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International Journal of Food Microbiology 42 (1998) 39–44

International Journal of Food Microbiology

Effect of a probiotic formula on intestinal immunoglobulin A production in healthy children

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Received 8 August 1997; received in revised form 28 December 1997; accepted 31 March 1998

Abstract

The anti-infectious effect of probiotics has recently been reported and one mechanism may be the non-specific stimulation of immunity. This study was performed to elucidate the influence of a probiotic formula on intestinal microflora and local immunity in healthy children. A follow-up formula containing viable bifidobacteria was given to seven healthy Japanese children (15 to 31 months old) for 21 days. During intake of the formula, the administered strain was detected in feces from five subjects (71%) and total fecal bifidobacteria slightly increased. Fecal levels of total IgA and anti-poliovirus IgA during intake of the formula were significantly higher than those before intake ($P < 0.05$). The increase in local IgA levels resulting from ingestion of the probiotic formula may contribute to enhancement of the mucosal resistance against gastrointestinal infections. © 1998 Elsevier Science B.V.

Keywords: Bifidobacteria; Fecal microflora; Follow-up formula; IgA; Local immunity; Probiotics

1. Introduction

Bifidobacteria are common anaerobes in the human intestinal microflora and are reported to play a significant role in promoting human health (Mitsuoka, 1984; Gibson and Roberfroid, 1995). These health-promoting functions can be obtained by oral administration of bifidobacteria (Benno and Mitsuoka, 1992; Hotta et al., 1987; Tomoda et al., 1991). The beneficial effects of the administration of viable microbes have recently been described as a probiotic effect (Fuller, 1991), and bifidobacteria can be considered as potential candidates for probiotic organisms.

Secretory immunoglobulin A (IgA) plays a central role in local immunity and has a significant function in creating a barrier against infections by pathogenic bacteria or viruses (Underdown, 1986; Ogra and Karzon, 1970). Breast milk contains IgA, which passively helps in preventing infections in breast-fed infants and results in a lower incidence of infectious

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0168-1605/98/$19.00
PII: S0168-1605(98)00056-7

disease in breast-fed infants compared to formula-fed infants (Howie et al., 1990). The relatively high incidence of infectious diarrhea in formula-fed infants can be reduced by feeding infant formulas containing viable bifidobacteria (Saavedra et al., 1994) or the other lactic acid bacteria (Brunser et al., 1989; Gonzalez et al., 1990). IgA is also actively produced in the intestine, and contributes to the elimination of infectious pathogens from the gastrointestinal tract (Underdown, 1986; Ogra and Karzon, 1970). Some bifidobacteria strains have recently been shown to stimulate IgA production in vitro (Yasui et al., 1992). Therefore, ingestion of bifidobacteria may stimulate active IgA production, thereby reducing infections. However, there is less evidence showing stimulation of local IgA production by probiotics in man.

The composition of the intestinal microflora depends on the age of the host. The population of bifidobacteria, which is predominant in the intestine of infants, decreases during and after weaning depending on the changes in diet (Mitsuoka, 1984). Children during and after weaning may lose the benefit of a microflora rich in bifidobacteria. Follow-up formula is a designed diet suitable for children during and after weaning (ESPGAN Committee on Nutrition, 1990). A follow-up formula with probiotic functions may have great potential for promoting health in those children. In this study, we performed a feeding trial of a follow-up formula containing a viable bifidobacterium in healthy Japanese children to elucidate the influence of the formula on the intestinal microflora and local production of IgA.

2. Materials and methods

2.1. Study design

Seven healthy children (four boys and three girls) aged 15 to 31 months participated in the study. Informed consent was obtained from the parents or guardians of eligible children. Routine immunization with polio vaccine is recommended in Japan, and all the subjects had completed the oral vaccination by 12 months of age. None of the children had a history of gastrointestinal diseases. The study was undertaken in accordance with the Helsinki Declaration of 1975 as revised in 1983.

A powdered cow milk-based follow-up formula for children (NAN BF, Nestlé Japan) containing *Bifidobacterium lactis* Bb-12 (Chr. Hansen, Horsholm, Denmark, previously identified as *Bifidobacteria bifidum*) was tested. *B. lactis* Bb-12 showed good stability in the formula; the viable number did not change for at least 18 months in the sealed can and we found no detectable changes in the viable number for at least 30 days after the formula can was opened (unpublished data).

At least 200 ml of reconstituted NAN BF containing approximately 10^9 of *B. lactis* Bb-12 was scheduled to be ingested by each subject daily from day 0 to day 20. Subjects were not given yogurt, any food or drugs with viable cultures, or any other follow-up formula from day −10 to day 27. The volume of NAN BF intake and consumption of all food by the subjects, the overall health condition, fecal properties and any treatment with drugs or other therapies were recorded. Fresh fecal samples were collected from each subject four days before intake (day −4), during intake (days 3, 8, and 20), and 7 days after intake (day 27). Fecal samples from one subject on day 3 and another on day 20 were not obtained, because they did not evacuate on time for the limited duration for sample transportation.

2.2. Bacteriological analysis

The fecal samples were immediately stored in anaerobic pouches (BBL Gaspak, Nippon Becton Dickinson, Tokyo) at 4°C, and fecal flora was analyzed within 24 h of sample collection. Fecal flora was analyzed using the method and media of Mitsuoka et al. (1976) including three non-selective media (EG, BL, and TS agar media) and nine selective media (BS, ES, NBGT, NEN, VS, LBS, DHL, TATAC, and PEES agar media). Media were purchased from Nissui Pharmaceutical (Tokyo) and Nippon Becton Dickinson (Tokyo). Microbial groups were identified by Gram staining, colony and cellular morphology, spore formation, aerobic growth, and selected biochemical characteristics. Bacteroidaceae, bifidobacteria, eubacteria, anaerobic gram-positive cocci, clostridia, enterobacteriaceae, streptococci, and staphylococci were enumerated. *B. lactis* Bb-12 was differentiated from other bifidobacteria strains by colonial and cellular morphology. Colonies

thought to be *B. lactis* Bb-12 were re-cultured and compared with the reference culture. *B. lactis* Bb-12 or similar colonies were not found in feces from any subject before intake of NAN BF and such colonies were never found in fecal samples from children who did not drink NAN BF in a preliminary study.

2.3. Fecal IgA analysis

The fecal extracts were used for measuring fecal IgA after appropriate dilution. The total fecal IgA level was determined by sandwich-type enzyme-linked immunosorbent assay (ELISA), in which anti-human IgA antibody was coated on an ELISA plate and detected with peroxidase-labeled anti-human secretory component of IgA. Fecal anti-poliovirus IgA was also determined by ELISA, in which poliomyelitis V.1 CF antigen (Alfa Biotech, Amsterdam) was coated on the plate and detected with peroxidase-labeled anti-human IgA antibody.

2.4. Statistical analysis

The results are expressed as mean value with standard errors. All comparisons were made with the levels of the initial samples obtained before NAN BF intake as a control, which had similar characteristics (e.g., microflora and IgA levels) to those of preliminary samples previously obtained from children who were fed a general diet including follow-up formula without viable bacteria. Statistical analyses were performed using the software SPSS (SPSS Japan, Tokyo). Statistical differences were calculated by the Mann–Whitney test in comparison with the value before intake of NAN BF.

3. Results

The test formula NAN BF was well accepted by all subjects, and each subject drank 189 to 470 ml/day^{-1} (median 259 ml) of NAN BF during the test period. The daily number of feces was almost the same before, during, and after intake for each subject. The food consumed by the subjects was similar to that consumed by adults.

Changes in the number of fecal bacteria are shown in Fig. 1. Before intake of NAN BF, bifidobacteria were the second most predominant bacteria at 10.1 ± 0.1 $\log_{10}$ number/g feces, which corresponded to 23% of the total bacteria in the fecal sample. *B. lactis* Bb-12 was not detected in samples before intake.

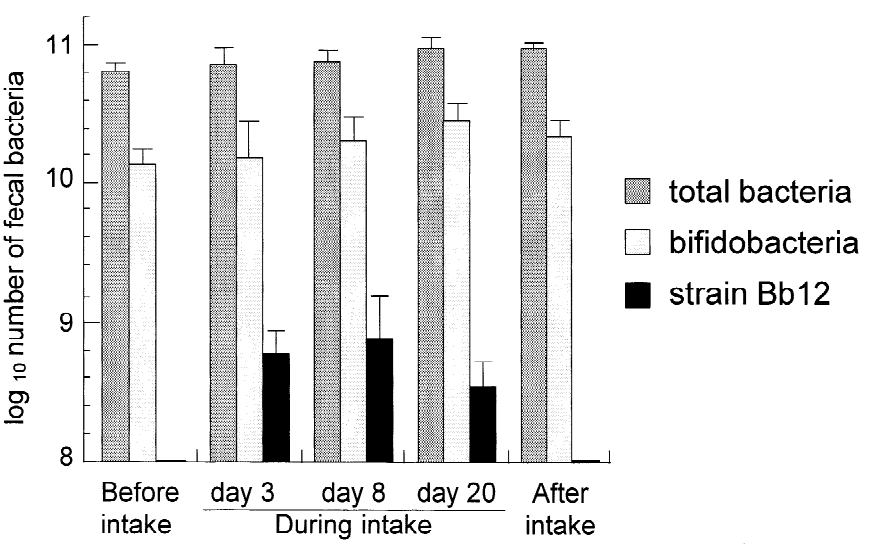

Fig. 1. Effect of probiotic formula for children on fecal microflora. Data are expressed as mean±SEM. The total number of fecal bacteria is the summation of the numbers of bacteroidaceae, bifidobacteria, eubacteria, anaerobic gram-positive cocci, clostridia, enterobacteriaceae, streptococci, and staphylococci. The number of children was seven except on day 3 and day 20 ($n = 6$), because fecal samples from two subjects were not obtained.

During intake, the number of bifidobacteria increased, reaching a maximum of $10.5 \pm 0.1 \log_{10}$ number/g feces on day 20 ($P = 0.07$), corresponding to 35% of the total number of fecal bacteria. The increase was not statistically significant compared to the level before intake of NAN BF ($P = 0.07$). *B. lactis* Bb-12 was found in 11 of 19 samples (53%) from five of seven subjects (71%) during intake. The number of *B. lactis* Bb-12 was 8.0 to $9.6 \log_{10}$ number/g feces, which accounted for 27% of the total bifidobacteria.

Seven days after cessation of intake (day 27), *B. lactis* Bb-12 was not detected in any sample, and the total number of bifidobacteria decreased. Bacteroidaceae were found to be the most predominant bacteria in all samples at approximately 40% of the total number of fecal bacteria during the entire experimental period (results not shown). There were no detectable changes observed in numbers of the other microorganisms throughout the experimental period and the other fecal bacteria showed little or almost no change in numbers (results not shown).

The levels of total fecal IgA and anti-poliovirus IgA are shown in Fig. 2. The total fecal IgA level was 1.49 ± 0.32 mg/g feces before intake of NAN BF. This level was similar to that from children not fed NAN BF in the previous study. During intake, the level of total fecal IgA was significantly increased, with the peak level occurring at 4.29 ± 1.09 mg/g feces on day 8 ($P < 0.05$), when the IgA level was 2.9-fold higher than the initial value. Anti-poliovirus IgA was detected in all fecal samples from the subjects. During intake of NAN BF, the level of anti-poliovirus IgA also increased significantly ($P < 0.05$). In two subjects, for which *B. lactis* Bb-12 was not found in any feces, an increase of IgA levels during intake was also found. After cessation of formula intake on day 27, the levels of IgA decreased and were close to the initial levels.

4. Discussion

The composition of the microflora in the intestines of healthy adults is known to be stable, and exerts resistance against colonization by exogenous pathogenic bacteria (Cortheier et al., 1985; Van der Waaij et al., 1971). In this study, fecal bifidobacteria in children aged one to three years were the second most predominant bacteria following bacteroidaceae, indicating that the composition of the intestinal microflora in these children was closer to that of adults rather than infants. During intake of NAN BF, the number of bifidobacteria slightly increased. However, the population of the other bacteria, especially bacteroidaceae, did not change greatly during intake of NAN BF, and bifidobacteria did not become predominant as in breast-fed infants. These results suggest that the intestinal microflora of young heal-

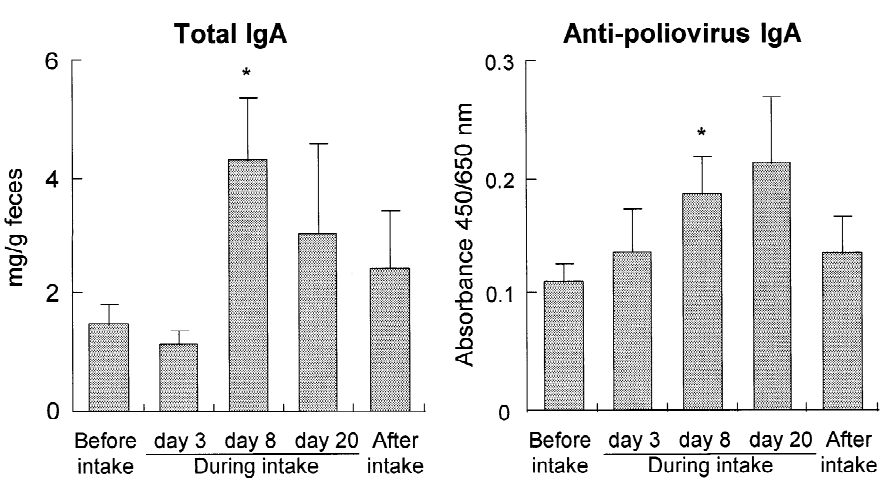

Fig. 2. Effect of probiotic formula for children on fecal IgA. Data are expressed as mean±SEM. Statistical differences were calculated by Mann–Whitney test by comparison with the value before intake (*$P < 0.05$). The number of children was the same as described in Fig. 1.

thy children seems to be as stable as that of adults, and the intake of probiotics does not drastically modify the microflora.

One of the beneficial effects of probiotics is to prevent infectious diseases (Saavedra et al., 1994; Brunser et al., 1989; Gonzalez et al., 1990). Saavedra et al. (1994) showed that a probiotic starter formula, which contains the same bifidobacterium strain as that in this study, reduced the incidence of acute diarrhea in hospitalized infants. They also showed that the shedding of rotavirus significantly decreased in the probiotic formula-fed infants. The mechanism of the prevention of infections by probiotics is likely to be elicited by an enhancement of the immunity of the host. Probiotics have been shown to activate macrophage activity in mice (Perdigón et al., 1986, 1988) and man (Schiffrin et al., 1994). Probiotics also enhance humoral immune responses by increasing IgA producing cells (Perdigón et al., 1990; Kaila et al., 1992) and stimulate antibody responses to some specific antigens (Perdigón et al., 1990; Yasui et al., 1992; Majamaa et al., 1995; Link-Amster et al., 1994). In this study, we found that the levels of total fecal IgA and anti-poliovirus IgA increased significantly during intake of the probiotic formula in healthy children. This suggests that ingestion of the formula containing bifidobacterium stimulated the production of IgA in the gastrointestinal tract of the children. The role of intestinal IgA is to eliminate invading pathogens from the gastrointestinal tract (Underdown, 1986). Anti-poliovirus IgA antibody prevents poliovirus multiplication in the gastrointestinal tract by inhibiting attachment to the mucosal surface (Ogra and Karzon, 1970). The increase of intestinal IgA by the formula feeding may enhance the efficacy of exclusion of pathogens from the gastrointestinal tract, and may partly explain the preventive effect on infectious diarrhea in some previous studies.

Colonization of bacteria in the gastrointestinal tract is an important characteristic of probiotics which results in beneficial effects for the host. During intake of NAN BF, *B. lactis* Bb-12 was found in the feces of most of the children. In some subjects, the number of *B. lactis* Bb-12 detected in the feces during intake was more than 10^9 per gram, which was sometimes more than the daily administration of *B. lactis* Bb-12 from NAN BF. This indicates that *B. lactis* Bb-12 could have reached the intestines and proliferated there. There is no direct evidence showing that *B. lactis* Bb-12 itself stimulated IgA production of the host. The population of *B. lactis* Bb-12 in the fecal samples was at most 27% of total bifidobacteria, which indicates that most bifidobacterial strains in the gastrointestinal tract, even during intake of the formula, were resident bifidobacteria. Some bifidobacteria strains were reported to stimulate IgA production in vitro (Yasui et al., 1992), implying the possibility that the stimulation of IgA production was elicited by resident bifidobacteria. However, the increase of IgA levels was observed only during NAN BF intake, when *B. lactis* Bb-12 appeared in the feces. The ingestion of the probiotic formula containing *B. lactis* Bb-12 and colonization by the strain could trigger IgA production by the host.

Weaned children cannot obtain IgA passively from breast milk, and therefore may be at risk from infectious diseases. Follow-up formula with probiotic bacteria may have great potential for preventing infections in children during and after weaning by enhancing the mucosal resistance to infections by active stimulation of local IgA production.

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