Feeding of *Bifidobacterium bifidum* and *Streptococcus thermophilus* to infants in hospital for prevention of diarrhoea and shedding of rotavirus

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Summary

Acute diarrhoea is a serious cause of infant morbidity and mortality, and the development of preventive measures remains an important goal. Bifidobacteria (which constitute the predominant intestinal flora of breastfed infants), as well as other lactic-acid-producing organisms such as *Streptococcus thermophilus,* are thought to have a protective effect against acute diarrhoeal disease. However, their efficacy has not been assessed in controlled trials.

In a double-blind, placebo-controlled trial, infants aged 5–24 months who were admitted to a chronic medical care hospital were randomised to receive a standard infant formula or the same formula supplemented with *Bifidobacterium bifidum* and *S thermophilus.* Patients were evaluated daily for occurrence of diarrhoea, and faecal samples, obtained weekly, were analysed for rotavirus antigen by enzyme immunoassay. Faecal samples were also obtained during an episode of diarrhoea for virological and bacteriological analyses. 55 subjects were evaluated for a total of 4447 patient-days during 17 months. 8 (31%) of the 26 patients who received the control formula and 2 (7%) of 29 who received the supplemented formula developed diarrhoea during the course of the study (p=0·035, Fisher's exact test, two-tailed). 10 (39%) of the subjects who received the control formula and 3 (10%) of those who received the supplemented formula shed rotavirus at some time during the study (p=0·025).

The supplementation of infant formula with *B bifidum* and *S thermophilus* can reduce the incidence of acute diarrhoea and rotavirus shedding in infants admitted to hospital.

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Introduction

Acute diarrhoea is a major cause of infant mortality in developing countries.[1,2] Furthermore, nosocomially acquired diarrhoeal disease in infants can lead to a prolonged stay in hospital and to increased medical costs. Although there are many microbial agents associated with gastroenteritis in this age group, rotavirus is the most important cause of the condition in infants admitted to hospital in the USA and in many other countries.[3] The development of effective methods to prevent acute gastroenteritis remains an important goal for infant health.[4]

The replication of pathogenic organisms within the gastrointestinal tract is determined by various microbial and host factors. One such factor is the composition of non-pathogenic intestinal flora. For example, the anaerobic bacteria of the genus *Bifidobacterium* constitute the predominant colonic flora of breastfed infants.[5] Bifidobacteria are thought to exert some of the protective effect against diarrhoea associated with breastfeeding.[6] Additionally, in laboratory animals bifidobacteria reduce viral shedding and delay the clinical onset of rotaviral infection.[7] Other non-pathogenic bacteria may also improve gastrointestinal function in infants. For example, lactic-acid-producing bacteria such as *Streptococcus thermophilus* replicate within the human gastrointestinal tract and generate lactase activity.[8] This enzymic activity facilitates the digestion of lactose in milk and can decrease the symptoms of malabsorption, which often accompany acute infectious diarrhoea.

Formulas and fermented milk products containing *B bifidum, S thermophilus,* and other bacterial cultures have been used for many years for their potential benefit on intestinal digestion and function.[9] However, the potential efficacy of the feeding of non-pathogenic bacteria for prevention of infantile gastroenteritis has not been assessed in controlled clinical trials. We did a double-blind, placebo-controlled trial to evaluate the efficacy of a formula containing *B bifidum* and *S thermophilus* for the prevention of acute diarrhoea in infants admitted to hospital.

Patients and methods

The study was carried out in the inpatient unit of the Mount Washington Pediatric Hospital, which is a facility for the long-term care of childen with chronic illnesses. The 46-bed unit consists of 13 rooms each housing 1–4 patients. Standard infection control procedures are used in accordance with policies of the Centers for Disease Control and Prevention. Study protocols and consent procedures were approved by the Joint

	Study formula	
	Supplemented	Control
Demographics		
No of subjects	29	26
Age (mo)*	10·9, 6·1	11·2, 5·7
Weight/age Z score*	−3·1, 1·6	−2·8, 1·7
Height/age Z score*	−3·6, 2·6	−3·7, 2·4
Primary underlying conditions		
Respiratory	12	13
Nutritional deficiency	7	5
Neurological	5	3
Immunological	3	4
Cardiac	2	1

*Mean, SD.

Table 1: **Subject characteristics**

Committee of Clinical Investigations of the Johns Hopkins Medical Institutions and the Mount Washington Pediatric Hospital.

Patients

All infants aged between 5 and 24 months who were admitted to the hospital from Jan 24, 1992, to June 14, 1993, were evaluated for inclusion in the study. Children were excluded if, at enrolment, they were being breast fed, they had a history of allergy to cow's milk, or they were receiving lactose-free, protein hydrolysate formulas for a malabsorptive disorder. Informed consent was obtained from the parent or guardian of eligible infants.

Study formulas

The two study formulas, prepared and supplied in powdered form by Carnation Nutritional Products (Glendale, CA) had the same nutrient composition (2·6 g protein, 4·1 g fat, 13·2 g carbohydrates per 100 kcal [4200 kJ]); they were casein based and contained vegetable oil, corn syrup solids, and lactose. The control formula consisted of this nutrient base alone, and the supplemented formula contained additional *B bifidum* ($1{\cdot}9\times10^8$ colony-forming units [CFU]/g powdered formula: $35{\cdot}8\times10^8$ CFU/100 kcal) and *S thermophilus* ($0{\cdot}14\times10^8$ CFU/g: $2{\cdot}69\times10^8$ CFU/100 kcal). Cultures of these organisms were from Christian Hansen's Laboratory, Denmark A/S. Bacteriological profile (to check identity and viability) was confirmed by quantification on standard culture media.

The formulas were diluted with sterile distilled water according to clinical needs as judged by the physician and nutritionist. The energy requirement for each infant was calculated by paediatric nutritionists who routinely evaluated all patients admitted to the hospital. Concentrations of formula ranged from 0·53 kcal/mL (2·2 kJ/mL) to 1 kcal/mL (4·2 kJ/mL).

Methods

Infants were randomised to receive either the supplemented formula or the control formula for the duration of their hospital stay. They were stratified at enrolment by age (<12 months, ≥12 months) and by energy intake from infant formula (≤50%, >50%), and were then assigned to the supplemented or control formula with a block randomisation procedure. For each substratum, 16 envelopes were filled randomly (8 envelopes each) with the code for the two formulas and sealed. As soon as all envelopes of each substratum had been taken, an additional 16 envelopes were prepared and used in an identical manner. Attending physicians, nutritionists, and study monitors were unaware of which formula was being administered.

If an infant was discharged but returned within 14 days, he or she was returned to the study and continued on the same study formula. If an infant was readmitted after more than 14 days, he or she was re-randomised and included in the analysis as a new study subject.

The following data were recorded weekly for each infant: weight, average daily energy intake, mean daily volume of formula intake, mean number of stools per day, total stool plus urine weight, mean daily number of episodes of regurgitation or vomiting, and changes in clinical status that affected the feeding regimen. In addition, infants were weighed daily and lengths were recorded each month.

Patients were also evaluated daily for stool consistency. Diarrhoea was defined as the passage of five or more liquid stools a day. An episode was defined as diarrhoea for 7 days or less. During episodes of diarrhoea, the formula was continued provided daily stool output was less than 75 mg/kg body weight. If output was greater, or if the infant had clinical signs of dehydration, the formula was withheld for 8 hours, while he or she was rehydrated with standard oral electrolytes (Rehydralyte, Ross Laboratories, Columbus, OH). The formula was then re-started with the addition of an electrolyte replacement solution (Pedialyte, Ross Laboratories) until the diarrhoeal episode resolved.

For microbiological evaluation, faecal samples, obtained daily during an episode of acute diarrhoea, were collected as whole stools or with cotton-tipped swabs. In the case of swabs, faecal material was emulsified in 1 mL sterile phosphate buffered saline (pH 7·4) before analysis. Samples were tested for rotavirus and enteric strains of adenovirus by enzyme immunoassay, and for selected pathogenic bacteria (salmonella, shigella, yersinia, and campylobacter) with standard microbiological methods. Rectal swabs were also obtained weekly irrespective of whether there was diarrhoea. Rotavirus antigen was sought in these samples with enzyme immunoassay methods.

Statistics

Statistical analyses were done with the Epi-Info Statistical Package (Centers for Disease Control and Prevention, Atlanta, GA), with two-way comparisons by the Fisher's exact test (two-tailed). The cumulative incidence of infection was calculated (Kaplan and Meier) with JMP 3.0 Software (SAS Institute, Cary, NC).

Results

Study population

Of 60 infants who were randomised, 5 received formula for less than 24 h and were excluded from further analyses. The 55 remaining subjects constitute the study population analysed; this included 8 infants who had two hospital admissions separated by more than 14 days. Therefore, the 55 study subjects consisted of 47 different infants. Of the 55 infants, 29 were randomised to receive supplemented formula and 26 to receive control formula.

At enrolment, there were no significant differences between the two groups with respect to age, height, weight, or diagnosis (table 1). The 55 infants were fed either supplemented or control formulas for 4447 patient-days. There was no difference in the mean (SD) number of days each formula was ingested per subject (supplemented formula 79·1 [8·1], control 82·8 [8·6], p>0·5). The groups were also similar with respect to other indices of formula intake—mean (SD) volume ingested

	Supplemented formula (n=29)	Control formula (n=26)	p
Diarrhoeal disease			
Episodes of disease	2 (6·9%)	8 (31%)	0·035
Duration per episode (days)*	4·0, 1·4	4·3, 1·4	>0·5
No of stools per day*	6·7, 0·5	6·7, 1·5	>0·5
Stool weight (g)*†	592, 78	523, 146	>0·5
Rotavirus shedding			
Prevalence	3 (10·3%)	10 (38·5%)	0·025‡
Shedding during diarrhoea	2 (6·8%)	5 (19·2%)	0·236‡

*Mean, SD. †Daily urine and stool output. ‡Fisher's exact test (two-tailed).

Table 2: **Diarrhoeal disease and rotaviral shedding**

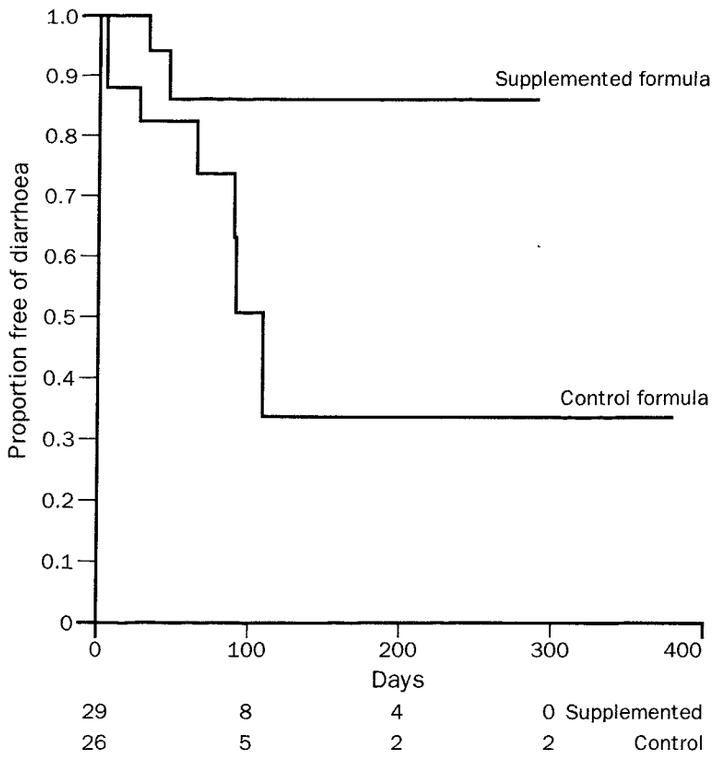

Figure: **Cumulative incidence of diarrhoea in infants receiving formula supplemented with *Bifidobacterium bifidum* and *Streptococcus thermophilus* (supplemented) and the same formula without these bacteria (control)**

(mL/day) was 701 (212) in the supplemented formula group and 767 (234) in the control group; mean (SD) daily energy intake per kg body weight was, respectively, 354·5 kJ (151·2) and 343·1 (154·1); and energy density of the formulas ingested by the infants was 4·0 (0·5) kJ/g and 3·8 (0·6) kJ/g.

All of the infants maintained or improved their nutritional status during the study. There were no technical or mechanical problems associated with the preparation or delivery of the formulas. There were no adverse effects judged to be associated with the feeding of either the supplemented or the control formula, and adequate growth was recorded in all infants.

Effect of supplemented formula on diarrhoeal disease

Significantly fewer infants receiving supplemented formula than receiving control formula developed acute diarrhoeal disease during their hospital stay (table 2). Furthermore, the cumulative incidence of diarrhoea was significantly reduced in infants receiving the supplemented formula (χ^2 4·65, p=0·031) (figure). None of the children who developed diarrhoea had more than one episode. Severity of diarrhoea, as judged by duration, number of stools per day, or stool weight per day, was similar between the two groups (table 2).

Seven of the ten episodes of diarrhoeal disease were associated with the shedding of rotavirus. Five of the episodes of rotavirus gastroenteritis occurred in children receiving control formula and two occurred in children receiving supplemented formula (p=0·24, Fisher's exact test). There were no other viral or bacterial pathogens in any of the samples obtained from the infants with diarrhoea. Overall, 10 (38%) of the children receiving control formula shed rotavirus at some time during their hospital stay compared with 3 (10%) children receiving the supplemented formula (p=0·025, Fisher's exact test).

Discussion

We have shown that the feeding of a formula supplemented with *B bifidum* and *S thermophilus* substantially reduces the incidence of acute diarrhoeal disease in infants in hospital. Our study supports the findings of previous uncontrolled studies, in which the feeding of bifidobacteria reduced the incidence of diarrhoea in infants.[10,11] Since bifidobacteria are major components of the flora of breastfed infants, our study also supports the notion that these bacteria may provide some of the protective or ameliorating effect of breastfeeding against acute enteritis.[12,13] Bifidobacteria ingested orally can be recovered in stools, in which high concentrations can be maintained while the organisms continue to be consumed.[14] However, we did not determine the intestinal viability of the ingested organisms, or address possible mechanisms of action.

Rotavirus was the most common causal agent of gastroenteritis identified in our study population. This finding accords with previous studies showing that rotavirus is the major agent of diarrhoeal disease in infants admitted to hospital in the age group we studied.[15] We found that the feeding of milk formula containing *B bifidum* and *S thermophilus* reduced the rate of rotavirus-associated diarrhoea from 19% to 7%, although statistical significance for the reduction in the rate of rotavirus diarrhoea could not be achieved because of the small number of infants. Larger studies should be done to ascertain the effect of the feeding of these organisms on the rate of gastroenteritis caused by rotavirus and other pathogens.

We also found that the feeding of *B bifidum* and *S thermophilus* led to a significantly lower rate of rotaviral shedding with respect to the number of infected children and the duration of detectable faecal shedding. Our data are consistent with animal-model studies showing that daily ingestion of *B bifidum* reduces shedding and clinical onset of rotaviral infection.[7] Thus, in addition to lowering the rate of diarrhoeal disease, the feeding of *B bifidum* and *S thermophilus* lowers the rate of rotaviral shedding into the environment. A decrease in rotavirus shedding may lead to less environmental exposure and, therefore, a lower rate of hospital-acquired infection in infants at risk of gastroenteritis.

The hypothesis that ingestion of acid-producing probiotic bacteria could be used to prevent diarrhoea and other intestinal diseases was first suggested by Metchnikoff in 1908. Our double-blind, placebo-controlled study confirms that the feeding of milk formula containing specific bacteria can result in a decrease in the rate of diarrhoea in high-risk paediatric inpatients. It is noteworthy that the formula containing *B bifidum* and *S thermophilus* was well tolerated by the children, many of whom were initially malnourished or immunocompromised because of underlying disease. Furthermore, although we added *B bifidum* and *S thermophilus* to infant formula, these or similar bacteria can be cultivated cheaply and added to other foods suitable for ingestion by children and adults. The use of these or similar microbial preparations may provide a practical method for the prevention of diarrhoeal disease in a wide range of clinical and environmental conditions.

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The role of tyrosinase in autoimmune vitiligo

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Summary

Vitiligo is a common depigmenting skin disease, associated with certain autoimmune endocrinopathies, and autoantibodies to several antigens can be found in melanoma cells. We set out to identify the antigens.

We examined 26 patients with vitiligo and associated endocrine disease. Of these, 18 patients (77%) and 8 immediate family members had autoantibodies specific for a 69 kDa protein in HTB-70 human melanoma cells that was not seen in control cells. The autoantibody-positive patient sera reacted with recombinant human tyrosinase expressed in *Escherichia coli* seen by western blots, as did antibodies raised in rabbits against hamster tyrosinase, but not to recombinant tyrosinase-related protein. Not one of 31 normal controls or 8 patients with alopecia or systemic lupus erythematosus had tyrosinase autoantibodies but a small proportion (12%) of 42 patients with autoimmune endocrine disease without a history of vitiligo had them.

The results show that tyrosinase, an enzyme important in melanin formation, is a principal autoantigen of autoimmune vitiligo.

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Introduction

Human vitiligo is a common skin disorder characterised by patchy areas of depigmentation due to loss of melanin-forming cells or melanocytes in the epidermis.[1] The aetiology is not known, but there is evidence for an autoimmune-mediated disease mechanism. Vitiligo is seen in several autoimmune endocrinopathies[2] and vitiligo-associated anti-melanocyte antibodies which can lyse cultured melanocytes by both complement activation and antibody-dependent cellular cytotoxicity have been reported in patients[3–5] and in the Smyth chicken model of the disease.[3]

Naughton et al[4–7] showed that anti-melanocyte antibodies were present in vitiligo patients. Sera from patients with vitiligo reacted with melanocyte surface and cytoplasmic antigens.[8] In the Smyth chicken model, melanocyte autoantibodies were detected in the sera of affected chicks several weeks before depigmentation, and the autoantibodies identified bound to multiple melanocyte proteins of between 65 and 80 kDa.[9]

Patients with vitiligo have specific losses of integumentary melanocytes, so we explored the possibility that one or more key enzymes involved in melanin synthesis could be important autoantigens in the disease pathogenesis. The key enzymes involved in melanin synthesis are tyrosinase and tyrosinase-related protein, both of which have a calculated molecular weight of 62 kDa. However, the native form of tyrosinase has a molecular weight of approximately 70 kDa.[10]

Patients and methods

We looked at sera from 26 vitiligo patients (table 1). Of these, 17 patients had vitiligo and an autoimmune endocrinopathy,