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Strain LB Against Nonrotavirus Diarrhea**

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Pediatrics 2007;120:e795-e803; originally published online Sep 3, 2007;
DOI: 10.1542/peds.2006-2930

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An Experimental Study and a Randomized, Double-Blind, Placebo-Controlled Clinical Trial to Evaluate the Antisecretory Activity of *Lactobacillus acidophilus* Strain LB Against Nonrotavirus Diarrhea

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Financial Disclosure: Laboratoire du Lactéol (Houdan, France) provided strain LB and batches of lyophilized, heat-killed LB bacteria plus their culture medium to Dr Servin and Lactéol Fort sachets and placebo sachets to Dr Sarrazin-Davila. Dr Liévin-Le Moal indicated she has no financial relationships relevant to this article to disclose.

ABSTRACT

OBJECTIVE. Previous studies have shown that selected strains of *Lactobacillus* have the capacity to antagonize rotavirus-induced diarrhea. However, only a few reports have documented their efficacy against nonrotavirus diarrhea. This study involved an experimental investigation and a clinical trial of the antisecretory activity of *Lactobacillus acidophilus* strain LB in the context of nonrotavirus diarrhea.

METHODS. The activity of a culture of *L. acidophilus* LB or of the lyophilized, heat-killed *L. acidophilus* LB bacteria plus their spent culture medium was tested in inhibiting the formation of fluid-formed domes in cultured human intestinal Caco-2/TC7 cell monolayers infected with diarrheagenic, diffusely adhering Afa/Dr *Escherichia coli* C1845 bacteria. A randomized, double-blind, placebo-controlled clinical trial of male or female children who were 10 months of age and presented with nonrotavirus, well-established diarrhea was conducted to evaluate the therapeutic efficacy of a pharmaceutical preparation that contains 10 billion heat-killed *L. acidophilus* LB plus 160 mg of spent culture medium.

RESULTS. Infection of the cells with C1845 bacteria that were treated with *L. acidophilus* LB culture or the lyophilized, heat-killed *L. acidophilus* LB bacteria plus their culture medium produced a dosage-dependent decrease in the number of fluid-formed domes as compared with cells that were infected with untreated C1845 bacteria. The clinical results show that in selected and controlled homogeneous groups of children with well-established, nonrotavirus diarrhea, adding lyophilized, heat-killed *L. acidophilus* LB bacteria plus their culture medium to a solution of oral rehydration solution shortened by 1 day the recovery time (ie, the time until the first normal stool was passed) as compared with children who received placebo oral rehydration solution.

CONCLUSIONS. Heat-killed *L. acidophilus* LB plus its culture medium antagonizes the C1845-induced increase in paracellular permeability in intestinal Caco-2/TC7 cells and produces a clinically significant benefit in the management of children with nonrotavirus, well-established diarrhea.

www.pediatrics.org/cgi/doi/10.1542/peds.2006-2930

doi:10.1542/peds.2006-2930

Key Words

children, nonrotavirus diarrhea, *Lactobacillus*, anti-secretory activity

Abbreviations

ORS—oral rehydration solution
DAEC—diffusely adhering *Escherichia coli*
PBS—phosphate-buffered saline
Sat—secreted autotransporter toxin
MRS—De Man, Rogosa, Sharpe
CFCS—cell-free culture supernatant
CFU—colony-forming units
LDH—lactate dehydrogenase

Accepted for publication Feb 23, 2007

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PEDIATRICS (ISSN Numbers: Print, 0031-4005; Online, 1098-4275). Copyright © 2007 by the American Academy of Pediatrics

IN DEVELOPING COUNTRIES, acute diarrhea is a major cause of morbidity and mortality, as a result of severe socioeconomic difficulties and the high prevalence of childhood malnutrition. Probiotic agents have been recently redefined as live microorganisms that, when consumed in adequate amounts as part of the diet, confer a health benefit to the host.¹ Clinical reports have documented the therapeutic efficacy of live, selected *Lactobacillus* strains used as probiotic agents against infectious diarrhea.^{2,3} In particular, *Lactobacillus rhamnosus* strain GG,⁴⁻¹⁰ *Lactobacillus casei* strain DN-114 001,^{11,12} and *Lactobacillus reuteri* strain 2112^{13,14} have demonstrated their therapeutic efficacy against diarrhea mainly of rotavirus origin. Van Niel et al¹⁵ and Szajewska et al¹⁶ conducted systematic reviews of published, randomized, double-blind, placebo-controlled trials on probiotic agents and concluded that the duration of rotavirus-induced diarrhea was significantly shorter in children who received live *Lactobacillus* strains than in those who received the placebo. Administering the nonprobiotic, lyophilized, heat-killed *Lactobacillus acidophilus* LB bacteria plus their spent culture medium in addition to oral rehydration solution (ORS) reduced the duration of rotavirus-induced, acute, watery diarrhea in children.^{17,18} However, it should be noted that probiotic agents have not shown any beneficial effect in children with severe disease.^{19,20}

In the past 10 years, it has been demonstrated using cellular and animal models that *L acidophilus* strain LB exhibits antibacterial activity.²¹⁻²⁶ This study involved clinical and experimental investigations of the antisecretory activity of *L acidophilus* strain LB in the context of nonrotavirus diarrhea. An experimental study was conducted to demonstrate the antisecretory activity of the heat-killed, lyophilized *L acidophilus* LB bacteria plus their spent culture medium, in a context of increased paracellular permeability leading to the inducing of fluid accumulation in cultured human intestinal Caco-2/TC7 cell monolayers by the diarrheagenic, diffusely adhering Afa/Dr *Escherichia coli* (Afa/Dr DAEC) strain C1845.²⁷ In addition, to assess the therapeutic efficacy of lyophilized, heat-killed *L acidophilus* LB bacteria plus their spent culture medium as an adjunct to ORS, we conducted a clinical trial in children who had nonrotavirus, well-established, acute diarrhea and were hospitalized at the Department of Pediatrics, Hospital Clínica Kennedy (Guayaquil, Ecuador).

METHODS

Bacterial Strains

The *L acidophilus* strain LB was provided by the Laboratoire du Lactéol (Houdan, France). The wild-type Afa/Dr DAEC strain C1845²⁷ was a gift of S. Moseley (Washington University, Seattle, WA). The recombinant *Escherichia coli* strain AAEC185 expressing the secreted auto-transporter toxin (Sat; AAEC185 *psat*) was used.²⁸ The

stock culture was maintained on 10% glycerol at -80°C . Before the experiments, the bacterial strain was transferred onto fresh Luria-Bertani agar (Difco Laboratories, Detroit, MI) and incubated at 37°C for 24 hours. For each experiment, bacteria were subcultured in Luria-Bertani broth (Difco Laboratories) at 37°C for 18 hours. For the experiment, the bacteria were washed 3 times with sterile phosphate-buffered saline (PBS) and recovered in an appropriate medium as described. Bacterial cells were counted in a Salubini chamber and then adjusted to the desired concentration. *L acidophilus* strain LB was grown in De Man, Rogosa, Sharpe (MRS) broth (Difco Laboratories) for 18 hours at 37°C . *Lactobacillus* cell-free culture supernatant (LB-CFCS) was obtained by centrifuging at $10\,000g$, for 30 minutes at 4°C . After being centrifuged, CFCS was passed through a sterile $0.22\text{-}\mu\text{m}$ Millex GS filter unit (Millipore, Molsheim, France). Batches of heated and lyophilized *L acidophilus* LB culture plus their spent culture medium were provided by Laboratoire du Lactéol. The lyophilized, heat-killed LB culture was reconstituted before use by dissolving 1000 mg of a batch preparation in 5 mL of sterile PBS.

Cell Culture

The cells used were human intestinal Caco-2/TC7 cells.²⁹ These cells were routinely grown using Dulbecco's modified Eagle's minimal essential medium (Invitrogen, Cergy-Pontoise, France; 25 mM glucose), supplemented with 15% heat-inactivated (30 minutes, 56°C) fetal calf serum (Invitrogen) and 1% nonessential amino acids. For maintenance purposes, cells were passaged weekly using 0.02% trypsin in $\text{Ca}^{2+}\text{Mg}^{2+}$ -free PBS that contained 3 mM EDTA. The experiments and cell maintenance were conducted at 37°C in an atmosphere of 10% $\text{CO}_2/90\%$ air. The culture medium was changed daily.

Cell Infection

For assays of infection, fully differentiated Caco-2/TC7 cell monolayers were used at postconfluence after 15 days in culture.²⁴ Briefly, the cell monolayers were washed twice with sterile PBS. Infecting bacteria were suspended in cell culture medium, and a total of 10^8 colony-forming units (CFU) per well of this suspension was added to each well of the tissue culture plate. The infection assay was conducted in the presence of 1% mannose to prevent type 1 fimbriae-mediated binding. The plates were incubated at 37°C in 10% $\text{CO}_2/90\%$ air for 3 hours. The monolayers were then washed 3 times with sterile PBS. Each assay was conducted in triplicate, with 3 successive passages of Caco-2/TC7 cells.

The inhibition of C1845-induced dome formation was determined by pretreating the bacteria (10^8 CFU/mL) for 1 hour with *L acidophilus* LB culture, or lyophilized, heat-killed LB culture, or not. The bacterial co-culture was centrifuged at $5500g$ for 5 minutes at 4°C , the cul-

ture medium was discarded, and the bacteria were washed once with sterile PBS before being resuspended in cell culture medium. Caco-2/TC7 monolayers were infected with untreated C1845 or *L. acidophilus* LB culture-treated or lyophilized, heat-killed LB culture-treated C1845 bacteria for 3 hours at 37°C in 10% CO₂/90% air.

Measurement of Cell Integrity

Cell integrity was determined by measuring the lactate dehydrogenase (LDH) activity in the culture medium (Enzyline LDH kit, Biomérieux, Dardilly, France) according to the manufacturer's instructions. Proteins were assayed by the bicinchonic acid protein assay. Results were expressed as milliunits of LDH activity per milligram of protein.

Bacterial Adhesion

For determination of the levels of cell-associated C1845 bacteria, infected Caco-2/TC7 cell monolayers were lysed with H₂O₂ for 10 minutes. Bacteria were suspended by vigorous pipetting, and bacterial CFU/mL of the lysates were determined by plating serially diluted aliquots on tryptic soil agar.

Determination of Fluid-Formed Domes in Caco-2/TC7 Cell Monolayers

For visualization of the domes that formed in control or infected Caco-2/TC7 cell monolayers, the cells were fixed, embedded in Epon, and then reembedded to cut sections perpendicular to the bottom of the flask. Semi-thin sections were examined by light microscopy. In addition, the number of domes was counted in 20 random microscopic fields per monolayer under phase-contrast microscopy. Domes were counted by 2 different technicians to eliminate bias.

Clinical Study

Children who were hospitalized at the Department of Pediatrics, Hospital Clínica Kennedy, were selected on the basis of their having acute diarrhea (at least 4 liquid stools passed during the last 24 hours) of suspected infectious origin (viral or bacterial) that had developed recently (<72 hours before inclusion). The severity of the diarrhea was assessed according to the following parameters: number of stools and their consistency, the presence or absence of mucus, bloating, pain, vomiting, dehydration, fever, and blood in stools. Rotavirus was detected by a latex agglutination test.

The study protocol was designed in accordance with the regulations that govern this type of study at the Hospital Clínica Kennedy and was approved by an internal review board. Parents or legal guardians of eligible children were informed, and their consent was obtained. The study was performed in accordance with the Decla-

ration of Helsinki. Ninety-three patients were screened, and 80 of them were enrolled. In accordance with World Health Organization guidelines,³⁰ all children enrolled received ORS (at least 100 mL/kg). The randomized, placebo-controlled, double-blind trial involved the use of the pharmaceutical preparation Lactéol Fort sachet (Laboratoire du Lactéol), which contains 10 billion heat-killed *L. acidophilus* LB plus 160 mg of spent culture medium. Placebo sachets, identical in appearance to the Lactéol Fort sachets, were specially prepared for this study and contained sucrose, ferrous oxides, silicic acid, and banana-orange flavoring but no LB bacteria or spent culture medium. The Lactéol Fort sachets and placebo sachets were provided free of charge for the study by Laboratoire du Lactéol. The patients were divided into 2 groups (*L. acidophilus* LB-ORS and placebo-ORS). The sachets were numerically coded to ensure sequential distribution. Both groups of patients received a 72-hour treatment that consisted of an initial dose of 2 sachets (*L. acidophilus* LB-ORS or placebo-ORS) and subsequently 1 sachet every 12 hours, with a total of 8 sachets ingested between meals. The final assessment was made after 96 hours and was based on the clinical signs displayed by the patients. The influence of the treatment on the duration of the disease was assessed in terms of recovery time, which was defined as the time from inclusion until the first normal stool was passed. For determination of this end point, the time and the consistency of every stool were recorded.

Statistical Analyses

For the experimental studies, Student's *t* test was used. For the clinical study, the statistical analyses performed depended on the treatment administered (*L. acidophilus* LB-ORS or placebo-ORS). All mean values are expressed as means \pm SD. The χ^2 test and a nonparametric test (Mann-Whitney) were used to compare the results obtained. We also performed a survival analysis on the main criterion, with a log rank test. The level of significance was 5%.

RESULTS

L. acidophilus LB Inhibits the C1845-Induced Increase in the Fluid Domes That Formed in Cultured Human Intestinal Caco-2/TC7 Monolayers

Cultured human intestinal Caco-2/TC7 cell monolayers form fluid-formed domes (henceforth referred to as fluid domes).³¹ This cell model can be used to evaluate anti-secretory activity. The wild-type Afa/Dr DAEC strain C1845, isolated from an infant who had diarrhea in Mexico,²⁷ was chosen for use as the test diarrheagenic bacterium inducing an increase in fluid accumulation, because Peiffer et al³² previously demonstrated that this enterovirulent *E. coli* strain increased the paracellular passage of fluid and electrolytes in Caco-2 cells. Inter-

ferential phase-contrast light microscopic examination of a Caco-2/TC7 cell monolayer reveals a fluid dome in C1845-infected cells (Fig 1A). Figure 1B shows that strain C1845 increased the number of fluid domes formed in Caco-2/TC7 cell monolayers. We wanted to

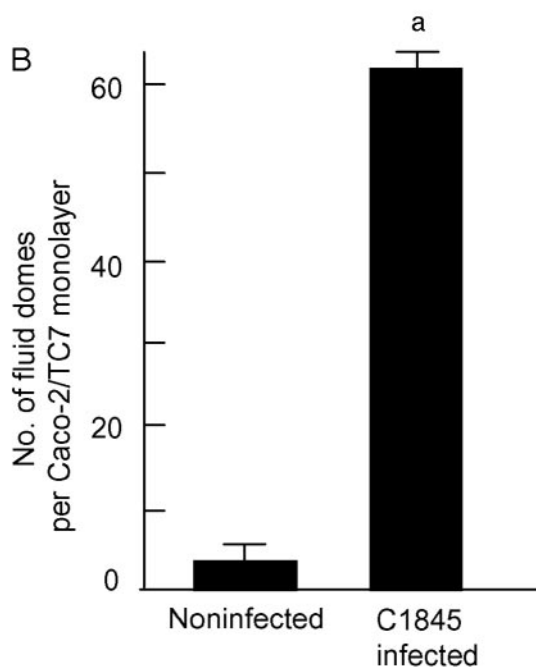
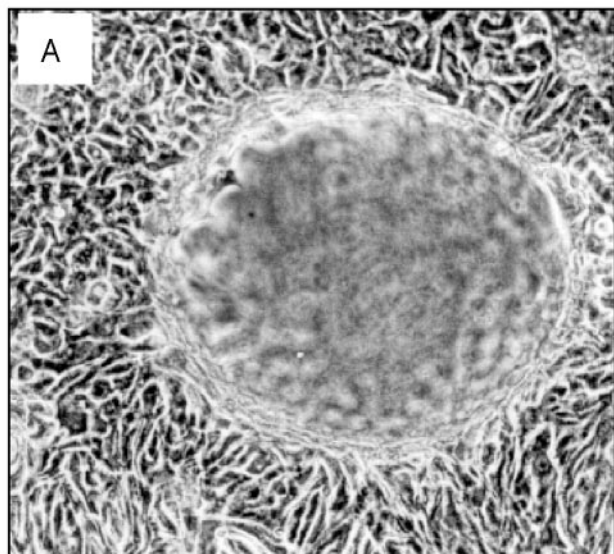
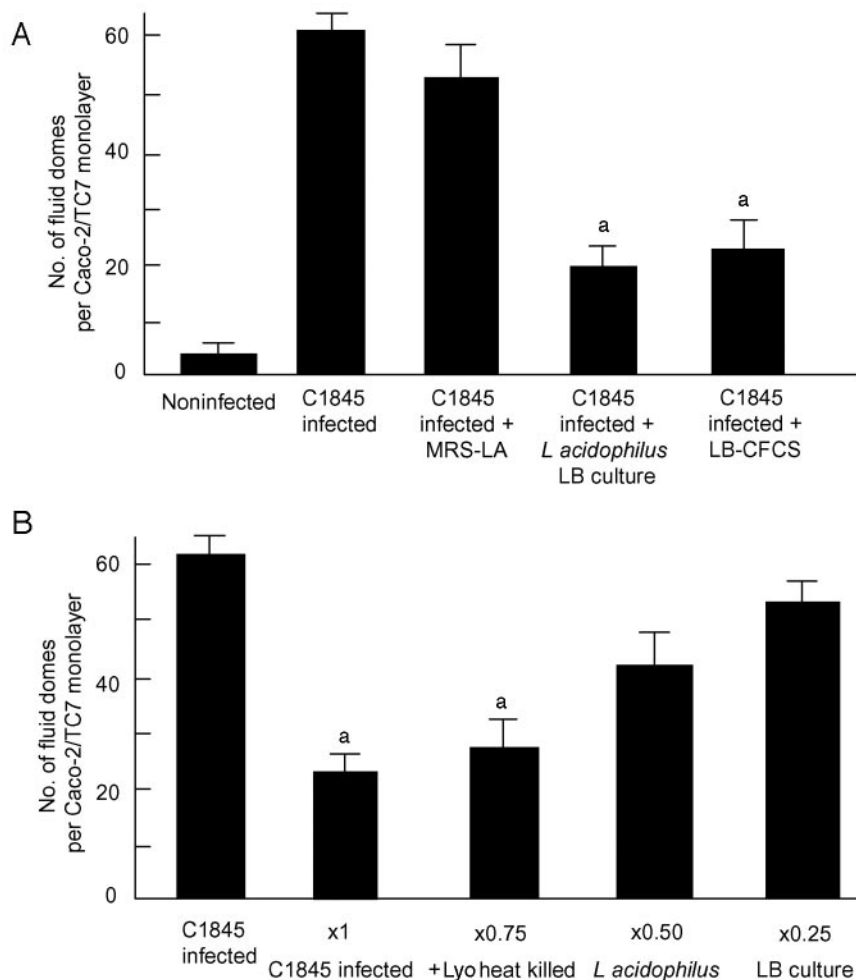


FIGURE 1 Increase in the number of fluid domes in C1845-infected Caco-2/TC7 monolayers. Caco-2/TC7 cell monolayers were infected with 10^8 CFU per well of C1845 bacteria for 3 hours at 37°C in 10% CO_2 /90% air. The fluid domes were counted in 20 randomly selected microscopy fields by examining monolayers by phase-contrast light microscopy. A, Fluid dome observed by phase-contrast microscopy in a C1845-infected Caco-2/TC7 monolayer (magnification: $\times 100$). B, Number of fluid domes formed in C1845-infected Caco-2/TC7 monolayers. Each value shown is the mean \pm SD from 3 experiments, each conducted in triplicate. ^a Values were significantly different than the noninfected value ($P < .01$).

find out whether the *L acidophilus* LB culture and/or the lyophilized, heat-killed LB culture would inhibit the C1845-induced increase in the number of fluid domes formed. Because *L acidophilus* LB cultures and heat-killed LB cultures are known to display killing activity against diarrheagenic Gram-negative bacteria,^{23–26} we used concentrations of the *L acidophilus* LB culture or the lyophilized, heat-killed LB culture with no killing activity (bacterial counts after 1 hour of contact C1845 + MRS: 8.20 ± 0.2 log; C1845 + *L acidophilus* LB culture: 8.02 ± 0.4 ; C1845 + lyophilized heat-killed LB culture: 8.11 ± 0.4 log CFU/mL). When the wild-type C1845 bacteria had been pretreated before being infected with the *L acidophilus* LB culture, a very significantly fewer fluid domes were observed in the infected cells than in cells that were infected with untreated, wild-type C1845 bacteria (Fig 2A). It was noted that no change in the number of fluid domes develops in noninfected cell monolayers that are treated with the *L acidophilus* strain LB. Treating the wild-type C1845 bacteria with MRS that contained lactic acid at the concentration found in the LB cultures (MRS-LA, 60 mM, pH 4.5) did not affect the number of C1845-induced domes (Fig 2A). A highly significant reduction in the number of fluid domes was observed in the cells that were infected with C1845 that had been pretreated before infection with the LB-CFCS (Fig 2A). The reduced number of fluid domes observed in the cells that were infected with LB-CFCS-treated C1845 was the same as that observed in the cells that were infected with the LB culture-treated C1845 bacteria (Fig 2A). When the wild-type C1845 bacteria had been pretreated for 1 hour with increasing concentrations of lyophilized, heat-killed LB culture, a dosage-dependent decrease in the number of fluid domes formed was observed (Fig 2B). Moreover, observation under light microscopy of semithin sections of Caco-2/TC7 cell monolayers that were infected with lyophilized, heat-killed LB culture-treated, wild-type C1845 showed that the fluid domes that were formed (Fig 3B) were much smaller than those that were formed in Caco-2/TC7 monolayers that were infected with untreated, wild-type C1845 bacteria (Fig 3A). To demonstrate that the blocking effect observed did not result from a reduction of the adhesiveness of the C1845 bacteria as a result of exposure to the *L acidophilus* LB bacteria, we checked the levels of C1845 bacteria that adhered to the cultured cells. We found that the levels of bacterial adhesion were the same for the untreated wild-type, C1845 bacteria; the C1845 bacteria that were treated with the *L acidophilus* LB culture; and the C1845 bacteria that were treated with lyophilized, heat-killed LB culture (untreated C1845: 7.45 ± 0.4 log CFU/mL; *L acidophilus* LB culture-treated C1845: 7.02 ± 0.7 ; lyophilized, heat-killed LB culture-treated C1845: 7.11 ± 0.6 log CFU/mL).

FIGURE 2

L. acidophilus LB culture and lyophilized, heat-killed LB culture reduced the number of fluid domes in C1845-infected Caco-2/TC7 cell monolayers. Before cell infection, the C1845 bacteria (10^8 CFU per well) were exposed for 1 hour at 37°C to *L. acidophilus* LB culture or to increasing concentrations of lyophilized, heat-killed LB culture. The cell monolayers were infected and processed for phase-contrast light microscopy as described for Fig 1. A, Number of fluid domes formed in Caco-2/TC7 monolayers that were infected with LB culture-treated C1845 bacteria. B, Number of fluid domes formed in Caco-2/TC7 monolayers that were infected with C1845 treated with increasing concentrations of lyophilized, heat-killed LB culture. Each value shown is the mean \pm SD from 3 experiments, each conducted in triplicate. ^a Values were significantly different from those for the C1845-infected cells ($P < .01$).



L. acidophilus LB Inhibits the Sat-Induced Increase in Fluid Domes in Cultured Human Intestinal Caco-2/TC7 Monolayers

Bacterial pathogens promote changes in fluid and ion transport, leading to diarrhea, mainly as a result of their toxins.^{33,34} Our group recently reported that the Sat expressed by Afa/Dr DAEC, including strain C1845, increases the paracellular passage of fluids and nonionic macromolecules in monolayers of Caco-2/TC7 cells.²⁸ An experiment was conducted to find out whether the blockade of the wild-type C1845 induced an increase in paracellular permeability by *L. acidophilus* LB culture or by the lyophilized, heat-killed LB culture was attributable to inhibition of the Sat-induced effect. To do this, Caco-2/TC7 cell monolayers were infected with the recombinant *E. coli* AAEC185 *psat*. This recombinant *E. coli* lacks adhesive factor³⁵ and so did not adhere to the Caco-2/TC7 cells and is known to secrete the Sat.²⁸ As for the C845, the *E. coli* AAEC1845 *psat* was pretreated before cell infection with or without the *L. acidophilus* LB culture or the lyophilized, heat-killed LB culture. Counting the fluid domes formed showed that there were significantly fewer domes in the *E. coli* AAEC185 *psat*-infected Caco-2/TC7 monolayers in the presence of

L. acidophilus LB culture or lyophilized, heat-killed LB than in cells that were infected with *E. coli* AAEC185 *psat* alone (Fig 4).

L. acidophilus LB Ameliorates the Outcome of Nonrotavirus Diarrhea in Children From Ecuador

The antisecretory effect of *L. acidophilus* LB reported could be just an in vitro effect. Because of the high human specificity of the virulence factors expressed by the Afa/Dr DAEC strains,³⁶ it was impossible to use an animal model to examine the antisecretory effect of the *L. acidophilus* LB culture or lyophilized, heat-killed LB. So to evaluate the in vivo efficacy of *L. acidophilus* strain LB plus its spent culture medium, we conducted a double-blind, placebo-controlled, and randomized study in a population of children who had nonrotavirus, acute, watery diarrhea and were hospitalized at the Department of Pediatrics, Hospital Clínica Kennedy. This clinical study investigated the effect of the lyophilized, heat-killed *L. acidophilus* LB bacteria plus its spent culture medium as an adjunct to ORS. In total, 93 children who had well-established diarrhea (<24 hours) were screened for enrollment in the study (Table 1). The

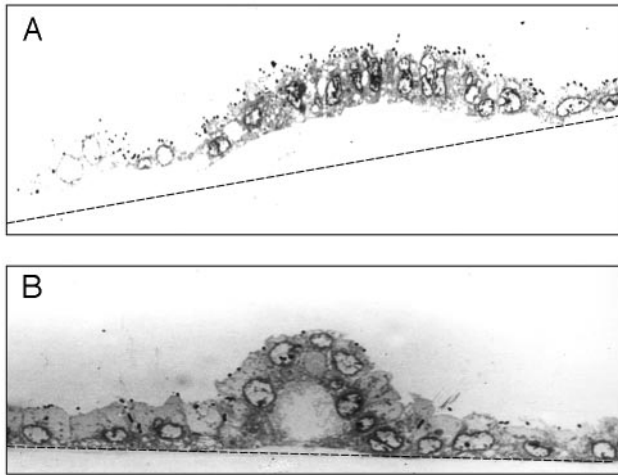


FIGURE 3
 Observation of fluid domes in Caco-2/TC7 cell monolayers that were infected with C1845 bacteria or infected with lyophilized, heat-killed LB culture-treated C1845 bacteria. Cell monolayers were processed for light-microscopic examination of semithin sections perpendicular to the bottom of the flask (magnification: $\times 100$). A, Fluid dome in the Caco-2/TC7 cell monolayer infected with C1845 bacteria. B, Fluid dome in the Caco-2/TC7 cell monolayer infected with lyophilized, heat-killed LB culture-treated C1845 bacteria. The hatched line indicates the bottom of the culture cell. The micrographs are representative of 2 experiments.

infection status showed that only 12 of the children screened were positive for rotavirus infection, agreeing with previous epidemiologic studies in Ecuador.^{37–39} The 12 children who were positive for rotavirus were not subsequently enrolled, and 1 other child was not enrolled because the parents did not give their consent. The 80 children who fulfilled the study criteria were assigned to treatment groups (Table 2). Our findings show that when administered in addition to ORS, the lyophilized, heat-killed *L acidophilus* LB bacteria plus its spent culture medium led to a significant reduction in the duration of diarrhea (Table 3). Indeed, the diarrhea lasted 1 day less in the group of infants who received the *L acidophilus* LB-ORS than in the placebo-ORS group. The diarrhea was resolved in a significantly higher proportion of children in the *L acidophilus*-ORS group than in the placebo-ORS group. Moreover, examination of the consistency of the stools showed that for the children in the *L acidophilus* LB-ORS group, the stools were formed, whereas those of the children of the placebo-ORS group tended to remain fluid or semifluid.

DISCUSSION

Very little information is available about the potential role of probiotic agents in nonrotavirus diarrheal episodes. We chose to investigate here the antisecretory activity of the *L acidophilus* strain LB against the Afa/Dr DAEC strain C1845, which belongs to class 6 of human enterovirulent *E coli*.⁴⁰ Epidemiologic studies have demonstrated an age-related incidence for Afa/Dr DAEC in diarrhea in children.^{41–45} At the brush border of enterocytes, Afa/Dr DAEC recognizes the human decay accel-

erating factor (CD55) and the human carcinoembryonic antigen (CD66e) as receptors. It has been previously reported that wild-type Afa/Dr DAEC strain C1845 promotes structural and functional lesions in the brush border, opens the junctional domain of polarized cells, and induces a proinflammatory response.³⁶ In particular, it has been demonstrated that Afa/Dr DAEC increases the paracellular permeability in human intestinal Caco-2/TC7 cells that form monolayers in culture and that this in turn promotes an increase in the formation of fluid domes as a result of an accumulation of fluids.^{28,32} Here, using the Caco-2/TC7 cells, we demonstrate that the live *L acidophilus* strain LB, LB-CFCS, and the lyophilized, heat-killed *L acidophilus* LB bacteria plus their culture medium exhibited antisecretory activity. Indeed, after treatment of the pathogen with *L acidophilus* LB culture, LB-CFCS, or the lyophilized, heat-killed *L acidophilus* LB bacteria plus their culture medium, we observed a decrease in the C1845-induced formation of fluid domes. Similar activity was previously reported for a few number of probiotic *Lactobacillus* strains. For example, *L casei* strain DN-114 001 is able to reduce the increase in paracellular permeability induced by the prototype EPEC strain E2348/69 in monolayer-forming, cultured, human, intestinal T84 colonic cells.⁴⁶ *Lactobacillus plantarum* strain 299v abolished the *E coli*-induced increase in intestinal permeability in rats.⁴⁷ However, the mechanism(s) of action supporting the inhibitory activity of these probiotic strains remain(s) to be determined.

Diarrheagenic bacteria are known to act by various mechanisms to induce a common intestinal injury characterized by a dramatic increase in the secretion of fluid and electrolytes.^{33,34} We conducted experiments to identify the mechanism by which *L acidophilus* strain LB blocks the C1845-induced increase in fluid domes. The wild-type strain C1845 expresses a toxin, known as Sat,⁴⁸ that displays enterotoxic effects that are characterized by a pronounced accumulation of intestinal fluid⁴⁹ and an increase in the paracellular permeability of Caco-2/TC7 cells.²⁸ We show that both the live *L acidophilus* strain LB and the lyophilized, heat-killed *L acidophilus* LB bacteria plus their culture medium inhibit the increase in the number of fluid domes in Caco-2 cell monolayers that are infected with a recombinant strain of *E coli* AAEC185, that secretes Sat.²⁸ It is tempting to hypothesize that the molecule(s) present in the CFCS of the *L acidophilus* strain LB could block the secretion of the Sat by the bacteria. Another hypothesis is that the molecule(s) present in the CFCS of the *L acidophilus* strain LB block the Sat-induced signaling necessary for the changes in the organization of the junctional domain that controls paracellular passage in monolayers of polarized cells.²⁸ We are attempting to demonstrate the mechanism that uses the molecule(s) present in the CFCS of the *L acidophilus* strain LB.

As has recently been pointed out,^{2,3} it is difficult to

FIGURE 4

L. acidophilus LB culture and lyophilized, heat-killed LB culture reduced the number of fluid domes formed in Caco-2/TC7 cell monolayers that were infected with the recombinant *E. coli* AAEC185 expressing the *sat* gene encoding for the Sat. Caco-2/TC7 cell monolayers were infected with untreated, *L. acidophilus* LB culture–treated or lyophilized, heat-killed LB culture–treated *E. coli* AAEC185 *psat* (10^8 CFU per well) for 3 hours at 37°C in 10% CO₂/90% air. The cell monolayers were processed for counting the fluid domes as described for Fig 1. Each value shown is the mean \pm SD from 3 experiments, each conducted in duplicate. ^a Values were significantly different from those for the C1845-infected cells ($P < .01$).

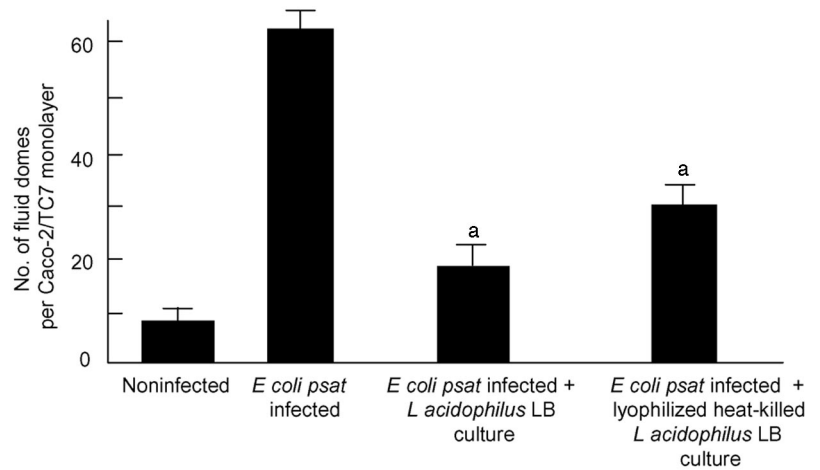


TABLE 1 Clinical Characteristics of the Children Screened

| Characteristic | Value |
|--|-------------|
| Total, <i>n</i> | 93 |
| Gender (male/female), <i>n</i> | 45/48 |
| Age, mo | |
| 1–12 | 59 |
| 13–24 | 34 |
| Age, mean (SD), mo | 11.0 (6.1) |
| Body weight, mean (SD), g | 8591 (2558) |
| Illness duration (SD), d | 2.1 (0.6) |
| Stool frequency, mean (SD) | 5.75 (2.2) |
| Stool consistency, <i>n</i> | |
| Lumpy | 5 |
| Semifluid | 27 |
| Fluid | 61 |
| Mucus absent/present, <i>n</i> | 62/31 |
| Bloating, <i>n</i> | |
| None or slight | 61 |
| Moderate | 18 |
| Intense | 14 |
| Pain, <i>n</i> | |
| None or slight | 69 |
| Moderate | 14 |
| Intense | 10 |
| Vomiting absent/present, <i>n</i> | 47/46 |
| Degree of dehydration, <i>n</i> | |
| Grade 0 | 75 |
| Grade I | 17 |
| Grade II | 1 |
| Fever, <i>n</i> | |
| None | 5 |
| 37.6°C–38.5°C | 64 |
| 38.6°C–39.5°C | 24 |
| Blood in stools absent/present, <i>n</i> | 77/16 |
| Rotavirus-positive, <i>n</i> (%) | 12 (12.5) |

TABLE 2 Clinical Characteristics of the Children Enrolled

| Characteristic | <i>L. acidophilus</i> LB-ORS (<i>n</i> = 42) | Placebo-ORS (<i>n</i> = 38) | <i>P</i> |
|-----------------------------------|--|---------------------------------|----------|
| Gender, <i>n</i> | | | |
| Male | 20 | 16 | .621 |
| Female | 22 | 22 | |
| Age, mean (SD), mo | 10.2 (5.3) | 10.1 (6.4) | .918 |
| Body weight, mean (SD), g | 8542 (2140) | 8138 (3003) | .383 |
| Duration of illness, mean (SD), d | 2.1 (0.6) | 2.1 (0.7) | .524 |
| Stool frequency, mean (SD) | 5.1 (1.9) | 5.9 (2.3) | .097 |
| Stool consistency, <i>n</i> | | | |
| Lumpy | 3 | 2 | .205 |
| Semifluid | 8 | 14 | |
| Fluid | 31 | 22 | |
| Bloating, <i>n</i> | | | |
| None or slight | 30 | 25 | .851 |
| Moderate | 7 | 8 | |
| Intense | 5 | 5 | |
| Pain, <i>n</i> | | | |
| None or slight | 33 | 29 | .619 |
| Moderate | 6 | 4 | |
| Intense | 3 | 5 | |
| Vomiting absent/present, <i>n</i> | 30/12 | 14/23 | .002 |
| Dehydration status, <i>n</i> | | | |
| Grade 0 | 38 | 30 | .277 |
| Grade I | 4 | 7 | |
| Grade II | 0 | 1 | |
| Fever, <i>n</i> | | | |
| None | 5 | 0 | .080 |
| 37.6°C–38.5°C | 29 | 28 | |
| 38.6°C–39.5°C | 8 | 10 | |

extrapolate from in vitro effects to the in vivo gastrointestinal situation. The resident microbiota and mucus and the peristaltic flow that continuously washes over the gastrointestinal epithelium could considerably modify or even abolish the effects of exogenous probiotic lactic acid bacteria and/or their active metabolites. We therefore conducted a clinical trial to evaluate the clin-

ical efficacy of the heat-killed *L. acidophilus* LB bacteria plus their culture medium against nonrotavirus diarrhea. Our results show that when administered concomitantly with ORS, the lyophilized, heat-killed *L. acidophilus* LB bacteria plus their culture medium were able to halve the duration of diarrhea in children who had nonrotavirus diarrhea. The shortening by 1 day of the recovery time from diarrhea was statistically significant, despite the relatively small number of infants in our study. A similar finding was recently reported, showing

TABLE 3 Comparison of Outcome Measures of All Children Treated With *L acidophilus* LB-ORS or Placebo-ORS

| Parameter | <i>L acidophilus</i> LB-ORS (n = 42) | Placebo-ORS (n = 38) | P |
|----------------------------------|---|-------------------------|------|
| Duration of diarrhea, h | 39.5 ± 10.5 | 63.4 ± 14.9 | <.01 |
| Cessation of diarrhea, yes/no, n | 36/6 | 20/18 | |

that in a subgroup in a randomized, double-blind, placebo-controlled clinical trial, conducted in infants and young children who were 4 to 24 months of age and presented with diarrhea, the live *Lactobacillus paracasei* strain ST11 significantly shortened the duration of non-rotavirus-induced diarrhea but had no effect against the rotavirus-induced diarrhea.²⁰

CONCLUSIONS

We have shown experimentally that *L acidophilus* LB culture or heat-killed *L acidophilus* LB bacteria plus spent culture medium were able to induce dosage-dependent blockade of the C1845-induced increase in the number of fluid domes in cultured, human intestinal Caco-2/TC7 cells. We have also shown that the lyophilized, heat-killed *L acidophilus* LB bacteria plus spent culture medium reduced the duration of nonrotavirus diarrhea in children who had bacteria-induced diarrhea. It is interesting that it was noted that the antisecretory effect observed was attributable to heat-resistant molecules that were present in the CFCS of the *L acidophilus* strain LB, because the lyophilized, heat-killed *L acidophilus* LB bacteria plus spent culture medium had the same effect as the *L acidophilus* LB culture. This is an important finding, because the variable storage quality of live probiotic cultures results in unpredictable losses of dose and activity in the preparations administered to patients with acute watery diarrhea, and this is a technologic problem that is difficult to solve, particularly in developing countries. Lyophilized, heat-killed *L acidophilus* LB bacteria plus spent culture medium has the advantage of providing a potentially stable pharmaceutical preparation with reproducible activity.

ACKNOWLEDGMENTS

We are grateful to R. Amsellem for assistance with the cell cultures. We are also indebted to J. Guignot for the generous gift of recombinant *E coli* AAEC185 strain expressing the *sat* gene.

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An Experimental Study and a Randomized, Double-Blind, Placebo-Controlled Clinical Trial to Evaluate the Antisecretory Activity of *Lactobacillus acidophilus* Strain LB Against Nonrotavirus Diarrhea

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Pediatrics 2007;120:e795-e803; originally published online Sep 3, 2007;
DOI: 10.1542/peds.2006-2930

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