Antimicrobial Activity of Intraurethrally Administered Probiotic 
*Lactobacillus casei* in a Murine Model of *Escherichia coli* Urinary Tract Infection

TAKASHI ASAHARA, KOJI NOMOTO,* MASAAKI WATANUKI, AND TERUO YOKOKURA

Yakult Central Institute for Microbiological Research, Kunitachi, Tokyo 186-8650, Japan

Received 8 September 2000/Returned for modification 21 December 2000/Accepted 2 March 2001

The antimicrobial activity of the intraurethrally administered probiotic *Lactobacillus casei* strain Shirota against *Escherichia coli* in a murine urinary tract infection (UTI) model was examined. UTI was induced by intraurethral administration of *Escherichia coli* strain HU-1 (a clinical isolate from a UTI patient, positive for type 1 and P fimbriae), at a dose of $1 \times 10^6$ to $2 \times 10^6$ CFU in 20 ml of saline, into a C3H/HeN mouse bladder, which had been traumatized with 0.1 N HCl followed immediately by neutralization with 0.1 N NaOH 24 h before the challenge infection. Chronic infection with the pathogen at $10^6$ CFU in the urinary tract (bladder and kidneys) was maintained for more than 3 weeks after the challenge, and the number of polymorphonuclear leukocytes and myeloperoxidase activity in the urine were markedly elevated during the infection period. A single administration of *L. casei* Shirota at a dose of $10^6$ CFU 24 h before the challenge infection dramatically inhibited *E. coli* growth and inflammatory responses in the urinary tract. Multiple daily treatments with *L. casei* Shirota during the postinfection period also showed antimicrobial activity in this UTI model. A heat-killed preparation of *L. casei* Shirota exerted significant antimicrobial effects not only with a single pretreatment (100 µg/mouse) but also with multiple daily treatments during the postinfection period. The other *Lactobacillus* strains tested, i.e., *L. fermentum* ATCC 14931T, *L. jensenii* ATCC 25258T, *L. plantarum* ATCC 14917T, and *L. reuteri* JCM 1112T, had no significant antimicrobial activity. Taken together, these results suggest that the probiotic *L. casei* strain Shirota is a potent therapeutic agent for UTI.

Urinary tract infection (UTI) is the most common bacterial infection seen in clinical practice. Human UTI comprises disease entities such as acute pyelonephritis with renal parenchymal involvement, cystitis limited to the urinary bladder, and asymptomatic bacteruria. Enterobacteriaceae such as *Escherichia coli*, which are normal inhabitants of human intestines, account for the vast majority of these uncomplicated infections (37, 65). Appropriate hygiene and cleanliness of the genital area are therefore recommended for prevention of UTI. On the other hand, studies have shown a correlation between a loss or disruption of the normal genital microflora, in particular *Lactobacillus* species, and an increased incidence of genital and bladder infections (57). Preclinical and clinical reports have focused on lactobacillus strains, their possible prophylactic effects against experimental *E. coli* infection, and the use of these strains for the prevention of human urogenital infections (7, 12, 17, 59, 60).

Suitable animal experimental models are required for appropriate preclinical studies of UTIs. Hagberg et al. were the first to show that mice could be challenged intravesically (by introducing pathogens directly into the bladder) without further manipulations of the urinary tract (18), and the murine model of ascending pyelonephritis has served as an excellent tool for defining the roles of individual virulence factors in the pathogenesis of UTI (18, 23, 25, 26, 28, 61). It should be noted, however, that the inoculum doses used in murine models are very high ($10^6$ CFU). Furthermore, high bladder infection levels reportedly persisted over the 14-day study period only in C3H/HeJ and C3H/OuJ mice, which are lipopolysaccharide (LPS) nonresponder strains, while strains such as C3H/HeN, C57BL/6, BALB/c, DBA.1, DBA.2, and AKR showed progressive resolution of bladder infections over a 14-day period (23, 24). Therefore, an appropriate model in which chronic UTI can be induced with a lower inoculum of *E. coli*, regardless of differences in genetic backgrounds, is needed.

In the present report, we first describe an improved murine chronic infection model of UTI, in which the infection was induced by traumatization of the bladder mucosa with inorganic acid and subsequent neutralization, followed by a single infusion of only $1 \times 10^6$ to $2 \times 10^6$ CFU of *E. coli* into the bladder. Chemical pretreatment of the bladder cavity ensured persistent infection without induction of systemic infection, and chronic infection was equally inducible in C3H/HeN and C3H/HeJ strains, which have been shown to differ in susceptibility to UTI (23). Using the improved murine urethral infection model, we investigated the antimicrobial effects of intravesically administered *Lactobacillus casei* strain Shirota, which is a well-documented probiotic strain (40). Intraurethral treatment with *L. casei* Shirota ($10^6$ CFU/day) inhibited pathogen growth in the urinary tract and suppressed infection-induced inflammatory responses. The characteristics of this antimicrobial activity included (i) a heat-killed (HK) preparation of *L. casei* Shirota effectively lowering levels of infectious bacteria and (ii) effectiveness of treatment during the postinfection period. These results suggest that the probiotic *L. casei* strain Shirota is potentially useful for both preventive and therapeutic treatment of UTI.

* Corresponding author. Mailing address: Yakult Central Institute for Microbiological Research, 1796 Yaho, Kunitachi, Tokyo 186-8650, Japan. Phone: 81-425-77-8962. Fax: 81-425-77-3020. E-mail: koji-nomoto@yakult.co.jp.
MATERIALS AND METHODS

Pathogens. Three E. coli strains were used. Two strains of E. coli (HU-1 and HU-2) isolated individually from urine cultures of patients with clinical symp-
toms were kindly provided by Toshikiko Mayumi, School of Medicine, Nagoya University, Nagoya, Japan. One strain (RI-1) was isolated from a rabbit intestine. The following characteristics of the bacterial strains were assessed: type I fim-
briae (48), by mannose-sensitive agglutination of guinea pig erythrocytes; P fim-
briae, by mannose-resistant agglutination of human type O erythrocytes (48); production of hemolysin, by qualitative evaluation using 5% sheep blood agar plates; capsule formation, by capsule stain (6). For the challenge experiments, E. coli strains were incubated overnight in brain heart infusion broth (Difco Laboratories, Detroit, Mich.). After being washed with phosphate-buffered saline (PBS) by centrifugation, bacterial cells were resuspended in PBS and adjusted to approximately 5 × 10^9 to 10 × 10^9 CFU/ml by comparison to McFarland turbidity standards confirmed by enumeration using the spread plate technique.

Murine model of UTI. Specific-pathogen-free female C57/Hc/Nc and C57/Hc mice at 7 weeks of age were purchased from Japan SLC, Inc., Shizuoka, Japan. The mice were housed under barrier-sustained conditions, with temperature (25 ± 0.5°C), humidity (55 ± 5%), and light (14 h of light and 10 h of darkness) automatically controlled, and were kept in polycarbonate cages (CLEA Japan, Inc., Tokyo, Japan) with stainless steel lids and sterilized bedding. The mice were maintained on an MF diet (Oriental Yeast Co., Ltd., Tokyo, Japan) and steril-
ed water (120°C for 30 min), which contained C2 at a final concentration of 1.5 ppm (1.5 μg/ml).

The mice were anesthetized by administration of sodium pentobarbital (0.05 mg/g of body weight). After sterilization of the periurethral area with 70% ethanol, a sterile 24-gauge Teflon catheter (outer diameter, 0.7 mm; length, 19 cm) was inserted into the bladder through the urethra. Before inoculation of bacteria, the bladder mucosa was traumatized by infusing 100 μl of 0.1 N HCl solution for 45 s, followed by neutralization with 100 μl of 0.1 N KOH and flushing with sterile saline into a tuberculin syringe (8). A 20-μl inoculum containing 1 × 10^6 to 2 × 10^6 organisms was then infused into the bladder through a catheter over 30 s through a microsyringe (GASTIGHT syringe; Hamilton Company, Reno, Nev.) 24 h after the bladder mucosa traumatization.

Lactobacilli. L. casei strain Shirota, L. fermentum ATCC 19437T, L. plantarum ATCC 14974T, and L. reuteri ICMP 11123T were used. Each lactobacillus strain was cultivated in MRS broth (Difco) at 37°C for 24 h, washed with distilled water, and resuspended in saline at a concentration of 5 × 10^9 to 10 × 10^9 CFU/ml. For preparation of an HK bacterial suspension, the harvested cells of lactobacillus suspended in distilled water at a concentration of 5 × 10^7 to 10 × 10^8 CFU/ml were heated at 100°C for 30 min and then cooled on ice. A 20-μl inoculum of the lactobacillus suspension was infused into the bladder through the catheter over 30 s using a microsyringe. As treatment prior to the infection with E. coli, mice received a single administration of lactobacilli 15 min after the chemical treatment. As postinfection treatments, mice received a single administration of lactobacilli 15 min after the chemical treatment. As postinfection treatments, mice received a single administration of lactobacilli 15 min after the chemical treatment. As postinfection treatments, mice received a single administration of lactobacilli 15 min after the chemical treatment. As postinfection treatments, mice received a single administration of lactobacilli 15 min after the chemical treatment. As postinfection treatments, mice received a single administration of lactobacilli 15 min after the chemical treatment. As postinfection treatments, mice received a single administration of lactobacilli 15 min after the chemical treatment. As postinfection treatments, mice received a single administration of lactobacilli 15 min after the chemical treatment.
was considered significant.

the bladder 24 h before the bacterial challenge. A previous infection were dramatically increased by chemical treatment of mice. The counts of viable bacteria in the bladder (A) and kidneys (B) were determined on days 1, 4, 7, 10, 14, and 24 after the challenge. Results are expressed as the means and SDs for six mice. Significant differences between the untreated controls and the treated group: *, P < 0.05; **, P < 0.01; ***, P < 0.001.

FIG. 1. A murine chronic UTI model. C3H/HeN mice were divided into two groups. Traumatization of the bladder was performed as described in Materials and Methods for one group (○), and another group (control) (□) was mock treated with saline. E. coli strain HU-1 at a dose of 2 × 10^6 CFU was infused into the bladders of anesthetized mice. The counts of viable bacteria in the bladder (A) and kidneys (B) were determined on days 1, 4, 7, 10, 14, and 24 after the challenge. Results are expressed as the means and SDs for six mice. Significant differences between the untreated controls and the treated group: *, P < 0.05; **, P < 0.01; ***, P < 0.001.

Myeloperoxidase (MPO) activity in urine was measured as that solubilized with hexadecyl trimethylammonium bromide (36). In brief, urination was achieved in nine mice in different groups under anesthesia, and 20-μl portions of the urine were detected in both strains at the chronic phases (Table 1), and more than 98% of the leukocytes were found by Giemsa staining to be neutrophils (data not shown).

Preventive effect of intraurethrally administered L. casei Shirota on UTIs in mice. A single infusion of L. casei Shirota at a dose of 10^6 CFU 24 h before infection markedly inhibited E. coli growth in the urinary tract. Viable counts of E. coli in the bladder decreased to less than 1/100 of those in the control group at 24 h and approached the lower detection limit on day 7 after the challenge infection (Fig. 2A). Renal infection also subsided significantly with L. casei Shirota treatment (Fig. 2B). The total viable count of L. casei Shirota detected in the bladder and kidneys immediately after infection was about 1/100 of the inoculum and decreased logarithmically, reaching undetectable levels by day 7 (Fig. 2C). No viable E. coli or L. casei Shirota organisms were detected in the liver (data not shown). Three strains of E. coli, which have different T and P fimbrial expression patterns (all of them were hemolysin negative and not capsulated), showed somewhat different intensities of pathogenicity in this infection model. The pathogenicity of strain HU-1, which expresses both types of fimbriae, was strongest, while strain RI-1, which expresses neither type, showed significantly less pathogenicity (Fig. 2D). L. casei Shirota exerted potent antimicrobial activity against all three E. coli strains, regardless of the differences in fimbrial expression patterns.

In order to assess whether or not the antimicrobial activity shown by L. casei Shirota was shared by the other lactobacillus strains, four strains which have different adhesive properties were tested. The results, shown in Table 2, clearly dem-

TABLE 1. Induction of UTI with E. coli in C3H/HeN

<table>
<thead>
<tr>
<th>Days post-infection</th>
<th>Log_{10} viable bacteria in bladder (mean ± SD)</th>
<th>Leukocyte concn in urine (10^6 cells/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C3H/HeN</td>
<td>C3H/HeJ</td>
</tr>
<tr>
<td>4</td>
<td>5.0 ± 0.1</td>
<td>7.0 ± 0.6</td>
</tr>
<tr>
<td>14</td>
<td>3.9 ± 0.6</td>
<td>5.3 ± 1.3</td>
</tr>
<tr>
<td>28</td>
<td>3.6 ± 1.1</td>
<td>5.1 ± 1.5</td>
</tr>
</tbody>
</table>

a Mice were infected with E. coli strain HU-1 at an inoculum of 2 × 10^6 CFU and dissected on days 4, 14 and 28.

b For eight mice per group.

c Mean ± SD. Three mice per group were used. NT, not tested.

Improved murine model of E. coli UTI. Without pretraumatization of the bladder, infusion of E. coli strain HU-1 at an inoculum size of 2 × 10^6 CFU into the bladder induced a local transitory infection in the mice, which terminated within a week after the challenge (Fig. 1). The intensity and duration of infection were dramatically increased by chemical treatment of the bladder 24 h before the bacterial challenge. A previous argument concerning the influence of inoculum size on possible reflux of the inoculated bacteria to the kidneys (27) prompted us to adopt an inoculum size of 20 μl, as well as to use a microsyringe for infusion, in order to minimize reflux. The viable count in the kidneys on day 1 after instillation was less than 1/100 of that in the bladder, suggesting that reflux of the pathogen by infusion treatment is minimal in this model. No viable counts were detected in the liver throughout the experimental period, suggesting that the infection was restricted to the urinary tract rather than becoming systemic (data not shown). The severity of UTI in the C3H/HeJ strain was greater than that in the C3H/HeN strain during both the initial phase (day 4) and the chronic phases (days 14 and 28 [Table 1]). Although the numbers of leukocytes in the urine before infection were under detectable levels in both strains (data not shown), increases in the number of leukocytes infiltrating the urine were detected in both strains at the chronic phases (Table 1), and more than 98% of the leukocytes were found by Giemsa staining to be neutrophils (data not shown).

Statistical analysis. Statistical differences between the control group and the treated group were evaluated with Fisher’s exact probability test for the incidence of infection and Student’s t-test for other benchmarks. A P value of <0.05 was considered significant.

RESULTS

Improved murine model of E. coli UTI. Without pretraumatization of the bladder, infusion of E. coli strain HU-1 at an inoculum size of 2 × 10^6 CFU into the bladder induced a local transitory infection in the mice, which terminated within a week after the challenge (Fig. 1). The intensity and duration of infection were dramatically increased by chemical treatment of the bladder 24 h before the bacterial challenge. A previous...
Stratified differences in antimicrobial activity among the strains tested and showed that the type strains, e.g., *L. fermentum*, *L. jensenii*, *L. plantarum*, and *L. reuteri*, did not exert significant antimicrobial activity. The results also clearly showed that strains with strong adhesive properties, such as adhesion to murine bladder epithelial cells (Fig. 3), platelet aggregation, fibronectin binding, hydrophobicity, and salivary aggregation (Table 2), do not necessarily have strong antimicrobial activity.

Studies concerning the influence of the inoculum dose on antimicrobial activity have shown that an *L. casei* Shirota inoculum of $10^7$ CFU exerted less pronounced antimicrobial activity, while more than $10^8$ CFU exerted potent antimicrobial activity. In contrast, *L. fermentum* at doses ranging from $10^7$ to $10^9$ CFU had no significant antimicrobial activity (data not shown).

**Effects of *L. casei* Shirota on inflammatory responses in the urinary tract during infection.** Histopathological analysis of

![Fig. 2](image-url) Preventive effect of *L. casei* Shirota against chronic UTI. (A through C) Either *L. casei* Shirota at a dose of $1.2 \times 10^8$ CFU in 20 μl of saline (○) or saline alone (control) (●) was infused into the bladder 15 min after traumatization, and *E. coli* strain HU-1 at a dose of $1.8 \times 10^8$ CFU was infused into the bladder 24 h later. On days 0 (just after infection), 1, 4, and 7 after the challenge infection, eight mice per period were dissected for bacteriological determination in the bladder (A) and kidneys (B). (A and B) changes in viable *E. coli* counts, (C) changes in viable *L. casei* Shirota counts in the bladder (△) and kidneys (★). (D) Mice pretreated with saline (solid bars) or *L. casei* Shirota at a dose of $10^9$ CFU 24 h earlier (open bars) were infected intravesically with *E. coli* strain HU-1 ($2.1 \times 10^8$ CFU), HU-2 ($2.5 \times 10^6$ CFU), or RI-1 ($2.2 \times 10^6$ CFU) and dissected for bacterial examination 24 h after infection. Results are expressed as the means and SDs for eight mice. Plus symbols and minus symbols in parentheses in panel D indicate whether or not the strain expresses type 1 fimbriae (first symbol) and P fimbriae (second symbol). Significant differences between untreated controls and the treated group: *, *P < 0.05; **, *P < 0.01.

**TABLE 2. Comparison of antimicrobial activity and adhesive activities among different Lactobacillus strains**

<table>
<thead>
<tr>
<th>Lactobacillus†</th>
<th>Log₁₀ difference in bacterial countb</th>
<th>Adhesive property†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH 7.3</td>
<td>pH 5.4</td>
</tr>
<tr>
<td><em>L. casei</em> strain Shirota</td>
<td>2.2*</td>
<td></td>
</tr>
<tr>
<td><em>L. fermentum</em> ATCC 14931T</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td><em>L. jensenii</em> ATCC 25258T</td>
<td>−0.4</td>
<td></td>
</tr>
<tr>
<td><em>L. plantarum</em> ATCC 14917T</td>
<td>−0.6</td>
<td></td>
</tr>
<tr>
<td><em>L. reuteri</em> JCM 1112T</td>
<td>0.5</td>
<td></td>
</tr>
</tbody>
</table>

* Each strain used was grown in MRS broth for 24 h at 37°C.

b Viable cells of each strain at an inoculum (*L. casei* Shirota, $1.8 \times 10^8$ CFU; *L. fermentum*, $1.0 \times 10^6$ CFU; *L. jensenii*, $1.0 \times 10^6$ CFU; *L. plantarum*, $2.2 \times 10^6$ CFU; *L. reuteri*, $1.9 \times 10^6$ CFU) were instilled intravesically on day −1, and the mice were infected with *E. coli* strain HU-1 ($2 \times 10^5$ CFU) intravesically on day 0. Mice were dissected 24 h after the infection. The antimicrobial activity of each *Lactobacillus* strain is expressed as the log₁₀ difference in the bacterial count, calculated as the log₁₀ number of *E. coli* bacteria in the bladders of mice pretreated with saline minus the log₁₀ number of *E. coli* bacteria in the bladders of mice pretreated with *Lactobacillus* strains. Six mice per group were used. *, *P < 0.05.

† Determined as described in Materials and Methods.
L. casei Shirota served in the infection control (Fig. 4A and B) and bacteria adhering to the mucosal layer in the bladders of both cated by neutrophil infiltration and numerous gram-negative bacteria clearly showed the inflammatory responses indicated by bacterial metabolites appear to be mainly responsible for the results obtained in the present study. It is unlikely that the bactericidal substances produced by lactobacilli, such as lactic acid, hydrogen peroxide (15, 16), and several kinds of bacteriocin (3, 5, 46), contribute to the antimicrobial activity of L. casei Shirota. This is because viable counts of the strain in the urinary tract decreased dramatically after infection with E. coli, and a heat-killed preparation of L. casei Shirota exerts potent antimicrobial activity. Moreover, L. casei Shirota has been found not to produce hydrogen peroxide at detectable levels even under aerobic culture conditions (data not shown).

Characteristics of the antimicrobial activity of L. casei Shirota in the murine chronic UTI model include (i) a heat-killed preparation of the probiotic strain being effective against UTI (Fig. 6) and (ii) effectiveness of treatment during the postinfection period (Table 3; Fig. 6). Both of these characteristics appear to be quite important for safe and practical use of L. casei Shirota as a therapeutic agent for UTI patients. The mechanism by which L. casei Shirota exerts such unique and practical antimicrobial activity against UTI is still unclear from the results obtained in the present study. It is unlikely that the bacterial metabolites produced by lactobacilli, such as lactic acid, hydrogen peroxide, and several kinds of bacteriocin, contribute to the antimicrobial activity of L. casei Shirota. This is because viable counts of the strain in the urinary tract decreased dramatically after infection with E. coli, and a heat-killed preparation of L. casei Shirota exerts potent antimicrobial activity. Moreover, L. casei Shirota has been found not to produce hydrogen peroxide at detectable levels even under aerobic culture conditions (data not shown).

Therefore, antimicrobial mechanisms other than those driven by bacterial metabolites appear to be mainly responsible for the results obtained in this experimental model. Type 1 fimbriae are expressed by many members of the Enterobacteriaceae, and experimental evidence suggests that they mediate adherence in the bladder and thus probably contribute to the pathogenesis of lower UTI (9, 14, 38). On the other hand, numerous epidemiological studies have indicated that uropathogenic E. coli strains are much more likely to express P fimbriae than are fecal isolates of E. coli (13, 14). Indeed, the prevalence of P fimbriae among E. coli strains

FIG. 3. Adhesion of lactobacillus strains to MBT-2 cell cultures, presented as the number of bacteria bound per 100 MBT-2 cells versus the concentration of bacteria added (CFU per milliliter). Representative results from two separate experiments are shown. Symbols: ○, L. casei Shirota; ●, L. fermentum ATCC 14931T; △, L. jensenii ATCC 25258T; ▲, L. plantarum ATCC 14917T; □, L. reuteri JCM 1112T.

<table>
<thead>
<tr>
<th>Bacteria added (10^6 CFU/ml)</th>
<th>Bacteria bound (cells / 100 MBT-2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>1000</td>
</tr>
<tr>
<td>2</td>
<td>2000</td>
</tr>
<tr>
<td>3</td>
<td>3000</td>
</tr>
<tr>
<td>4</td>
<td>4000</td>
</tr>
<tr>
<td>5</td>
<td>5000</td>
</tr>
<tr>
<td>6</td>
<td>6000</td>
</tr>
</tbody>
</table>

The advantage of the infection model used herein is that a relatively smaller inoculum, only 1 x 10^6 to 2 x 10^6 CFU, induces chronic UTI (Fig. 1; Table 1). In contrast, inoculum doses of more than 10^8 CFU were needed to induce UTI in the experimental models employed in prior studies (18, 23–25, 26, 28, 61). Hopkins et al. reported that genetically distinct inbred mice differ in initial susceptibility to an E. coli UTI and in their ability to resolve the infection. Significant UTIs were induced in the majority of murine strains evaluated, and these infections gradually resolved with the exception of two LPS nonresponder strains, C3H/HeJ and C3H/OuJ (23, 24). The present results showed UTI infection to persist throughout the 28-day study period in the C3H/HeN strain (Fig. 1; Table 1), which was reported to undergo progressive UTI resolution in a previous study. These results were apparently attributable to pretreatment of the bladder mucosa before infusion of the pathogen, because infections without pretreatment resolved within a week (Fig. 1A). Histopathological examination revealed chemical treatment of the bladder to induce inflammatory hyperplasia of the mucosa (Fig. 4), which may create conditions conducive to E. coli infection, such as increased expression of extracellular matrix (ECM). It is well known that fimбриae, such as type 1, Pap, and S, bind to ECM molecules such as fibronectin, laminin, and type IV collagen (for reviews, see references 55 and 67).

Antimicrobial activity of L. casei Shirota with postinfection treatment. Because UTI with E. coli was sustained for more than 3 weeks after the challenge infection (Fig. 1; Table 1), L. casei Shirota treatment was initiated during the postinfection period to assess its therapeutic potential (Table 3). Multiple treatments of mice with L. casei Shirota significantly reduced viable E. coli counts in the bladder during the postinfection period.

Antimicrobial activity of an HK preparation of L. casei Shirota. As shown in Fig. 2, the antimicrobial effect of L. casei Shirota with a single infusion was maintained for a week after E. coli inoculation despite the dramatic decrease in viable L. casei Shirota counts. An HK preparation of L. casei Shirota was then assessed for antimicrobial activity in order to ascertain whether the metabolic activity of the lactobacillus is required for the antimicrobial activity (Fig. 6). HK L. casei Shirota at a dose of 100 µg per mouse, which is nearly equivalent to 10^6 CFU, clearly showed antimicrobial activity to be exerted in a fashion similar to that of the live preparation (Fig. 6A). HK L. casei Shirota showed antimicrobial activity with multiple treatments during the postinfection period (Fig. 6B).
FIG. 4. Effects of *L. casei* Shirota on histopathological changes in the urinary tract during *E. coli* infection. Mice were challenged intravesically with *E. coli* strain HU-1 at a dose of $2 \times 10^8$ CFU and were dissected for histopathological examination of the bladder 4 days later. Either saline (A and B), *L. casei* Shirota at a dose of $1.6 \times 10^8$ CFU (C and D), or *L. fermentum* ATCC 14931T at a dose of $1.0 \times 10^8$ CFU (E and F) was infused into the bladder 24 h before the challenge infection. Panels A, C, and E were stained with hematoxylin and eosin. Magnification, $\times 108$. Panels B, D, and F were Gram stained. Magnification, $\times 432$. 
against UTI in this murine model. On the other hand, L. casei than another probiotic, Caco-2 cells, intestinal mucus, and ileostomy glycoproteins Shirota has been shown to have higher adhesion affinity to involved in the mechanism of protection. L. rhamnosus lower than that for L. casei of Shirota at a dose of 1.6 × 10^8 CFU (57, 58), the present results suggest that these assumptions may not hold true for the antimicrobial activity of L. plantarum line, MBT-2, than the ineffective strains, such as L. jensenii much lower adhesive activity to a murine bladder epithelial cell strains despite their different fimbrial expressions (Fig. 2D), the mechanism of the antimicrobial activity appears to be unrelated to inhibition of the fimbria-mediated pathogenesis of E. coli. Recent reports have shown that there are differences in adhesion to intestinal epithelial cells (Caco-2 cells) among lactobacillus strains (35, 39, 60, 66), and that indigenous lactobacilli isolated from the urethral surfaces of healthy women block the adherence of gram-negative uropathogenic bacteria to uroepithelial cells from women without a history of UTI (59, 60). We, however, have found that L. casei Shirota does not have adhesive properties such as fibronectin binding, salivary aggregation, and platelet aggregation in vitro, while the ineffective strains, such as L. fermentum, L. jensenii, L. plantarum, and L. reuteri, show strong adhesive properties (Table 2). Moreover, L. casei Shirota exerted a much lower adhesive activity to a murine bladder epithelial cell line, MBT-2, than the ineffective strains, such as L. jensenii and L. plantarum (Fig. 3). Although the mechanisms underlying the antimicrobial activity of lactobacilli are believed to involve the production of inhibitory substances and competitive exclusion (57, 58), the present results suggest that these assumptions may not hold true for the antimicrobial activity of L. casei Shirota against UTI in this murine model. On the other hand, L. casei Shirota has been shown to have higher adhesion affinity to Caco-2 cells, intestinal mucus, and ileostomy glycoconjugates than another probiotic, L. rhamnosus GG, while the adhesion of L. casei Shirota at saturating cell concentrations was much lower than that for L. rhamnosus GG (39, 66). Therefore, further studies are required to establish whether the inhibition of E. coli adhesion to urinary tract epithelial cells may be involved in the mechanism of protection.

The magnitude of local inflammation elicited by bacteria in the urinary tract accounts for most of the clinical features of UTI (37, 65). Evidence from murine models suggests that the inflammatory response at the initial phase of infection (within 24 h of infection) is essential for clearance of bacteria from the urinary tract (19, 62). It has been shown that uropathogenic E. coli stimulates local production of proinflammatory cytokines and chemokines in the urinary tract. In studies of mice with experimental UTI and in human volunteers deliberately colonized with E. coli, there were marked increases in the levels of interleukin-6 (IL-6) and IL-8 (2, 10, 22). Moreover, it has been shown that uroepithelial cells, upon exposure to E. coli, secrete cytokines such as IL-1α, IL-6, and IL-8 (1). However, the maintenance of augmented inflammatory responses indicated by the dramatic increases in neutrophils and MPO activity in the urine during E. coli infection (Table 1; Fig. 5) appears to show vain host responses aimed at eliminating the pathogen in the chronic infection model. The exaggeration and protraction of host defense responses in the UTI model may instead cause tissue injury and maladaptive repair, leading to a sustained infection. There are reports indicating that virulent

![Fig. 5](image-url)

**TABLE 3.** Antimicrobial activity of L. casei Shirota by daily administration during the postinfection period

<table>
<thead>
<tr>
<th>Group</th>
<th>Log10 viable E. coli organisms in bladder (mean ± SD)</th>
<th>Days 1–11</th>
<th>Days 4–11</th>
<th>Days 7–11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. casei Shirota</td>
<td></td>
<td>6.6 ± 0.97</td>
<td>4.8 ± 0.7</td>
<td>4.8 ± 0.9</td>
</tr>
</tbody>
</table>

* Mice were treated with saline alone (control) or L. casei Shirota (1.2 × 10^8 to 1.6 × 10^8 CFU) once daily starting on day 1, 4, or 7 and continuing to day 11.

+ Mice were infected with E. coli strain HU-1 at an inoculum of 2 × 10^6 CFU on day 0 and dissected on day 12 after infection. Six to eight mice per group were used. *, P < 0.05.

The incidence or infection was 8 of 8 for the control group and 2 of 7 for the L. casei Shirota-treated group, respectively (P < 0.05 by Fisher’s exact probability test).
Inflammatory responses in the urinary tract were markedly inhibited in the L. casei Shirota-treated group (Figs. 4, 5), suggesting that inhibition of the growth of pathogenic bacteria resulted in the suppression of subsequent infection-induced inflammatory responses. L. casei Shirota reportedly exerts antitumor (4, 32, 45, 54) and antimicrobial (47, 52, 53, 70) activities in clinical and preclinical studies. Furthermore, nonspecific augmentation of components of the innate immune system, such as macrophages (34, 47, 63) and natural killer cells (33), has been thought to play important roles in these activities. Strains such as L. fermentum ATCC 14931\(^T\) and L. plantarum ATCC 14917\(^T\) have been reported to have much weaker activities than L. casei Shirota (63, 71). There are also reports indicating the importance of cell-mediated immune responses in UTI resolution (29, 49, 50). Morin et al. reported that treatment of mice with staphylococcal enterotoxin B, a superantigen, leads to enhanced UTI resolution through a mechanism that may include direct stimulation of effector cells in the bladder and the actions of cytokines such as IL-1, IL-6, GM-CSF, and tumor necrosis factor alpha (49). Jones-Carson et al. reported that knockout mice with \(\gamma\delta\) T-cell or gamma interferon deficiencies were more susceptible to UTI than immunocompetent mice and mice with immunodeficiencies in IL-10, IL-4, inducible nitric oxide synthase, or antibody production (29). Taken together, these results raise the possibility that local activation of the innate antimicrobial activity by L. casei Shirota may facilitate inhibition of pathogen growth in the urinary tract.

On the other hand, L. casei Shirota has been shown to exert potent preventive activity in a wide variety of inflammatory disease models such as autoimmune diabetes (43), chronic rheumatoid arthritis (31), and allergic bronchial asthma (44). The mechanisms underlying the anti-inflammatory activity of L. casei Shirota have therefore been recognized as being exerted via improvement of disrupted immune responses in the disease state (42, 51). Further investigation is required to determine whether the administration of L. casei Shirota in the bladder potentiates the innate protective immune responses during UTI.

FIG. 6. Antimicrobial activity of an HK preparation of L. casei Shirota. (A) Preventive activity. E. coli strain HU-1 at an inoculum of 1.8 \(\times\) 10\(^6\) CFU was infused 24 h after treatment with HK L. casei Shirota (100 \(\mu\)g/mouse), and viable E. coli counts were determined on days 0 (immediately after the challenge), 1, 4, and 7. (B) Therapeutic activity of HK L. casei Shirota with postinfection administration. Mice that had been infected intravesically with E. coli strain HU-1 at an inoculum of 2.0 \(\times\) 10\(^6\) CFU received 7 daily intravesical infusions of saline or HK L. casei Shirota at a dose of 100 \(\mu\)g/mouse starting on day 7 after the infection, and the mice were dissected for bacteriological examination on days 7 (controls only), 8, 11, and 14 after the challenge infection. Symbols: \(*\), saline-treated control; \(\circ\), group treated with HK L. casei Shirota. Results are expressed as the means and SDs for eight mice. *, \(P < 0.05\) for differences between untreated controls, and treatment groups.

ACKNOWLEDGMENTS

We thank Kazumi Uchida and Shoichi Kado for performing the histopathological analyses. The skillful assistance of Kensuke Shimizu and Tomomi Suzuki in animal experiments is gratefully acknowledged.

REFERENCES


to. 19:1–8.


bio. 48:585–617.


trophic requirement and bacterial clearance correlated with LPS responsive


dairy *Lactobacillus* strains to Caco-2 cell cultures. Int. J. Food Microbiol. 41:45–51.


