Preventive Effect of Probiotic Bifidobacteria against Shiga Toxin-Producing Escherichia Coli and Salmonella Infections

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Shiga toxin-producing Escherichia coli and Salmonella, causative bacteria of food poisoning (intestinal infectious disease) in humans, are still serious problems. Shiga toxin-producing E. coli O157:H7 (STEC) grows and produces Shiga toxin (Stx) in the intestine, and causes hemorrhagic enteritis. A typical etiologic agent of Salmonella food poisoning, Salmonella enterica serovar Typhimurium (S. Typhimurium), grows in the intestine and invades the body via the intestinal epithelium, causing inflammation. The importance of the prevention of STEC- and S. Typhimurium-induced food poisoning has been stressed because they frequently cause outbreaks, the course is rapid, and only a very small number of bacteria (10^1 to 10^3 CFU) is needed to induce a severe infection. Probiotics are defined as ‘Live microorganisms which when administered in adequate amounts confer a health benefit on the host’. Bifidobacteria, major constituents of the intestinal flora, are typical probiotics which are expected to help prevent intestinal infection. In this study, we investigated the anti-infectious activity of bifidobacteria against STEC and S. Typhimurium infections using a mouse intestinal infection model, and analyzed the infection-preventive mechanism. For STEC infection, a novel mouse fatal infection model was prepared by combining STEC infection at 5 x 10^3 CFU and Mitomycin C (MMC) treatment (for the induction of stx gene expression) in the late logarithmic phase of intestinal STEC growth. The anti-infectious activity of the orally administered probiotic Bifidobacterium breve strain Yakult (BbY) was investigated using this mouse intestinal STEC infection model. STEC-induced death was strongly inhibited in BbY-treated mice. Interestingly, STEC growth in the intestine was not inhibited, but stx gene expression and Stx production were strongly inhibited. In addition, the intestinal environment was improved in the BbY-treated mice through normalization of the intestinal level of acetic acid, a major organic acid in the intestine, and pH. When STEC was grown in vitro in a medium reproducing the acetic acid level and pH in the cecum, Stx production was completely inhibited, suggesting that the expression of this pathogenic factor was inhibited by BbY-induced improvement of the intestinal environment. In the mouse intestinal S. Typhimurium infection model, BbY inhibited the abnormal growth of S. Typhimurium and improved the intestinal environment, resulting in the inhibition of systemic S. Typhimurium infection. This study, using an experimental animal model, clarified the preventive effect of the probiotic BbY on food poisoning (intestinal infectious disease) caused by intestinal STEC and retardation of intestinal S. Typhimurium growth. These findings suggest the usefulness of probiotics as a novel preventive agent for human food poisoning (intestinal infectious disease).

Key words: Bifidobacterium breve strain Yakult, Shiga toxin-producing Escherichia coli, Salmonella enterica serovar Typhimurium, preventive effect, mouse

INTRODUCTION

Shiga toxin-producing Escherichia coli and Salmonella, Causative bacteria of food poisoning (intestinal infectious disease) in humans, are still serious problems (6, 13, 27, 35, 39, 43, 44). Shiga toxin-producing E. coli O157:H7 (STEC) grows and produces Shiga toxin (Stx) in the intestine, and causes hemorrhagic enteritis. Stx produced by STEC includes Stx2. Stx2 exhibits similar biological properties to but different immunological and physicochemical properties from those of Stx1, which has the same structure as the Shiga toxin produced by Shigella dysenteriae. In severe STEC-induced infectious disease, Stx causes hemolytic uremic syndrome (HUS) and encephalopathy, resulting in fatal outcomes (36). A typical etiologic agent of Salmonella food poisoning, Salmonella enterica serovar

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Typhimurium (S. Typhimurium), grows in the intestine and invades the body via the intestinal epithelium, causing inflammation (9). The importance of the prevention of STEC- and S. Typhimurium-induced food poisoning has been stressed because they frequently cause outbreaks, the course is rapid, and only a very small number of bacteria (10³ CFU) is needed to induce a severe infection (16, 25, 30, 38).

Animal models may be useful for evaluating the preventive effects of agents and foods on intestinal infection with STEC and S. Typhimurium. However, no study has prepared any infection model at a bacterial count of 10⁴ to 10³, corresponding to the bacterial count in the presence of infection in clinical practice. Also, for STEC infection, no study has analyzed the relationship between a fatal outcome in mice and the intestinal level of Stx. Therefore, a new animal model should be prepared.

In clinical practice, antibiotics are routinely administered to prevent intestinal infection. However, antibiotic treatments have limitations: antibiotic stimulation of STEC increases Stx production (46), and S. Typhimurium strains, such as S. Typhimurium DT104 (37) and S. Typhimurium Newport (17), have become resistant to multiple drugs.

Probiotics are defined as ‘Live microorganisms which when administered in adequate amounts confer a health benefit on the host’ (31). Bifidobacteria, a major constituent of the intestinal flora, is a typical probiotic which is expected to help prevent intestinal infection. Furthermore, acetic acid, the main metabolite of bifidobacteria, exhibits bactericidal activity against various types of pathogenic gram-negative bacilli in vitro, and is considered to be a protective factor against intestinal infection. However, the anti-infectious activity of bifidobacteria against STEC and S. Typhimurium remains to be clarified.

In this study, we developed a new mouse intestinal STEC infection model, investigated the anti-infectious activity of bifidobacteria against STEC, and analyzed the infection-preventive mechanism. In addition, we examined the efficacy of bifidobacteria’s bactericidal activity against intestinal infection with S. Typhimurium, the pathogenesis of which differs from that of STEC infection.

DEVELOPMENT OF A MOUSE FATAL INTESTINAL STEC INFECTION MODEL (34)

Mice, to which water containing streptomycin sulfate (SM, 5 mg/ml) was given, were orally infected with 5 × 10⁴ CFU of SM-resistant STEC. The STEC count reached 10⁹ CFU per g feces within 24 hr after infection via marked proliferation in the intestine (Fig. 1-1-A). Thereafter, bacterial excretion at the same level persisted. Since bacterial cell metabolic reactions are most active in the logarithmic growth phase, stx gene-inducing mitomycin C (MMC) was intraperitoneally injected into SM-treated mice 3 times every 3 hr (0.25 mg/kg Bw × 3), starting in the late logarithmic growth phase of STEC (18 hr after STEC infection) or immediately after the end of the logarithmic growth phase (36 hr after STEC infection). The body weight markedly decreased, and all mice died within 9 days of infection (Fig. 1-1-B, C). In the cecums of these mice, the levels of Stx1 and Stx2 transiently and markedly increased 6 to 9 hours after the end of MMC inoculation (Fig. 1-2-A, B). In particular, the level of Stx2 was 200 times higher than that of Stx1 when the production of Stx1 and Stx2 induced by STEC reached a peak (Fig. 1-2-C, D). In histological analysis, when the production of Stx by STEC in the intestine peaked, the murine ileal epithelium showed apoptosis in a large number of crypt cells compare to normal mice (Data not shown). In the mice immediately before death, infectious injury in the ileal region had subsided. However, injuries to a hematopoietic organ, the bone marrow (erythroblastopenia), the mesenteric lymph nodes (apoptosis) as well as tubular dilatation with degeneration, necrosis, and exfoliation of the epithelial cells of the renal cortex distal uriniferous tubule were observed. Hematological showed increases in blood urea nitrogen (BUN) and creatinine levels (Data not shown). We also compared the pathogenicity among 11 strains of STEC derived from various stx gene variants (stx1, stx2, and stx2c) using this STEC infection model (Table 1). There were no differences in the level of STEC colonization in the cecum 18 hr after STEC infection among the strains. However, there were marked differences in the mortality of mice after MMC inoculation; 5 to 6 of 6 mice died after challenge with STEC strains producing a high level (approximately 200 μg/g or more) of Stx2 in the intestine, regardless of the stx gene variants. However, no mouse died after challenge with STEC strains producing a low level (10 μg/g or less) of Stx2.

These results suggest that fatal intestinal infection can be induced by infection with STEC at 5 × 10⁵ CFU, corresponding to the infectious bacterial count in clinical practice, followed by 3 inoculations of MMC in the late logarithmic proliferative phase of STEC in the intestine. In addition, there was a transient but marked increase in Stx2 production induced by STEC in the murine intestine after MMC inoculation, which led to a fatal outcome.
PROTECTIVE EFFECT OF BIFIDOBACTERIA ON INTESTINAL INFECTION WITH STEC (2)

Using the mouse intestinal STEC infection model that we newly developed, we investigated the anti-infectious activity of bifidobacteria and its protective mechanism. In the STEC-infected control group (STEC count: $5 \times 10^3$ CFU, MMC: 0.25 mg/kg BW x 3 times), STEC proliferated in the intestine to a bacterial count of $10^9$ CFU/g, and 12 of 14 mice died (Fig. 2-1). In the Bifidobacterium breve strain Yakult (BbY)-treated
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In the STEC-infected control group, the cecal expressions of STEC \(\text{stx1} \) and \(\text{stx2} \) mRNAs increased 3 hr after the end of MMC inoculation, then gradually decreased (Fig. 2-2-A, B). \(\text{Stx1} \) mRNA expression was approximately 5 times higher than that prior to MMC inoculation (18 hr after infection). \(\text{Stx2} \) mRNA expression was approximately 50 times higher than the pre-inoculation value. \(\text{Stx1} \) and \(\text{Stx2} \) levels in the cecal contents transiently increased, and reached a peak 6 hr after the end of MMC inoculation (Fig. 2-2-C, D). The peak level of \(\text{Stx2} \) was approximately 200 times higher than that of \(\text{Stx1} \). In the

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Table 1. Virulence of various STEC strains in the infection model

| Strain   | Virulence gene | Stx concentration in the cecal contents | No. of deaths | Survival time (day, mean ± SD) |
|----------|----------------|------------------------------------------|---------------|-------------------------------|
|          | stx1 | stx2 | stx2c | stx2d | stx2e | eae | hly | Stx1 | Stx2 |               |               |
| V354     |     |     |     |     |     |     |     |     |     |     | ND=           | 45.1 ± 49.1   | 5/6 | 9.2 ± 1.1 |
| V406     |     |     |     |     |     |     |     |     |     |     | ND            | 201.9 ± 146.8 | 5/6 | 9.0 ± 1.2 |
| CDC EDL 933 |     |     |     |     |     |     |     |     |     |     | 0.3 ± 0.2   | 299.7 ± 226.8 | 6/6 | 8.5 ± 0.0 |
| 89020087 |     |     |     |     |     |     |     |     |     |     | 0.8 ± 0.5   | 198.3 ± 82.2 | 6/6 | 7.3 ± 1.0 |
| V356     |     |     |     |     |     |     |     |     |     |     | 0.7 ± 0.6   | 205.1 ± 190.7 | 6/6 | 8.8 ± 0.9 |
| TT-18    |     |     |     |     |     |     |     |     |     |     | ND           | 10.7 ± 6.5   | 1/6 | 9            |
| V20      |     |     |     |     |     |     |     |     |     |     | ND           | 1.0 ± 0.9    | 0/6 | —            |
| V50      |     |     |     |     |     |     |     |     |     |     | ND           | 1.2 ± 1.0    | 0/6 | —            |
| TR226A   |     |     |     |     |     |     |     |     |     |     | 0.4 ± 0.2   | 8.6 ± 5.3    | 0/6 | —            |
| EDL 931  |     |     |     |     |     |     |     |     |     |     | 0.2 ± 0.1   | 44.4 ± 40.4  | 2/6 | 8.5          |
| O-1      |     |     |     |     |     |     |     |     |     |     | 0.3 ± 0.2   | 198.3 ± 172.4 | 1/6 | 8.0          |

\(a\) By RT-PCR using specific primers for virulence genes (19–12, 32, 40, 41, 47).

\(b\) Mice were orally infected with STEC at an inoculum dose of \(2.2–4.9 \times 10^3 \text{CFU}\) on day 6 after starting SM treatment. MMC at a dose of 0.25 mg/kg was injected three times intraperitoneally to the infected mice every 3 hr starting from 18 hr after infection. STEC viable counts per g feces (Log10: mean and SD) at 18 hr (30 hr) after the STEC challenge were: V354: 9.1 ± 0.2 (9.0 ± 0.2), V406: 9.1 ± 0.2 (8.8 ± 0.2), CDC EDL 933: 8.8 ± 0.4 (8.8 ± 0.2), 89020087: 9.0 ± 0.2 (9.4 ± 0.2), V356: 9.3 ± 0.1 (9.2 ± 0.2), TT-18: 9.1 ± 0.2 (8.7 ± 0.3), V20: 9.0 ± 0.3 (8.4 ± 0.4), V50: 8.6 ± 0.2 (8.6 ± 0.2), TR226A: 8.1 ± 0.2 (9.0 ± 0.1), EDL 931: 8.8 ± 0.1 (9.1 ± 0.3), O-1: 8.0 ± 1.1 (8.8 ± 0.2).

\(c\) Mice were sacrificed 6 hr after the last MMC shot, and the Stx concentration in the cecal contents was determined using a reversed passive latex agglutination (RPLA) kit. Results are expressed as the mean ± SD of the Stx concentration (\(\mu\)g) per g of cecal contents for six mice.

\(d\) Mice were observed for survival for 16 days after STEC challenge.

\(e\) ND: Not detected. The lower detection level was 0.04 \(\mu\)g/g cecal contents.
BbY-treated group, increases in the STEC stx1 and stx2 mRNA expressions in the cecum after MMC inoculation and the production of Stx1 and Stx2 were markedly inhibited (Fig. 2-2). There were no marked differences in the mRNA expressions of eaeA, espA, and espB, which are involved in the adhesion of STEC to the intestinal epithelium, or hly, which is involved in hemolysin production, between the STEC-infected control and the BbY-treated groups (Data not shown). Pathologically, although infectious injury in the intestinal epithelium, kidneys, bone marrow, and mesenteric lymph nodes were observed in the control group, they were not seen in the BbY-treated group (Data not shown).

Subsequently, we compared the anti-infectious activity among 4 strains of bifidobacteria of different origins with a strong natural resistance to SM (MIC >4 mg/ml) (Table 2). In the BbY-treated and B. pseudocatenulatum DSM 20439-treated groups, the intestinal proliferation of STEC was not inhibited in comparison with the STEC-infected control group. However, we observed marked anti-infectious activities such as an increase in the survival rate, reduced body weight loss, and inhibition of stx mRNA expression and Stx production. However, in the B. bifidum ATCC 15696-treated and B. catenulatum ATCC 27539T-treated groups, there was no anti-infectious activity. In the STEC-infected control group, the cecum level of acetic acid decreased to 28 mM, and the pH value increased to 7.15 (Fig. 2-3). In the groups treated with Bifidobacterium strains exhibiting anti-infectious activities, the cecum level of acetic acid was 56

Table 2. Comparison of the anti-toxic activities of several strains of bifidobacteria with natural resistance to SM

| Treatment with | No. of deaths/ Total no. of mice a,b,c,d (Survival time, [day, mean ± SD]) | STEC viable counts / g feces at 18 hr after infection (Log10 mean ± SD) a,b | Stx concentration in the cecal contents a,b,c,e | stx mRNA in the cecal contents a,b,c,f |
|---------------|-------------------------------------------------|-------------------------------------------------|---------------------------------------------|---------------------------------------------|
| Untreated control | 8/10 (8.0 ± 1.2) | 9.2 ± 0.2 | 0.7 ± 0.5 | 0.7 ± 0.5 |
| BbY | 0/10 | 9.0 ± 0.3 | 0.1 ± 0.1* | 1.9 ± 1.2** |
| B. pseudocatenulatum DSM 20439 | 0/10 | 9.1 ± 0.3 | 0.1 ± 0.1* | 1.4 ± 1.4** |
| B. bifidum ATCC 15696 | 8/10 (8.6 ± 1.4) | 9.1 ± 0.2 | 0.6 ± 0.6 | 141.3 ± 99.7 |
| B. catenulatum ATCC 27539T | 7/10 (8.6 ± 1.4) | 9.0 ± 0.3 | 0.8 ± 0.3 | 155.7 ± 128.6 |

a SM at a concentration of 5 mg/ml in drinking water was given to mice from day –6 until day 16. Bifidobacterial strains (1×10^8 CFU/mouse/day) at an inoculum size of 0.1 ml/mouse were administered to separate groups of mice (10 mice/group) once a day from day –5 until day 3.

Population levels of bifidobacteria in feces at the time of STEC infection (Log10 mean ± SD): BbY: 9.7 ± 0.1, B. pseudocatenulatum DSM 20439: 9.7 ± 0.1, B. bifidum ATCC 15696: 9.6 ± 0.2, B. catenulatum ATCC 27539T: 9.8 ± 0.3.

b Mice were orally infected with STEC at a dose of 8.1×10^{11} CFU on day 0.

c MMC (0.25 mg/kg BW) was administered a total of 3 times at 18, 21, and 24 hr post-infection.

d Mice were observed for survival for 16 days after STEC infection.

Significant difference between the Bifidobacterium-treated and untreated control mice (*p<0.05, **p<0.01).
mM, and the pH value was 6.75; abnormalities in the enteric environment observed in the STEC-infected control group, were markedly reduced. However, in the groups treated with *Bifidobacterium* strains without anti-infectious activities, there was no improvement in the enteric environment.

In addition, in a medium in which the cecum level of acetic acid and pH value of the groups treated bifidobacteria with anti-infectious activities were reproduced, Stx2 production induced by STEC after MMC stimulation was inhibited to less than one thirtieth of that of the STEC-infected control group, although the proliferation of STEC was not inhibited (Fig. 2-4).

Thus, in the STEC-infected mice, there were marked differences in the anti-infectious activities of the bifidobacteria strains. *Bifidobacterium* strains that improved the enteric environment via an increase in the acetic acid level and a decrease in pH, inhibited STEC stx gene expression and Stx production, and exhibited an anti-infectious activity without inhibiting the intestinal proliferation of STEC.

**PROTECTIVE EFFECT OF THE SYNBIOTIC ADMINISTRATION OF BIFIDOBACTERIA AND TRANSGALACTOSYLATED OLIGOSACCHARIDES ON SYSTEMIC INFECTION WITH *Salmonella* (1)**

Prebiotics are nondigestible food constituents that selectively alter the growth or activity of one or a limited number of bacterial species in the colon in a manner that potentially improves the health of the host (7, 15). Used in combination, pro- and prebiotics are called synbiotics (7). We investigated the effects of the synbiotic administration of probiotics (bifidobacteria) and prebiotics (transgalactosylated oligosaccharides (TOS)) on intestinal infection with *S. Typhimurium* LT-2 (LT-2). Mice in which the stable intestinal colonization of BbY at $10^{10}$ CFU/g was achieved by the oral administration of BbY (10⁸ CFU/mouse) under SM treatment, were orally infected with $10^{2}$ CFU of the SM-resistant LT-2. Explosive intestinal growth of LT-2 ($10^{9}$ CFU/g), as observed in the infected control group, was markedly inhibited (Fig. 3-1). Combination therapy of a selective bifidobacteria growth factor, TOS, and BbY enhanced BbY’s inhibitory effects on LT-2 growth (Fig. 3-1). In addition, in the BbY-treated and BbY + TOS-treated
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Inhibition of opportunistic intestinal infection with S. Typhimurium LT-2 (LT-2) by BbY colonization in SM-treated mice. SM at a concentration of 2 mg/ml in drinking water was given to mice from day –5 until day 7. BbY (1-3 × 10^8 CFU/mouse/day) and transgalactosylated oligosaccharides (TOS, 10 mg/mouse/day) at an inoculum dose of 0.1 ml/mouse were administered to mice once a day from day –7 until day 7. Mice were infected orally with LT-2 (1 × 10^8 CFU) on day 0. Feces were obtained on days 1, 3, 5, and 7 after infection, and the counts of viable LT-2 (panel A) and BbY (panel B) were examined using selective media. Results are expressed as the mean and standard deviation of six mice. Symbols for panel A: ○: infection control, ●: TOS, △: BbY, □: BbY + TOS. Symbols for panel B: △: BbY, □: BbY + TOS. a: The values were below the lower detection limit (100 CFU).

Significant difference between the treated groups and untreated control: ** p<0.01.

Fig. 3-1. Inhibition of opportunistic intestinal infection with S. Typhimurium LT-2 (LT-2) by BbY colonization in SM-treated mice. SM at a concentration of 2 mg/ml in drinking water was given to mice from day –5 until day 7. BbY (1-3 × 10^8 CFU/mouse/day) and transgalactosylated oligosaccharides (TOS, 10 mg/mouse/day) at an inoculum dose of 0.1 ml/mouse were administered to mice once a day from day –7 until day 7. Mice were infected orally with LT-2 (1 × 10^8 CFU) on day 0. Feces were obtained on days 1, 3, 5, and 7 after infection, and the counts of viable LT-2 (panel A) and BbY (panel B) were examined using selective media. Results are expressed as the mean and standard deviation of six mice. Symbols for panel A: ○: infection control, ●: TOS, △: BbY, □: BbY + TOS. Symbols for panel B: △: BbY, □: BbY + TOS. a: The values were below the lower detection limit (100 CFU).

Significant difference between the treated groups and untreated control: ** p<0.01.

Fig. 3-2. Importance of undissociated acetic acid in the intestine in the growth-inhibition of LT-2. The pH and concentration of acetic acid (A.A.) were adjusted in the growth medium so that conditions were the same as in the normal cecum (●: pH 6.4, A.A.: 60 mM), BbY-colonized cecum (○: pH 6.75, A.A.: 45 mM), and B. bifidum ATCC 15696-colonized cecum (△: pH 6.8, A.A.: 29 mM). Then, LT-2 at a concentration of 10^4 CFU/ml was added to each medium and cultivated at 37 °C for 0, 1, 3, or 6 h, and viable bacterial counts were determined. Results are expressed as the percent growth.

Table 3. Comparison of anti-infectious activities of SM-resistant Bifidobacterium strains in combination with TOS

| Treatment a | Number of LT-2 in organ b |
|-------------|--------------------------|
|             | Spleen | MLN | Cecum |
| Saline(Control) | 3.8 ± 0.3c (6/6)d | 3.9 ± 0.2 (6/6) | 8.2 ± 0.4 (6/6) |
| TOS | 3.7 ± 0.7 (6/6) | 3.6 ± 0.3 (6/6) | 8.2 ± 0.6 (6/6) |
| BbY | 2.9 (1/6) | 3.2 (1/6) | 6.9 ± 0.8* (6/6) |
| B. pseudocatenulatum DSM20439 | 3.0 (1/6) | 3.4 (1/6) | 6.9 ± 0.9* (6/6) |
| B. bifidum ATCC15696 | 3.5 ± 0.5 (5/6) | 3.2 ± 0.4 (6/6) | 8.5 ± 0.4 (6/6) |
| B. catenulatum ATCC27539T | 3.5 ± 0.5 (5/6) | 3.4 ± 0.5 (6/6) | 8.0 ± 0.6 (6/6) |

a Bifidobacterium strains at a dose of 1-3 × 10^8 CFU/mouse and TOS at a dose of 10 mg were orally administered to mice once a day from day –7 until day 7. Population levels of bifidobacteria at the time of LT-2 infection. (Log10: mean ± SD): BbY: 9.3 ± 0.2, B. pseudocatenulatum DSM 20439: 8.9 ± 0.4, B. bifidum ATCC 15696: 9.5 ± 0.2, B. catenulatum ATCC 27539T: 9.1 ± 0.8.

b Mice were infected with LT-2 at 10^2 CFU on day 5 after starting SM treatment, and were dissected 7 days later.

c The results are expressed as the mean Log10 CFU and SD per entire organ (spleen and MLN) or g cecal contents.

d Numbers of organs in which LT-2 were detected / number of organs tested.

Significant difference between the Bifidobacterium-treated and control group (* p<0.05).
In conclusion, the oral administration of *Bifidobacterium* strains that improve the enteric environment inhibited the abnormal growth of *Salmonella* in the intestine, preventing systemic infection with *Salmonella*. This growth-inhibitory effect was further strengthened by synbiotics.

CONCLUSION

In this study, we newly developed a mouse fatal intestinal infection model using a small inoculum of STEC. We found that bacterial proliferation and Stx2 production were closely involved in the appearance of pathogenicity in this model. This mouse model is simpler than previously reported experimental animal models, such as gnotobiotic mice, malnourished mice, gnotobiotic piglets, chickens, and newborn rabbits, with regard to treatment and maintenance conditions, and large-scale maintenance is possible. In addition, the mortality of this mouse model is very high, infection is established by oral infection with a small number of infectious bacteria, and fatal Stx production by STEC in the intestine can be analyzed in detail, suggesting that this model will be very useful as an evaluation system for antibiotics, vaccines, Stx adsorbents, and food.

Regarding the mechanisms of the inhibition of pathogenic bacterial infection by bifidobacteria and lactobacilli, the inhibition of enteropathogen-cell interactions, growth inhibition by competition for nutrients, inhibition of toxin binding to receptors, action of antibacterial factors, such as bacteriocin, and immune activation have been reported. The infection-preventive effect of bifidobacteria may be exerted by improving antimicrobial agent-induced abnormal intestinal environments (disruption of colonization resistance), different from the previously reported mechanisms. Of the organic acids serving as indices of the intestinal environment, acetic, lactic, and citric acids have been shown to exhibit more potent bactericidal activity against pathogens than inorganic acids, such as hydrochloric acid. The bactericidal activity of organic acids has been reported to reside mainly in the non-dissociated forms. Thus, an elevated concentration of acetic acid, which is the main organic acid in the intestine as well as the main metabolic product of bifidobacteria, and a reduced pH may be very important in the infection-preventive action. There were 2 characteristics of the intestinal environment-improving effect-based prevention of infection by bifidobacteria. One was inhibition of pathogenic factors observed in intestinal STEC infection (inhibition of pathogenic factors of the pathogen harmful to the host without reducing the number of infectious bacteria), and the other was growth inhibition of intestinal *S. Typhimurium* infection (development of infectious disease was inhibited by suppressing the growth of the pathogen and decreasing its count). The infection-preventive mechanism of bifidobacteria against the causative bacteria of intestinal infectious diseases may be different corresponding to the pathogenesis and disease development mechanism of the pathogens.

This study also clarified that the infection-preventive effect of bifidobacteria markedly varied among strains. Not all strains are suitable for clinical application as probiotics, and the usefulness of strains should be scientifically demonstrated through basic research and clinical studies. The results regarding the anti-infectious activity of bifidobacteria in these mouse intestinal STEC and *S. Typhimurium* infection models suggest the usefulness of probiotics in the prevention of intestinal infection in humans.

APPLICATION FOR THE PREVENTION OF GASTROINTESTINAL INFECTIOUS DISEASES IN THE CLINICAL PROBIOTICS FIELD

Clinical studies on the intestinal environment-improving, infection-preventive action of probiotics including bifidobacteria have recently been reported. In the digestive surgery field, the prevention of postoperative infectious complications is a major issue. Liver and extrahepatic bile duct resection with hepatojejunostomy for biliary cancer is very invasive, and infectious complications, such as wound infection, intra-abdominal abscess, and bacteremia, often occur after surgery. The translocation of intestinal bacteria is considered to be one cause. Kanazawa et al. reported that the number of useful bacteria, such as *Bifidobacterium* and *Lactobacillus*, markedly decrease in the intestine after surgery in patients with biliary cancer, whereas the counts of bacteria which may cause infectious diseases, *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and *Candida*, increase, and the intestinal total organic acid and acetic acid concentrations markedly decrease. They also investigated the infection-preventive effect of the addition of synbiotics (BbY, *L. casei* strain Shirota, and galactooligosaccharides) to postoperative enteric nutrition in patients with biliary cancer, and found that synbiotic treatment markedly improved the intestinal bacterial flora and intestinal environment (total organic acid and acetic acid concentrations) and significantly reduced the incidence
of infectious complications in patients after surgery for biliary cancer. Furthermore, the quality of life of patients was improved through shortening of the postoperative hospital stay and duration of antibiotic therapy. Sugawara et al. (36) also clarified that synbiotic treatment not only before but also after surgery for biliary cancer has beneficial effects.

Neonates of pediatric surgery field requiring surgery and intensive care immediately after birth (short-bowel syndrome, hirschsprung’s disease, and laryngotracheoesophageal cleft) are treated with antibiotics and surgery under the restriction of oral ingestion immediately after birth, which markedly aggravates the intestinal bacterial flora and leads to malnutrition and susceptibility to infection. Kanamori et al. (19–22) performed symbiotic therapy for these patients (enteric or oral administration), and achieved improvements in the intestinal bacterial flora, environment (short-chain fatty acid (SCFA) concentrations), function (peristalsis and fecal properties), nutritional condition (body weight gain) and reduced the incidences of enteritis and systemic infectious disease. In the emergency medical care field, infectious complications are fatal in systemic inflammatory response syndrome (SIRS) patients. Shimizu et al. (23) reported that the symbiotic treatment of SIRS patients markedly improved the abnormal intestinal flora and environment, i.e. increased the number of Bifidobacterium, decreased pH, and increased the SCFA concentration, and reduced the incidence of infectious complications, such as bacteremia, enteritis, and pneumonia.

It may be important to perform many basic researches and clinical studies following medical criteria, as described above. The accumulation and systematization of information may further improve the clinical applicability of probiotics and synbiotics.

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