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Effect of Administration of *Lactobacillus casei* Strain GG on the Gastrointestinal Microbiota of Newborns

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The aim of the study was to determine whether *Lactobacillus casei* strain GG could colonise the intestine of newborns and the influence of its administration on establishment of the microbiota. The faecal bacterial population of 25 under 1 mth old newborns was studied: in addition to breastfeeding, 15 babies (GG group) received for 2 wk immediately after birth *Lactobacillus* GG supplement as freeze-dried powder diluted in water in a dose of 10^{10}–10^{11} c.f.u./g; 10 newborns (control group) did not receive any supplement to breastfeeding. The faecal bacterial composition of meconium was similar in both groups studied. Ten newborns (67 per cent) excreted *Lactobacillus* GG, while in eight cases (53·3 per cent) *Lactobacillus* GG was found even 2 wk after the administration was stopped. The faecal concentrations and the relative proportion of *Lactobacillus* GG were individually different. In 3–4 d, 5–7 d and 1 mth old newborns of the GG group the faecal concentrations of lactobacilli exceeded those of the control group. The faecal microorganisms predominance pattern did not differ in the case of 1 wk and 1 mth old newborns of the GG group. The study shows that 2 wk administration of *Lactobacillus* GG, which starts right after birth, increases intestinal lactobacilli concentrations and does not impair the establishment of a normal faecal bacterial microbiota.

KEY WORDS—*Lactobacillus casei* GG; Neonatal faecal bacteria.

INTRODUCTION

The early intestinal colonisation by bacteria of newborns has been followed in several studies.[13,21,28] However, the factors that determine which bacterial strains persist in the infants' digestive tract are as yet not completely known.[24]

The aetiological relation between the incidence of pyoseptic infections and the disturbances of indigenous microbiota formation of newborns has been commented upon.[2,3] The protective function of the intestinal microbiota has been mainly associated with establishment of bifidobacteria in the newborn's intestine.[4,5,19] However, recently Hall and coworkers[9] have reported that lactobacilli may become an important part of the faecal microbiota in early infancy and that modern methods of neonatal care are associated with their delayed or deficient colonisation.

Together with the issue of enhancing the beneficial relationship between host and intestinal microbial populations, there is a renewed interest in ingestion of dairy products fermented by lactobacilli.[7,8,22] However, it has not been ascertained whether large doses of live microorganisms can disturb the establishment of the normal balance of gastrointestinal bacteria in newborns.

The *Lactobacillus casei* strain GG has successfully colonised the gastrointestinal tract of healthy adults and rotavirus sick infants.[12,23] The aim of the present study was to estimate the ability of *Lactobacillus* GG to colonise the intestine of healthy newborns and the influence of its administration on normal microbiota establishment.

SUBJECTS AND METHODS

Faecal samples of 25 random full-term infants born at Tampere University Hospital (Finland) were studied during their first month of life. The healthy neonates were recruited into the study within the first days of life after informed parental consent had been obtained.

The median birthweight of infants was 3590 g (range 2630–4110 g). They were all breast-fed during the first week of life. Of the 25 babies studied, 15

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received *Lactobacillus* GG supplement (GG group) and 10 did not get any supplementation to the breast feeding (control group). *Lactobacillus* GG was administered as a freeze-dried powder diluted in about 5 ml of water, as a dose of 10^{10}–10^{11} colony forming units (c.f.u.)/g during the first 2 wk of life. During the first month of life either breast feeding was continued or mixed feeding was introduced—breast milk plus formula (three cases of GG group). None of the newborns was treated with antibiotics nor had their mothers received any antibiotic treatment.

At first the meconium of 21 children was studied, then the faeces were repeatedly investigated on days 3–4, 5–7 and 28–32 in 15, 17 and 23 infants respectively.

Approximately 1 g of voided stool was collected into plastic containers by the ward staff, and later by the parents into a plastic container. In hospital it was immediately taken to the laboratory where it was kept at −20°C until investigated (maximally after 5 mth). At home the samples were put into a domestic refrigerator until being taken on the same day to the laboratory for study.

Bacteriological studies

The quantitative composition of faecal bacteria was estimated.[17] The weighed samples of faeces were serially diluted under a stream of CO_2 in prereduced phosphate buffer (pH 7·2). The faecal concentrations of bacteria ($\log_{10}$ c.f.u./g) were determined by seeding the serial dilutions on different freshly prepared media: MRS agar (Merck Diagnostics; pH 6·0) for lactobacilli and streptococci, Columbia anaerobe agar with 5 per cent propionic acid[1] for bifidobacteria, MacConkey agar for coliforms, FAST-agar (Lab M) for bacteroides.

For lactobacilli and streptococci the MRS plates were incubated for 72 h in 10 per cent CO_2 at 37°C. The plates for cultivation of bifidobacteria and bacteroids were incubated for up to 5 d in an anaerobic glovebox (N_2: 85 per cent, CO_2: 10 per cent, H_2: 5 per cent; Don Whitley Scientific, Shipley, UK). The MacConkey plates were incubated aerobically at 37°C for 24 h. The number of colonies from the last two emerging growth dilutions on different media were counted.

The isolated microorganisms were identified only to genus level. The coliforms were identified using standard methods (Kliegler-agar, oxidase-test), the anaerobes (bifidobacteria, bacteroides) from the last two dilutions' emerging growth were identified by colonial and cellular morphology and Gram's stain reaction after checking their inability to grow on blood agar medium in aerobic conditions, and the lactobacilli and streptococci were identified by their cellular morphology and negative catalase production.[11]

The relative proportion of different microorganisms was calculated as a percentage of the total concentration from each sample. Microorganisms were considered predominant if they made up more than 50 per cent of the total count.

Lactobacillus *GG estimation in faecal samples*

For *Lactobacillus* GG identification, colonial and cellular morphology was primarily helpful. The number of large, creamy white and convex colonies were counted if in Gram-stained smears uniform short gram-positive rods, grouped mainly in curved chains, were present. Also some physiological–biochemical characteristics of 5–10 typical colonies from each sample (altogether 132 estimations) were studied: the ability to grow at 15°C in 0·15 per cent agar containing MRS broth; the inability to ferment lactose in PY medium[11] with 1 per cent of lactose and chlor-phenol red indicator); the inability to produce gas from dextrose in MRS–agar medium with 1 per cent of dextrose and grow by 0·4 per cent teepol (Sigma, St Louis, MO, USA) containing MRS–agar medium.[14]

Statistical analysis

To compare the concentrations of microorganisms of GG group with those of the control group on different days of estimation, the Mann–Whitney's rank sum test (U-test) for unpaired data was used.

RESULTS

Colonisation by Lactobacillus *GG*

Lactobacillus GG was detected in the faeces of 10 of 15 newborns of the GG-group (67 per cent), but not in the other five (the detection level was 3·0 $\log_{10}$ c.f.u./g). From one newborn we repeatedly isolated an *L. casei* lactose non-fermenting strain, although according to the protocol of the study the child had not received the GG preparation.

L. casei strain GG was never recovered from meconium samples. In nine cases the *Lactobacillus* GG strain was isolated at the first week of life (Table 1) and in eight cases at the age of 1 mth (Table 1). *Lactobacillus* GG was present in seven newborns at

Table 1. Faecal concentrations of total lactobacilli and *Lactobacillus casei* strain GG in neonates ($\log_{10}$ c.f.u./g)

Individual neonates harbouring *L. casei* strain GG	3–7 d Total lactobacilli	3–7 d *L. casei* strain GG	28–31 d Total lactobacilli	28–31 d *L. casei* strain GG
1	8·5	8	9·4	9
2	5	4	9	8·9
3	8·7	5·6	4	0
4	5	0	8·8	8·8
5	6·9	6·9	9·5	9
6	8·4	8	8·9	0
7	8·7	8·5	9·5	9
8	9·6	8	8·6	7·5
9	8·5	8·4	8·4	7·4
10	8·7	8·4	9·8	8·5

least twice and only in three newborns in one single analysis.

The *Lactobacillus* GG counts proved individually different, their range at the age of 3–7 d being $\log_{10}$ 4·0–8·5, median $\log_{10}$ 8·0 c.f.u./g. These counts increased significantly at the age of 1 mth ($\log_{10}$ 7·4–9·0, median $\log_{10}$ 8·7 c.f.u./g, $P<0{\cdot}01$). The relative proportion of *Lactobacillus* GG to total lactobacilli was also individually different (range 0·8–100 per cent), and also varied in different samples of an individual.

Establishment of intestinal microbiota of neonates during the first week of life

In meconium the most frequently isolated groups of microorganisms were coliforms (in 26 per cent of neonates), bacteroides (27 per cent) and bifidobacteria (28 per cent). The maximal counts of coliforms ($\log_{10}$ 11·1 c.f.u./g) and bifidobacteria ($\log_{10}$ 10·1 c.f.u./g) exceeded that of bacteroides ($\log_{10}$ 9·1 c.f.u./g). Lactobacilli were seldom present (11 per cent). There was no significant difference in the faecal microbiota composition of meconium between the two study groups of neonates.

In 3–4 d old newborns of the GG group, the coliforms (67 per cent) and lactobacilli (89 per cent) were frequently present, and their concentrations significantly exceeded those observed in the control group (Figure 1). At the age of 5–7 d, the *Lactobacillus* GG group newborns had a higher level of lactobacilli and coliforms compared to the control group. At the end of the first month, the newborns of the *Lactobacillus* GG group were also more intensively colonised with bifidobacteria than the control group infants (Figure 1).

For the evaluation of the state of an individual's faecal bacterial microbiota, the predominant bacteria are described (Table 2). In different cases either bifidobacteria, bacteroides, lactobacilli and/or streptococci dominated. There was no significant difference in the predominant faecal bacteria to genus level between the *Lactobacillus* GG group and control group newborns at the age of 5–7 d and 1 mth. In no case was *Lactobacillus* GG the only predominant organism (Table 2).

DISCUSSION

In this study we have shown that *L. casei* strain GG is able to survive in the intestine of newborns if its oral administration is started soon after birth and continues during the first 2 wk of life, i.e. it could be recovered from the faecal samples of 10 of 15 (67 per cent) newborns who received the supplement. Furthermore, it persisted in eight infants (53·3 per cent) for at least 2 wk after cessation of supplementation.

As a rule, due to the active clearance mechanisms of the gastrointestinal microecosystem, artificially introduced strains of microorganisms are quickly eliminated from the gut. However, the persistence of *L. casei* GG meets a definition of colonisation.[26] It has been suggested that the microbes become part of the indigenous microbiota whose immunogenic capacity is low due to induction of tolerance towards the first contaminants of neonates.[6,10,25]

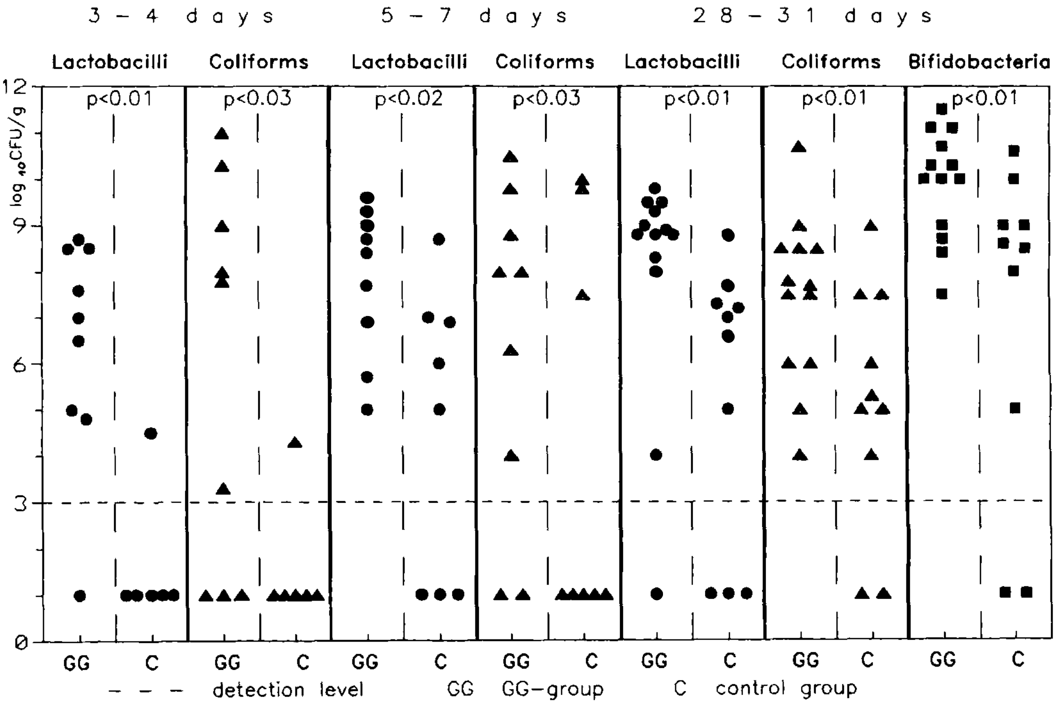

Figure 1. Significant changes in the intestinal microbiota of newborns after administration of *L. casei* strain GG

Table 2. Predominant microorganisms of faecal microbiota of newborns excreting *Lactobacillus* GG

Study group	Predominant microorganisms*		
	Bifidobacteria	Bacteroides	Lactobacilli
5–7 d			
GG-group ($n=9$)	5	1	—
Control-group ($n=8$)	4	1	—
28–31 d			
GG-group ($n=8$)	6	1	1†
Control-group ($n=10$)	4	2	1

*Predominant microorganisms represent cases where the concentrations of some microbes formed > 50 per cent of the total.

†Lactobacilli + streptococci were both present as equally predominant microbes.

The faecal concentrations of *L. casei* GG differed between individuals, varying from one newborn to another by a factor of nearly 10^5. The relative proportion of *L. casei* GG as a component of the total lactobacillus concentration also differed (from 0·8 per cent up to 100 per cent). This individual specificity of species and quantitative composition has previously been commented upon.[14,18] It has been suggested that the individuality of indigenous microorganisms can be explained in part by the genetic characteristics of the host[15,16] which determine the individually different composition of several human secretions,[20,27] which may serve as specific nutrients for intestinal microorganisms. The absence of colonisation with *Lactobacillus* GG in almost half of the newborns who received the supplement might be explained either by the unsuitability of a particular newborn's mother's secretions for the metabolism and growth of *L. casei* GG or by the individually specific immunoregulation of the intestinal microbiota by the host.[25] The possibility that *L. casei* GG persisted in those patients in a quantity below our detection level cannot be excluded.

In one newborn who did not receive supplement we repeatedly found lactobacilli resembling *L. casei* GG. Indeed, in some rare cases (in some 5 per cent of adults) lactose non-fermenting strains of *L. casei* may form the indigenous lactobacillus component of the faecal microbiota.[14] It cannot be excluded that the newborn obtained these microorganisms during cross-contamination in the hospital.

The early administration of *L. casei* GG influenced the newborn's intestinal microbiota. In the *L. casei* GG-group the concentrations of lactobacilli, bifidobacteria and coliforms were significantly higher than in the control group at the ages of 1 wk and 1 mth. However, the predominant populations of microbes (>50 per cent of the total) at the age of 1 mth were similar in both study groups. Up to the end of the first month there was a more frequent predominance of bifidobacteria in the *L. casei* GG-group infants.

This finding indicates that the large quantities of *L. casei* GG which were administered did not outcompete the indigenous microbiota of newborns. This is in accordance with the results of other authors, who failed to observe an inverse relationship between concentrations of lactobacilli and those of either bifidobacteria or coliforms.[9,19] However, we cannot agree with Hall and coworkers[9] who claim that lactobacilli form the predominant component of the microbiota of newborns.

Thus, the administration of live lactobacilli increased their concentration but did not alter establishment of the normal microbiota.

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