

Viable versus inactivated lactobacillus strain GG in acute rotavirus diarrhoea

Minna Kaila, Erika Isolauri, Maija Saxelin, Heikki Arvilommi, Timo Vesikari

Abstract

The effect of viable or heat inactivated human *Lactobacillus casei* strain GG on rotavirus immune responses in patients with rotavirus diarrhoea was assessed. Rotavirus serum IgA enzyme immunoassay antibody responses were higher in infants treated with viable *L casei* strain GG than in those treated with inactivated *L casei* strain GG. There was a significant difference at convalescence with rotavirus specific IgA secreting cells found in 10/12 infants receiving viable but only 2/13 infants receiving inactivated *L casei* strain GG. The results indicate that viable *L casei* strain GG stimulate rotavirus specific IgA antibody responses, theoretically significant in the prevention of reinfections.

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The treatment of acute diarrhoea begins with correction and maintenance of rehydration, but the appropriate diet thereafter is debated.¹ It has been suggested that the optimal diet should include fermented milks as they are low in lactose, their proteins are more easily digested, and they seem to have advantageous effects on the immune responses.² In a previous study, the peroral administration of *Lactobacillus casei* strain GG during acute diarrhoea was associated with an enhanced immune response to rotavirus as measured by circulating IgM and IgA producing cells at the acute stage of the infection.³ Using measurement of this immune response as a tool, the present randomised double blind two cell clinical trial was designed to address specifically the question whether viable lactobacilli are required for stimulation of rotavirus specific immune responses.

Patients and methods

The inclusion criteria were admission for acute gastroenteritis of less than seven days' duration and age less than 4 years. During the period of the rotavirus epidemic season, 41 well nourished children (mean age 12.8 months, range 1.3-38.4) were enrolled.

On admission the children were clinically examined, and their treatment followed the usual clinical guidelines of oral rehydration and rapid refeeding.⁴ The children were weighed daily, and the attending nurses noted the quality (watery, loose, or solid) and

number of stools. Together with the rapid refeeding regimen appropriate for age, the patients received in a randomised, double blind fashion twice daily either viable *L casei* strain GG, 10¹⁰⁻¹¹ colony forming units (cfu) (n=20, group A), or heat inactivated *L casei* strain GG (n=21, group B), each for five days. The patients were discharged according to the judgment of the attending paediatrician. The patients were seen by a physician (MK), who was blinded to the treatment group, at convalescence one month later.

Twenty six of 41 patients fulfilled the inclusion criteria and were rotavirus positive, therefore the final study population was 13 for group A and 13 for group B.

The preparations of *L casei* strain GG were manufactured by the Research and Development Centre of Valio Ltd, Helsinki, Finland, as follows. *L casei* strain GG was concentrated, washed with water, and the concentrate divided into two. One half was heated to 85-100°C for 10 min. Saccharose was added as a cryoprotectant into both halves (about 50% of dry matter), and both preparations were lyophilised. The viable half, that was not heat treated, contained 1×10¹¹ cfu/g *L casei* strain GG, whereas the heat inactivated half did not contain living bacteria. Both preparations were divided into 0.1 g aliquots and stored at -18°C until reconstitution and administration in tap water (about 5 ml). The appearance and taste of these solutions were indistinguishable.

Heparinised blood was drawn for the enzyme linked immunospot (ELISPOT) assay during the acute phase (day 1 after admission) (n=12 in group A, n=11 in group B), and at follow up one month later (n=12 in group A, n=13 in group B). During diarrhoea measurement of the rotavirus specific response was possible in nine and eight patients, respectively. For rotavirus antibody studies paired sera were available from 11 patients in group A and six patients in group B.

The ELISPOT assays measuring the number of lymphocytes secreting immunoglobulins of isotypes IgM, IgA, and IgG (immunoglobulin secreting cells) and the number of lymphocytes secreting specific antibodies to rotavirus (specific antibody secreting cells) were carried out as described previously.³

Rotavirus IgA and IgG serum antibodies were measured using an enzyme linked immunosorbent assay (ELISA) method, which was a single serum dilution modification of the test described by Midthun *et al.*⁵ The antigen was a rhesus human reassortant rotavirus strain D×RRV (a reassortant between D

University of
Tampere, Department
of Clinical Medicine
M Kaila
E Isolauri

Department of
Biomedical Sciences
T Vesikari

Research and
Development Centre,
Valio Ltd, Helsinki
M Saxelin

National Public Health
Institute, Turku,
Finland
H Arvilommi

Correspondence to:
Dr M Kaila, Department of
Clinical Medicine, University
of Tampere, PO Box 607,
33101 Tampere, Finland.

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Table 1 Clinical description of the patients receiving viable (group A) or inactivated (group B) *L casei* strain GG on admission and on discharge; data are mean (SD)

	Group A (n=13)	Group B (n=13)	p Value*
On admission			
Age (months)	12.7 (4.8)	16.2 (8.5)	0.21
Duration of diarrhoea (days)	2.5 (1.4)	2.9 (2.0)	0.53
Weight (g)	9750 (2340)	10130 (2040)	0.66
Weight loss (g)	373 (123)	472 (218)	0.15
Serum sodium (mmol/l)	137 (5)	138 (5)	0.56
pH	7.35 (0.06)	7.35 (0.07)	0.88
On discharge			
Weight (g)	9870 (2100)	10450 (2100)	0.51
Mean (95% CI) duration of diarrhoea (days)	1.5 (1.0 to 2.2)	1.6 (1.1 to 2.3)	0.83

*Student's *t* test.

strain human rotavirus and rhesus rotavirus (RRV) grown in MA-104 cells; this virus has the VP7 serotype specificity of human rotavirus serotype 1. Briefly, microtitre plates were coated with hyperimmune rabbit anti-rotavirus serum (Dakopatts a/s) and subsequently with the supernatant of D×RRV infected MA-104 cell culture. The plates were blocked with 5% fetal bovine serum dilutions in phosphate buffer Tween. Single serum dilutions (1:50) were placed in duplicate wells. Alkaline phosphatase conjugated rabbit IgG to human α -chain and γ -chain (for IgA and IgG, respectively) followed by *p*-nitrophenyl phosphate substrate were added, and the absorbance read at 405 nm. Known positive and negative control sera were included. The results were expressed in enzyme immune units after comparison with positive and negative control sera. The value of a given serum was its percentage of the absorbance value of the positive reference serum corrected by background.

Rotavirus in stools was tested with an enzyme immunoassay (Rotazyme, Abbott Laboratories).

STATISTICAL ANALYSES

Because of skewed distribution of data natural logarithmic transformation was used, and the results are given as geometric means (95% confidence interval, CI). The Student's two tailed independent *t* test was used as appropriate. The χ^2 test was used in statistical comparisons.

Results

CLINICAL OUTCOME

The clinical characteristics on admission were consistent with mild to moderate dehydration and metabolic acidosis and did not differ between groups. The duration of diarrhoea after admission was short in both groups, and

Table 2 Number of patients showing a rotavirus specific IgA specific antibody secreting cell response, and the level of rotavirus specific IgA serum antibodies at the acute and convalescent stage of rotavirus gastroenteritis, in infants receiving viable (group A) or inactivated (group B) *L casei* strain GG

	Group A	Group B	p Value
Specific antibody secreting cells (No with >0.5/10 ⁶ cells/No of patients)			
Acute	2/9	2/8	0.66*
Convalescent	10/12	2/13	0.002*
Mean (95% CI) antibodies (EIU)			
Acute	0.04 (0.01 to 0.3)	0.1 (0.01 to 0.5)	0.52†
Convalescent	50.7 (29.4 to 87.2)	22.4 (11.7 to 43.0)	0.04†

*The χ^2 test; †Student's *t* test. EIU=enzyme immune units.

there was no difference between the patients receiving viable and inactivated *L casei* strain GG (table 1).

IMMUNOLOGICAL OUTCOME

At the acute stage, the concentrations of immunoglobulin secreting cells of isotypes IgM, IgA, and IgG were high in both treatment groups. There was a tendency to a greater response in group A than in group B, although the difference was not statistically significant. The transient nature of this response was demonstrated by a significant decrease in the number of immunoglobulin secreting cells by convalescence compared with values during diarrhoea; the two treatment groups were alike (data not shown).

During diarrhoea, the mean numbers of specific antibody secreting cells to rotavirus/10⁶ cells were similar in both groups (data not shown). The IgM and IgG specific antibody secreting cells to rotavirus could not be detected at convalescence in either treatment group.

Very low numbers of IgA secreting cells to rotavirus were seen during diarrhoea in both treatment groups alike. By convalescence, there was a 5.4-fold increase in the number of these cells in group A compared with group B, with a risk ratio of 5.4 (95% CI from 1.5 to 19.9). Most patients in group A, but not in group B, showed a detectable rotavirus specific antibody secreting cells response at the convalescent stage.

All patients had a detectable ELISA IgA response to rotavirus from the acute to the convalescent stage (table 2). The mean IgA antibody concentrations at the acute stage were low, and similar in the two groups. The infants in group A showed a more vigorous IgA serum antibody response to rotavirus and had higher antibody values at convalescence than infants in group B.

Discussion

A recent study showed that even in infants under the age of 6 months, a previously tolerated milk can safely be included in the diet during acute diarrhoea.⁶ Moreover, milk fermented with lactic acid bacteria has been shown to shorten the duration of acute diarrhoea.³ The usefulness of such milks in the diet during diarrhoea can be linked with viability of the lactic acid bacteria, and therefore their ability to survive the gut transit, to adhere to the mucosa, and transiently to colonise the gut. The adherent property is significant in enhancing the local immune response, and in stabilising the mucosal barrier to decrease inadvertent transmission of antigens from the gut.^{7,8} In contrast to the alleged importance of viability, immunostimulation and a beneficial clinical effect have also been demonstrated using a supernatant obtained after centrifugation of lactic acid bacteria.⁹

The most interesting result of the present study is that viable *L casei* strain GG enhanced

the rotavirus specific IgA secreting cell response and the serum IgA antibody response to rotavirus. Therefore, using viable *L casei* strain GG as an adjunct in the diet during acute rotavirus diarrhoea may promote development of immunity against reinfections.¹⁰ In this study, clinical recovery from rotavirus diarrhoea was equal in the two groups receiving viable or heat inactivated *L casei* strain GG during the diarrhoea. However, in developing countries using heat inactivated *L casei* strain GG might obviate the need to preserve viability of the bacteria, while maintaining the beneficial clinical effect.

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