ABSTRACT

Fermented milk supplemented with 2 probiotic strains, Bifidobacterium lactis Bi-07 and Lactobacillus acidophilus NCFM, and a prebiotic, isomaltooligosaccharide, was orally administered to 100 healthy adults at 480 g/d for 2 wk in a randomized controlled trial. The fecal bacterial compositions of these subjects were examined by culture before and after the intervention. The same fermented milk was also orally fed to BALB/c mice, and immune as well as fecal bacteria analyses were conducted using the same culturing methods. After the intervention, increases in fecal bifidobacteria and lactobacilli were observed among the subjects compared with the subjects in the control group. In contrast, after the intervention, fecal enterobacteria were significantly decreased in the test group compared with the control group. The same effects on the composition of the intestinal microbiota were observed in mice. Furthermore, the tested mice were found to have significantly increased delayed-type hypersensitivity, plaque-forming cells, and half-hemolysis values after the intervention with the fermented milk. In summary, the synbiotic fermented milk containing probiotics and a prebiotic may contribute to improve intestinal health and may have a positive effect on the humoral and cell-mediated immunity of host animals.

Key words: fermented milk, symbiotic, intestinal microbiota, immune response

INTRODUCTION

Because more than 400 species of microbes connected by a complicated system of communication have been estimated to reside in the human gut, the human intestinal microbiota have been referred to as the “forgotten organ” (Mitsuoka, 1980; Quigley, 2010b). Intestinal microbiota on the whole are closely associated with nutrient absorption, vitamin production, and metabolism (Sekirov et al., 2010). Emerging scientific research has also demonstrated that intestinal microbes can influence human immunity by cross-talk with gut-associated immune systems (Quigley, 2010a). Abnormal intestinal microbiota have been linked to many human diseases, such as inflammatory bowel disease, allergies, diabetes, and colon cancer, as well as various autoimmune disorders, such as rheumatoid arthritis (Moore and Moore, 1995; Kalliomäki and Isolauri, 2003; Kanauchi et al., 2003; Manichanh et al., 2006; Penders et al., 2007; Wen et al., 2008; Davis and Milner, 2009). Therefore, normalization of the intestinal microbiota may be an important strategy in managing public health.

The lactic acid bacteria (LAB) and their fermented milk have long been used as traditional foods worldwide (Khurana and Kanawjia, 2007). They are generally believed to have beneficial health effects in humans. Since the 1980s, increasing scientific evidence from well-designed epidemiological and clinical studies has demonstrated the potent health-promoting effects of LAB and fermented milk, such as colonization resistance against various infection-causing pathogens (Bernet-Camard et al., 1997), stabilization of the gastrointestinal barrier function (Gotteland et al., 2001), and modulation of gastrointestinal immune activity (Haller et al., 2000; Delcenserie et al., 2008; Ruemmele et al., 2009; Salva et al., 2010). Among the suggested mechanisms by which LAB express these health-promoting effects is their potent ability to contribute to the homeostasis of intestinal microbiota (Turnbaugh et al., 2006; Galdeano et al., 2009). The LAB (principally through their involvement in the fermentation of milk) have been used as dietary supplements to enhance intestinal health (Tannock et al., 2000; Garrido et al., 2005; Mohan et al., 2006; Ouwehand et al., 2009). However, LAB, lactobacilli, and bifidobacteria, which have been widely used as probiotics, have also been shown to differ from each other in terms of their effects on human health and in their underlying mechanisms (Tannock et al., 2000; Garrido et al., 2005; Mohan et al., 2006; Ouwehand et al., 2009). Therefore, the combination of different probiotic strains has recently been suggested as a practical means of improving the potency of probiotic therapies.
to promote human intestinal health (Tannock et al., 2000; Garrido et al., 2005; Mohan et al., 2006; Ouwehand et al., 2009).

A prebiotic is currently defined as a selectively fermented preparation that induces specific changes in the composition or activity of the gastrointestinal microflora, or both, and confers health-related benefits to the host (Roberfroid, 2007). Prebiotics can be carbohydrates, such as oligosaccharides, or noncarbohydrates. Many clinical studies have shown that a combination therapy with prebiotics can enhance the health-promoting effects of probiotic fermented milk (Rafter et al., 2007; Kukkonen et al., 2008; Knight et al., 2009; Ooi et al., 2010a,b; Wallace et al., 2011).

The aim of this study was to test the potent health-promoting effects of symbiotic fermented milk prepared with a combination of a prebiotic and probiotics on host animals. A randomized controlled trial with 100 healthy subjects was conducted to test the effects of fermented milk supplemented with 2 probiotic strains and a prebiotic on the human intestinal microbiota. In addition, identical tests were performed with a mouse model to reproduce the effects of the tested fermented milk on the intestinal microbiota and to examine its immunoregulatory effect on host animals.

MATERIALS AND METHODS

Preparation of the Fermented Milk and a Placebo Yogurt

The tested fermented milk (SFM) was prepared using fresh milk, sugar, and a stabilizer, which was fermented with a starter culture, *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* (Danisco, Copenhagen, Denmark), supplemented with the probiotic strains *Lactobacillus acidophilus* NCFM and *Bifidobacterium lactis* Bi-07 (Danisco), and the prebiotic isomaltooligosaccharide (IMO; Shandong Tianmei Biotechnology Co. Ltd., Heze, Shandong, China).

To prepare the SFM, the sugar, stabilizer, and IMO were first dissolved in fresh milk (2.8% protein, 3.0% milk fat), and the milk mixture was heated to 41°C, followed by homogenization (60°C, 20 MPa) and sterilization (95°C, 5 min) before being cooled to 42°C. The starter culture and probiotic bacteria were then inoculated, and fermentation was completed at 42°C after approximately 5 h. The final preparation contained *B. lactis* (8.0 × 10⁷ cfu/g), *Streptococcus thermophilus* (1.5 × 10⁸ cfu/g), *Lactobacillus* (6.6 × 10⁷ cfu/g), IMO (0.6%), protein (2.5%), fat (2.7%), and soluble solids (13%).

For the animal tests, SFM was modified by adding 30-fold more of the functional ingredients (probiotic bacteria and IMO) compared with the human study. The test samples were prepared for BALB/c mice by diluting the samples with sterile water. A common commercial yogurt fermented with *L. delbrueckii* ssp. *bulgaricus* and *Strep. thermophilus* was used as a placebo.

Human Subjects

A total of 100 healthy adult volunteers (50 male, 50 female, mean age 35 ± 5 yr) were recruited for the present study. Volunteers were selected according to strict criteria, and all subjects were 1) healthy adults who had not used any antibiotic and had no history of gastrointestinal disease in the preceding month; 2) not on any medication that could affect their judgment or functions; 3) did not have any severe cardiovascular, cerebrovascular, liver, kidney, or psychological disorders; and (4) agreed to complete the test efficiently. The human study was conducted in accordance with the Declaration of Helsinki. The study was approved by the Ethics Committee of Shijiazhuang Junlebao Dairy Co. Ltd. (Shijiazhuang, Hebei, China), and all subjects provided their written informed consent.

The subjects were randomly divided into test and control groups. The subjects in the test group ingested SFM at 480 g/d for 14 d, and the control group maintained their diet without any change. During the investigation period, subjects were not permitted any medication, although their diet was not restricted. Medical examinations were conducted before and after the intervention, including assessments of mental condition, sleep, blood pressure, feces, urine, blood biochemical indices, chest x-ray, and type-B ultrasound of the liver, gallbladder, spleen, and kidneys.

Test Animals

Forty specific-pathogen-free grade male BALB/c mice (BW of 18.0 to 20.4 g) were purchased from Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China). Mice were housed under constant environmental conditions (temperature: 21 to 22°C; humidity: 40 to 50%) on a 12-h light-dark cycle.

After 1 wk, the mice were randomly divided into 4 groups and were fed sterile water (control) or the test milk, the latter at doses of 0.4 g/10 g of BW (low dose), 0.8 g/10 g of BW (medium dose), or 2.4 g/10 g of BW (high dose), through a gastric tube for 14 d.

Analysis of Fecal Bacteria

Fecal samples were collected from subjects for microbiological analyses before and after oral administration.
of the test milk. Fresh stools from the human subjects and mice were collected and immediately stored at 4°C. All the collected samples were analyzed under sterile conditions within 4 to 6 h. Fecal bacteria were analyzed using the method recommended by the Ministry of Health of the People’s Republic of China (2003). Briefly, BBL agar medium (Oxoid Ltd., Basingstoke, UK) was used for *Bifidobacterium* counts, eosin-methylene blue agar (Oxoid Ltd.) was used for *Enterobacteriaceae*, enterococcosel agar (bile esculin azide agar, self control) was used for *Enterococcus*, de Man, Rogosa, Sharpe agar (Oxoid Ltd.) was used for all lactobacilli, tryptose sulfite cycloserine agar (Merck KGaA, Darmstadt, Germany) was used for *Clostridium perfringens*, and modified Gifu anaerobic medium (Merck KGaA) agar was used for *Bacteroides* species. Tryptose sulfite cycloserine agar cultures were anaerobically incubated at 37°C for 24 h; de Man, Rogosa, Sharpe cultures were anaerobically incubated at 37°C for 48 h; BBL agar and modified Gifu anaerobic medium agar cultures were anaerobically incubated at 37°C for 48 h; and eosin-methylene blue and enterococcosel agar cultures were aerobically incubated at 37°C for 24 and 48 h, respectively.

**Analysis of Immunological Response**

A total of 240 female Kunming mice (BW of 19 to 22 g) were obtained from the Vital River Laboratory Animal Technology Co. Ltd. Mice were housed under constant environmental conditions (temperature: 21 to 22°C; humidity: 40 to 50%) on a 12-h light-dark cycle. Mice were randomly divided into 4 groups (60 in each group). One group was used for assessing the weight of the immune organs, delayed-type hypersensitivity (DTH), plaque-forming cells (PFC), and half-hemolysis (HC$_{50}$) values. The other groups were used for assessing phagocytosis of peritoneal macrophages, carbon granule clearance, lymphocyte transformation, and natural killer (NK) cell activity.

Each group was randomly divided into 5 subgroups and fed deionized water (control) or the test sample at 0.08 g/10 g of BW (low dose), 0.8 g/10 g of BW (medium dose), or 2.4 g/10 g of BW (high dose). The test sample was administered through a gastric tube for 30 d. Immune parameters were determined according to the method recommended by the Ministry of Health of the People’s Republic of China (2003).

**DTH Assays**

After the final administration of the test sample, the first group of tested mice was injected with 2% (vol/vol) sheep red blood cells (SRBC). After 4 d, the left metapedes voix pedis of the mice subjected to SRBC challenge was measured. The difference in voix pedis thickness before and 24 h after injection was calculated and compared with those of the different groups.

**Measurement of the Immune Organs, HC$_{50}$, and PFC**

The first group of test mice was anesthetized, and blood samples were collected by enucleation of the eyeball 5 d after intraperitoneal injection of SRBC, and the HC$_{50}$ was determined according to the formula

$$\text{HC}_{50} = \left( \frac{\text{OD of sample}}{\text{OD of half SRBC hemolysis}} \right) \times \text{dilution multiple},$$

where OD is optical density. The thymus and spleen were weighed after blood collection to calculate the organ:body ratio. A spleen cell suspension was prepared for the PFC assay.

**Measurement of the Phagocytic Capacity of Peritoneal Macrophages**

After the final administration of the test sample, the second group of mice was injected with 2% (vol/vol) SRBC. Two milliliters of Hanks’ solution was administered to the animals by intraperitoneal injection after 4 d. The animals were then anesthetized and the abdomen was pressed gently for 30 s. The cavitas abdominalis was opened and the peritoneal lavage fluid was collected; 0.25 mL was placed on a slide to which 0.25 mL of chicken red blood cell suspension was added. After incubation at 37°C for 30 min, the slides were subjected to methanol fixation and Giemsa staining; 100 macrophages were counted through immersion objective observation, and the phagocytosis ratio and phagocytosis index were calculated.

**Carbon Granule Clearance Test**

The third group of mice was administered 0.2 mL of a 5-fold dilution of India ink through normal saline by intravenous injection. After 1 and 10 min of administration, a 20-μL blood sample was collected from the canthus venous plexus. The sample was then added to 2 mL of 0.1% (wt/vol) Na$_2$CO$_3$ solution before the optical density was measured at 600 nm with a BT224 semiautomatic biochemistry analyzer (Biotecnica Instruments SpA, Rome, Italy). Finally, the animals were anesthetized, and the liver and spleen were weighed to calculate the phagocytic index (a) using the following formula:
\[ K = \frac{\lg \text{OD}_1 - \lg \text{OD}_2}{(t_2 - t_1)}; \]

phagocytic index (a) = \( \frac{\text{BW}}{\text{liver weight} + \text{spleen weight}} \times \sqrt[3]{K}; \)

where \( K \) is the index of phagocytosis, \( \lg \) is the logarithm, \( \text{OD} \) is optical density, and \( t \) is time (\( t_2 - t_1 \) is the sampling time interval difference).

**Lymphocyte Transformation Test and Measurement of NK Cell Activity**

The fourth group of mice was anesthetized and their spleens were collected under sterile conditions. A spleen cell suspension at a concentration of \( 2 \times 10^6/\text{mL} \) was prepared from an RPMI 1640 complete culture solution. The optical densities at 570 nm of wells to which concanavalin A was added and those to which it was not added were measured according to the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide method. The multiplication of lymphocytes was represented by the difference in values between these wells.

Target cells (YAC-1 cells) were cultured 24 h before the test, and a \( 5 \times 10^6/\text{mL} \) spleen cell suspension was prepared from an RPMI 1640 complete culture solution under sterile conditions. The optical density at 490 nm was measured according to the lactic acid dehydrogenase method with enzyme-labeled instruments; NK cell activity was calculated using the following formula:

\[
\text{NK cell activity} (\%) = \left( \frac{\text{OD}_{\text{reaction}} - \text{OD}_{\text{natural release}}}{\text{OD}_{\text{the largest release}} - \text{OD}_{\text{natural release}}} \right) \times 100,
\]

where \( \text{OD} \) is optical density.

**Statistical Analyses**

Data were expressed as mean \( \pm \) SE, and analyses were performed using SPSS (IBM, Armonk, NY). The homogeneity of variance of the data was confirmed before ANOVA. Calculating the \( F \)-value revealed no significant differences between groups. An \( F \geq 0.05 \) and \( P \leq 0.05 \) comparison of group means and pairwise comparison were performed. Data with heterogeneity of variance or abnormal distributions were analyzed after variable transition. The rank-sum test was used if the variable transition was unsuitable. Data relating to the phagocytic index and NK cell activity were transformed by \( \sin^{-1}\sqrt{p} \), and homogeneity of variance was tested before ANOVA.

**RESULTS**

**Effect of SFM on the Intestinal Bacterial Composition of Human Subjects**

After 2 wk of intervention, all subjects completed the study with no complaints of health problems (Tables 1 and 2). Fecal bacteria from the subjects were analyzed before and after the intervention (Figure 1). After the intervention, fecal populations of lactobacilli and bifidobacteria were significantly increased in the test group compared with the control group. A significant decrease was observed in the number of enterobacilli (\( P < 0.01 \)). Enterobacilli were also significantly decreased in the mice administered a lower dose of SFM (\( P < 0.05 \)). However, no significant change was found in the control group.

**Effect of SFM on the Intestinal Bacterial Composition of Mice**

The 2-wk SFM intervention had no adverse effects on the mice. Before administration, the treated and control groups were not statistically significant (Table 3). Populations of lactobacilli and bifidobacteria were significantly increased in a dose-dependent manner in the mice administered SFM compared with the control.

**Table 1. Blood biochemical indices of subjects**

<table>
<thead>
<tr>
<th>Blood biochemical index</th>
<th>Range of normal values</th>
<th>Before test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Males (n = 50)</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>3.9–6.1</td>
<td>5.0 ± 0.6</td>
</tr>
<tr>
<td>Alanine aminotransferase (U/L)</td>
<td>5–40</td>
<td>16 ± 10</td>
</tr>
<tr>
<td>Blood urea nitrogen (mmol/L)</td>
<td>2.14–7.14</td>
<td>5.38 ± 1.02</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>3.1–5.7</td>
<td>4.2 ± 0.6</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.6–1.7</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td>Total protein (mmol/L)</td>
<td>60–80</td>
<td>73 ± 3</td>
</tr>
<tr>
<td>Albumin (mmol/L)</td>
<td>35–55</td>
<td>47 ± 2</td>
</tr>
<tr>
<td>Creatinine (μmol/L)</td>
<td>44–97</td>
<td>83 ± 8</td>
</tr>
</tbody>
</table>
An increase in bifidobacteria was observed in both the medium-dosage ($P < 0.05$) and high-dosage ($P < 0.01$) groups, and these differences were significant compared with bifidobacteria observed in the control group. The increase in lactobacilli was significantly different in the test groups ($P < 0.05$ for the medium-dosage group, and $P < 0.01$ for the high-dosage group) compared with the control group. A reduction in the number of enterobacilli was also observed in the test group, but this was not significant.

Among the treated groups, populations of bifidobacteria were significantly increased after the SFM intervention in the medium-dosage ($P < 0.05$) and high-dosage ($P < 0.01$) groups, and a significant increase in lactobacilli occurred in the high-dosage group ($P < 0.01$). A significant reduction in enterobacilli also occurred in the medium-dosage ($P < 0.05$) and high-dosage ($P < 0.01$) groups after the intervention.

### Effect of SFM on Immune Regulation in Mice

The effects of SFM on the immune response were studied in specific-pathogen-free grade mice (Kunming species). Cellular immunity was evaluated in terms of T-lymphocyte proliferation and DTH, humoral immunity was estimated by the PFC assay and $HC_{50}$ value, and nonspecific immunity was measured based on phagocytosis of chicken red blood cells by peritoneal macrophages, carbon clearance, and NK cell activity. After 4 wk of SFM administration, only 3 parameters were significantly different: DTH, $HC_{50}$, and PFC.

![Figure 1. Composition of fecal bacteria in human subjects. Bifidobacteria, lactobacilli, enterobacilli, enterococci, bacteroids, and *Clostridium perfringens* were analyzed by a culture method in fecal samples of volunteers before and after 2 wk of receiving the tested fermented milk (SFM; log10 cfu/g of fresh feces). Results are expressed as mean ± SEM (log10 cfu/g of fresh feces) for each group (n = 50; control and SFM groups). Significant differences from the control group were noted for enterobacilli ($P < 0.01$), lactobacilli ($P < 0.01$), and bifidobacteria ($P < 0.01$).](image)

<table>
<thead>
<tr>
<th>Blood index</th>
<th>Range of normal values</th>
<th>Before test</th>
<th>After test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red blood cells ($\times 10^{12}$ cells/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male: 4.0–5.5</td>
<td>4.6 ± 0.3</td>
<td>4.6 ± 0.3</td>
<td>4.6 ± 0.3</td>
</tr>
<tr>
<td>Female: 3.5–5.0</td>
<td>4.2 ± 0.3</td>
<td>4.3 ± 0.3</td>
<td>4.3 ± 0.2</td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male: 120–160</td>
<td>138 ± 12</td>
<td>137 ± 10</td>
<td>138 ± 11</td>
</tr>
<tr>
<td>Female: 110–150</td>
<td>121 ± 7</td>
<td>120 ± 9</td>
<td>124 ± 8</td>
</tr>
<tr>
<td>White blood cells ($\times 10^{9}$ cells/L)</td>
<td>4.0–10.0</td>
<td>6.6 ± 1.5</td>
<td>6.7 ± 1.5</td>
</tr>
</tbody>
</table>

*SFM = the tested fermented milk.*
In the test of DTH triggered by SRBC challenge, the increase in thickness of the left metapedes voix pedis in the SFM groups was greater than that observed in the control and common yogurt groups (Figure 2). Comparison of the thickness increase between the SFM and control groups revealed a significant difference in both the medium- and high-dosage groups (P < 0.01 and P < 0.05, respectively). Similar results were observed between the SFM and common yogurt groups (P < 0.01 for the medium-dosage group, P < 0.01 for the high-dosage group).

The PFC assay results are shown in Figure 3. Compared with the control and common yogurt groups, the number of lacunae increased in the SFM groups with an increase in dosage. However, only the difference between the high-dosage group and the common yogurt group was significant (P < 0.05). Significant differences between the low-dosage (P < 0.05) and high-dosage (P < 0.01) groups and the control group were also observed.

The HC50 value in the SFM groups showed a dose-dependent increase, with clearly significant differences compared with the control group for all dosages (P < 0.01 for all SFM groups vs. the control group; Figure 4). Compared with the common yogurt group, significant differences were found for the medium- and high-dosage groups (P < 0.01 for both). The mean value for the medium-dosage group was higher than that for the high-dosage group, but this difference was not significant. The T-lymphocyte proliferation, peritoneal macrophage phagocytosis, carbon clearance, and NK cell activity did not differ significantly among the groups.

**DISCUSSION**

The gastrointestinal tract is one of the largest interfaces between the human body and the external environment (Mitsuoka, 1980). Numerous endogenous microbes are known to colonize the surface of the gastrointestinal tract throughout the life of the host, comprising a complex internal community known as the intestinal microbiota. In healthy adults, microbial cells are estimated to outnumber somatic and germ cells by a ratio of 10:1 (Mitsuoka, 1980). Furthermore, the habitats of the intestinal microbiota vary in different parts of the human gastrointestinal tract, and each has a different symbiotic relationship with the host.

Lactobacilli and bifidobacteria are among the main components of the human intestinal microbiota. These microbes have a deep symbiotic relationship with their host. Successful colonization and activities of these microbes in the gastrointestinal tract have been shown to be necessary for the health of the host. Therefore, many lactobacilli and bifidobacteria are potential probiotic...
candidates, including *B. lactis* Bi-07 and *L. acidophilus* NCFM, which were first isolated from the human intestine (Sanders and Klaenhammer, 2001). However, lactobacilli and bifidobacteria have different ecological distributions in the human gastrointestinal tract. Lactobacilli are generally found in the small intestine, whereas bifidobacteria reside in the colon because of differences in adaptability to anaerobic conditions and other undeciphered reasons. Therefore, lactobacilli and bifidobacteria may play different roles in human health, although evidence is limited to date. It is hypothesized that greater health-promoting effects can be obtained from the administration of a combination of lactobacilli and bifidobacteria than by the use of either alone.

In the present study, oral administration of a fermented milk product (SFM) prepared with *B. lactis*
Bi-07 and *L. acidophilus* NCFM significantly increased the fecal lactobacilli and bifidobacteria populations of the subjects compared with those of the control group. Furthermore, populations of enterobacilli were significantly decreased in these subjects. These changes suggest that SFM can enhance the growth of beneficial bacteria and suppress the proliferation of harmful bacteria in the intestinal microbiota, thereby contributing to improving intestinal health. *Lactobacillus acidophilus* NCFM is one of the most frequently researched probiotic strains, and it is widely used in the dairy and nutrition industry (Sanders and Klaenhammer, 2001). This bacterium adheres well to human cells in vitro, and the successful passing and colonization of this bacterium in the human intestine have been demonstrated in several human studies (Sanders and Klaenhammer, 2001). In light of these studies, the increase in the fecal lactobacilli population observed here is believed to have been the result of the orally administered SFM. These results further illustrate that *L. acidophilus* NCFM can survive in and colonize, at least temporally, the intestines of human subjects. *Bifidobacterium lactis* Bi-07 is also a common probiotic strain (Quigley, 2011; Ringel-Kulka et al., 2011). Although few scientific studies have been conducted on the health-promoting effects of *B. lactis* Bi-07, this bacterium was found to bind effectively to intestinal mucus and to improve the intestinal environment of infants and the elderly (Candela et al., 2007). Therefore, the increase in fecal bifidobacteria observed in our subjects may be due, at least in part, to oral administration of this bacterium. In contrast, IMO is a multifunctional prebiotic that has positive effects on human digestive health. Isomaltooligosaccharide is not digested in the small intestine and is instead fermented by *Bifidobacterium* species in the human colon, consequently enhancing proliferation of bifidobacteria (Yen et al., 2011). Therefore, the significant increase in fecal bifidobacteria may also have resulted from IMO-stimulated proliferation of endogenous bifidobacteria. Several recent studies have indicated that orally administered *L. acidophilus* NCFM and *B. lactis* Bi-07 have very limited effects on the intestinal microbiota and do not have any significant effects on other microbial groups (Larsen et al., 2011; Lahtinen et al., 2012). Therefore, this study is the first to demonstrate that oral administration of this combination may inhibit the growth of enterobacilli in the human gastrointestinal tract.

Emerging scientific data indicate that LAB and milk fermented by LAB can stimulate or modify the immune response of host animals, or both (Haller et al., 2000; Mohan et al., 2006; Delcenserie et al., 2008; Ouwehand et al., 2009; Ruemmele et al., 2009; Salva et al., 2010). Because the immunoregulatory effects of LAB are strain dependent, administering a combination of various probiotic strains may be a good way to increase their immunoregulatory effects. Here, the SFM supplemented with the 2 probiotics and a prebiotic was expected to induce immunoregulatory effects.

The immunoregulatory effects of functional foods and related ingredients can be tested by DTH, the PFC assay, HC50 value, phagocytic capacity of peritoneal macrophages, carbon clearance, lymphocyte transformation, and NK cell activity (Yang et al., 2008; Liu et al., 2010).
al., 2009; Qin et al., 2010). A combination of these tests can directly or indirectly analyze various aspects of the cell-mediated humoral and nonspecific immunity of host animals. In the present study, these analyses were used to monitor the effect of the fermented milk on the immunity of host animals. However, these analyses could not be performed on human subjects; therefore, a mouse model was used. Results should be interpreted with caution because it is well known that not all the results from animal models can be extrapolated to humans.

Before immune analysis, the potent effect of SFM on the intestinal microbiota observed in the human study was checked again with mice. The oral administration of SFM significantly increased fecal lactobacilli and bifidobacteria populations in a dose-dependent manner compared with the control group. These results suggested that the effects of SFM on the human intestinal microbiota were successfully reproduced in the mouse model. In the subsequent immunological studies, oral administration of SFM significantly increased the DTH, HC50, and PFC of the tested mice. However, T-lymphocyte proliferation, peritoneal macrophage phagocytosis, carbon clearance, and NK cell activity were not significantly altered. Although the mechanism behind the increase in DTH remains unclear, DTH is generally considered not to be an antibody mediated, but rather a cell-mediated response. In contrast, HC50 and PFC are closely associated with humoral immunity. These results suggest that the tested fermented milk may have influenced the humoral and cellular immunity of the host animal, at least in part. Humoral and cell-mediated immunity are 2 basic immune mechanisms in humans. The former is mediated by secreted antibodies produced in cells of the B-lymphocyte lineage, whereas the latter involves T lymphocytes. Enhancement of humoral and cell-mediated immunity may contribute to human health (Gill, 1998). Therefore, these results suggest that SFM could be used as a functional food to promote immune responses.

Oral administration of SFM significantly increased the populations of lactobacilli and bifidobacteria in both human subjects and mice. These populations are considered to consist, at least in part, of the 2 probiotic strains administered through the milk. Therefore, L. acidophilus NCFM and B. lactis Bi-07 can be considered to have successfully colonized the intestines of the host animals and are likely key components in the alteration of immunity. Lactobacillus acidophilus NCFM has previously been shown to possess potent immunoregulatory effects in a murine dendritic cell model (Weiss et al., 2010). However, no significant changes in the immune markers of subjects were observed in several human studies (Andreasen et al., 2010; Larsen et al., 2011; Lahtinen et al., 2012). Therefore, the changes in immunity of the tested mice may also have resulted partly from other components in the tested fermented milk or from a change in the prevalence of other intestinal microbes. Further studies are required to confirm the immunoregulatory effects of SFM in humans as well as to elucidate the associated immunoregulatory factors and underlying mechanisms.

REFERENCES


