



Probiotics in Children

Benjamin Kligler, MD, MPH^{a,b,*},
Patrick Hanaway, MD^c, Andreas Cohrssen, MD^{a,d}

^a*Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10463, USA*

^b*Continuum Center for Health and Healing, 245 Fifth Avenue, Second Floor,
New York, NY 10016, USA*

^c*Genova Diagnostics, 63 Zillicoa Street, Asheville, NC 28801, USA*

^d*Beth Israel Residency Program in Urban Family Practice, 16 East 16th Street,
New York, NY, USA*

The gastrointestinal microflora has been a subject of interest in medical science since the early twentieth century when Nobel laureate Metchnikoff [1] proposed that many diseases were related to the action of gut bacteria and that consuming beneficial lactic acid-producing bacteria (by drinking fermented milk) was health promoting because it prevented growth of putrefactive bacteria in the gut. Recent scientific advances have reawakened interest in these clinical observations, highlighting the role of the gut flora in metabolism, protection, and immune regulation. The authors examine the development of this postnatal organ, review its role in health and disease, and discuss the clinical opportunities to modify its expression through the use of probiotics.

Gastrointestinal flora and probiotics: the development of a postnatal organ

At birth, the human body is sterile. Colonization of the mucosal membranes starts during delivery before the first breath and evolves quickly over the first days of life [2], but quantity and species vary markedly over the first 2 years of life [3]. There is a rapid succession of bacteria, depending on environmental influences including maternal flora, place of delivery, type of delivery (cesarean section or vaginal), age at birth, hygiene measures,

* Corresponding author. Continuum Center for Health and Healing, 245 Fifth Avenue, Second Floor, New York, NY 10016.

E-mail address: bkligler@chpnet.org (B. Kligler).

antibiotics, birth order, and type of feeding. As the child moves to solid food, the gut flora becomes more adultlike in composition [4].

The variations in the gastrointestinal microbiota over the first 2 years of life have implications for the functional ability of gut flora to optimize its myriad activities in nutrition/metabolism, defense, and education of the immune system. Many species have evolved to peacefully coexist within the gastrointestinal tract. More than 500 species have been noted, each with numerous strains identified by molecular probes [5]. Overall, the number of bacteria present in the gastrointestinal lumen is tenfold the number of cells in the human body [2]. These species interact with the innate immune system and play a critical role in the dynamic education of the adaptive immune system, promoting balance and strength in the developing immune system.

The probiotic theory was championed by Metchnikoff [1], who recognized the value of fermented foods in promoting optimal digestive health. Today, the term *probiotics* (Greek, *pro*, “for,” and *biosis*, “life”) has been defined as nonpathogenic microorganisms that, when ingested, exert a positive influence on host health or physiology [6]. A probiotic must be of human origin, be resistant to destruction by gastric acid and bile, adhere to intestinal epithelial tissue, and be able to colonize the gastrointestinal tract (if only for a short period) [7]. Beneficial gut flora can also be stimulated by nondigestible foods, which are known as prebiotics [8]. Clinical studies show how these prebiotic and probiotic agents can be used to promote balance in the gut flora, creating benefit for a number of diseases.

Development of the gut flora

The child in utero is swimming in and drinking amniotic fluid, which is sterile. With delivery, environmental exposure to the maternal birth canal, fecal material, skin, and birthing assistants/nurses determines the initial make-up of facultative aerobic organisms that first colonize. This milieu rapidly gives way to *Enterobacter* and *Streptococcus* spp over the first few days of life and then to *Bifidobacterium* and *Bacteroides* spp by the end of the first week [9]. Factors that influence the succession of gut flora from that time forward include type of delivery [10], feeding habits [11], gestational age [10], hospitalization [3], and infant antibiotic use [12].

It is well established that the type of delivery has an impact on gut flora that goes beyond the first few days of life. Cesarean section is associated with a decrease in the beneficial obligate anaerobes *Bacteroides* and *Bifidobacterium*, along with an increase in *Clostridium* sp [13]. Of interest, these differences in gut flora are not found in developing countries where early colonization with enterobacteria is the norm in vaginal and in abdominal births [14].

Pronounced changes are again noted in differentiating gut flora in breast-fed versus bottle-fed infants [10]. Breast-fed infants derive benefit from the immunoregulatory nutrients present; they also benefit from the metabolic

(essential fatty acid) and prebiotic (oligosaccharide) effects of breast milk. Bottle-fed infants have a higher amount of commensal flora overall, along with a broader and more heterogeneous distribution of gut flora, including coliforms, *Streptococcus* sp, *Clostridium difficile*, and other clostridial species. As many as 50% to 60% of bottle-fed infants have *Clostridium difficile* (versus 6%–20% of breast-fed control infants), but this bacteria does not have the same degree of pathogenicity in infants as it does in older children and adults. With age, the *Clostridium difficile* declines and the pattern of distribution becomes more adultlike.

Additional changes are noted independently with the use of antibiotics in infancy, although no clear relationship to intrapartum or prenatal maternal antibiotic use has been established. Infants receiving antibiotics change the succession by increasing *Klebsiella*, *Citrobacter*, and *Enterobacter*; by decreasing coliforms, *Bacteroides*, and *Bifidobacterium*; by decreasing short-chain fatty acids; and by increasing *Candida albicans* [15]. Antibiotics also disrupt individual species, leading to colonization resistance—an inability of beneficial flora to effectively colonize and competitively exclude less-desirable microbes. Other environmental factors, including more aggressive standards of hygiene, have led to a delayed development pattern and even the absence of certain groups of bacteria in the commensal flora of neonates [9].

Other factors influencing the succession of gut flora include gestational age and birth order. Preterm neonates have significantly altered microbiota, over and above that predicted by extensive hygiene, hospitalization, and antibiotic therapy. *Clostridium difficile* is present in most premature infants, and it takes over 6 months for the beneficial *Bifidobacterium* to reach normal values. The presence of an older sibling in the household helps to decrease the risk of altered gut flora by promoting an increase in *Bifidobacterium* [10].

Although the subspecies and quantities of bacteria vary over time, the families and species of commensal flora remain relatively constant within an individual over the course of a lifetime. Factors including weight gain [16], inflammation [17], and antibiotic use [18] cause disruptions in the balance of the gut flora through the remainder of childhood and into adulthood. Furthermore, in many cases, alterations in gut flora precede the development of illness, including atopic diseases [19]. The gastrointestinal microflora can be modified by diet and environmental factors to affect health [20].

Physiologic functions of the gut flora

Humans and bacteria have coevolved to offer mutual benefit. With more than 100 trillion bacteria present in the gastrointestinal tract, the necessity of a mutually beneficial relationship is clear [21]. The Intersection Human Genome Sequencing Consortium [22] identified 223 proteins from bacterial origin that have now been established as part of the human genome. The positive health contributions of these gut microflora are in the areas of nutrition, metabolism, protection, and immune regulation. Imbalances in

the gut flora and alterations in the biologic terrain can have a deleterious effect on normal function in any of these areas.

Alterations in diet, including glycemic load, fiber content, essential fatty acid composition, pH balance, and macronutrient/micronutrient composition all have tremendous effects on the balance of commensal flora within the gastrointestinal tract [23]. The critical metabolic and nutritive functions of the gut flora include digestion, absorption, fermentation, vitamin synthesis, biotransformation, and energy production. This array of activities is required for normal human physiologic functioning. It is postulated that the short-chain fatty acid synthesis that provides energy to the gut epithelium may also be involved in the “cross-talk” that influences the development of humoral and cell-mediated portions of the mucosal immune system.

The “defense and protection” function of the microflora is mediated by a number of mechanisms, including competitive exclusion (competing for nutrients, space, and adherence); ensuring normal intestinal barrier function; stimulating immunoglobulin (Ig)A production; creating the mucoid bi-film layer; and producing antimicrobial substances such as bacteriocidins to actively stop infection [24]. Further trophic stimulation of the gut epithelium and modulation of intestinal permeability are also part of the inherent defense system.

Most important of the functions of the gut flora early in life are the development, education, and modulation of the mucosal immune system. New evidence is evolving that the persistent interactions between the host and its bacteria that take place in the gut may constantly reshape the immune system [5]. Nearly 70% of the human immune system is localized in the digestive tract. The mucosal surface of the gastrointestinal tract is 200 times the surface area of the skin. Defense against microbes is mediated by the early reactions of innate immunity, followed by the reaction of adaptive immunity. Innate immunity includes the skin, mucosal epithelia, cytokines, and phagocytes. These nonspecific defense mechanisms provide defense against common pathogens, but the innate immune response stimulates the adaptive immune system and influences the nature of the adaptive response [25]. The process of “oral tolerance” is an important example of this [26].

Through the process of coevolution, the body has developed a number of methods to identify microbes and modulate the adaptive immune system, based on the proper timing and presence of the bacterial stimuli. The body responds differentially to bacterial stimuli and responds to a variety of structural components on each bacterium.

The succession of gut flora, described earlier, offers early education to the innate immune system to begin to recognize “self.” There are molecules of recognition—pattern recognition receptors, toll-like receptors, and pathogen-associated molecular patterns—that facilitate awareness of the bacterial environment and determine the release of stimulating or suppressive cytokines. Recent evidence demonstrates that immunostimulatory DNA [27] may derive from the copious amounts of bacterial DNA present in the

gastrointestinal tract. The epithelial mucosa is equipped with pattern recognition receptors that recognize bacterial DNA from commensal bacteria and effectively modulate immune function [28]. Dendritic cells (DCs) also sample the gut milieu to define local antigens that induce IgA production. Pathogenic bacteria will up-regulate the adaptive immune system (by way of interleukin [IL]-12) within the Peyer's patches and the mesenteric lymph nodes, inducing nuclear factor-kappa β (NF- κ B) activation of the inflammatory cascade. Conversely, the normal gut microflora promotes immune modulation (by way of IL-10) and has anti-inflammatory properties.

Oral tolerance is mediated by regulatory T (T_{reg}) cells, which have anti-inflammatory capabilities. Precursor T cells are transformed into T_{reg} cells when DCs have not been exposed to inflammation. Precursor T cells are transformed into $T_{effector}$ cells (T_{H1} or T_{H2}) in the setting in which the DCs are mature (ie, activated by inflammatory signals) [29]. Gut flora (like *Lactobacillus*), however, can down-regulate DC maturation, thus preventing the activation of $T_{effector}$ cells [30]. Disruption of gut flora disrupts oral tolerance. Correction of gut flora improves oral tolerance. Thus, our immune system is dynamically educated by the presence of bacteria at the interface of the intestinal epithelium. The gut flora interacts with our innate immunity and influences the adaptive immune response in an important dialog between our immune system and the environment. Commensal bacteria are also able to modulate the expression of host genes involved in important intestinal functions including nutrient absorption, mucosal stimulation, xenobiotic metabolism, and intestinal maturation [31].

Different bacteria induce different immunologic responses. Nonpathogenic bacteria also elicit different cytokine responses from epithelial cells, inducing differential effects on the gut-associated lymphoid tissue (GALT) and on the adaptive immune system [32]. Because of this dynamic interplay between the gut flora and the GALT, the immunologic response system can be modified based on dietary change (in the form of prebiotics) and beneficial bacteria (in the form of probiotics) (Box 1).

Clinical applications

A wide range of probiotic strains and combinations of strains have been examined in clinical trials over the past 2 to 3 decades for indications ranging from prevention of antibiotic-associated diarrhea (AAD) to treatment of atopic dermatitis to infantile colic and irritable bowel syndrome. The most widely studied strains include *Lactobacillus* sp (including *L acidophilus*, *L rhamnosus*, *L bulgaricus*, *L reuteri*, and *L casei*, among others), *Bifidobacterium* sp, and *Saccharomyces boulardii*, a nonpathogenic yeast.

Prevention of antibiotic-associated diarrhea

There are a large number of clinical trials and several meta-analyses evaluating the effects of probiotics in preventing AAD. Many antibiotics

Box 1. "Hygiene hypothesis" versus "old friends"

In 1989, Strachan [33] described in the "hygiene hypothesis" that the increased prevalence of allergy and atopic illness in industrialized countries is a result of the decrease in exposure to common infections during early life, secondary to smaller family size. The theory tells us that there is a relative increase in T_H2 (humoral) activity due to the lack of T_H1 (cell-mediated) stimulation. Many have attempted to extend this hypothesis, based on epidemiologic evidence, to include the role of antibiotics, vaccines, and antimicrobial soaps, but their effects have not been proven [34]. Current research focuses on the role of nutrition, timing, and gut flora maturation on immunologic development [35].

More recent analyses have questioned the hygiene hypothesis' emphasis on T_H1/T_H2 imbalance, given the epidemic rises in allergic (T_H2) and autoimmune diseases (T_H1). Evolution has kept us in close contact with microorganisms including bacteria, viruses, parasites, and helminths. It appears that our innate immune system has evolved to recognize these "old friends" as harmless. Paradoxically, persons in affluent countries may not have the necessary "friends" present to consistently stimulate the maturation of T_{reg} cells. Thus, immunoregulation, as determined by the $T_{effector}/T_{reg}$ balance, may be more important than the T_H1/T_H2 balance [34].

Hooper and Gordon [36] highlighted the effects of imbalance within this complex ecosystem. The increasing prevalence of allergy and atopy is associated with alterations of intestinal colonization and decreased tolerance to common food proteins and inhaled allergens. Treatment with probiotics has helped to shift these symptoms back to normal [37]. Overall, we see that these critical environmental interactions highlight immunologic dysregulation arising from the combination of varied bacterial species (commensal and pathogenic), altered adaptive immune system activation, and multiple antigenic stimuli.

selectively eradicate lactobacilli and bifidobacteria, leaving enterotoxic *Escherichia coli* and *Clostridium difficile* to flourish. In some patients, this leads to an overgrowth of the more pathogenic bacteria and, subsequently, to diarrheal episodes [38,39]. Administration of probiotics is meant to reverse this overgrowth and rebalance the intestinal flora. Although there is still controversy regarding the optimal combination and dose of probiotic strains for this indication, there is consensus in the literature that a variety

of probiotics, when started at or soon after the initiation of antibiotics, can reduce the incidence and the severity of AAD.

Kotowska and colleagues [40] studied 269 children in Poland aged 6 months to 14 years who were placed on antibiotics in a double-blind, randomized placebo-controlled trial. These children received 250 mg of *Saccharomyces boulardii* or a placebo twice daily for the duration of antibiotic treatment. These investigators found a significantly reduced prevalence of diarrheal episodes (defined as three or more loose or watery stools per day for 48 hours or more, occurring during or up to 2 weeks after the antibiotic therapy) in children treated with *Saccharomyces boulardii* compared with controls subjects (9/119 [8%] versus 29/127 [23%]; relative risk [RR]: 0.3; 95% confidence interval [CI]: 0.2–0.7).

A smaller randomized controlled trial (RCT) in 157 patients from Brazil came to a similar conclusion. Correa and colleagues [41] gave infants aged 6 to 36 months a commercial formula containing *Bifidobacterium lactis* and *Streptococcus thermophilus* for a total of 15 days at the initiation of antibiotic therapy. They found substantially more diarrhea in the placebo-supplemented infants (32%) compared with the treatment group (16%). A Turkish study by Erdevé and colleagues [42] found similar benefits in 466 children aged 1 to 5 years randomized to receive sulbactam-ampicillin or azithromycin with placebo or *Saccharomyces boulardii*. AAD was observed in 42 of 222 patients (18.7%) in the placebo group versus only 4.7% in the probiotic group.

A meta-analysis by Szajewska and colleagues [43] in 2006 of six RCTs ($n = 766$) concluded that probiotics reduced the risk of AAD in children from 28.5% to 11.9% (RR: 0.44; 95% CI: 0.25–0.77) compared with placebo. The risk reduction was similar regardless of the type of probiotic used (*Lactobacillus GG* [LGG], *Saccharomyces boulardii*, or *Bifidobacterium lactis* plus *Streptococcus thermophilus*). These investigators concluded that for every seven patients who would develop diarrhea while being treated with antibiotics, one fewer would develop AAD if he or she also received probiotics. A second meta-analysis, by Johnston and colleagues [44], examined six RCTs ($n = 707$) and found that although a per-protocol analysis showed benefit for probiotics over placebo, a more sensitive intention-to-treat analysis did not show a significant benefit in reducing the incidence of AAD. The investigators attribute this lack of significant effect to excessive loss of subjects to follow-up in several of the trials. The investigators, however, found a significant benefit (RR: 0.36; 95% CI: 0.25–0.53) in a subgroup analysis of four studies that used at least 5 billion colony-forming units (CFUs) daily of LGG, *L sporogens*, or *Saccharomyces boulardii*, suggesting that inadequate dosing may be an important factor in the trials that do not show an effect.

In conclusion, the data to date support the use of probiotics in the prevention of AAD. Doses of 5 to 10 billion CFUs should be used, and various probiotic strains appear to be equally effective.

Treatment of acute diarrhea

Probiotics have also been extensively studied in the treatment of all-cause acute diarrhea in children. Many of these studies have methodological problems, and the heterogeneity in causes of acute diarrhea makes drawing definitive conclusions difficult. Nevertheless, it appears that probiotics may be effective in at least some cases of acute diarrhea.

For example, Sarker and colleagues [45], using *L paracasei* strain ST11 in 230 male infants and young children aged 4 to 24 months in Bangladesh, found a reduction of stool output (225 ± 218 mL/kg versus 381 ± 240 mL/kg), stool frequency (27.9 ± 17 versus 42.5 ± 26), and oral rehydration solution intake (180 ± 207 mL/kg versus 331 ± 236 mL/kg) in children who had moderate nonrotavirus diarrhea, but found no benefit in the treatment of severe rotaviral infection. Billoo and colleagues [46] studied 100 children aged 2 months to 12 years in Pakistan who received 250 mg of *Saccharomyces boulardii* for 5 days or placebo and found a reduction in diarrheal episodes to from 4.2/d (on day 3 of treatment) in the placebo group to 2.7/d in the probiotic group. This benefit was sustained for 2 follow-up months, with additional diarrheal episodes of 0.54 in the treatment group and 1.08 in the placebo group.

Szymanski and colleagues [47] randomized 87 infants and children aged 2 months to 6 years to *L rhamnosus* strains 573L/1 at a dosage of 12 billion CFUs twice daily for 5 days or to placebo. They found a nonsignificant trend in the nonrotavirus infection group (84 hours of diarrhea in the treatment group versus 96 hours in the placebo group), but a significant benefit in the outcome for the rotavirus infection group (duration of diarrhea 76 hours in the treatment group versus 115 hours in the placebo group). A Peruvian RCT by Salazar-Lindo and colleagues [48] in 179 infants and children aged 3 to 36 months did not find benefits of *L rhamnosus* LGG using an enriched milk formula compared with placebo. The investigators speculate that post-diarrheal lactose intolerance may have contributed to the lack of effect.

Several studies have looked at the impact of probiotics on duration of hospital stay in children who have acute diarrhea. For example, Krugol and Koturoglu [49] in Turkey published an RCT ($n = 200$) showing that 250 mg of *Saccharomyces boulardii* for 5 days reduced the length of the hospital stay from 3.9 days to 2.9 days.

One systematic review and one recent meta-analysis concluded that probiotics are probably effective in treatment of children who have acute diarrhea. In 2005, Allen and colleagues [50] examined 23 studies of probiotic use for acute diarrhea in adults and children ($n = 1917$). In a subset of 12 studies performed in infants and children, mean duration of diarrhea was reduced by 29.2 hours in subjects taking probiotics (95% CI: 25.1–33.2; $P < .00001$). Because a variety of probiotics were used in these trials, because the causes of diarrhea in these studies were so heterogeneous, and because the outcomes were often measured by parents rather than by the

investigators, questions remain regarding the exact magnitude of the potential benefit. Nevertheless, the investigators concluded that probiotics “appear to be a useful adjunct to rehydration therapy in treating acute, infectious diarrhea in adults and children” [50].

Finally, Szajewska and colleagues [51] examined *Saccharomyces boulardii* in a meta-analysis for the treatment of acute gastroenteritis in children. These investigators combined data from four RCTs ($n = 619$) and found a significant reduction in duration of diarrhea (-1.1 days; 95% CI: -1.3 to -0.8) in children taking *Saccharomyces boulardii* compared with placebo. These investigators, however, qualified their conclusions with a caveat that their results should be interpreted with caution due to the methodological limitations of the included studies.

Given their wide margin of safety, probiotics should play a role in the treatment of acute infectious diarrhea despite the limitations of the literature to date. Some researchers further refine this observation and believe that LGG is the most effective strain for this indication and maintain that probiotics are most effective in treating rotaviral diarrhea [52]. These claims are not yet proven, and additional research is needed to clarify these questions. It is clear that probiotics should be started as early as possible in the course of the illness and that a dose of approximately 5 to 10 billion CFUs per day is probably appropriate.

Prevention of community-acquired diarrhea

Several studies have examined the utility of probiotics for preventing acute diarrheal episodes in the community setting. Given the facts that community-acquired diarrhea remains a major cause of death among children in the developing world and that probiotic-fortified formulas can be manufactured easily and cheaply, this research is of particular importance. For example, in a study of undernourished children aged 1 month to 2 years in Peru ($n = 204$), participants were randomized to LGG or placebo for 15 months. Children in the LGG group had significantly fewer diarrheal episodes (5.2 per child per year versus 6.0 in the placebo group, $P < .03$) [53].

Rio and colleagues [54] studied 135 children in Argentina to specifically examine the impact of underlying nutritional status on the effectiveness of probiotics. They compared undernourished children with well-nourished children, and used fermented milk, which provided *L acidophilus* and *L casei* ($1-10 \times 10^7$ CFUs/mL), versus regular milk (placebo). They found a reduced frequency of diarrheal episodes over a 3-month period in the well-nourished treated versus placebo groups (20 episodes in 35 actively treated patients versus 35 episodes in 27 patients in the placebo group). This difference was not seen in the undernourished treated group compared with the undernourished placebo group. Both treated groups, however, fared better in the prevention of protracted diarrhea than the placebo groups (0 episodes of diarrhea lasting over 14 days versus 12 episodes).

A number of studies have also been done in developed countries. Chour-aqui and colleagues [55] studied 90 healthy children younger than 8 months living in residential or foster care settings in France and gave them acidified milk formula containing *Bifidobacterium lactis* BB12 or a conventional formula. They found a significant difference in the daily probability of diarrhea in the treated group (0.84) versus the placebo group (2.3). There was no significant difference, however, between the groups in terms of the overall prevalence of diarrhea during the study period.

Thibault and colleagues [56] examined the prevalence of diarrhea in a sample of over 900 healthy infants aged 4 to 6 months in child care settings given a formula enriched with *Bifidobacterium brevis* plus *Streptococcus thermophilus* 065 or standard formula. These investigators found no difference in incidence or duration of diarrheal episodes or hospital admissions but found that diarrheal episodes were less severe in the probiotic group, as measured by significantly fewer cases of dehydration, fewer formula changes, fewer prescriptions for oral rehydration salts, and fewer medical consultations.

Weizman and colleagues [57] studied 201 infants aged 4 to 8 months in 14 child care centers in Israel over a 12-week period. Children were randomized to a formula with *Bifidobacterium lactis* BB12 (1×10^7 CFUs/mL of formula), to a formula with *L reuteri* (1×10^7 CFUs/mL of formula), or to no probiotics. Infants on probiotics had significantly fewer febrile illnesses (0.27 versus 0.42) and fewer diarrheal episodes (0.13 versus 0.31) than the untreated controls. The effects were more prominent with *L reuteri*.

Sazawal and colleagues [58] published a meta-analysis of 34 RCTs examining probiotics of various types for prevention of acute diarrhea of all causes. In the 12 trials with data on children, Sazawal and colleagues [58] found an overall reduction in risk of developing diarrhea of 57% (95% CI: 35–71; $P < .001$). This analysis combined AAD with community and hospital-acquired diarrhea, making it difficult to evaluate the specific impact of probiotics on either of these relatively distinct clinical entities.

Finally, in another prevention-oriented study, Vendt and colleagues [59] examined the effect of probiotic supplementation on growth during the first 6 months of life. Infants up to age 2 months ($n = 120$) were randomized to LGG-enriched formula or to standard formula in a double-blind fashion. The treated group showed significantly greater height and weight than the standard formula group at age 6 months. As in the study by Weizman and colleagues [57], this effect was demonstrated in a well-nourished cohort in a developed country (Finland); if this finding could be replicated in a less well nourished group of children in a developing country, the implications for probiotics as a strategy for prevention and health promotion would be significant.

Prevention of necrotizing enterocolitis in premature children

Probiotics seem to hold substantial promise in the prevention of necrotizing enterocolitis (NEC) in premature infants. Bin-Nun and colleagues [60]

randomized 155 premature infants having a birth weight lower than 1500 g to receive a probiotic-supplemented formula containing *Bifidobacterium infantis*, *Streptococcus thermophilus*, and *Bifidobacterium bifidus* or standard formula. The incidence of NEC was significantly reduced in the treatment group (4% versus 16.4%; $P = .03$). Three of 15 babies who developed NEC died, and all NEC-related deaths occurred in control infants.

A similar result was obtained by Lin and colleagues [61], who studied 367 very low birth weight infants (<1500 g) in China. These investigators added a mix of *L acidophilus* and *Bifidobacterium infantis* to breast milk. The risk of NEC was significantly reduced in the treatment group compared with the control group. The risk of death was 7 in 180 in the treatment group versus 14 in 187 in the control group, and the risk of NEC was 9 in 180 versus 24 in 187, respectively.

Irritable bowel syndrome and constipation

Although in clinical practice, probiotics are commonly used in the treatment of irritable bowel syndrome, to date, clinical trials have not shown substantial efficacy. An RCT by Bausserman and colleagues [62] ($n = 50$) in children fulfilling Rome II criteria for irritable bowel syndrome who were treated with LGG or placebo for 6 weeks found no significant benefit in the abdominal pain scale (44% response in the treatment group versus 40% response in placebo). There was, however, a significant effect on abdominal distention scores. Another study by Banaskiewicz and colleagues [63] in 84 children aged 2 to 16 years who had severe constipation examined LGG versus placebo as an adjunct to lactulose. The treatment group and the placebo group in this trial showed improvement in about two thirds of patients, making it difficult to draw definite conclusions.

Infantile colic

Recently, Savino and colleagues [64] compared the use of *L reuteri* with simethicone in the treatment of infantile colic. Simethicone, although not distinguishable in efficacy from placebo, has been the treatment of choice for colic for many years. Ten billion CFUs of probiotics were given daily to 41 breast-fed infants who met the clinical criteria for colic versus 60 mg of simethicone per day. Beneficial effects began to be noted in the probiotic group within 24 hours, and by 28 days, the average crying time was decreased by 65% in the probiotic group. In addition, 95% of all infants receiving probiotics responded positively compared with only 7% responders in the simethicone group.

Atopic dermatitis: treatment

Because of the apparent role of probiotics early in life in regulating systemic immunologic development, interest has recently developed in the impact of probiotics on atopic dermatitis in young children. In one placebo-controlled

RCT, for example, Weston and colleagues [65] treated 56 children (aged 6–18 months) who had moderate to severe atopic dermatitis with 1×10^9 CFUs of *L fermentum* VRI-033 twice daily for 8 weeks. At the end of the study at 16 weeks, children in the probiotic group showed a significant reduction in the Severity Scoring of Atopic Dermatitis (SCORAD) index ($P = .03$) that was not seen in the placebo group. In this study, 92% of children in the treatment group had a SCORAD index that was better than baseline values at week 16 compared with 63% of children in the placebo group. This difference was also significant ($P = .01$). In an additional analysis of the children in the study, these investigators found that the probiotic group showed a significant increase over baseline in T_H1 cytokine interferon- γ responses at the end of the 8-week supplementation period. The placebo group showed no such change. Furthermore, this increase in interferon- γ responses was directly proportional to the decrease in the SCORAD index ($r = -0.445$; $P = .026$) during the intervention period, and the effect was still present 2 months after supplementation had ended [66].

Another study exploring possible mechanisms was performed by Rosenfeldt and colleagues [67]. In a double-blind, placebo-controlled crossover study ($n = 41$), these investigators used the lactulose/mannitol test—a validated measure of intestinal permeability—and found that improvement in atopic dermatitis was significantly correlated with decreased intestinal permeability ($P = .001$). These findings, according to the investigators, may suggest that “impairment of the intestinal mucosal barrier appears to be involved in the pathogenesis of atopic dermatitis.”

Two other studies suggest that probiotics may be effective for atopic dermatitis only in children who have previous food sensitization. In a double-blind study of children who had suspected cow’s milk allergy ($n = 230$), Viljanen and colleagues [68] found that LGG was only effective for atopic dermatitis in children who had documented IgE hypersensitivity.

A small randomized study ($n = 59$) from New Zealand, which treated children for 12 weeks with *L rhamnosus* and *Bifidobacterium lactis*, similarly showed an effect only in those who had documented food sensitization [69].

Finally, Passeron and colleagues [70] compared the effectiveness of a preparation containing only “prebiotics”—substances that “selectively stimulate the growth and/or the activity of a limited number of bacterial strains already established in the gut”—with a preparation containing prebiotics plus probiotics (*L rhamnosus* Lcr35 1.2×10^9 three times daily) in a double-blind RCT of children aged 2 years and older who had atopic dermatitis ($n = 48$). After 3 months of treatment, both groups showed significant improvement in the SCORAD index, with no statistical difference between groups.

Atopic dermatitis: prevention

A Finnish RCT of infants at high risk for atopic disease based on family history ($n = 132$) examined the role of prenatal and postnatal

administration of LGG in preventing the development of eczema. In the active group, mothers were given LGG (1×10^{10} CFUs daily) for 4 weeks prenatally and for 6 months postnatally if breastfeeding; babies who were not breastfeeding were given the same dose orally. At age 2 years, atopic eczema was diagnosed in 31 of 68 in the placebo group but only in 15 of 64 in the active group (RR: 0.51; 95% CI: 0.32–0.84) [71]. These beneficial effects persisted at 4 years without any additional treatment [37].

A recent Australian placebo-controlled RCT ($n = 178$) examined the effectiveness of *L acidophilus* LAVRI-A1 in preventing the onset of atopic dermatitis in newborns at high risk for this condition. In this study, newborns received 3×10^9 CFUs plus maltodextrin daily or maltodextrin alone from birth to 6 months; no difference in incidence of atopic dermatitis was found at age 6 months or 12 months [72]. Furthermore, subjects in the treatment group had a higher incidence of sensitization to milk protein at the end of the study than children in the placebo group. A potential confounder in this study was the use of maltodextrin as a placebo; because this substance is a prebiotic, it may exert its own beneficial effects. In addition, this study did not include a prenatal period of probiotic administration.

Other clinical indications

Probiotics have shown some promise for a number of other clinical indications in children, including acne [73], irritability in infants [74], and severe gingivitis (in a chewing gum preparation) [75]. LGG has also been examined as an adjunct to conventional therapy in children who have Crohn's disease and, although well tolerated, it did not change the time to relapse [76]. All these indications require further study before treatment recommendations can be made.

Safety considerations

Despite the extensive clinical trial literature described in this article, there are few reports of significant complications or adverse effects from the use of probiotics. Mild abdominal discomfort and flatulence are the only adverse effects reported regularly in most of the trials.

There have been concerns raised regarding the risk of septicemia from the use of probiotic supplementation. This can certainly occur, and there are case reports of bacteremia with probiotic species following oral administration. These cases are rare and without exception occurred in severely ill or immunocompromised hosts or in children who had short-gut syndrome or indwelling catheters. For example, De Groote and colleagues [77] reported a case of LGG bacteremia in a child who had short-gut syndrome and an indwelling Broviac catheter and gastrostomy tube. Alternately, Srinivasan and colleagues [78] examined the safety of *L casei* shirota in a group of critically ill children ($n = 28$) in a pediatric ICU and found no evidence of

bacteremia. Likewise, the studies cited earlier using probiotics for the prevention of NEC in premature infants did not report any cases of bacteremia using the probiotic organisms. Given the potential benefits in this type of vulnerable patient and the awareness that there may be a risk of bacteremia, however small, it is advisable for decisions to be made on a case-by-case basis in children who are immunocompromised or have indwelling catheters.

In healthy children, it appears that the risk of bacteremia is almost nonexistent. Hammerman and colleagues [79] examined the safety of two specific strains—*L rhamnosus* GG and *Bifidobacterium* sp—in a recent systematic review and concluded that although case reports of sepsis do exist and deserve attention, the risk, at least with these two specific organisms, is extremely low. These investigators found no cases of sepsis reported in any prospective clinical trial. There are no reports of sepsis or other pathologic colonization in healthy patients.

Dosage

Given the wide range of types of probiotic combinations and dosages examined in the clinical trial literature to date, it is difficult to make definitive statements regarding specific choice of preparation and exact dosages. Doses ranging from several million CFUs per day to 600 billion CFUs (for chronic pouchitis) and 3.6 trillion CFUs (for remission of ulcerative colitis) have been used in published studies. Regarding *Saccharomyces boulardii*, the dose used in most studies is between 250 and 500 mg daily. Regarding LGG, *Bifidobacterium* sp, and combinations of other bacterial strains, it appears that, at least for the best-studied indications of prevention of AAD and prevention and treatment of acute infectious diarrhea, the minimum daily dose should be in the range of 5 to 10 billion CFUs regardless of the specific preparation. For example, the Canadian meta-analysis of AAD studies found strong evidence of effectiveness, even using intent-to-treat analysis, in the studies that used a dose of over 5 billion CFUs daily regardless of the specific strain. Given the wide safety margin of probiotics, in particular, and the lack of evidence that higher doses lead to any increase in risk, it is probably wise to err on the side of higher dosage ranges.

Regarding the less extensively studied indications such as atopic dermatitis, the optimal dose and preparation have not been clarified to date. Doses of 5 billion CFUs daily and higher are commonly used in clinical practice, even with very young infants, for these other indications.

Probiotics are available in a wide variety of preparations, including powders, capsules, and liquids. Although most studies use twice-daily dosing, there is no evidence to date that once-daily dosing is not adequate. Practitioners and patients should be aware that as with many other dietary supplements, the specific dose of active ingredient (in this case, CFUs per day) in a given amount of powder or liquid can vary tremendously between brands. Thus, careful reading of the label is paramount for parents, and practitioners

should counsel on this point specifically. Quality is also a major issue in using probiotics in clinical practice. Many probiotic preparations do not contain the number of viable CFUs stated on the label. ConsumerLab.com, an independent laboratory that performs quality control assessments of dietary supplements, recently found in an analysis of probiotic products that 5 of 19 brands analyzed did not contain the number of live organisms claimed on the label [80]. Practitioners planning to use probiotics regularly in clinical practice should consult this site to familiarize themselves with several of the high-quality products available. Most probiotic preparations should be kept refrigerated, and in the case of prevention of AAD, probiotics should probably be taken at least 2 hours before or after the antibiotic, if possible.

Recently, a number of products have been developed to deliver probiotics in foods, most commonly yogurt. Conventional yogurt products generally contain only small amounts of live bacteria not adequate to deliver a therapeutic dose. This has led to the development of “therapeutic” yogurts, which can contain 1 billion or more CFUs, per 4-oz container. Although some of these products have been shown to decrease intestinal transit time [81]—suggesting that they may be helpful in treating constipation—none has been studied to date for a specific clinical problem.

The question of which strain or strains of probiotic to use for a given clinical situation is a challenging one. In the few cases in which a specific strain has been shown to be effective for a specific condition, there are likely few studies of other strains for the same indication. In the case of the best-studied indications—AAD and acute infectious diarrhea—meta-analyses show no significant difference in effectiveness among LGG, *Saccharomyces boulardii*, and a variety of combination products including *Bifidobacterium* sp and other strains. Thus, it appears that choosing the proper strain or combination of strains is less important than recommending a sufficiently high dose. Head-to-head trials comparing the effectiveness of specific probiotic strains or combinations for specific clinical indications will be needed to determine whether specific combinations are superior for specific situations. To date, few such trials have been published.

Summary

As should be obvious from this review, there is now an extensive body of medical literature on the use of probiotics in children. Several important points for the practicing clinician clearly emerge:

1. The gastrointestinal flora plays a complex and important role in the development of healthy immunologic and digestive function in young children. Understanding this role and assisting in the development of a healthy gastrointestinal flora (eg, by encouraging breastfeeding and minimizing the use of antibiotics whenever possible) is an important function for clinicians caring for young children.

2. Probiotics are extremely safe in healthy children, and even in immunocompromised or seriously ill children, significant complications are rare.
3. Probiotics are almost certainly effective in reducing the risk of AAD and in reducing the duration of acute infectious diarrhea.
4. Probiotics may also be effective in preventing community-acquired diarrheal infections and in reducing the risk of NEC in premature infants. They may also be helpful in the prevention and treatment of atopic dermatitis.
5. The exact strain or combination of strains most effective for common clinical indications has yet to be determined; for now, the exact strain used seems less important than whether an adequate dose is used.
6. Doses in the range of 5 to 10 billion CFUs per day or higher are appropriate for most clinical indications in children.
7. There is a wide range in quality among products on the market; clinicians should familiarize themselves with several of the widely available high-quality products.

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