to determine the significance of association between age and the ELISPOT response.

RESULTS

Clinical characteristics of patients. Altogether, 39 (88%) patients were rotavirus-positive, and they formed the actual study ($n = 22$) and placebo ($n = 17$) groups. The clinical picture upon admission was comparable in both groups with iso-osmolal dehydration and metabolic acidosis (Table 1). The ages of the patients in the two groups were comparable, with the difference between means being 3.2 mo (95% CI –0.6, 7.0).

Clinical outcome. During a short stay in hospital, the patients were rehydrated and realimented, with weight gain resulting in both groups (Table 2). The duration of diarrhea was significantly reduced in the study group compared to the placebo group. During follow-up, there were no recurrences of diarrhea.

Antigen-nonspecific immune response. A prominent ISC response was measured during the acute phase of diarrhea in both groups, especially in cells secreting IgM (Fig. 1). This transient response in the study group was almost twice that in the placebo group in all Ig classes. At the convalescent phase, the difference between the two groups was no longer seen. The magnitude of the ISC responses did not correlate with age; for IgM ISC, $\rho = 0.09$ and $p = 0.65$.

Antigen-specific immune response to rotavirus. During diarrhea, patients in both the study group and the placebo group showed a similar sASC response to rotavirus in IgM class [95 (95% CI 46, 198) and 82 (95% CI 32, 210) sASC/ 10^6 cells,

Table 1. Clinical characteristics on admission of patients hospitalized for acute rotavirus gastroenteritis receiving rapid realimentation and either *Lactobacillus* GG (study group) or placebo yogurt

	Study group ($n = 22$)		Placebo group ($n = 17$)		<i>t</i> test <i>p</i>
	Mean	(SD)	Mean	(SD)	
Age (mo)	17.5	(6.5)	14.3	(4.4)	0.09
Weight on admission (g)	10 134	(1 920)	9 660	(1 990)	0.46
Dehydration (%)	4.7	(1.6)	5.0	(2.8)	0.54
Base excess (mmol/L)	–8.5	(3.8)	–7.2	(3.2)	0.37
Serum sodium (mmol/L)	138	(3)	138	(4)	0.77

Table 2. Clinical outcome of patients with acute rotavirus diarrhea during hospitalization given either *Lactobacillus* GG (study group) or placebo yogurt

	Study group ($n = 22$)	Placebo group ($n = 17$)	<i>p</i>
Weight gain (g)*	285 (114, 711)	275 (167, 454)	0.95†
Diarrheal stools (%)‡			
Day 1	91	94	
Day 3	9	53	0.002§
Duration of diarrhea (d)	1.1 (0.6)	2.5 (1.4)	0.001†

* Geometric mean (95% CI).

† *t* test.

‡ % of patients.

§ χ^2 test.

|| Mean (SD).

The intestinal mucosa constitutes a major host barrier against foreign antigens encountered by the enteral route. The predominant mucosal Ig is IgA. It is responsible for immune exclusion of luminal antigens and may dampen local inflammatory reactions (16). In this study, the rotavirus-specific immune response, as measured by the ELISPOT method, was manifest at convalescence, which is in keeping with studies in animals (4) and humans (17, 18). Previous animal studies support the notion that antibodies of IgA isotype in the intestinal lumen are crucial for recovery and protection from rotavirus infection (4).

It is interesting that *Lactobacillus* GG enhances the IgA response to rotavirus. The augmentation of the immune response by *Lactobacillus* GG is similar to the adjuvant action previously demonstrated for cholera toxin (19). *Lactobacillus* GG has been shown to adhere to a human colon carcinoma cell line (20) and to colonize the gastrointestinal tract (21). It has been demonstrated that the adherence properties of the cholera toxin B subunit are related to the intensity of the mucosal immune response (22) and that colonization with commensal bacteria promotes the production of IgA (23). The most likely explanation for the enhancement of the IgA response is the adherent property of *Lactobacillus* GG. *Lactobacillus* GG may influence permeation of antigens through Peyer's patches, thereby stimulating the numerous IgA-committed B cell population therein (16).

The ISC response during diarrhea may reflect rapid recruitment of local nonspecific immune mechanisms to limit the spread of the infection. The specific response to BLG during diarrhea suggests that it is partly directed against dietary antigens concomitantly present within the gut. Intestinal permeability is acutely increased during a rotavirus infection (24), and a local immune response to bystander antigens may provide protection against sequelae of rotavirus diarrhea, *i.e.* protracted diarrhea (25) and gastrointestinal allergy (26). The abrupt effect of *Lactobacillus* GG on the ISC response is in support of the hypothesis of adjuvant action, where a temporal closeness between antigen and adjuvant administration is crucial (27), whereas the effect on the intensity of the response may be attributed to its adherent quality.

In conclusion, it is suggested that *Lactobacillus* GG may be a beneficial supplement of nutritional therapy of acute gastroenteritis in that it promotes recovery and gut immune defense mechanisms.

Acknowledgment. The authors thank Sinikka Suur-Nuuja for excellent technical assistance in determination of serum antibodies to milk proteins.

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