Probiotic Agents: Clinical Applications in Infants and Children

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Interest in the use of live microbial agents for health maintenance and disease prevention or treatment has exploded over the last few years. Many of these organisms, under the generic name of probiotics, are being proposed as remedies for a large number of gastrointestinal and other systemic conditions ranging from diarrheal disease to allergy to cancer prevention. Recently several books and reviews have been dedicated to the topic (1–3).

The term “probiotic,” as an antonym of the term “antibiotic,” was originally proposed in 1965 by Lilley and Stillwell (4), to be used for substances that favor the growth of microorganisms. More than 20 years later, Fuller (5) broadly defined them as “a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance.” The concept is not new. At the turn of the century, Metchnikoff (6) suggested that the consumption of live microbes in fermented milk products to maintain this balance between pathogenic and nonpathogenic bacteria may, at least in part, be an explanation for why certain ethnic groups live longer than others.

The human body lives in a heavily contaminated bacterial environment, and symbiosis with these microbes seems a condition of survival—a human has more prokaryotic organisms associated with skin, lung, and gut surfaces than eukaryotic human cells. The role and importance of the intestinal flora in maintaining the gut luminal milieu and its effects on colonic epithelium, mucosal integrity, vitamin and nutrient metabolism, absorption, and so on have been well described. A logical management approach to situations that alter our microbial environment (dietary, environmental, antibiotics) would be to increase our association deliberately with specific nonpathogenic organisms to counter that alteration. Thus conceptually, the use of probiotics constitutes a purposeful attempt to modify the relation with the immediate microbial environment in ways that may benefit human health.

PROBIOTICS AND INTESTINAL FLORA IN INFANCY

The differences in microflora between children who are breast-fed and those who are not have been evaluated in many studies. There is ample evidence that breast feeding
protects against diarrheal disease, urinary tract infections, septicemia, and possibly necrotizing enterocolitis. The protection conferred by breast milk is probably multifactorial, involving secretory immunoglobulin (Ig)A, lysozyme, the presence of lactoferrin, low casein and phosphate content, and possibly complex oligosaccharides (7). The cellular and humoral components of human milk may have an effect not only in modulating the population of intestinal flora, but also in protecting against the normal bowel lumen inhabitants. In animal models, breast milk can reduce counts of enterobacteria in the small bowel (8) and also can decrease translocation of *Escherichia coli* through the epithelium (9,10).

As breast feeding can affect the virulence of colonizing pathogens, much attention has been paid to the differences in intestinal flora between breast-fed and bottle-fed infants. Several studies on breast-fed infants have documented the high levels of bifidobacteria, which form the predominant component of the normal intestinal flora (11,12). Although strong evidence for this is lacking, it is claimed that differences in the fecal microflora, particularly the dominant bifidobacteria in the gut lumen, may play a role in the protective effect in breast-fed infants. The mechanisms determining specific profiles of gut microflora are still unclear. Some factors in breast milk that may enhance the selective growth of bifidobacteria include the presence of N-acetylglucosamine, glucose, lactoferrin, galactose, fructose, and other less-well-described "bifidogenic" factors (13–15). The low protein content, which results in a reduced buffering capacity in human milk, also may facilitate bifidobacterial proliferation (16). Other differences observed in breast-fed infants compared with bottle-fed infants include generally lower counts of clostridia and enterococci (17–21) and higher counts of staphylococci (17,22).

These observations, however, are not entirely consistent. Other studies have reported similar levels of bifidobacteria in breast-fed and bottle-fed infants (10,18,22–24). In some of these studies, bifidobacteria were found in very low counts in both populations of children (22,25). Moreover, it has not been fully demonstrated that high bifidobacterial counts are always associated with lower levels of other enterobacteria in breast-fed children (19), and the occurrence of bifidobacteria does not always correlate with the uniformly low fecal pH found in breast-fed infants (17,25,26). Intestinal levels of lactobacilli and bacteroides organisms seem to be less affected by breast feeding or bottle feeding (19,27).

In some cases, other more complex mechanisms may be involved. For example, *E. coli* isolated from breast-fed infants are more sensitive to the antibacterial effect of human serum (28), and less often carry the K1 virulence-associated antigen than do *E. coli* from bottle-fed infants (21). Recent studies have shown that breast feeding promotes the expression of the mannose-binding type 1 fimbrial adhesin by intestinal *E. coli* strains (29), probably because of an interaction between type 1 fimbriae and mannose-containing carbohydrate chains on secretory IgA in human milk. In breast-fed infants, however, *E. coli*–expressing mannose-resistant adhesins occur less often, possibly as a result of specific cell activity (30). The type I fimbriae of *E. coli* have not been linked to pathogenic activity, whereas mannose-resistant adhesins are considered virulence factors (31). Thus it appears that a combination of in-
increased bifidobacterial counts and decreased levels of other enterobacteria, as well as other luminal host factors, may play a role in protection from diarrheal disease in premature babies or newborn infants, and individuals in general.

The potential modification of intestinal flora to increase the predominance of specific nonpathogenic bacteria and to modify the intestinal milieu seems a reasonable alternative to attain a prophylactic or therapeutic effect against enteropathogens. Even though there is anecdotal information about the use of probiotics in newborn infants, controlled studies have not yet been published. In one study, *Lactobacillus GG* given in infant formula persistently colonized four of seven premature neonates, with relatively little effect on enterobacteria, yeasts, or staphylococci (32). Studies in which *Lactobacillus GG* was given to premature infants for short periods (1 week) did not show a reduction in stool intestinal pathogens or an apparent clinical benefit in this population (32,33). Current knowledge suggests a theoretic role for these agents in preterm babies, but much work remains to be done.

**CLOSTRIDIUM DIFFICILE DIARRHEA**

*Clostridium difficile* diarrhea is an opportunistic infection that typically follows the disruption of the intestinal flora by antibiotics, although infrequently it also can occur spontaneously. It usually responds to treatment with vancomycin or metronidazole. Relapses, however, are common. Several investigators have reported the resolution of *C. difficile* diarrhea with oral supplements of *Lactobacillus GG* or with the use of fecal enemas in adults (34) and in four children (35). Another study reported similar potential effects in a larger number of adults (36). *Lactobacillus GG* was originally isolated from a healthy human, and it appears to have a superior capability for establishing itself in the colon after regular ingestion. An antimicrobial substance that inhibits *C. difficile in vitro*, as well as other enteropathogens, has been identified (36).

In another study, the administration of another lactobacillus (*L. acidophilus* strain NCDO 1748) did not have any effect on patients with *C. difficile*-associated colitis (37). Although the evidence is strong, placebo-controlled studies in children showing the superiority of these agents over standard treatment must be done.

*Saccharomyces boulardii*, a nonpathogenic yeast, has been successful in prophylactic use in adults for antibiotic-associated diarrhea (38) and in the treatment of adults with *C. difficile disease* (39). Buts *et al.* (40) used *S. boulardii* successfully to treat 19 children with *C. difficile disease* in a small open trial. Of the 19 children (median age, 8 months), 18 (95%) had resolution of diarrhea within 1 week of starting *S. boulardii* treatment (40). No placebo-controlled prophylactic studies with *S. boulardii* are yet available in children.

**ANTIBIOTIC-ASSOCIATED DIARRHEA**

The use of antibiotics constitutes an assault on the gastrointestinal flora, accepted as one of the risks taken in managing severe infections. Probiotics would be a reasonable alternative to help the gastrointestinal flora resist this aggression. Numerous
reports, as well as some controlled studies, testify to the benefit of several agents in
the management of antibiotic-associated diarrhea not connected with C. difficile.
These include bifidobacteria (19), Enterococcus faecium-SF68 (41), Lactobacillus
GG (42), L. acidophilus, L. bulgaricus (43,44), and S. boulardii (38,45).

More common is the change in bowel habit and diarrhea associated with antibiotic
use. Recently Vanderhoof et al. (46) evaluated the effect on diarrheal occurrence of
concomitant ingestion of Lactobacillus GG in children given antibiotics for several
types of acute illness. They found that diarrhea, defined as at least two liquid stools
per day, occurred in only 8%, versus 25% in children given placebo, with a decrease
in duration of diarrhea by 1.1 days in the treated group. Stool consistency was eval-
uated by using an analogue scale, and 48% versus 17% of the children had an aver-
age stool consistency of better than "soft to loose." From the standpoint of clinical
relevance, the significance of a reduction in diarrhea by 1 day over a 10-day period
might be questioned, whereas the occurrence of two "soft to loose" stools per day
might, for some clinicians, barely rate the definition of "diarrhea." These considera-
tions aside, this study does show that in this population of children, the ingestion of
Lactobacillus GG can mitigate the effect on bowel habit (frequency and consistency)
caused by antibiotics. The impact that this reduction would have on day-care absent-
eeism, health care-seeking behaviors, or compliance with antibiotic treatment could
be significant, but must remain speculative until a study is done to address these ques-
tions specifically.

The population studied was made up of healthy children with very mild diarrheal
disease, in whom the probiotic effect might not have been particularly marked. It
could be expected that studies in children in higher-risk categories, particularly those
in whom gastrointestinal function or flora may already be partially or chronically dis-
rupted, might yield clinical benefits of a greater magnitude or significance. For ex-
ample, in a study in undernourished children (47), Lactobacillus GG decreased the
incidence of diarrheal disease in non-breast-fed children but had little effect on those
who were being breast-fed.

TREATMENT OF DIARRHEAL DISEASE IN CHILDREN

There is a rapidly growing body of published reports in the area of management of
acute infantile diarrheal disease with probiotic agents. Boudra et al. (48) and
Touhami et al. (49) showed an improved clinical outcome (measured by stool output
and weight gain) in children with persistent diarrhea fed yogurt as opposed to milk.
These studies and other earlier ones, although provocative, were done in an open
fashion, with relatively little control over intake and potential differences in diet.

Well-controlled studies have recently been published. Isolauri et al. (50) studied
71 children admitted to hospital with acute diarrhea. The patients were randomly as-
signed to receive Lactobacillus GG–fermented milk, or Lactobacillus GG as a
freeze-dried powder, or were given pasteurized yogurt as placebo, for 5 days after
oral rehydration therapy. Diarrheal duration was reduced from 2.4 days in the
placebo group to 1.4 days in the supplemented group. Of all subjects, 82% had
rotavirus infection, and more interestingly, the reduction in diarrhea associated with *Lactobacillus* GG was greater when only patients with confirmed rotaviral infection were analyzed. The same group of investigators showed that at convalescence, 90% of a group treated with lactobacilli versus 45% in the placebo group developed a specific IgA antibody-secreting cell response to rotavirus (51).

*Lactobacillus* GG also was used by Raza *et al.* (52) in another prospective placebo-controlled trial (52). Forty children in Pakistan were enrolled after oral rehydration to receive *Lactobacillus* GG or placebo twice daily for 2 days. Most had varying degrees of malnutrition and moderate to severe diarrhea. Watery diarrhea persisted at 48 hours in 31% in the treated group versus 75% in the placebo group. No differences were found in those children with bloody diarrhea or in the actual duration of diarrhea. In a recent trial, Guarino *et al.* (53) gave *Lactobacillus* GG or placebo to 100 children with diarrhea who were seen in clinics for ambulatory patients by family pediatricians. The dose was $3 \times 10^7$ colony-forming units (CFU). In this group of children, all of whom had mild to moderate dehydration, the duration of diarrhea—as perceived by the parents—was reduced from 6 days to 3 days in the children treated with *Lactobacillus* GG. The reduction was independent of whether the rotaviral diarrhea or diarrhea of another etiology did or did not develop in the children. Six days after the onset of diarrhea, a significantly smaller number of treated children continued to shed rotavirus. In another controlled trial, Majamaa *et al.* (54) also showed a small but significant shortening of the course of rotaviral diarrhea (2.8 to 1.8 days) and a higher secretory IgA response in 49 children treated with *Lactobacillus* GG compared with that in groups receiving *L. rhamosus* or a combination of *Streptococcus thermophilus* and *L. delbruckii*.

Saccharomyces boulardii has been used in several clinical trials. In one study, a dose of 0.5 g daily for 5 days given with standard oral rehydration therapy (ORT) was compared with ORT alone in 38 children with diarrhea (55). The treated group showed a decrease in stool weight and an increase in transit time as measured by carmine red. In another large placebo-controlled, double-blind study in 130 Mexican children with acute diarrhea, *S. boulardii* reduced stool output and improved overall treatment by several different measures (56).

In another recent trial, Shornikova *et al.* (57) randomized 40 patients aged between 6 and 36 months who were admitted to hospital for acute diarrhea to receive *L. reuteri*. The dose was $10^{10} - 10^{11}$ CFU daily, with a matching placebo, throughout their period of hospital stay for $\leq 5$ days (57). Duration of diarrhea was decreased from an average of 2.9 days to 1.7 days in the treatment group (not significant, NS). However, when taking watery diarrhea during the second day of treatment as a measure of clinical outcome, only 26% of children in the treatment group versus 81% in the placebo group still had watery stools. Rotavirus IgA antibodies were studied in both groups at entry and 4 weeks later, and showed no significant differences.

Only a few controlled studies showing marginal or negative results are available. Hotta *et al.* (58) used a commercially available bifidus yogurt and oral preparations of *Bifidobacterium breve* to demonstrate eradication of *Campylobacter jejuni* from stools in children with enteritis, although this occurred less rapidly than in patients
treated with standard erythromycin (58). In a study by Pearce and Hamilton (59), 94 children admitted to hospital for acute diarrhea were given a mixture of *Streptococcus salivarius* SSP, *S. thermophilus*, and *L. delbruekii*, providing $10^8$–$10^9$ bacteria/day, but did not show any improvement in their disease.

**PREVENTION OF DIARRHEAL DISEASE IN CHILDREN**

Most studies have documented potential benefits of probiotics in the treatment of diarrheal disease. Much less is known about the prophylactic effects of probiotics. Saavedra *et al.* (60) conducted a double-blind, placebo-controlled trial in infants aged 5 to 24 months, in long hospital stays for nongastrointestinal conditions. Fifty-five subjects were randomized to receive a standard infant formula or the same formula supplemented with bifidobacteria and *S. thermophilus*. Over a 17-month period, in 31% of the patients receiving control formula but in only 7% of those receiving the supplemented formula did diarrhea develop. Interestingly—and independent of the occurrence of diarrhea—39% of the subjects who received the control formula but only 10% of those who received the supplemented formula shed rotavirus at some time during their hospital stay. This was the first study that has shown a preventive effect against pediatric diarrheal disease of continued administration of probiotics and also an effect on the occurrence of viral pathogens.

In a more recent study (47), 204 undernourished children aged 6 to 24 months from an indigent periurban Peruvian population received either *Lactobacillus* GG or placebo in flavored gelatin once daily, 6 days per week, for 15 months. Children in the *Lactobacillus* GG group had significantly fewer episodes of diarrhea (5.21 episodes of diarrhea/child/year in the *Lactobacillus* GG group vs. 6.02 in the placebo group). The decreased incidence of diarrhea in the *Lactobacillus* GG group was greatest in the 18- to 29-month age group and was largely limited to non-breast-fed children. The duration of diarrheal episodes and the causes of diarrhea were similar in both groups, except that adenovirus was more common in the placebo group.

The potential effects of several of these agents in the management of viral diseases are particularly exciting, as they do not seem to be mediated by a simple modification of intestinal bacterial flora but instead reflect a more complicated system that prevents infection by a virus rather than a bacterium. Recently Isolauri *et al.* (61) showed an improved immunogenicity of oral rotavirus vaccine in 2- to 5-month-old infants when they received vaccine together with *Lactobacillus* GG. The treated infants showed an increased response in rotavirus-specific IgM-secreting cells on day 8 after vaccination. IgM seroconversion was not significantly different in the groups, but IgA seroconversion was reported as 93% *versus* 74%, which was significantly different. In a previous study in children with acute rotaviral diarrhea, this same group of investigators showed that treatment with *Lactobacillus* GG was associated with an enhanced nonspecific humoral response during the acute phase of the infection, reflected in IgG, IgA, and IgM immunoglobulin-secreting cell numbers (51). At convalescence, 90% of the study group *versus* 46% of the placebo group had developed an IgA-specific antibody-secreting cell response to rotavirus. These and other
studies suggest that certain biologic agents may promote recovery from rotaviral diarrhea by augmenting the local immune defense. However, depending on the timing of treatment and response, the greatest effect may be in protecting the child from future episodes of infection.

The possibility that biologic agents such as bifidobacteria and Lactobacillus GG decrease rotaviral shedding by enhancing the immune response also is of conceptual importance, signifying a departure from the simplistic concept that “good bacteria” override “bad bacteria” to promote intestinal health. Interestingly, other studies have recently documented the effectiveness of bifidobacteria in ameliorating rotavirus diarrhea and in decreasing rotaviral shedding in animals (62).

Recent animal studies suggest alternative ways in which probiotics could be used prophylactically. Yasui et al. (63) showed passive protection against rotavirus-induced diarrhea in mouse pups born to and nursed by dams fed B. breve (63). This passive protection was associated with increased levels of antirotavirus IgA in the milk of the dams who were fed B. breve and immunized orally with rotavirus, compared with those of the dams immunized with rotavirus and not supplemented with B. breve. It is an intriguing possibility that regular ingestion of some of these agents may enhance antigen-specific IgA antibody production in the milk of mother–infant pairs and thus provide a more efficient mechanism for protection from rotaviral as well as from other infectious diseases.

SAFETY OF PROBIOTICS IN INFANTS AND CHILDREN

Safety does not appear to be a significant concern. Centuries of use of these lactic acid–producing bacteria and lack of significant adverse effects with most strains currently in use are reassuring. A recent review identified 143 human clinical trials between 1961 and 1998, involving more than 7,500 subjects, with no adverse events reported (3). Nevertheless, we should remain vigilant.

There is only one controlled follow-up study so far (64,65) evaluating the effects of long-term consumption of infant formulas containing probiotic agents. In all, 131 healthy infants between ages 3 and 24 months, recruited from 27 day-care centers, were randomized to receive two standard formulas supplemented with bifidobacteria and S. thermophilus at a concentration of \( \sim 10^7 \) CFU/g or \( 10^6 \) CFU/g, respectively, or an unsupplemented formula. The mean follow-up was 7 months of continuous formula intake. All subjects had normal growth throughout the study. No adverse effects were identified in any of the indices assessed, which included day-care absenteeism and the need for medical attention. Average bacterial load consumed per subject per day was \( 4.0 \times 10^8 \) and \( 3.1 \times 10^7 \) for the high and low groups, respectively. It is of note that, despite lower formula intakes, up to the end of the study, the lowest consumption per subject was >\( 10^5 \) CFU/day, even in the older subjects. Both supplemented groups showed a significant change in bowel habits compared with the placebo group, characterized by an increased frequency of soft stools, less frequent reporting of hard stools, a lower frequency of daily bowel movements, with decreased variability in frequency and no increase in constipation or discomfort. In
addition, there was a 25% decrease in diaper rash in the group supplemented at the higher level. Thus formula supplementation with these organisms appears to act as an "intestinal regulator" of function and motility, leading to what could be considered a more desirable bowel habit. The effects noted were generally more evident with the group supplemented at the higher level, suggesting dose dependence for some of these effects.

The use of probiotic products for their potential beneficial effects has a cost. The development of candidate strains for specific effects and the clinical trials necessary to demonstrate these effects are needed. In addition, adequate quality control of any products sold can be complex and costly. This, together with the relative safety of these agents, has been at least in part responsible for the lack of regulation of probiotic products. Many over-the-counter probiotics now widely available in health food stores are neither reliable as products nor effective as remedies (66). They are sold under the general umbrella of "probiotics," with disguised or sometimes overt claims. Sensible regulation of products and claims, as well as responsibility on the part of the industry, will be extremely important in the appropriate use of these agents in the future.

REFERENCES


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DISCUSSION

*Dr. Zoppi:* When we did experiments combining probiotics with antibiotics, we found that the dose of probiotics should be enhanced to several billions per day, not millions. Do you agree with that?

*Dr. Saavedra:* That goes back to a question that was raised earlier about the dose effect. I believe that when we talk about a particular effect, we should always specify what dose was used to obtain that effect. I think dose probably is important, and it has to do with the specific...
effect we are looking for. If the effect we are looking for is long-term stimulation of the gastrointestinal tract, we probably do not need a very large number of bacteria—we just need to make sure the stimulus is constant. If we are trying to neutralize the profound microbiologic change that the use of an antibiotic induces, we probably do need a larger dose of a particular organism. Until we understand exactly what mechanism operates for each specific benefit, it will be hard to answer your question in a more precise fashion.

Dr. Vigi: I have a question concerning safety. I believe that, as with vaccination, we must be prepared to take some risks because the purpose is to achieve a beneficial effect, so maybe a low level of risk is acceptable. I do not think we have enough information on the safety of probiotics, especially the lactobacilli. It is true that there have not been reports of adverse effects with bifidus, but there have been some papers describing effects of lactobacilli that were not without importance for the recipients (1–3). Do we really want persisting colonization, or would we be better off with a temporary effect? Perhaps killed bacteria are the answer to the problem of safety. As we heard earlier, beneficial effects can be obtained by using killed probiotic bacteria. Could that be a way of achieving a favorable effect without potential adverse effects, especially with small premature babies whose immune systems are immature? I think there should still be concerns in the minds of pediatricians about the use of probiotics in the general population.

Dr. Saavedra: The question of safety is of course important, and there have indeed been reports of bacteremia caused by lactobacilli. We have to remember that the average individual has several billions of lactobacilli in the colon to begin with, so that native lactobacillus is there whether or not we give any more by mouth. Because a few bacteremias caused by these apparently nonpathogenic lactobacilli have been reported does not necessarily mean the patients were eating yogurts at the time. Most of these reports have not been in individuals who were purposely taking large amounts of these lactobacilli. Second, we will always have the problem of taxonomy—identifying which specific lactobacilli are potential pathogens in particular individuals. But I do share your concern that before moving ahead with recommendations about giving a specific strain to a large population of individuals; long-term studies must be done to assure safety.

As you say, the problem also might be circumvented by giving nonliving organisms, and again this will probably become clearer once we understand what the exact mechanism is. I believe, however, that there are some effects—particularly the inhibition of other bacterial pathogens—that may well require a live agent. Conversely, if benefit can be obtained by nonspecific stimulation of certain parts of the gut-associated immune system, it is possible that components of the bacterium, once it is killed, will still produce specific immunostimulation. Overall, from the studies that have been done, it does appear that you can get some effects with nonviable bacteria; however, these will always be less than those with live organisms.

Dr. Delmau: You did not mention *Saccharomyces boulardii*. Do you think it is not safe?

Dr. Saavedra: I did not get into the discussion of *Saccharomyces boulardii*, so I appreciate the question. This organism has been used for a number of specific potential clinical benefits. Some well-controlled trials seem to show benefit in antibiotic-associated diarrhea and traveler’s diarrhea. It has not always been possible to replicate the effects, but there seems to be good evidence that they do have the same kind of biologic effect. From the point of view of safety, there are fewer well-controlled studies of saccharomyces in infants and children than there are of other bacterial probiotic agents, but it does seem to be safe. However, until we have more data on the use of saccharomyces, particularly in young infants and children, it would be difficult to be dogmatic about the safety issue.
Dr. Raihã: You mentioned food sterility. There is an interesting study comparing the frequency of allergy between Baltic and Scandinavian countries, clearly showing that there is less allergy in the Baltic countries. The difference seems to be that we are giving our infants very sterile, prepacked, factory-made food in Scandinavia, and they are not doing that. Do you think it is detrimental to be too sterile? Could we avoid having to consider probiotics by simply being a little less concerned about sterility?

Dr. Saavedra: I would not dare to say that, because unfortunately, there are pathogens out there, and we need to avoid them; we must not go back to the Stone Age, we must keep moving forward! It may be, however, that we have exaggerated the need to truly sterilize the food supply. Conversely, of course, it is very difficult to be specific about what bacteria we do allow and what bacteria we do not allow in food. So what we are doing now is to reestablish our experience with the microbial environment that we have deprived ourselves of, but in a controlled fashion—that is, with probiotics or prebiotics. One of the things we may have focused on too much is the attempt to replicate breast milk. Breast milk is relatively sterile, except for the organisms around the nipple of the mother, so from that point of view, we should sterilize milk. However, breast milk does induce the growth of specific microorganisms that we have not been able to reproduce yet with artificial feeding. So maybe our reference should not be breast milk, but the breast-fed baby. When we can reproduce—by our products, mechanisms, and medical interventions—the ecology in the gut of a breast-fed baby, then that should be our reference, not the actual product that we are giving the baby. We have learned over time that problems can arise if we really try to reproduce breast milk—for example, if we put the same amount of iron in formula as in breast milk, we get a lot of anemic babies. We learned very quickly that we cannot just copy breast milk. We have to copy the result of breast milk, which is the breast-fed child, so our reference should be the baby.

Dr. Marini: You have studied something like 120 babies fed with probiotics up to age 2 years. In that normal population, I would expect there to be an incidence of allergic disease of ~30–35%. What in fact was the incidence of allergy?

Dr. Saavedra: I would love to answer that question. Unfortunately, we did not follow that as an outcome variable, although obviously it would have been extremely interesting. Unfortunately the population was not selected for any particular high risk of allergy.

Dr. Marini: The incidence in a normal population would be ~30%. In a high-risk population, it would probably be as high as 60% in the first 2 years of life. The socioeconomic level of your population is probably also important, considering that hygiene may be lower in the group you studied.

Dr. Saavedra: It is certainly an aspect that should be looked into. To add to those epidemiologic observations about allergy in developed countries versus less-developed countries, the same thing probably holds true for inflammatory bowel disease. The incidence of both Crohn's disease and ulcerative colitis, for example, has increased as we have moved toward a more sterile environment. Whether that means that probiotics or prebiotics could ultimately be used in the prevention of inflammatory bowel disease is something that Dr. Guandalini will be talking about, I think.

Dr. Endres: I have a question concerning your study with nonhospitalized infants, on the protective effect on diaper rash and constipation. In your abstract, you published a nice table showing that the lower dose of 10^6 organisms was more effective than 10^7 organisms in one of these conditions—I forget whether it was diaper rash or constipation. Have you an explanation for that?

Dr. Saavedra: It was constipation. It was interesting that those children had a reduced frequency of stooling but also less "constipation." if we define that as having hard stools with
discomfort. It appears that probiotic agents have a regulatory effect on intestinal transit, similar to the role of fiber in the adult population; the water content in the stool—which is what ultimately determines stool consistency—seems to be much more stable and less variable in children receiving probiotics over an extended period than in children who are constantly changing their diets, particularly around the time of weaning. Concerning the dose effect, we are coming to realize that dose may be important for some clinical effects but not for others in most of the children who are given probiotics over an extended period.

Dr. Alliett: As you have pointed out, each probiotic strain has its own immunologic properties. Some enhance the Th1 response; others have more effect on the Th2 response. More and more diseases are now classified as either Th1 or Th2 predominant. What would happen if you were to give a Th1-enhancing strain in a Th1-predominant disease, or a Th2-enhancing strain in a Th2-predominant disease?

Dr. Saavedra: The short answer is that we do not know; we can only speculate. This goes back to whether we give just one organism or several, whether we give a probiotic with a prebiotic, in what dose we give them, and what the host is. I guess it is always possible that we could make things worse, and that is why when we look at specific agents we need to make sure that we have investigated all the potential clinical outcomes if we are concerned about specific disorders—whether they be Th1-, or Th2-, or IgE-mediated, or whether there is some other immunologic disturbance in that individual. At this point, we can only speculate, but I do not think there are data to suggest that we are likely to aggravate any of these disorders. Given the fact that most of these bacteria have been ingested so widely for such a long time, the chance that we are going to see any significant negative response is probably very low.

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