Nutrient Interactions and Toxicity

Bifidobacterium animalis Protects Intestine from Damage Induced by Zinc Deficiency in Rats

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ABSTRACT We investigated the potential beneficial effects of Bifidobacterium animalis on intestinal damage using zinc-deficient (ZD) rats as a model for intestinal alterations. The ZD rats were fed diets containing 1 mg Zn/kg for 20 (ZD20) or 40 (ZD40) d to induce damage that differed in severity. Subgroups of these rats, the ZD20 + B and ZD40 + B groups, received a suspension of B. animalis (3.5 × 10^10 colony forming units) daily for the last 10 d. Another subgroup, the ZD40 + B + 7 d group, was fed the ZD diet for 7 d after the B. animalis treatment period. Zinc deficiency induced ulcerations, edema, inflammatory cell infiltration and dilatation of blood vessels in duodenum, jejunum and ileum, with increasing severity between 20 and 40 d after zinc deficiency. The mucosa of the ZD20 + B group was well preserved, and most of the morphologic alterations induced by zinc deficiency were normalized in the ZD40 + B group. The high fecal concentrations of B. animalis in the ZD40 + B and ZD40 + B + 7 d groups indicate that these bifidobacteria survived passage through the gastrointestinal tract and proliferated. Electron microscopy confirmed the elevated numbers of bifidobacteria in cecum. Treatment with B. animalis resulted in greater epithelial cell proliferation and disaccharidase activities in the ZD40 + B group compared with the ZD40 group. These findings indicate that B. animalis can protect the intestine from alterations induced by zinc deficiency, suggesting that this bacterium may play a role in intestinal mucosal defense. J. Nutr. 129: 2251–2257, 1999.

KEY WORDS: • Bifidobacterium animalis • zinc deficiency • intestine • rats • epithelial proliferation

Special interest has been focused recently on the use of bifidobacteria and lactic acid bacteria as probiotics, microorganisms that affect the health of human and animals in a beneficial way. The several benefits ascribed to the ingestion of these microorganisms include the following: 1) maintenance of proper balance in the human intestinal microbiota by the formation of a barrier against proliferation of exogenous pathogens; 2) stimulation of intestinal immune system; 3) a possible role in cholesterol reduction and in prevention of carcinogenic activity; 4) improvement of lactose tolerance; 5) the conveying of nutrients of special importance for the intestinal mucosa, such as short-chain fatty acids; and 6) production of micronutrients such as B-complex vitamins and antioxidants (Ballongue 1993, Fuller 1989, Salminen et al. 1996, Sanders 1993, Walker and Duffy 1998).

Probiotics have been used in both humans and experimental animals in the treatment or prevention of gastrointestinal disease (Bengmark 1996, Macfarlane and Cummings 1999, Salminen et al. 1996). However, the results are not sufficiently clear. In addition, only a few strains with beneficial effects have been identified. A study of a gnotobiotic murine model suggested that oral inoculation of Lactobacillus salivarius prevented Helicobacter pylori infection (Kabir et al. 1997). The exogenous administration of Lactobacillus reuteri was shown to prevent the development (and enhance the healing) of acetic acid–induced colitis (Fabia et al. 1993). Lactobacillus reuteri were also demonstrated to prevent bacteremia in a rat model of peritonitis (Bengmark 1996). Other strains, such as Bifidobacterium bifidum and Streptococcus thermophilus, have been used for the prevention of diarrhea and shedding of rotavirus in hospitalized infants (Saavedra et al. 1994, Sanders 1993). In previous studies, we have reported that ulcerations, edema, inflammatory cell infiltration and dilatation of blood vessels developed in the small intestine of zinc-deficient (ZD)2 rats, depending on the severity of the deficiency (Nobili et al. 1997, Virgili et al. 1999). In this study, we have investigated the potential beneficial effect of Bifidobacterium animalis on intestinal damage by using ZD rats as a model in which intestinal alterations of different severity may be induced. Valuable selection criteria for probiotic bacteria are resistance to gastric acidity, adhesion to enterocytes or proliferation in large number (Bengmark 1996, Schiffrin et al. 1997). It has already been shown that different strains of bifidobacteria can survive the passage through the stomach and small intestine of humans (Biavati et al. 1995, Marteau et al. 1990, Pochart et al. 1992).
In this study, we used *B. animalis* as a species able to survive and proliferate through the gastrointestinal tract (Bivati et al. 1995). This species is ingested by large numbers of consumers of fermented milk containing bifidobacteria (Bivati et al. 1992).

**MATERIALS AND METHODS**

**Bacteria.** *Bifidobacterium animalis*, strain MB5, was grown for 24 h at 37°C in TPY (tryptone-phytone-yeast extract) broth medium, in anaerobic conditions as previously reported (Biavati et al. 1991). *Escherichia coli* strain K12, ATCC14928 (American Type Culture Collection, Rockville, MD), was cultured in LB broth medium (10 g tryptone, 5 g yeast extract and 5 g NaCl/L), and incubated overnight at 37°C in moderate rotation. The cells were collected by centrifugation at 12,000 X g for 10 min, resuspended in sucrose solution (120 g/L) and lyophilized.

**Animals.** Male Sprague-Dawley rats (Charles River, Como, Italy) weighing 150–160 g were housed in stainless steel cages and maintained at 23°C in a 12-h light:dark cycle. Zinc-deficient (ZD) rats (n = 70) were fed a purified diet containing 1 mg Zn/kg diet. They were divided in ZD20 and ZD40 groups and fed the ZD diet for 20 or 40 d, respectively, to induce intestinal damage of different severity. Fourteen rats remained in each group; the other rats were divided in three subgroups. The subgroups ZD20 + B and ZD40 + B (n = 14/group) received orally, by gavage, a suspension of pure viable *B. animalis* in saline, at the dose of 3.5 X 10⁸ colony forming units (cfu), daily for the last 10 d of the dietary treatment (i.e., after 10 or 30 d of zinc deficiency, respectively). The subgroup ZD40 + B + ZD7 1 (n = 6) was treated as the subgroup ZD40 + B and then fed the ZD diet for another 7 d after the ingestion period of *B. animalis*. As negative controls, two other subgroups (n = 4/group) of the ZD40 group were used. The subgroup ZD40 + E received a suspension of *E. coli* (3.5 X 10⁸ cfu) daily for the last 10 d of the experiment. The subgroup ZD4 + Zn received by gavage, for the last 10 d, 5 μg zinc as zinc carbonate dissolved in deionized, distilled water, corresponding to the amount of zinc present in the suspension of bifidobacteria, as determined by flame atomic absorption spectrophotometry after a complete bacterial lysis. The control (C) group (n = 14) was fed the ZD diet supplemented with zinc carbonate to have an adequate level of zinc (50 mg Zn/kg).

All experiments were approved by the Animal Care and Ethics Committee of the Istituto Nazionale della Nutrizione, Rome, Italy and conformed to published guidelines (National Health and Medical Research Council 1985).

**Histology.** Pieces of duodenum, jejunum and ileum (~2.5 cm) were immersed in Bouin's fixative for 12 h, washed in PBS for 24 h, embedded in paraffin at 58°C and sectioned at 7 μm. After Mallory's staining, the intestinal sections were examined without knowledge of origin under a light microscope. The variables selected to establish the extent of the most evident tissue modifications were ulcerations, edema, inflammatory cell infiltration (IC) and dilatation of blood vessels. A score from 0 to 4 was assigned to the histologic variables in relation to the severity of alterations as follows: 0 = normal; 1 = mild; 2 = moderate; 3 = marked; 4 = severe.

**Fecal analysis.** Pieces of the C, ZD20, ZD40 + B and ZD40 + B + ZD7 1 rats were collected on the last day of their experimental periods. Samples were serially diluted and plated onto selective agar medium (Biavati et al. 1991). After incubation in anaerobic jars for 3–4 d at 37°C, the colonies were counted and checked for cell morphology. Some colonies were transferred into TPY broth medium, and *B. animalis* was identified by morphology, electrophoretic pattern of total cellular proteins, presence of fructose-6-phosphate-phosphoketolase (Biavati et al. 1991) and DNA homology with MB5 by DNA-DNA hybridization as previously described (Biavati et al. 1992).

**Scanning electron microscopy.** Pieces of colon from the C, ZD20, and ZD40 + B rats were fixed in 50 g/L glutaraldehyde in 0.1 mol/L phosphate buffer, pH 7.2, for 15 min and dehydrated in graded ethanol series for 15 min at 5°C. Samples were "critical point" dried, coated with gold-palladium film and observed under a Philips 515 scanning electron microscope ( Milan, Italy) at 9–10 kV.

**Hydrophobicity assay.** The hydrophobicity of *B. animalis* was assayed by the salt aggregation test (SAT), as described by Lindahl et al. (1981); the SAT is based on precipitation of cells by salts, that is the higher the hydrophobicity of the proteins, the lower the salt concentration required to aggregate the cells.

**Assessment of epithelial cell proliferation.** Epithelial cell proliferation was expressed as a labeling index (LI). Rats were injected intraperitoneally with vincristine (1 mg/kg) to arrest cells in metaphase. After 6 h, the intestine was removed and sections of duodenum, jejunum and ileum were processed as described above for histology. The LI was estimated in 20 crypts under light microscope by the mean number of nuclei arrested in metaphase and was calculated by the following formula: number of arrested metaphases X 100/ number of cells per crypt column. Crypt column was defined by the number of epithelial nuclei in the left column of the crypt.

**Enzyme and protein assays.** Sucrase and maltase activities were assayed in the three intestinal regions by the method of Messer and Dahlqvist (1966). Protein content was measured by the method of Lowry et al. (1951).

**Zinc analysis.** Zinc concentration in rat serum and suspension of bifidobacteria, previously mineralized with nitric acid in the presence of hydrogen peroxide, was determined by flame atomic absorption spectrophotometry using a SpectrAA-400 model atomic absorption spectrometer (Varian, Sunnyvale, Melbourne, Australia).

**Statistical analysis.** Data are the mean ± SEM of at least six experiments. The significance of the difference was evaluated by one-way ANOVA followed by Fisher's test (Winer 1971). Differences with P < 0.05 were considered significant.

**RESULTS**

**Body weight and serum zinc concentration.** The body weights of both the ZD20 and ZD40 rats were dramatically lower than that of the C rats (P < 0.05; Table 1). The serum zinc concentration was similarly lower in the ZD20 and ZD40 rats compared with the C rats. The body weights and serum zinc concentrations of the ZD20 + B and ZD40 + B did not differ from those of the ZD20 and ZD40 rats, respectively.

**Morphology.** As previously reported (Nobili et al. 1997, Virgili et al. 1999), zinc deficiency induced several alterations in the morphology of the three intestinal regions (Fig. 1). The intestine of the ZD20 rats had a few ulcerations in duodenum.

### Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body weight</th>
<th>Serum zinc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g mmol/L</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>364 ± 57a</td>
<td>19.6 ± 1.9a</td>
</tr>
<tr>
<td>ZD20</td>
<td>190 ± 22b</td>
<td>4.1 ± 0.6b</td>
</tr>
<tr>
<td>ZD20 + B</td>
<td>206 ± 21b</td>
<td>4.1 ± 0.46b</td>
</tr>
<tr>
<td>ZD40</td>
<td>189 ± 21b</td>
<td>4.6 ± 0.48b</td>
</tr>
<tr>
<td>ZD40 + B</td>
<td>191 ± 20b</td>
<td>4.6 ± 0.48b</td>
</tr>
</tbody>
</table>

1 The ZD rats were fed a zinc-deficient diet for 20 (ZD20) or 40 d (ZD40) and treated for the last 10 d with B. animalis (ZD20 + B and ZD40 + B).

2 Values are the mean ± SEM, n = 10. Within a column, values with different superscripts are significantly different. P ≤ 0.01.
and jejunum, and a marked edema and moderate ICI in all three regions (score: from 1 to 2). The intestine of the ZD40 rats had developed widespread ulcerations, severe edema, dense ICI and severe dilatation of blood vessels (score: from 2 to 4). The most severely damaged region was the jejunum, with a score of 4, whereas the ileum was less affected, with a score of 2. We had also made a histologic examination of intestine at the beginning of each treatment with bifidobacteria (i.e., after 10 or 30 d of zinc deficiency). After 10 of zinc deficiency the mucosa was normal, whereas after 30 d, the duodenum and ileum developed a few ulcerations, moderate edema and ICI, and the jejunum appeared already severely damaged (not shown). After B. animalis administration, the morphologic alterations induced by zinc deficiency did not develop or were notably less. In fact, the mucosa of ZD20 + B rats was well preserved, although mild edema in jejunum and mild ICI in the three regions were seen (score: ≤ 1). In addition, the intestine of the ZD40 + B rats had developed only very rare ulcerations and moderate edema in jejunum, and mild ICI in the three regions (score from ≤ 1 to ≤ 2). The beneficial effect of B. animalis persisted after the end of the ingestion period because the mucosa of the ZD40 + B + 7 rats did not differ from that of the ZD40 + B rats. Histologic scores are presented in Table 2. Treatment with either E. coli or zinc solution did not induce any effect because the mucosa of the ZD + E and ZD + Zn rats did not differ from that of the ZD40 rats (not shown). Thus, the results presented highlight that B. animalis can protect the intestine from the morphologic damage induced by zinc deficiency.

B. animalis enumeration. Light microscope observation and assay of fructose-6-phosphate-phosphoketolase identified several isolates ascribable to Bifidobacterium spp. in the feces of the ZD40 + B rats but not in the feces of either the C or ZD rats. Gel electrophoresis of cellular proteins from bifidobacter-
ria isolated from the ZD40+B rats showed a protein pattern that corresponded to that of B. animalis strain MB5 (not shown). The same identification was obtained by DNA-DNA hybridization. The homology of the isolates with MB5 was always close to 100%. The recovery of B. animalis in the ZD40+B rats at the end of treatment was 10.3 ± 0.8 × 10^9 cfu (Table 3), a value even higher than the total amount of bifidobacteria ingested for the 10 d of treatment (3.5 × 10^9 cfu). The concentration of B. animalis was not reduced 7 d after ingestion stopped. Thus, these data indicate that B. animalis survived and proliferated along the digestive tract.

**Electron microscopy.** The electron microscope scanning of cecum (Fig. 2) confirmed the presence of numerous bifidobacterium-like bacteria in the cecum of the ZD40+B but not in the cecum of the ZD40 rats.

**Hydrophobicity property of B. animalis.** The SAT revealed that B. animalis aggregated at low salt concentrations (i.e., 0.85 mol/L ammonium sulfate). It has been shown that bacterial aggregation caused by low salt concentrations (from 0.01 to 2.0 mol/L) is an index of high surface hydrophobicity (Lindahl et al. 1981). Therefore, our results indicate high hydrophobicity of B. animalis; according to some authors (Weerkamp et al. 1985), this suggests adhesion capacity of this bacterium (see also Discussion).

**Epithelial cell proliferation.** As shown in a previous study (Nobili et al. 1997), the LI was markedly affected by 40 d of zinc deficiency (Fig. 3). In this study, we also measured the epithelial cell proliferation after a less severe zinc deficiency. No difference was seen in LI among the ZD20+B, ZD40 and C rats. On the other hand, the LI of the ZD40+B rats was higher than that of the ZD40 rats in all intestinal regions, and was not different from the other rats. Thus, these data indicate

### TABLE 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Duodenum</th>
<th>Jejunum</th>
<th>Ileum</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZD20</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>ZD20 + B</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>ZD40</td>
<td>4</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>ZD40 + B</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>ZD40 + B + 7 d</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>C</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

1 The ZD rats were fed a zinc-deficient diet for 20 (ZD20) or 40 d (ZD40) and treated for the last 10 d with B. animalis (ZD20 + B and ZD40 + B). C = control rats.

2 The extent of alterations (ulcerations, edema, inflammatory cell infiltration and dilatation of blood vessels) was graded as follows: 0 = absent; 1 = mild; 2 = moderate; 3 = marked; 4 = severe.

### TABLE 3

Recovery of Bifidobacterium animalis from feces of zinc-deficient (ZD) rats at the end of treatment and 7 d after the ingestion period of B. animalis.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>cFU/g feces</th>
<th>cFU/g total feces</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>ZD40</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>ZD40 + B</td>
<td>6.2 ± 0.6</td>
<td>10.3 ± 0.8</td>
</tr>
<tr>
<td>ZD40 + B + 7 d</td>
<td>7.1 ± 1.2</td>
<td>11.2 ± 2.0</td>
</tr>
</tbody>
</table>

1 Feces were collected from the following rats: control (C), ZD fed a zinc-deficient diet for 40 d (ZD40), ZD40 treated for the last 10 d with B. animalis (ZD40 + B), and ZD40 + B maintained on the zinc-deficient diet for another 7 d after the ingestion period of B. animalis (ZD40 + B + 7 d).

2 Values are means ± SEM, n ≥ 6. cfu, colony forming units; ND, not detectable.
that treatment with B. animalis normalized the reduced crypt cell proliferation induced by severe zinc deficiency.

Disaccharidase activities. We confirmed previous results that the activities of sucrase and maltase were significantly affected by 40 d of zinc deficiency (Fig. 4; Nobili et al. 1997). In addition, the disaccharidase activities of the ZD20 rats did not differ from those of the C rats. After the administration of B. animalis, the disaccharidase activities of the ZD20 + B rats did not differ from those of the C and ZD20 rats. On the other hand, both the sucrase and maltase activities of the ZD40 + B rats were significantly greater than those of the ZD40 rats. The jejunal maltase of the ZD40 + B rats was significantly higher than that of the C rats. Thus, the results indicate that B. animalis can also affect some functional activities of intestine.

**DISCUSSION**

There is increasing evidence that lactic acid bacteria can induce health benefits. However, the effects on the health of humans and experimental animals require better documentation. Only a few studies have been conducted to investigate the potential beneficial effect of probiotics on gastrointestinal diseases (Bengmark 1996 and 1998, Salminen et al. 1996). In this study, using ZD rats as a model for intestinal damage, we showed that exogenous administration of B. animalis, strain MB5, may have beneficial effects by either preventing or normalizing intestinal alterations. In fact, we found that this treatment almost completely prevented the development of alterations in the ZD rats. Moreover, most of the ulcerialations, inflammation and edema that appeared between 20 and 40 d of zinc deficiency were prevented or normalized by treatment with B. animalis.

Crucial for the successful treatment with probiotic bacteria is its survival in the gastrointestinal tract, resistance to gastric acidity, capacity to adhere to mucosal surface and transient colonization above a critical level. It has been shown that some species of probiotics survive the passage through the gastrointestinal tract, whereas others do not (Pedrosa et al. 1995). Bouhnik et al. (1992) showed fecal recovery in humans of viable Bifidobacterium sp. ingested in fermented milk. Other authors found that Lactobacillus acidophilus, Lactobacillus bulgaricus and B. infantis were usually but not always recovered in the small intestine for 3 h after their ingestion (Clements et al. 1983). A recent study by Biavati et al. (1995) reported that B. animalis survived passage through the gastrointestinal tract when administered to humans. That study was conducted among healthy individuals. Thus, the possibility that B. animalis did not survive transit through the damaged intestine of the ZD rats could not be excluded. However, our data on cecal and fecal bifidobacteria clearly indicate that B. animalis survived the gastrointestinal transit of the ZD rats and colonized. The survival and resulting high fecal concentrations of B. animalis are compatible with potential probiotic activity (Bouhnik et al. 1992). This bacterium was able to proliferate 7 d after the end of the ingestion period. It is interesting that after this period, the intestine was still protected from the ZD-induced morphologic alterations. Prolonging the post-estimation period decreased the recovery of B. animalis. We had done both fecal analysis and histology of small intestine 14 and 21 d after the end of the ingestion period (data not shown). After 21 d postingestion, when bacteria were no longer detectable in feces, all morphologic damage was again present in the small intestine. Thus, the beneficial effect appeared to be dependent on the presence of viable bifidobacteria. Our results are in agreement with several studies showing that the numbers of fecal bifidobacteria and lactobacilli remain high for a certain period after the end of exogenous administration of such bacteria (Biavati et al. 1995, Goldin et al. 1992, Wagner et al. 1997).
A certain number of bifidobacteria possibly could not survive gastrointestinal passage, thus releasing their zinc. Consequently, the beneficial effect observed after the administration of bifidobacteria could be due to the bacterial zinc. However, both serum zinc concentration and body weight did not increase after the administration of B. animalis, as would be expected after a zinc supplementation derived from bacterial lysis. In addition, the concentration of zinc in the B. animalis suspension was very low. Despite these considerations, we treated the ZD rats with a solution of zinc sulfate containing an amount of zinc equivalent to that measured in the bacterial suspension. We also tested the treatment of the ZD rats with another bacterium, by administering E. coli at the same concentration as that of B. animalis and for the same period of time. Neither the zinc solution nor E. coli showed any beneficial effect, indicating the specificity of the activity of viable B. animalis in the intestine of ZD rats.

It has been suggested that the ability of probiotic bacteria to adhere to intestinal epithelial cells may be important for colonization in the gastrointestinal tract and optimal function (Bengmark 1996, Fuller 1989). Some strains of bifidobacteria are able to adhere to intestinal cells, whereas others, such as Bifidobacterium sp., are not or are only weakly adhesive in vitro (Bernet et al. 1993, Perez et al. 1998, Wagner et al. 1997). A recent study showed that B. bifidum with agglutinating capacity can adhere to epithelial cells in vitro (Perez et al. 1998). This study also indicated that high hydrophobicity was necessary for adhesion and autoagglutination, in agreement with the findings of other investigators (Weerkamp et al. 1985). On the basis of these data, our results of the high surface hydrophobicity of B. animalis suggest that this bacterial strain has the capacity for cell adhesion. In addition, a previous study has shown adhesion of B. animalis to the keratinized stomach of congenitally immunodeficient mice (Wagner et al. 1997).

Regardless of the capacity to adhere, several effects have been reported concerning different bifidobacterial species. Schiffrin et al. (1997), using L. acidophilus and B. bifidum for their high adhesion and colonization capacity, respectively, demonstrated an immune modulation of blood leukocytes by both lactic acid bacteria. Thoreux et al. (1995) reported that fermented milk with different strains of lactobacilli and Bifidobacterium sp. modulates cell proliferation and differentiation of IEC-6 intestinal cells, independently of the capacity of adhesion. On the contrary, Bernet et al. (1993) demonstrated an inhibition in vitro of enteropathogen-cell interaction by those bifidobacterial strains, such as B. breve and B. infantis, which showed high adhesiveness to intestinal epithelial cells. The results of this study indicate the ability of another bacterial strain of the Bifidobacterium species (one that likely has adhesion capacity) to affect epithelial intestinal cells in a beneficial way.

Rat enterocytes are continuously exfoliated from the tips of the villi and replaced with new cells migrating out of the crypts to reach the villous apex in 2–3 d (Leblond 1981). In this study, we showed for the first time to our knowledge, that B. animalis can normalize reduced crypt cell proliferation. Few studies have shown that lactobacilli and bifidobacteria can regulate cell proliferation under certain conditions. Some authors reported that lactic acid bacterial cell wall components, peptidoglycans, have the unique ability to both inhibit the growth of tumor cells and stimulate the proliferation of normal cells (Bengmark 1998). Other authors have found, both in vivo and in vitro, that intestinal epithelial cells are stimulated to proliferate after treatment with lactobacilli or bifidobacteria (Thoreux et al. 1995 and 1998). In this study, the epithelial cell proliferation of the ZD20 rats was unaffected by treatment with bifidobacteria (ZD20 + B rats), whereas it was stimulated in the ZD40 + B rats. Considering that diffusely severe necrotic mucosa was generated in the ZD40 and not in the ZD20 rats, it is likely that a significant enhancement of epithelial cell proliferation by bifidobacteria was generated only in a damaged tissue in which a great replacement with new cells was required.

The disaccharidase activities were affected by 40 d of zinc deficiency. Treatment with bifidobacteria effectively increased both sucrase and maltase activities. At the end of treatment with bifidobacteria, the intestinal mucosa of the ZD40 + B rats was almost completely normalized morphologically; the higher sucrase and maltase activities may be a consequence of this normalization rather than a direct effect of B. animalis. However, the disaccharidase activities of the ZD40 + B rats tended to be greater than those of the C rats, suggesting that B. animalis can have a specific effect on these enzymes. Consistent with our data, some authors have demonstrated that treatment with Saccharomyces boulardii (Buts et al. 1994) or milk fermented by L. casei (Thoreux et al. 1998) enhanced brush border enzyme activities of rats.

The mechanisms by which B. animalis exerts its beneficial effects must be clarified. In previous studies, we suggested that inflammation plays an important part in intestinal damage induced by zinc deficiency; treatment with dexamethasone, thyroxine or cyclosporin A may counteract the inflammation (Nobili et al. 1997, Virgili et al. 1999). Moreover, we found that the expression of proinflammatory interleukin-1β reverted to the normal level after treatment of ZD rats with cyclosporin A (Vignolini et al. 1998). Considering that probiotic species are able to stimulate the immune system and cytokine production (Marin et al. 1996, Schiffir et al. 1997), we hypothesized that the beneficial effect of B. animalis results from its ability to exert an anti-inflammatory activity by modulating cytokine expression. We are now analyzing the proinflammatory cytokine expression in intestine of ZD rats treated with B. animalis; preliminary results indicate that the level of interleukin-1β and interleukin-8 are greater in ZD rats and are decreased after treatment with B. animalis. Thus, among other potential mechanisms of action, an anti-inflammatory activity may be fundamental to the protective function of B. animalis.

The increased cell proliferation and disaccharidase activities may be secondary to the reconstituted integrity of intestine that follows the reduced inflammation, although a specific effect on these enzymes seems to be exerted by B. animalis.

In conclusion, the results presented in this study indicate that B. animalis exerts beneficial effects by either preventing or normalizing the intestinal alterations induced by zinc deficiency. Such protection of B. animalis supports the concept that bifidobacteria play a role in the intestinal mucosal defense and gives new information on the probiotic properties of B. animalis. Better understanding of the mechanisms by which bifidobacteria exert the beneficial effects is a necessary step in clarifying their role in zinc deficiency and may provide insights into their role in treating gastrointestinal diseases.

ACKNOWLEDGMENTS

The authors thank G. Di Lullo for expert technical assistance in the analysis of zinc, R. Rami and P. Rami for excellent care of animals and A. Rauseo for valuable help in the preparation of figures.

LITERATURE CITED

Intestinal protection by B. animalis in zinc deficiency


Prevention of Lactobacillus acidophilus and Streptococcus thermophilus to infants in hospital for prevention of diarrhea and shedding of rotavirus. Lancet. 344: 1046–1049.


