Oral Administration of *Butyrivibrio fibrisolvens*, a Butyrate-Producing Bacterium, Decreases the Formation of Aberrant Crypt Foci in the Colon and Rectum of Mice¹,²

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ABSTRACT *Butyrivibrio fibrisolvens*, a butyrate-producing ruminal bacterium, was evaluated for use as a probiotic to prevent colorectal cancer. Oral administration to Jcl:ICR mice of a new strain of *B. fibrisolvens* (MDT-1) that produces butyrate at a high rate (10⁹ cfu/dose) increased the rate of butyrate production by fecal microbes, suggesting that MDT-1 can grow in the gut. The number of colorectal aberrant crypt foci (ACF), putative preneoplastic lesions induced by 1,2-dimethylhydrazine, was reduced after MDT-1 administration (10⁹ cfu/dose, 3 times/wk for 4 wk). The number of aberrant crypts (ACs), number of foci having 3 or 4 ACs per focus, and the percentage of mice having 3 or 4 ACFs per focus were also reduced, suggesting that the progression of lesions was suppressed by MDT-1. Interestingly, the MDT-1 cell homogenate did not have a similar beneficial effect. MDT-1 had low β-glucuronidase activity, and administration of MDT-1 reduced the β-glucuronidase activity in the colorectal contents. The numbers of natural killer (NK) and NKT cells in the spleen were markedly enhanced in response to MDT-1. Decreased β-glucuronidase activity and increased numbers of NK and NKT cells and butyrate production may explain in part why MDT-1 administration suppressed ACF formation. These results suggest that colorectal cancer may be prevented or suppressed by the utilization of MDT-1 as a probiotic. Administration of MDT-1 had no harmful effect on the health of mice at least for 3 mo. J. Nutr. 135: 2878–2883, 2005.

KEY WORDS: • *Butyrivibrio fibrisolvens* • intestinal bacteria • probiotic • colon cancer • butyrate

SCFA, particularly butyrate, produced by intestinal microbiota play important roles in the physiology and metabolism of the intestine and intestinal mucosa (1,2). In addition to serving as a preferred energy source for colonocytes (3–5), butyrate has been implicated in protection against colon cancer and ulcerative colitis (6). The incidence of human colorectal cancer is increasing in developed countries (7–9), and large intestinal disease is also a common problem in small animal practice (10). Thus, augmentation of butyrate production in the intestine would be desirable for the maintenance of colonic health in both humans and pet animals.

Butyrate is produced by intestinal bacteria from carbohydrates such as resistant starch, dietary fiber, and fructooligosaccharides (8,9) that escape digestion in the small intestine. The effects of increasing quantities of low-digestible dietary carbohydrates on the supply of butyrate were investigated in experimental animals, including pet animals (10,11). Similar studies in rodents also investigated the ability of low-digestable carbohydrates to protect against experimentally induced tumors (8,9,12). The results of these studies suggest that a stable butyrate-producing colonic ecosystem resulting from resistant starch and fiber in the diet can reduce the incidence of colonic diseases (9,13).

Among butyrate-producing bacteria, *Clostridium butyricum* has been used in humans and animals of far-east Asia to ameliorate diarrhea and constipation (14,15). Recently, administration of this bacterium was shown to suppress chemically induced aberrant crypt foci (ACF),⁴ putative preneoplastic lesions, in the rat colon (16) and experimental colitis in rats (17,18). Increased production of butyrate was suggested to be one of the reasons (17,18) for the beneficial effects of *C. butyricum*.

We are interested in the utilization of *Butyrivibrio fibrisolvens*, a representative butyrate-producing ruminal bacterium with a high capacity to produce butyrate (19), as a probiotic. The ability of *B. fibrisolvens* to produce conjugated linoleic acid (CLA) (20) may also benefit health (21) by prevention of carcinogenesis (22), atherosclerosis (23), and tumorigenesis (24,25), and improvement of hyperinsulinemia (26) and immune functions (27,28).

*B. fibrisolvens* also resides in the intestine of humans (29).

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² Supplemental Tables 1 and 2 are available as Online Supporting Material with the online posting of this paper at www.nutrition.org.

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⁴ Abbreviations used: ACF, aberrant crypt foci; AC, aberrant crypt; BSA, bovine serum albumin; cfu, colony-forming unit; CLA, conjugated linoleic acid; DMH, 1,2-dimethylhydrazine; FITC, fluorescein isothiocyanate; NK, natural killer; NKT cell, natural killer T cell; PE, phycoerythrin.
MATERIALS AND METHODS

Bacterial strains and culture conditions. B. fibrisolvens strains (ATCC19171, ATCC51255, OB156, A38, and TH1) were obtained as described previously (31). Another strain of B. fibrisolvens, called MDT-1, was newly isolated from the rumen of a Japanese native goat by the roll tube method (32), and identified according to Bryant (33). C. butyricum MIYAIRI 588 was provided by Meiji Seika Kaisha.

All bacterial strains were grown in a growth medium for B. fibrisolvens, which consisted of a basal medium (31) and mixtures of SCFA, vitamins, and trace minerals (34). Details of culture conditions and procedures were as described previously (31). Cell growth was estimated by optical density at 600 nm (OD600). Cells were harvested at OD600 1.3–1.5 (late-log growth), and immediately cooled on ice. Viable cells were counted using Hungate’s roll tube method in a medium containing 2% agar (32). Five tubes were measured for each sample, and colony numbers were counted.

Mice. Male Jcl:ICR mice (4 wk old; CLEA Japan) were used in all experiments. Mice were housed in plastic cages with wire tops, and permitted free access to a commercial diet (CE-2, CLEA Japan) and water. The diet contained 249 g crude protein, 46 g crude fat, 31 g crude fiber, 67 g ash, 514 g nitrogen-free extracts, and 14.51 MJ/kg. General animal handling and care procedures were carried out according to the “Guideline for the Care and Use of Laboratory Animals, Meiji University.”

Oral administration of bacteria to mice. B. fibrisolvens MDT-1 and C. butyricum grown to late-log stage were collected by centrifugation (5000 × g, 10 min, 4°C) and washed with 0.8% NaCl. Washed cells, resuspended in 0.8% NaCl (10^13 cfu/L), were used as described previously (31). Addition of probiotics to a culture of mixed fecal microbes of dogs resulted in increased butyrate production (30), introduction of B. fibrisolvens as a probiotic, or stimulation of its growth, may increase butyrate and CLA production in the gut. We hypothesized that administration of probiotic B. fibrisolvens to humans will also increase butyrate and CLA production in the gut.

Group A: administration of saline; Group B: administration of DMH (D), Group C: administration of DMH and intact B. fibrisolvens MDT-1 (Bi) 3 times/wk; Group D: Administration of DMH and intact MDT-1 concentrate (Bi) 3 times/wk; Group E: Administration of DMH and MDT-1 cell homogenates (Bh) 3 times/wk; Group F: administration of DMH and intact C. butyricum (Ci) 3 times/wk; A: Autopsy.

The numbers of specific leukocyte subtypes were measured by immunofluorescent antibody staining using a flow cytometer (FACSCalibur, Becton Dickinson). Lymphocyte subpopulations were identified and gated using forward vs. side scatter characteristics. T cells were identified using fluorescein isothiocyanate (FITC)-conjugated rat anti-mouse CD3 (clone KT3). B cells and natural killer (NK) cells were identified using phycoerythrin (PE)-conjugated rat anti-mouse B19 (6D5) and PE-conjugated rat anti-mouse NK1.1 (PK136), respectively. All monoclonals were obtained from Beckman Coulter. Cell suspensions were incubated with antibody for 20 min at room temperature, washed with PBS, and read on the FACSCalibur; 5000–5000 events were acquired from each preparation. Control samples matched for each fluorochrome and each antibody isotype were used to set negative staining criteria. Data were analyzed using CELLquest software (Becton Dickinson).

Assay of β-glucuronidase activity. Bacterial cultures were centrifuged (5000 × g, 10 min, 4°C), and the supernatant was assayed to measure extracellular β-glucuronidase activity. The cells resuspended in 50 µL of 0.1 mol/L sodium acetate buffer (pH 5.0) containing 0.2% (wt/v) BSA were disrupted by sonication (<4°C), and the supernatant (cell homogenate) was assayed for intracellular β-glucuronidase activity.

Results

EFFECT OF B. FIBRISOLVEN S ON ACF

Among the 6 groups, group C, DMH and cell homogenates of MDT-1 3 times/wk; and group F, DMH and cell homogenates of MDT-1 3 times/wk; and group F, DMH and intact MDT-1 concentrate (Bi) 3 times/wk, were found to have significantly (P < 0.05) reduced the number of ACF compared to the control group. The number of ACF in group F was significantly (P < 0.05) reduced compared to group D, which was treated with DMH and intact MDT-1 concentrate (Bi) 3 times/wk.

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RESULTS

Butyrate-producing ability of B. fibrisolvens MDT-1. A new strain of B. fibrisolvens, MDT-1, produced a higher proportion of butyrate than other strains of B. fibrisolvens and C. butyricum, \( P < 0.05 \) (Supplemental Table 1). The growth rate of MDT-1 did not differ from the rates of B. fibrisolvens TH1 and C. butyricum MIYARI 588, and was higher than those of the other strains of B. fibrisolvens \( (P < 0.05) \). Thus, MDT-1 produced butyrate at a higher rate than other strains examined.

Activity of \( \beta \)-glucuronidase in B. fibrisolvens. \( \beta \)-Glucuronidase activity was not detected in the cell homogenate of 6 strains of B. fibrisolvens, whereas the activity in the culture supernatant was 1.5–3.1 nmol/(h · mg cell-N), indicating that B. fibrisolvens secretes \( \beta \)-glucuronidase (Supplemental Table 2). Similar results were obtained with C. butyricum MIYARI 588. \( \beta \)-Glucuronidase activities in C. perfringens 1290 and E. coli K12 were ~9-fold higher than from B. fibrisolvens. Furthermore, these 2 bacteria demonstrated cell-associated \( \beta \)-glucuronidase activities that were 430- and 150-fold higher, respectively, than levels of the secreted enzyme. Thus, \( \beta \)-glucuronidase activity in B. fibrisolvens appeared to be generally low, and MDT-1 had the lowest activity.

Effect of MDT-1 on SCFA production in the intestine (Expt. 1). Administration of MDT-1 to mice resulted in a gradual increase in the initial rate of butyrate production by fecal microbes during the first 7 d (Table 1). Assuming that fecal microbes represent intestinal microbiota, and that the initial rate of butyrate production reflects the initial numbers of butyrate-producing bacteria, this increase indicates that MDT-1 administration resulted in more butyrate-producing bacteria in the gut than in the controls. The elevated rate of butyrate production was maintained until d 14, and then decreased slowly to the basal value by d 21. In contrast, when C. butyricum was administered, the initial rate of butyrate production was significantly increased on the next day, but returned to the basal level by d 3 (Table 1).

Administration of MDT-1 increased the rate of propionate production and decreased the rate of succinate production (Table 1). However, C. butyricum did not affect the production of these organic acids.

Effect of MDT-1 on ACF formation (Expt. 2). No ACF were observed in mice that were not treated with DMH. After administration of intact MDT-1 cells 1 and 3 times/wk, the

<table>
<thead>
<tr>
<th>Bacterium administered (1 × 10^8 cfu/mouse)</th>
<th>Succinate (mmol/L · h)</th>
<th>Lactate (mmol/L · h)</th>
<th>Acetate (mmol/L · h)</th>
<th>Propionate (mmol/L · h)</th>
<th>Butyrate (mmol/L · h)</th>
<th>Total (mmol/L · h)</th>
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<td></td>
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<tr>
<td>Day 0</td>
<td>0.45 ± 0.06</td>
<td>9.74 ± 0.69</td>
<td>1.58 ± 0.33</td>
<td>0.56 ± 0.17</td>
<td>0.17 ± 0.01</td>
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<td>0.18 ± 0.02</td>
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<td>9.01 ± 1.08</td>
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<td>0.40 ± 0.12</td>
<td>0.16 ± 0.02</td>
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<td>7</td>
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<td>0.55 ± 0.11</td>
<td>0.17 ± 0.02</td>
<td>11.26 ± 1.03</td>
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<td>8.79 ± 1.40</td>
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<td>0.67 ± 0.12</td>
<td>0.16 ± 0.02</td>
<td>11.59 ± 1.49</td>
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<td>9.32 ± 0.82</td>
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<td>0.64 ± 0.14</td>
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<td>12.28 ± 0.93</td>
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<td>B. fibrisolvens</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
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<td>9.67 ± 0.89</td>
<td>1.43 ± 0.41</td>
<td>0.63 ± 0.18</td>
<td>0.17 ± 0.02</td>
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<td>0.18 ± 0.02</td>
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<td>0.50 ± 0.12</td>
<td>9.30 ± 0.93</td>
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<td>11.77 ± 0.98</td>
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<td>0.50 ± 0.18</td>
<td>0.20 ± 0.02</td>
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<td>0.46 ± 0.19</td>
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<td>11.63 ± 1.64</td>
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<td>0.37 ± 0.13</td>
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<td>1.73 ± 0.31</td>
<td>0.33 ± 0.18</td>
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\( P \)-value

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<tr>
<th>Bacterium</th>
<th>Day</th>
<th>Bacterium × Day</th>
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<tr>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&lt;0.0001</td>
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</table>

1 Values are means ± SEM, \( n = 5 \). Means in a column with superscripts without a common letter differ, \( P < 0.05 \).

2 Bacteria were administered on d 0 (1 dose).
numbers of DMH-induced ACF per mouse were reduced from 24.0 to 17.9 (75%) and 9.4 (40%), respectively (Table 2). More pronounced amelioration by intact MDT-1 was noted in the numbers of ACs per mouse, which decreased to 60% (1 time/wk) and 30% (3 times/wk) of control mice. Intact MDT-1 cells also reduced the number of foci having 3 or 4 ACs per focus. The percentage of mice having 3 or 4 ACs per focus was reduced from 70% in the control to 20% after treatment 3 times/wk (Expt. 3). Interestingly, MDT-1 cell homogenates did not reduce the incidence of ACF, suggesting that intact or viable cells are required for this effect. However, intact cells of C. butyricum had no effect on the frequency of ACF formation or the numbers of AC per focus.

Administration of DMH decreased the body weight gain, cecum weight, colorectum weight, colorectum length, and the weight per length of colorectum of mice, and increased the spleen weight (data not shown, P < 0.05). Administration of intact MDT-1 cells 3 times/wk lessened these effects (P < 0.05), but neither intact MDT-1 cell homogenate nor intact cells of C. butyricum alleviated these effects (data not shown). Thus, live MDT-1 appears to mitigate the detrimental effects of DMH.

$\beta$-Glucuronidase activity in the cecal and colonic contents of mice treated with DMH were 50 and 140% greater, respectively, than in control mice, and provision of intact MDT-1 cells alleviated the effect of $\beta$-glucuronidase activity (Table 3). Neither the MDT-1 cell homogenate nor intact C. butyricum cells affected $\beta$-glucuronidase activities in the cecal and colonic contents.

Effect of MDT-1 on the splenic lymphocyte population (Expt. 3). Administration of intact MDT-1 cells (1 dose) did not affect the numbers of T and B cells in the spleen after 1 wk, but the numbers of NK and NKT cells (CD3/NK1.1 double positive cells) were 3.5- and 3.9-fold, respectively, greater than those of control mice (data not shown, P < 0.05).

**DISCUSSION**

Administration of live MDT-1 to mice reduced the number of DMH-induced ACF (Table 2), suggesting that the presence of MDT-1 reduces the incidence of lesions (41). MDT-1 also decreased the number of ACs, the number of foci having 3 or 4 ACs per focus, and the percentage of mice having 3 or 3 ACs per focus, which suggests that the growth of lesions is suppressed by administering MDT-1 (41). However, MDT-1 cell homogenates did not reduce the incidence of ACF. It is conceivable that increased butyrate production and/or modified composition of intestinal bacteria due to live or growing MDT-1 cells alleviated the effect of DMH.

When live MDT-1 was administered, the rate of butyrate production by fecal microbes increased gradually (Table 1), suggesting that MDT-1 cell numbers increased slowly in the gut. Administration of MDT-1 increased propionate production and decreased succinate production (Table 1), suggesting that the number of bacteria producing propionate via the succinate pathway increased. Thus, the overall composition of microbial members may be altered by the growth of MDT-1.

Administration of live MDT-1 reduced $\beta$-glucuronidase activity in the contents of the colon and rectum (Table 3). Why B. fibrisolvens reduced the total $\beta$-glucuronidase activity of intestinal microbiota is not clear, but it is possible that its administration suppressed the growth of other bacteria with $\beta$-glucuronidase activity.

**TABLE 2**

*Effect of the administration of B. fibrisolvens MDT-1 on the number of ACF in mice (Expt. 2)*

<table>
<thead>
<tr>
<th>Administration of DMH</th>
<th>Bacterium</th>
<th>Number of ACs/$^1$</th>
<th>Number of ACs/$^2$</th>
<th>1 or 2 ACs/focus/$^1$</th>
<th>3 or 4 ACs/focus/$^1$</th>
<th>Mice with 3 or 4 ACs/focus/$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>No</td>
<td>24.0 ± 4.2$^a$</td>
<td>30.9 ± 4.3$^a$</td>
<td>22.7 ± 4.0$^a$</td>
<td>1.3 ± 0.1$^a$</td>
<td>70$^a$</td>
</tr>
<tr>
<td>Yes</td>
<td>B. fibrisolvens (intact cells), 1 time/wk</td>
<td>17.9 ± 3.0$^b$</td>
<td>21.9 ± 2.8$^b$</td>
<td>17.4 ± 2.8$^b$</td>
<td>0.5 ± 0.2$^b$</td>
<td>50$^{ab}$</td>
</tr>
<tr>
<td>Yes</td>
<td>B. fibrisolvens (intact cells), 3 times/wk</td>
<td>9.4 ± 1.3$^c$</td>
<td>11.5 ± 1.7$^c$</td>
<td>9.0 ± 1.3$^c$</td>
<td>0.4 ± 0.2$^c$</td>
<td>20$^c$</td>
</tr>
<tr>
<td>Yes</td>
<td>B. fibrisolvens (cell homogenate), 3 times/wk</td>
<td>19.7 ± 2.7$^{ab}$</td>
<td>25.0 ± 3.1$^{ab}$</td>
<td>18.7 ± 2.1$^c$</td>
<td>0.9 ± 0.3$^{ab}$</td>
<td>70$^a$</td>
</tr>
<tr>
<td>Yes</td>
<td>C. butyricum (intact cells), 3 times/wk</td>
<td>25.6 ± 4.8$^a$</td>
<td>32.6 ± 4.4$^a$</td>
<td>24.2 ± 3.8$^a$</td>
<td>1.4 ± 0.2$^a$</td>
<td>70$^a$</td>
</tr>
</tbody>
</table>

$^1$ Values (n/mouse) are means ± SEM, n = 10. Values in a column without a common letter differ, P < 0.05.

$^2$ Percentage of total mice having ACF. Data were evaluated at P < 0.05 by Fisher’s Exact Test.

**TABLE 3**

*Effect of the administration of B. fibrisolvens MDT-1 on $\beta$-glucuronidase activity in the cecal and colonic contents of mice (Expt. 2)*

<table>
<thead>
<tr>
<th>Administration of</th>
<th>Bacterium</th>
<th>Cecal content</th>
<th>Colonic content</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMH</td>
<td></td>
<td>µmol/(h · g wet contents)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>No</td>
<td>22.6 ± 4.4$^c$</td>
<td>10.9 ± 1.4$^c$</td>
</tr>
<tr>
<td>Yes</td>
<td>No</td>
<td>34.1 ± 3.1$^{ab}$</td>
<td>26.5 ± 5.7$^{ab}$</td>
</tr>
<tr>
<td>Yes</td>
<td>B. fibrisolvens (intact cells), 1 time/wk</td>
<td>29.6 ± 3.2$^{bc}$</td>
<td>24.3 ± 1.9$^c$</td>
</tr>
<tr>
<td>Yes</td>
<td>B. fibrisolvens (intact cells), 3 times/wk</td>
<td>26.7 ± 4.1$^c$</td>
<td>15.7 ± 3.4$^{bc}$</td>
</tr>
<tr>
<td>Yes</td>
<td>B. fibrisolvens (cell homogenate), 3 times/wk</td>
<td>36.2 ± 3.2$^a$</td>
<td>29.3 ± 3.0$^a$</td>
</tr>
<tr>
<td>Yes</td>
<td>C. butyricum (intact cells), 3 times/wk</td>
<td>39.6 ± 2.0$^a$</td>
<td>30.4 ± 2.0$^a$</td>
</tr>
</tbody>
</table>

$^1$ Values are means ± SEM, n = 10. Means in a column without a common letter differ, P < 0.05.
high β-glucuronidase activity, because \( B. \) \( \text{fibrisolvens} \) produces bacteriocins (42).

DMH is a colon carcinogen that conjugates with glucuronic acid in the liver and is excreted into the intestine via the bile (43). Because the glucuronic conjugate is hydrolyzed by β-glucuronidase in the intestine, resulting in reversion to carcinogenic DMH (44), β-glucuronidase plays an important role in the induction of ACF. It was reported that a β-glucuronidase inhibitor suppressed azoxymethane (AOM)-induced carcinogenesis in the rat colon (45), and that there was a positive correlation between fecal β-glucuronidase activity and the number of AOM-induced ACF in the rat colon (46). These findings suggest that the formation of ACF is suppressed by a decrease in β-glucuronidase activity in the colon. The reduction in β-glucuronidase activity in the colorectal contents in response to MDT-1 administration appears to be one of the reasons why ACF formation was suppressed.

Great increases in the numbers of NK and NKT cells were counted in the spleen of mice administered live MDT-1. NK cells constitute a separate lineage of lymphocytes capable of mediating early innate immune responses to viral infections as well as recognizing transformed malignant cells (48,49). Roles for NK cells in tumor rejection and cytolytic activity against tumor cell lines in vitro are well recognized (50,51). The roles of NK cells in tumor rejection and cytolytic activity against tumor cell lines in vitro are well recognized (50,51). The administration of \( B. \) \( \text{fibrisolvens} \) may have an effect on the suppression of ACF formation. A number of studies indicate that administration of biotics, binding or degrading potential carcinogens, producing short chain fatty acids, increases in the numbers of NK and NKT cells, and perhaps enhanced butyrate production in the gut. It may be possible to prevent or suppress colorectal cancer in humans and pet animals by the utilization of MDT-1 as a probiotic.

**LITERATURE CITED**


