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Antagonistic Activity Exerted In Vitro and In Vivo by *Lactobacillus casei* (Strain GG) against *Salmonella typhimurium* C5 Infection

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The aim of this study was to compare the antagonistic properties of *Lactobacillus casei* GG exerted in vitro against *Salmonella typhimurium* C5 in a cellular model, cultured enterocyte-like Caco-2 cells, to those exerted in vivo in an animal model, C3H/He/Oujco mice. Our results show that a 1-h contact between the invading strain C5 and either the culture or the supernatant of *L. casei* GG impeded the invasion by the *Salmonella* strain in Caco-2 cells, without modifying the viability of the strain. After neutralization at pH 7, no inhibition of the invasion by C5 was observed. The antagonistic activity of *L. casei* GG was examined in C3H/He/Oujco mice orally infected with C5 as follows: (i) *L. casei* GG was given daily to conventional animals as a probiotic, and (ii) it was given once to germ-free animals in order to study the effect of the population of *L. casei* GG established in the different segments of the gut. In vivo experiments show that after a single challenge with C5, this strain survives and persists at a higher level in the feces of the untreated conventional mice than in those of the treated group. In *L. casei* GG germ-free mice, establishment of *L. casei* GG in the gut significantly delayed the occurrence of 100% mortality of the animals (15 days after C5 challenge versus 9 days in germ-free mice [$P < 0.01$]). Cecal colonization level and translocation rate of C5 to the mesenteric lymph nodes, spleen, and liver were significantly reduced during the first 2 days post-C5 challenge, although the *L. casei* GG population level in the gut dramatically decreased in these animals.

Recently, Klaenhammer (21) proposed that *Lactobacillus* strains with well-defined properties should be selected and characterized for specific use. Among *Lactobacillus* strains used in fermented milks, *Lactobacillus casei* GG has been shown to promote clinical recovery from acute rotavirus diarrhea in infants (17–20, 24) and from either antibiotic-associated or traveller's diarrhea in adults (27, 36). It has been previously reported that *L. casei* GG adheres to enterocyte-like cells in culture (11). It exerts an antagonistic effect against several bacteria and produces an unknown antimicrobial substance against *Escherichia coli* (37). But the mechanism of action of *L. casei* GG in vivo remains unknown. We have recently reported that selected, adhering human *L. acidophilus* strains inhibit both cell association and cell entry of a variety of enterovirulent bacteria within human cultured enterocyte-like Caco-2 cells (5–8). In the present work, we have compared the antagonistic effect observed in vitro in a cellular model (Caco-2 cells) to that observed in an in vivo animal model (C3H/He/Oujco mice). First, we studied whether *L. casei* GG can inhibit the invasion of cultured enterocyte-like Caco-2 cells by *Salmonella typhimurium* C5 (28). Second, the antagonistic activity of *L. casei* GG was examined in C3H/He/Oujco mice orally infected with *S. typhimurium* C5. Two experimental designs were used: (i) *L. casei* GG given daily to conventional animals as a probiotic, able to transit along the gut, and (ii) *L. casei* GG given once to germ-free animals in order to study the effect of the establishment of *L. casei* GG in the different segments of the gut.

MATERIALS AND METHODS

Bacterial strains. *S. typhimurium* C5 was kindly provided by M. Popoff (Institut Pasteur, Service des Enterobactéries, Paris, France) (28). It was grown on Luria broth for 18 h at 37°C. After centrifugation, the culture of *S. typhimurium* C5 was harvested in phosphate-buffered saline (PBS) and adjusted to the appropriate concentration in PBS (explained in the different assays). Viable bacteria were counted after plating of suitable dilutions on tryptic soy agar (TSA) (Difco, Detroit, Mich.) and incubation at 37°C for 18 h. *L. casei* GG was originally isolated from the fecal flora of a healthy human volunteer and was kindly supplied by Gorbach (13). It was grown on MRS broth (Difco) at pH 6.4 (9). After centrifugation, the bacteria were resuspended and adjusted to a concentration of 4×10^8 CFU/ml in supernatant. Spent culture supernatant of *L. casei* GG was sterilized by filtration (0.45-μm Millipore filters).

Mice. Both conventional (Iffa Credo, L'Arbresle, France) and germ-free animals were adult female C3H/He/Oujco mice of 7 to 8 weeks of age. Germ-free mice (Cesal, Orléans, France) were reared in Trexler-type isolators fitted with a rapid transfer system (La Calhène, Vélizy Villacoublay, France). They were given ad libitum a commercial diet, RO3 40 (UAR, Villemoisson/Orge, France), sterilized by gamma irradiation (40 kGy), and autoclaved demineralized water. Conventional mice were fed an identical but nonirradiated diet.

Caco-2 cell culture. Enterocyte-like Caco-2 cells (13, 30) were obtained from Alain Zweibaum (INSERM Unit 178, Villejuif, France). Cells were routinely grown in Dulbecco modified Eagle's minimal essential medium (25 mM glucose) (Eurobio, Paris, France) supplemented with 20% inactivated (30 min, 56°C) fetal calf serum (Boehringer, Mannheim, Germany) and 1% nonessential amino acids. For the adhesion assay of *L. casei* GG and for inhibition of cell association and cell invasion of *S. typhimurium*, monolayers of Caco-2 cells were prepared in six-well Corning tissue culture plates (Corning Glass Works, Corning, N.Y.). Cells were seeded at a concentration of 1.4×10^4 cells per cm^2. Maintenance of the cells and all experiments were carried out at 37°C in a 10% CO_2–90% air atmosphere. The culture medium was changed daily. Caco-2 cells were used after 60 to 90 cell passages. Cells were used for adherence assays at late postconfluence after 15 days in culture.

Infection of Caco-2 cells by *S. typhimurium* C5. The cell infection assay was conducted as previously reported (1, 7). Briefly, prior to infection, the Caco-2 monolayers were washed twice with PBS. One milliliter of a suspension of 2×10^8 CFU of C5 per ml in PBS and 1 ml of the cell culture medium were added to each well of the tissue culture plate. The plates were incubated for 60 min at 37°C in 10% CO_2–90% air and were then washed three times with sterile PBS. *S. typhimurium* internalization was determined by quantitative determination of bacteria located within the infected monolayers by the aminoglycoside antibiotic assay. After incubation, monolayers were washed twice with sterile PBS and afterwards incubated for 60 min in a medium containing 50 μg of gentamicin per ml. Bacteria adhering to the Caco-2 brush border were killed by this treatment, whereas those located within Caco-2 cells were not. The monolayers were washed three times with PBS and lysed with sterilized distilled water. Appropriate dilutions were plated on TSA to determine the number of viable intracellular

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bacteria by bacterial colony counts. Each assay was conducted in triplicate with three successive passages of Caco-2 cells.

Inhibition assays of *S. typhimurium* Caco-2 cell invasion. The inhibition of *S. typhimurium* C5 cell invasion by *L. casei* GG was determined by preincubating the pathogen as follows: 0.25-ml aliquots of 4×10^8 CFU of C5 per ml in PBS were incubated with 0.25-ml aliquots of each test sample and 0.5 ml of the Caco-2 cell culture medium for 1 h at 37°C. Cultured samples were brought to pH 7, as needed, with 1 to 3 drops of NaOH (5 N). Control experiments were conducted with PBS (pH 7). After centrifugation ($10,000 \times g$ for 7 min at 4°C), the pellet was resuspended with 1 ml of Caco-2 cell culture medium and with 1 ml of PBS and then counted in order to determine the number of surviving bacteria. Contact between the Caco-2 cells and the treated *S. typhimurium* and determination of the number of viable intracellular *S. typhimurium* organisms were conducted as described in the previous paragraph. After cell lysis, the *L. casei* GG organisms adhering to Caco-2 cells were counted in the appropriate dilutions, plated on MRS agar (pH 5.4).

Infection of mice. Conventional mice were infected with a single 0.2-ml dose of a known concentration of *S. typhimurium* C5 (10^8 CFU/ml for C3H mice) with a gastric probe. Prior to oral *Salmonella* infection, 0.2 ml of MRS broth or 0.2 ml of *L. casei* GG culture in MRS broth was given daily to the control group or to the *L. casei* GG-treated group at doses of 10^8 *L. casei* GG per mouse. The treatment was given daily for 7 days. The feces of the mice were individually collected after 1, 4, 7, and 11 days postinfection. Inoculation of C5 into germ-free animals was performed as follows: a single dose of 2×10^6 CFU per mouse in bottled water was given to the animals, who had been deprived of water since the day before. *L. casei* GG was inoculated into germ-free mice as a single dose of a 100-fold diluted fresh culture in bottled water, 1 week before challenge with C5.

Determination of *S. typhimurium* C5 in feces and tissues. Feces of *S. typhimurium* C5-infected conventional mice were individually collected after 1, 4, 7, and 11 days postinfection. The feces were weighed and diluted 10-fold in PBS. Viable C5 organisms were determined after serial 10-fold dilutions in PBS and plating on *Shigella* and *Salmonella* agar (SS agar medium; Difco) in order to differentiate *Salmonella* counts from those of the other enterobacteria. After incubation for 48 h at 37°C, colonies of *S. typhimurium* C5 were black and easy to distinguish from normal resident enterobacteria, which appear as red or pink colonies. Results were expressed as the mean concentration of viable bacteria (log CFU per gram of feces).

S. typhimurium C5-infected gnotobiotic mice were killed by cervical elongation. The mesenteric nodes were first collected, and the spleen and the liver were collected next. Then, a sterile swab was passed over the intestinal cavity in order to verify that the intestinal wall had not been damaged. This swab was soaked in 1 ml of PBS, which was poured in a plate with 15 ml of TSA. In cases of positive culture, the results for the corresponding mice were discarded. In order to determine the C5 level in the liver, spleen, and mesenteric nodes, the tissues were weighed, mixed with 2 ml of PBS by ULTRATURRAX for 2 min, and then diluted 10-fold. The dilutions were plated on TSA. Bacterial counts of C5 are given per gram of organ. The contents of the stomach, the small intestine, and the cecum were sampled. The small intestine was divided into three segments corresponding approximately to the duodenum, the jejunum, and the ileum.

Determination of *L. casei* GG in feces and tissues. Feces of *L. casei* GG-inoculated conventional or gnotobiotic mice were individually collected. The feces were weighed and diluted 10-fold in PBS. Fecal counts of *L. casei* GG were obtained by plating 0.1 ml of each 10-fold serial dilution on MRS agar (pH 5.4). The plates were incubated at 37°C for 48 h. Identification of *L. casei* GG in feces of conventional mice was performed as follows. On MRS agar, *L. casei* GG colonies are large, creamy white, and opaque, in contrast to the small transparent colonies which represented other lactobacillus strains. For each mouse, several colonies were picked up and further confirmation was obtained by additional Gram staining and sugar fermentation tests (37). For counting *L. casei* GG in *L. casei* GG-associated germ-free mice, the animals were killed 10 days after the inoculation of the *L. casei* GG strain. Intestinal contents and mesenteric lymph nodes were collected as described in the preceding paragraph for C5-associated mice, except that the medium used for the sterile swab was MRS agar. The contents of the stomach, the three small intestinal segments, the cecum, and the colon were also collected, weighed, and diluted 10-fold. The organs were then opened and gently washed with 10 successive, 5-ml sterile PBS aliquots. The tissues were drained, weighed, mixed with 2 ml of PBS by ULTRATURRAX for 2 min and then diluted 10-fold. Bacterial counts of *L. casei* GG are given per gram of organ, per gram of content, or per milliliter of the 10th wash with PBS.

Statistical analyses. Numbers of viable bacteria were compared by variance analysis in the form of the Student *t* test. Percentages of cumulative mortality were compared by the chi-square test as proposed by Yates (34).

RESULTS

Inhibition by *L. casei* GG of *S. typhimurium* cell invasion in cultured human enterocyte-like Caco-2 cells. Table 1 shows that the invasion of the Caco-2 cells by *S. typhimurium* C5 is strongly reduced when the pathogen has been in contact for 1 h with the culture or the filtered spent culture supernatant of *L. casei* GG, though the viability of the pathogen has not been significantly modified. When both the spent culture supernatant and the culture of *L. casei* GG were neutralized at pH 7, the number of *Salmonella* organisms invading cells was not different from that obtained with *Salmonella* organisms treated with neutralized MRS broth or with PBS. The adhesion of *L. casei* GG was similar with normal and neutralized cultures: 4.4 ± 0.3 and 4.2 ± 0.2 (log CFU/ml $\pm$ standard error of the mean [SEM]) per Caco-2 well, respectively. These results indicate that the filtered spent culture supernatant of *L. casei* GG modifies the ability of C5 to invade the enterocyte-like cells in vitro.

TABLE 1. Survival of *S. typhimurium* C5 after treatment with *L. casei* GG cultures and invasion in Caco-2 cells[a]

Treatment (no. of trials)	pH of samples	No. of bacteria	
		Surviving after 1 h of contact[b]	Invaded[c]
Control PBS (5)	7	8.3 ± 0.3	6 ± 0.2[d]
Culture of *L. casei* GG (5)	3.9 ± 0.1	8.2 ± 0.2	2.5 ± 0.5[e]
Neutralized culture of *L. casei* GG (4)	7	8.4 ± 0.1	6.2 ± 0.2[d]
Filtered supernatant of *L. casei* GG (5)	3.9 ± 0.1	8.2 ± 0.2	3.1 ± 0.2[e]
Neutralized filtered supernatant of *L. casei* GG (4)	7	8.4 ± 0	6.5 ± 0.1[d]
MRS broth (5)	4	8.4 ± 0.2	5.2 ± 0.2[f]
Neutralized MRS broth (4)	7	8.5 ± 0.2	6.3 ± 0.2[d]

[a] The C5 inoculum was 8.2 ± 0.1 (log CFU/ml).
[b] Bacteria which survived after a 1-h contact with each sample.
[c] Intracellular bacteria in the suspension of the lysed cells (see Materials and Methods).
[d] Significantly different from values with footnote *f* ($P < 0.05$).
[e] Significantly different from values with footnotes *d* and *f* ($P < 1.10^{-4}$).
[f] See footnotes *d* and *e*.

Effect of *L. casei* GG on the population level of *S. typhimurium* C5 organisms in infected conventional C3H/He/Oujco mice. The protective effect of *L. casei* GG against *Salmonella* infection was studied with an animal model, i.e., C3H/He/Oujco mice. The *S. typhimurium* C5 dose chosen to be given orally to the animals was 2.5×10^8 CFU per animal. *L. casei* GG was given daily to infected conventional animals as a probiotic able to transit along the gut. Figure 1 shows that the level of the viable C5 bacteria was lower in the *L. casei* GG-treated group of conventional mice than in the untreated group. The difference was significant at 4, 7, and 11 days postinoculation with C5. *L. casei* GG was detected in the feces of the animals at average levels of 5.7 ± 0.2 and 5 ± 0.3 at day 1 and day 7, respectively (log CFU/gram of feces $\pm$ SEM).

Population of *L. casei* GG in the intestinal contents and on the intestinal mucosa in *L. casei* GG-monoassociated C3H/He/Oujco mice (Fig. 2). In germ-free C3H mice, *L. casei* GG colonized the digestive tract. The population levels of GG were similar in the stomach and in the distal digestive tract (10^9 CFU/g). The number of *L. casei* GG organisms in aliquots of the last wash of each organ was always about 10^2 to 10^4 times lower than that remaining in the washed organ itself. This result indicates that by this technique, *L. casei* GG is associated with the mucosa of both the stomach and the intestine. This association to the mucosa increases from the proximal to the distal intestine. *L. casei* GG was also detected in the mesen-

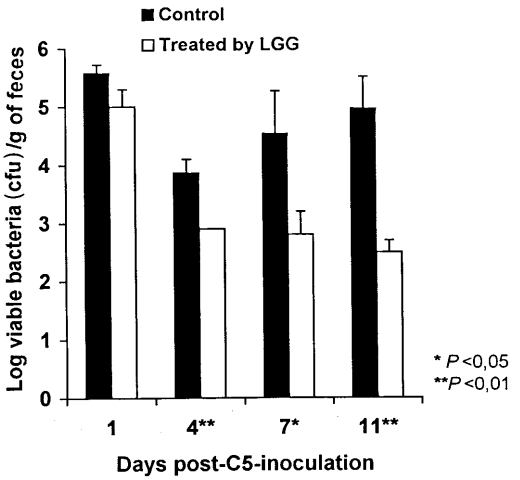

FIG. 1. Elimination of *S. typhimurium* C5 from conventional C3H/He/Oujco mice treated daily with *L. casei* GG. Each value is the mean ± SEM of the fecal counts obtained from six mice per group.

teric lymph nodes at about 100 bacteria per gram of organ, indicating a slight translocation of this strain.

Effect of *L. casei* GG on the population level of *S. typhimurium* C5 in infected germ-free C3H/He/Oujco mice. Germ-free C3H/He/Oujco mice were used to study the effect of *L. casei* GG when established at different levels of the gut. In one group, animals received *L. casei* GG as a single dose, 7 days before the inoculation of C5. *L. casei* GG bacteria were counted in the feces of each mouse the day before challenge with C5. According to this test, *L. casei* GG was established in the digestive tract at the level of 9.6 ± 0.2 (log CFU/g of feces ± SEM). Table 2 shows that the association with *L. casei* GG in germ-free C3H mice prolongs life in mice, since at 9 days post-C5 inoculation, all of the *L. casei* GG-associated mice survived while 100% of the nonassociated mice were already dead ($P < 0.01$, chi-square test). However, at 15 days, 100% cumulative mortality was reached for all the animals. At 1 day post-C5 inoculation, the population level of C5 in all the parts of the gut was lower, but it was not significantly different in treated and in nontreated mice. Figure 3A shows that cecum colonization levels were higher in nontreated mice than in GG-associated mice at 2 and 3 days postinoculation (P, <0.0001 and 0.02, respectively). Translocation of C5 in the mesenteric lymph nodes was also reduced at 1 day ($P < 0.05$) and at 2 days ($P < 0.01$) (Fig. 3A). It was significantly reduced in the liver and spleen at 2 days (P, <0.0001 and <0.001, respectively) (Fig. 3B). No significant difference between the two groups of mice could be observed later, until 9 days post-C5 inoculation, concerning gut colonization and translocation of *Salmonella.*

After the inoculation of *Salmonella*, the population of *L. casei* GG decreased dramatically, first in the stomach and then in the other parts of the gut (Fig. 4). It remained in the cecum

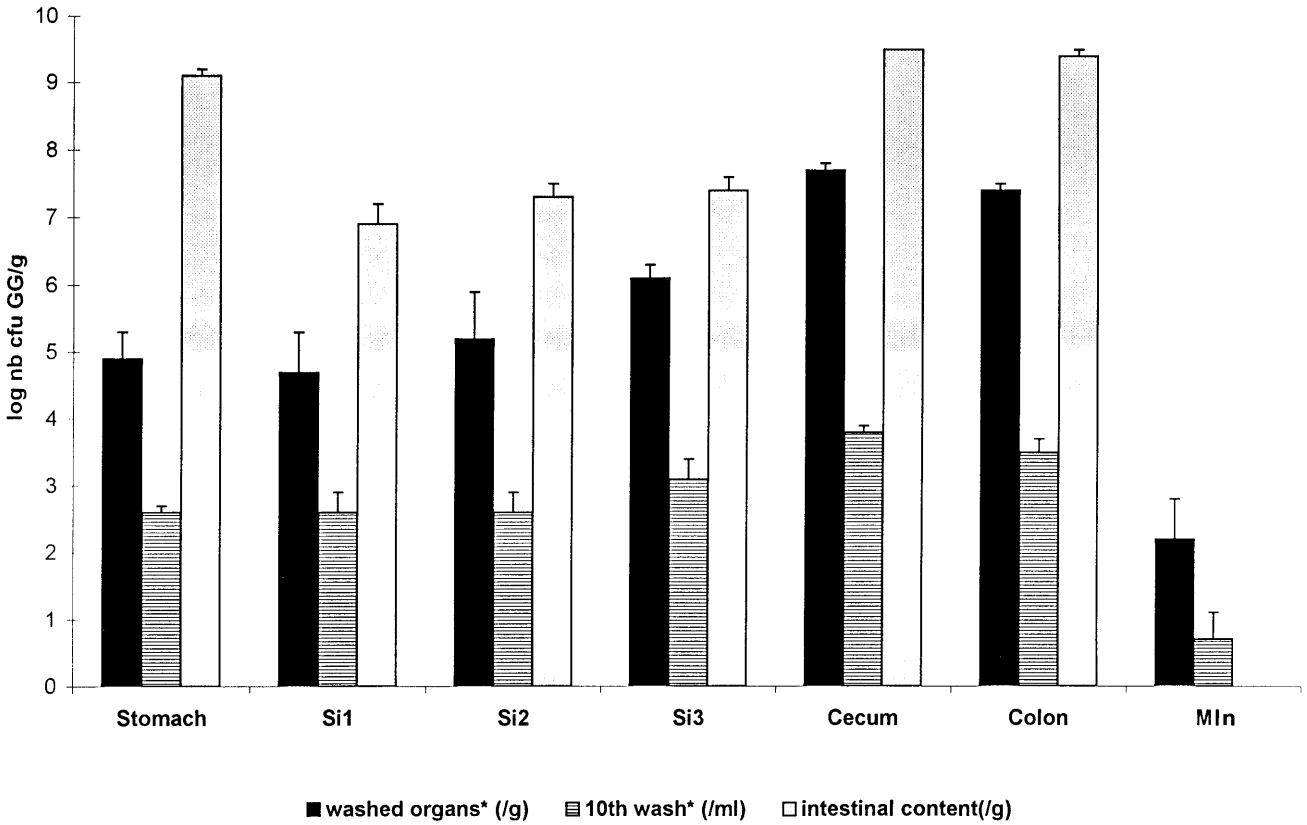

FIG. 2. Population of *L. casei* GG in the intestinal contents and on the intestinal mucosa of *L. casei* GG-monoassociated C3H/He/Oujco mice. Each bar represents the mean ± SEM for four mice per group. The mesenteric lymph nodes (MlN) were first removed. Second, the contents of the stomach; the three segments of the small intestine corresponding approximately to the duodenum, the jejunum and ileum; and the cecum and colon were also collected sampled. The organs were opened and gently washed with 10 successive 5-ml sterile PBS aliquots. Bacterial counts of *L. casei* GG are given per gram of washed organ, per gram of content, or per milliliter of the 10th wash with PBS.

TABLE 2. Mortality due to *S. typhimurium* C5 in germ-free C3H mice associated or not associated with *L. casei* GG

No. of days post-C5 inoculation[a]	No. of mice (%)[b]	
	Germ-free mice	Germ-free mice associated with *L. casei* GG
3	1 (17)	0
4	2 (33)	0
5	3 (50)	0
8	5 (83)	0
9	6 (100)[c]	0
12		4 (60)
15		6 (100)

[a] C5 was given in drinking water at 2.5×10^6 CFU per mouse; *L. casei* GG-monoassociated mice received *L. casei* GG 8 days before challenge with C5.
[b] Number of dead animals and percent cumulative mortality.
[c] Significantly different from the *L. casei* GG-monoassociated group ($P < 0.01$, chi-square test) (34).

between 2 and 9 days post-C5 inoculation at the level of 10^3 to 10^6 CFU/g.

DISCUSSION

It has been previously reported that *L. casei* GG has a protective effect in several clinical situations, e.g., with diarrhea due to rotavirus in infants (17–20, 24) and in traveller's diarrhea (27, 36). When this strain is given to infants, premature or not, it colonizes the intestinal tracts of more than 50% of the healthy full-term infants (25), but it does not decrease the potential reservoir of pathogens in them (25, 35). However, no evidence has been presented to date showing that the *L. casei* GG antimicrobial substance, found to be produced in vitro (37), is also produced in vivo and that the various beneficial effects mentioned above are indeed due to it.

We have previously described studies that show that adhesion of selected human *L. acidophilus* strains to Caco-2 cells leads to the inhibition of pathogen cell association and cell invasion in vitro (1, 5–8). Our results show that a culture containing *L. casei* GG, which adheres to the Caco-2 cells (11), exerts an antagonistic activity in vitro against *S. typhimurium* invading the cultured human enterocyte-like Caco-2 cells. Because the spent culture supernatant of *L. casei* GG contains an antimicrobial substance (37), we have examined whether the activity against the *S. typhimurium* Caco-2 cell infection is related to an antibacterial substance present in the spent culture supernatant of *L. casei* GG. We reported that the spent culture supernatant of *L. casei* GG is able to prevent the invasion of Caco-2 cells by C5, since the contact between C5 and the *L. casei* GG supernatant dramatically decreased the cell invasion rate of C5. Since the neutralized *L. casei* GG supernatant has no inhibitory effect, the activity of the *L. casei* GG supernatant could be related to the pH. However, our experiments show that the pH alone has no effect, since MRS broth at pH 4 had only a slight effect on the invasion rate of *S. typhimurium* C5. The mechanism of the antagonistic activity of *L. casei* GG seems to be dependent on an acidic environment, perhaps due to lactic acid itself or to a substance active at a low pH. Altogether, our results are in accordance with those of Silva et al. (37), showing that *L. casei* GG secretes into its culture supernatant an antimicrobial substance which develops its activity in the pH range from 3 to 5. Our results give additional information, since we show that a 1-h contact of C5 and the culture or the supernatant of *L. casei* GG modified the rate of invasion of the human cultured intestinal Caco-2 cells by the strain without modifying the viability of the pathogen.

We observed that in the nontreated conventional mice, in which many lactobacilli are attached to the squamous epithelium of the stomach (32), a high number of living C5 organisms are measured in the feces. In the conventional infected mice treated with the *L. casei* GG culture, the level of *S. typhimurium* C5 in the feces was decreased. This result means that *S. typhimurium* is able to survive and to persist at a higher level in the guts of control animals than in those of the treated group. Examining the activity of *L. casei* GG against *S. typhimurium* infection in vivo in the germ-free C3H mice, we find that our results show a significant diminution of the mortality. In the *L. casei* GG-associated germ-free animals, both the C5 colonization rate in the cecum and the translocation of C5 are reduced. One possible explanation is that the former effect is related to the competitive exclusion role of *L. casei* GG against C5, due to the antimicrobial substance produced by this strain. But this hypothesis remains to be demonstrated. The adhesion of *L. casei* GG to Caco-2 cells (11) has been said to provide

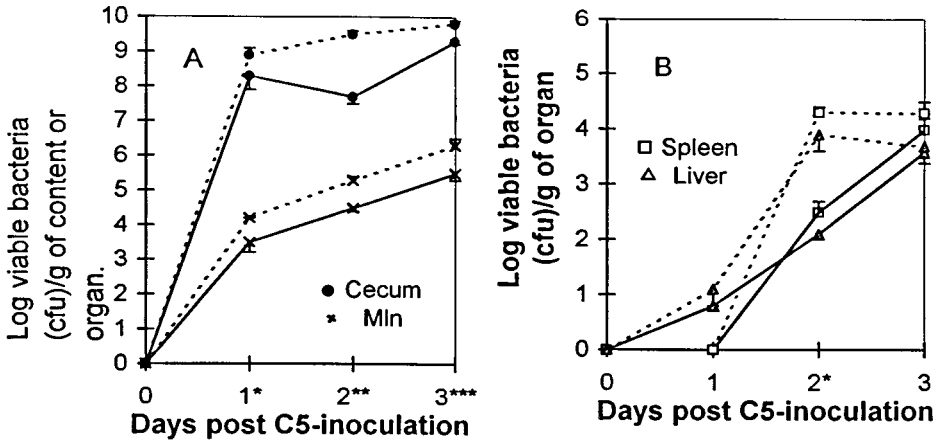

FIG. 3. Cecal colonization (A) and translocation rate (A and B) of *S. typhimurium* C5 in germ-free C3H/He/Oujco mice associated with *L. casei* GG. Each point represents the mean ± SEM for four mice per group. Dotted lines, germ-free animals; solid lines, monoassociated *L. casei* GG germ-free mice. (A) Cecum and mesenteric lymph nodes (Mln). *, Significantly reduced ($P < 0.05$) in Mln of *L. casei* GG-associated mice; **, significantly reduced in Mln ($P < 0.05$) and in the ceca ($P < 1.10^{-4}$) of *L. casei* GG-associated mice; ***, significantly reduced in the cecum ($P < 0.02$). (B) Liver and spleen. *, significantly reduced in the livers ($P < 1.10^{-4}$) and in the spleens ($P < 1.10^{-3}$) of *L. casei* GG-associated mice.

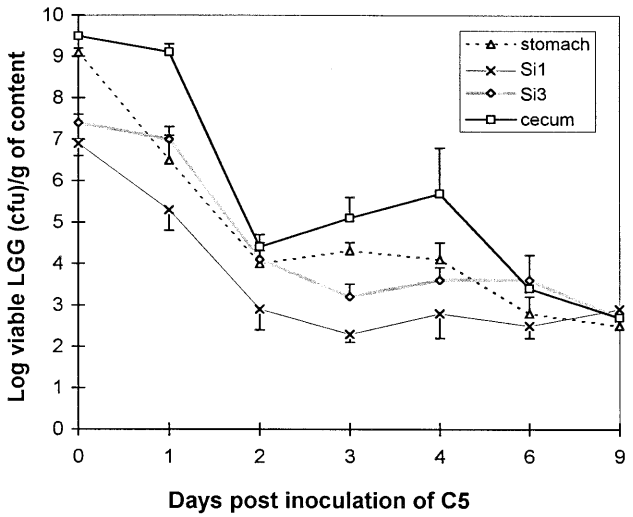

FIG. 4. Decrease in population of *L. casei* GG after inoculation of *S. typhimurium* C5 in guts of *L. casei* GG-monoassociated C3H/He/Oujco mice. Each point represents the mean ± SEM for four mice per group. The small intestine was divided in three segments, Si1, Si2, and Si3, corresponding approximately to the duodenum, the jejunum, and the ileum.

one explanation for the intestinal colonization ability of this strain. As a matter of fact, clinical studies show the persistence of the bacteria in fecal samples from human volunteers observed by Saxelin et al. (33), but the mechanism of this colonization remains unknown. Our results are in accordance with all of these observations, since we have shown that viable *L. casei* GG bacteria are recovered from the feces of conventional mice. The results shown in Fig. 2 further show that *L. casei* GG is associated with the intestinal wall in amounts progressively increasing from the stomachs to the colons of *L. casei* GG-monoassociated germ-free mice. However, despite a high colonization rate of *L. casei* GG in the intestines of the germ-free mice, the infection by *S. typhimurium* C5 led to a decrease of the colonization level of *L. casei* GG. Similar results have already been found with a strain of *L. casei* and a strain of *E. coli* in the gut of a human gnotobiotic infant (16). Our results indicate that the level of the remaining *L. casei* GG in the guts of the infected gnotobiotic mice correlates with a protective effect against *S. typhimurium* C5 infection, but the mechanism of action remains unknown.

Another mechanism could be postulated to explain the protective effect of *L. casei* GG. It has been reported previously that the adherent property is significant in enhancing the local immune response and in stabilizing the mucosal barrier to decrease inadvertant transmission of antigens from the gut (19). Recent results obtained by Kaila et al. (20) show that the level of the immune response in infants is higher with viable *L. casei* GG than with heat-killed *L. casei* GG, supporting the hypothesis that either adhesion or transient survival in the gut is necessary to stimulate the immune system. The diminution of the translocation rate could be the consequence of the diminution of the colonization rate in situ. The translocation rate could also be affected by the stimulation of the immune system after *L. casei* GG cell association and therefore decrease the number of C5 organisms able to survive inside the organism. In clinical cases of recovery from rotavirus gastroenteritis in which the diarrheal phase is shortened both by viable and by heat-killed *L. casei* GG, the improvement in the recovery could be related to the immune response against rotavirus, since an increase of serum antibodies, immunoglobulin-secreting cells, and specific antibody-secreting cells has been observed (19, 20, 24). It has been reported that other strains of *Lactobacillus* are able to stimulate the immune system in mice and humans (22, 23); Perdigon et al. (29) showed that the treatment of mice with an *L. casei* suspension for 3 days stimulated the immunoglobulin A anti-*Salmonella* antibodies and decreased the number of *S. typhimurium* organisms in the liver and the spleen. Moreover, other lactic bacteria, such as *Bifidobacterium bifidum*, have been shown to increase the immunological response of mice to antigens such as ovalbumin (26) and to shorten the diarrheal time due to rotavirus in suckling mice (10) and in infants (31).

It is well established that *S. typhimurium* invasiveness is multifactorial and that at least six distinct genetic loci are involved (2). *S. typhimurium* invades enterocyte-like Caco-2 cells (12). In vivo, the terminal ileum is the primary site of *S. typhimurium* invasion (4, 15); Peyer's patches and M cells of the small intestine are the first to be invaded (3). We have observed here that the main reservoirs of *L. casei* GG in mice are the stomach and cecum, whereas the ileum is colonized to a lesser degree. This discrepancy between the localization of the pathogen and *L. casei* GG could explain why monoassociated animals are not completely protected by the establishment of *L. casei* GG in the gut. In the case of *L. casei* GG, several cumulative mechanisms could be involved in the protective effect: (i) the slight decrease in the number of *S. typhimurium* organisms at the beginning of the infection, related to the presence of the antimicrobial substance or to lactic acid; (ii) the modification of the *S. typhimurium* surface, which decreases its ability to penetrate the intestinal cells; and (iii) the stimulation of the immunological system of the treated animals following the translocation of *L. casei* GG. Further experiments are required to establish the mechanism by which this strain affects directly *S. typhimurium* pathogenicity. In the future, the immunological aspects of the protective role of *L. casei* GG should be studied.

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