Use of Probiotics in Pediatrics: Rationale, Mechanisms of Action, and Practical Aspects

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Nutr Clin Pract 2007 22: 351
DOI: 10.1177/0115426507022003351

Invited Review

Use of Probiotics in Pediatrics: Rationale, Mechanisms of Action, and Practical Aspects

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ABSTRACT: The use of probiotics (ingested microbes that can modify intestinal microbial populations in a way that benefit the host) has moved from concept to actual demonstration of specific benefits by specific microorganisms for specific populations. It is increasingly clear that these benefits to the host are mostly mediated by the profound effect that intestinal microflora (microbiota) have on gut barrier function and host immune response.

Intestinal bacteria are more numerous than the human cells of the host that harbors them. Despite having many potential pathogens in this microflora, humans do not routinely get infected. It is no coincidence that gut-associated immune tissue constitutes approximately 80% of all immunologically active cells in the human host. The gut interacts with intestinal bacteria, both resident and ingested, to develop protective mechanisms (*via* improving gut barrier function and immune stimulation for defense) and appropriate, nonexaggerated responses (*via* immune modulation and immune tolerance) to support host health. The mechanisms of this interaction between host and bacteria are increasingly being unraveled and in great part explain the clinical benefits that have been reported with specific probiotic bacteria by enhancing host defense mechanisms (such as for treatment and prevention of viral diarrhea and reducing risk of necrotizing enterocolitis), mitigating antibiotic-associated diarrhea, and modulating host immune response (such as in allergic disease).

The year 2007 marks 100 years since Eli Metchnikoff suggested that the consumption of lactic acid bacteria may be of benefit to the human host.[1] However, not until the mid-1960s did the term *probiotic* come into vogue. Probiotics today have become a common term to clinicians, as well as the general public; there has been an explosion in interest, reflected in the number of scientific publications on the topic. Research is increasingly focusing on mechanisms that explain the clinical benefits reported with various specific microbes in pediatric populations. The work so far, as discussed below, underscores the profound effect that intestinal microflora (microbiota) have on gut barrier function and host immune response.

Intestinal bacteria (prokaryotic organisms) are more numerous by a factor of 100 to 10 than the eukaryotic cells of the human that harbors them. In addition, many of the 500+ species normally present in the microflora are pathogenic, and the intestinal mucosa is only less than a mm thick. Despite this, humans do not develop infections on a regular basis, thus demonstrating the effectiveness of gut immune-related mechanisms.

The gut-associated immune tissue constitutes approximately 80% of immunologically active cells in the human host.[2] Microbial flora is responsible for the abundance and activation of the mucosal immune system in healthy individuals.[3] For example, absence of microflora (as in germ-free-reared animals) leads to an atrophic mucosa and an underdeveloped cellular immune response, as well as inadequate maturation of antibody secretion, particularly immunoglobulin A (IgA).[3–6] Thus, the intestinal microflora profoundly influence the development of specific and nonspecific cellular and humoral gut mucosal immune responses.[7]

In summary, the gut needs exposure to bacteria to develop its immune defense and appropriate (nonexaggerated) immune responses, which support host health. The mechanisms of host-bacteria interaction are increasingly being unraveled and, in great part, explain the clinical effects certain ingested (probiotic) bacteria have on improving host health, including enhancing defense mechanisms (such as against viral infections), as well as modulating host immune response (such as in allergic disease).

Probiotics: Basic Concept and Characteristics

Intestinal microflora are composed of both well-established resident microbes and those ingested

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0884-5336/07/2203-0351$03.00/0
Nutrition in Clinical Practice 22:351–365, June 2007

orally, which transiently occupy the gastrointestinal (GI) tract. Probiotics are generally defined as nonpathogenic organisms in the food supply (ingested microbes) that are capable of conferring a health benefit to the host by modifying gut microbial ecology. The Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO), in a report on the topic, define probiotics as "live microorganisms which, when consumed in adequate amounts as part of food, confer a health benefit on the host."[8] Although certain yeasts, such as *Saccharomyces boulardii*, have been used as probiotic agents, the main focus of this review will be on probiotic bacteria.

Not all viable ingested bacteria in the food supply are probiotics. Many have no discernable benefit beyond serving as a way to ferment food products. In addition, not all those bacteria which have demonstrated one or another benefit are similar in their effects or mechanisms on the host. Therefore, no statements should generally refer to "probiotics" as "efficacious" or "safe"; this would be as inappropriate as stating "antibiotics" in general are efficacious or safe. We have postulated a more broad approach for the use of probiotics: the incorporation of specific microbes into the diet in a purposeful attempt to modify the relation of the host with its immediate microbial environment in ways that may benefit health.[9] Probiotic bacteria are a subset of specific organisms (identified by genus, species, and when appropriate by strain), which, when ingested, demonstrate specific positive effects or benefits on the host.

Fermented foods, particularly dairy products (like yogurt), have been consumed for centuries. Today, foods and beverages containing lactic-acid-producing bacteria (LAB) constitute up to 40% of the food supply worldwide.[10] LAB, particularly members of the genus *Lactobacilli*, *Bifidobacteria*, and *Streptococcus*, are the most widely used in the food supply, and they are rarely associated with infections or negative effects on humans. Only a small number of these bacteria have been studied, and some specifically have been shown to have a probiotic effect.

Since early reports in the mid-1960s, scientific interest has grown dramatically in probiotics, today averaging approximately 600 scientific publications per year retrievable from PubMed/MEDLINE (NLM, NIH). The more frequently studied probiotic agents are *Lactobacillus* species, including *L rhamnosus (GG)*, *L acidophilus*, *L casei*, *L johnsonii*, and *L reuteri. L rhamnosus (GG)* is the most frequently studied *Lactobacillus* in humans. Among *Bifidobacteria*, the commonly studied probiotic agents are *B breve*, *B infantis*, *B lactis*, and *B longum.* Care should also be taken with the names of these organisms, the taxonomy and names of which have evolved over time. For example, *B animalis* subspecies *lactis* (*B lactis*) has also been called *B bifidum*, *B animalis*, or *Bifidobacterium* strain Bb12. *Lactobacillus paracasei* subspecies *rhamnosus* is commonly referred to as *Lactobacillus GG*.

There are several generally accepted characteristics that define probiotic bacteria.[8,9,11]

Probiotics

- are microbial organisms;
- remain viable and stable after culture, manipulation, and storage before consumption;
- survive gastric, biliary, and pancreatic digestion;
- are able to induce a host response once they enter the intestinal microbial ecosystem (by adhering to gut epithelium or other mechanisms); and
- yield a functional or clinical benefit to the host when consumed.

Others have suggested that probiotic bacteria should be of "human origin" and that they should "colonize" the intestine.[9,11] However, we could not likely predict or assume that at some point, in the millions of years that hominids and prokaryotic microorganisms have coexisted, certain bacteria "became" part of the intestinal microflora of "humans." In fact, it is yet unclear whether many organisms we regularly find in the human colon are just transient because they are also ubiquitous in other animals and foods.[12] It is also clear, as discussed below, that once the intestinal microflora of a host is established, most *ingested* bacteria that exert a probiotic benefit only transiently "colonize" or occupy the gut[13,14] and do not typically become part of the permanent resident microflora of the host.

Probiotic bacteria are thus specific microorganisms of many in the food supply that, when ingested, transiently occupy the GI tract and lead to documented health benefits.

Establishment and Role of Intestinal Microflora in Infancy

The intestine of a newborn is essentially sterile. During the birthing process and during the first few days of life, the gut is inoculated with bacteria. Children born vaginally are thus exposed very early to maternal flora. The microflora develops rapidly after birth and is markedly dependent on genetic factors, mode of delivery (vaginal *vs* cesarean section), the mother's flora, type of feeding, and early environmental surroundings. During the first 2 days of life, an infant's intestinal tract is rapidly colonized with bacteria consisting mainly of *Enterobacteria*. In most breastfed infants, the *Bifidobacteria* counts increase rapidly to constitute 80%–90% of the total flora. *Lactobacilli* and *Bacteroides* increase to a lesser extent, and *Enterobacteria* decrease. Formula-fed infants, on the other hand, tend to have a flora that is more complex, consisting mostly of coliforms and *Bacteroides*, with significantly lower prevalence of *Bifidobacteria*.[15,16] After weaning, the microflora of children begins to resemble that of adults, with *Bacteroides*, *Veillonella*, and *Fusobacterium* on the

increase. At the same time, the number of unculturable microbes also increases. Although the composition of the microflora varies among individuals, the composition within each individual remains stable over prolonged periods.[16–18] The microflora constitutes a large metabolically active biomass of 10^{10} to 10^{12} prokaryotic organisms, close to 500 different species, many of which are known potential pathogens. Separating this large microbial mass (which coats the extensive surface of the gut) from the bloodstream are only a few layers of cells of the epithelium. How is it that infants (or adults, for that matter) are not infected or septic more often?

The fact that approximately 80% of all immunologically active cells of the body are in the gut-associated lymphoid tissue (GALT) is an affirmation of the importance of microbe–gut immune system interaction. It is now clear the normal microbial flora is necessary for the development of GALT.

Analysis of the gut tissues of germ-free mice reveals an underdeveloped sparse mucosal immune system, with small Peyer's patches without germinal centers, and small T cell zones. The lamina propria contains essentially no IgA plasma cells or CD4 cells, and intraepithelial lymphocytes also are rare.[3–6] In man, the best clue for the dependence of the mucosal immune system on gut bacteria comes from the relatively rare analyses of newborn gut showing similar characteristics. Thus, gut luminal microbes are responsible for mucosal immune system development in healthy infants. There is now abundant evidence that signaling through specific receptors, particularly toll-like receptors, intestinal bacteria affect epithelium cell function, which determines T-cell differentiation and antibody responses to T-cell-dependent antigens, regulating immune gut response. Colonization is thus apparently responsible for IgA responses to luminal antigens. Secretory IgA is among the most important component of antibody response to gut lumen protein and pathogen antigens. Colonization also induces modulation of the ratio of T-helper type 2 (Th2, proallergic) to T-helper type 1 (Th1, suppressive) responses, which could decrease the chances for immune hyperreactivity, such as in allergic disease, as further discussed below.[19,20]

Type of Intestinal Bacteria and Effects on Immune Function

Resident bacteria, particularly *Lactobacilli* and *Bifidobacteria*, can exert antimicrobial activities influencing both local and systemic immunity.[21] The lower incidence of infection and GI disturbances in breastfed infants may, in part, be related to differences in microflora between breastfed and formula-fed infants.

The resident *Bifidobacteria* and *Lactobacilli* in the gut can offer resistance (by competition or inhibition) to colonization by other potentially pathogenic microbes, thereby functioning as part of the gut defense barrier. They have also been associated with the secretion of substrates that have antimicrobial properties[22] and the secretion of mucins *via* activation of MUC2 and MUC3 genes, part of the intestinal barrier that can inhibit adherence of pathogenic bacteria.[23] Some *Bifidobacteria* and *Lactobacilli* given orally may enhance the production of a balanced T-helper-cell response[24,25] and stimulate production of interleukin (IL)-10 and transforming growth factor (TGF)-β,[26–28] both of which have a role in the development of immunologic tolerance to antigens and can decrease allergic-type immune responses.

Another major effect of intestinal bacteria is the enhancement of secretory immune function and intestinal flora. Secretory immunoglobulin A (sIgA), the most important and predominant immunoglobulin in mucosal surfaces, provides protection against antigens, potential pathogens, toxins, and virulence factors.[29] Intestinal sIgA synthesis is influenced by microflora. The development of IgA-producing plasmablasts in the intestinal mucosa, precursors for sIgA, are influenced greatly by the microflora.[7] Breast milk contains significant levels of sIgA that are transferred to the infant. In addition, *Bifidobacteria*, which predominates in breastfed infants, have been shown to stimulate the synthesis and secretion of IgA.[30–33] During the neonatal period, sIgA in the stool of formula-fed infants is essentially undetectable.[34,35] *Bifidobacteria* and *Lactobacillus* given orally have been shown to influence sIgA in a number of animal trials.[36–38] Infant studies that investigated the effects of specific *Lactobacilli* and *Bifidobacteria* supplementation on stimulating the mucosal immune response have reported similar positive results, as discussed below. In summary, association with specific types of bacterial species (particularly *Lactobacilli* and *Bifidobacteria*) helps the host maintain adequate gut barrier and immune mechanisms.

Gut Microflora Alterations in Disease

Given the effects gut microbial populations have on gut immunity, it would be expected that "alterations" in *resident* intestinal flora might be associated with conditions resulting from altered immune responses. This is increasingly being demonstrated. For example, increased fecal *Bacteroides* and *Clostridia* and lower and atypical *Bifidobacteria* counts have been associated with infants who develop atopic disease compared with normal controls.[39–41] As might be expected, premature infants are exposed to drastically abnormal microbial environments, as well as feeding regimens and antimicrobial use. Significant alterations in their intestinal microbiota, particularly predominance of nonbifidobacteria species, have been documented and provide a basis for intervention with probiotics for reduction of risk of conditions such as necrotizing enterocolitis (NEC).[42–44] Finally, in patients with

inflammatory bowel disease, decreased counts of *Lactobacillus* and *Bifidobacteria* and increase in other species such as *Bacteroides* species seem to be associated with occurrence or severity of disease.[45–47]

These alterations in the "balance" of intestinal microflora, particularly in immune and inflammatory-related diseases, coupled with the drastic reduction in the oral ingestion and exposure to microbes, have led to postulation of the "hygiene hypothesis." This theory suggests that a lower exposure in early childhood to bacterial and other antigens in industrialized societies has led to inadequate development and maturation of immune responses and appears responsible for the growing epidemic of asthma and allergies due to inadequate defensive and immune-modulating gut immune diseases.[48–50] Increasingly, it is being recognized that host-microbe interactions have an effect on atopic disease. Infants are born with a predominance of Th2 (T helper 2) lymphocyte activity, which predisposes them to an exaggerated response to allergens, with increased IgE production. Exposure to intestinal bacteria, on the other hand, stimulates Th1 (T helper 1) activity (which primarily reacts defensively to bacterial stimuli as part of the protective immune response). As a consequence, intestinal microbes (resident and ingested) can redirect this immune balance from a Th2-predominant response to a balanced Th1/Th2 response, decreasing the chances for a potential exaggerated allergic response. Finally, TReg (regulatory) cells release cytokines such as TGF-β, which can inhibit Th1 or Th2 overexpression and also play a role in adequately balancing the host response to bacterial or food antigens, and their activity seems to be increased by luminal microbes.[40,51–53]

The gut-microbe interactions and their effect on immune response thus provide a rationale for the use of probiotic bacteria in pediatric populations.

Effects of Probiotics on Gut Flora

Specific *Bifidobacteria* and *Lactobacilli*, when given orally, are successful in transiently colonizing the gut of infants and young children. Significant increases in fecal *Bifidobacteria* have been reported as early as 1 week after supplementation. In some cases, these counts can reach levels similar to those found in breastfed infants.[54–58] *Bifidobacteria* supplementation in premature infants has also been shown to positively modify the microflora of the infants' intestines.[59–61] Beneficial increases in stool short-chain fatty acids, reductions in stool pH, and decreases in fecal ammonia and indoles[57,58] and concentrations of *Bacteroides* and *E coli* have been documented[55,56] with *Bifidobacteria* supplementation. As a result, specific probiotic bacteria positively affect the ratio of favorable to unfavorable microflora in infants and children and lead to positive changes in gut luminal environment. It is important to emphasize that when probiotic bacteria (*Bifidobacteria* and *Lactobacilli*, which show a positive clinical effect) are ingested, they are not part of the resident microflora of the host, and their counts typically decrease or disappear once ingestion stops.

To summarize these aspects of gut-microbe interactions:

1. Acquisition and establishment of intestinal microflora are necessary for adequate development of gut barrier function and immune response of the host.
2. Certain types of resident bacteria, namely, *Lactobacilli* and *Bifidobacteria*, are not typically pathogenic and seem beneficial in fostering host immune development and response.
3. The composition of intestinal microflora of infants who are not breastfed or infants and children who develop diarrheal disease, atopic disease, and other inflammatory conditions appears altered when compared with the microflora of breastfed infants or healthy infants and children. Particularly, these affected infants show less *Bifidobacteria* and *Lactobacilli* relative to other components of microflora.
4. Specific *Lactobacilli* and *Bifidobacteria*, when ingested, can modify the composition of intestinal microbial ecology.
5. These ingested organisms have the potential of further supporting gut barrier function and appropriate host immune system development and response.

These interactions are the basis for the concept and applications of probiotic bacteria in infants and children discussed below.

Effects of Probiotics on Gut Barrier and Immune Function

An increasing number of clinical trials have documented effects of ingestion of specific probiotic bacteria on gut barrier function and immunity. For example, in both animal and human models, ingestion of *L casei*, *L bulgaricus*, and *L acidophilus* has been shown to activate production of macrophages and enhance phagocytosis.[17] Serum sCD14, a marker of immunologic maturation in the neonate, was significantly greater than placebo in infants provided probiotics. Additionally, decreased gut permeability with *Lactobacilli*,[62] and recently in premature infants receiving *Bifidobacteria*,[60] and is another mechanism by which probiotics may function.

In adults, consumption of *L acidophilus* La1 and *Bifidobacteria* increased specific and total secretory IgA to salmonella after *S typhi* oral vaccination to levels considered clinically relevant.[63] These same probiotics, as well as *B lactis*, have been shown to increase phagocytic activity against *E coli* species, as well as enhance natural killer cell activity.[64,65] Among the more consistently found effects of specific *Bifidobacteria* and *Lactobacilli* in pediatric populations is the effect on humoral immunity, particularly

on secretory IgA and other immunoglobulins. An increase in IgA-, IgM-, and IgG-secreting cells in circulation, as well as fecal IgA concentrations, has been reported.[31,66–69] Similarly, increased specific IgA to rotavirus after infection[68] or antipolio IgA after immunization was reported.[31] Recent reports indicate similar IgA increases in premature infants receiving *B lactis*.[70]

In addition, some probiotic bacteria have also been shown to exert beneficial effects on pro- and anti-inflammatory cytokine secretion.[17,31] Decreases in fecal 1 antitrypsin, urinary eosinophil protein X, tumor necrosis factor (TNF)-α,[26,28,67,71] and changes in TGF-β and other cytokines point to the potential these agents have to down-regulate inflammatory mediators, particularly in infants with exaggerated immune response, such as in atopy.

Some *Bifidobacteria* and *Lactobacilli* can influence the differentiation of T helper cells into Th1 or Th2 cells, responsible for secretion of distinct cytokines, which govern immune responses. Taken together, the overall immunomodulatory effects of probiotics demonstrate their potential for use in a variety of situations where the gut barrier may be compromised or an imbalance exists in cytokine and immune cell populations.[11,72]

In summary, effects have been documented with use of various probiotics on gut barrier function and immune response. These include effects on innate (nonspecific immune defense) and adaptive immunity (responses that require exposure to pathogens or antigens and that the immune system recognizes and "remembers"). Adequate adaptive responses are important for host defense, as well as to develop immune tolerance, which decreases chances for abnormal immune hyperreactivity or inflammation. As a summary, the following are documented effects on innate and adaptive immunity that have been reported:

Effects on innate nonspecific immunity reported with probiotic use

- Promote mucin production
- Compete with and inhibit growth of potential pathogens
- Decrease gut permeability
- Enhance natural killer cell activity, macrophage activation, and phagocytosis

Effects on adaptive immunity reported with probiotic use

- Increase IgA-, IgG-, and IgM-secreting cells
- Increase total and specific secretory IgA in serum and intestinal lumen
- Modulate inflammatory gut immune responses

These effects are supported by a large and growing body of evidence from *in vitro* and animal studies, which are too extensive to cover in this review. More important, however, these effects are now being documented in human studies, in both adults and pediatric populations. Table 1 summarizes reported effects of the use of specific probiotic bacteria on specific immunoprotective host mechanisms. Table 2 summarizes probiotic effects on relevant immunologic markers reported in infants and children, some related to host defense, others to immunomodulation. Studies in adults support similar findings, with most of the probiotic bacteria used in pediatric populations. The effects on gut barrier and immune function discussed above support and explain the various clinical benefits observed with different probiotic bacteria.

Clinical Benefits Reported With Probiotic Bacteria in Pediatrics

A detailed review of the individual clinical trials that support the use of specific probiotics is beyond the scope of this paper. The readers are referred to recent excellent summaries of the available evidence for prevention or management of various conditions in this age group.[73,74] As an adjunct to this literature, Table 3 lists the individual clinical trials that have been reported and that form the main body of work for each of the more common clinical situations studied in pediatric patients: prevention and treatment of diarrheal disease, atopic disease, antibiotic-associated diarrhea, and NEC. A brief discussion of each follows. Clinical trials of probiotic use in inflammatory bowel disease and irritable bowel syndrome are in their vast majority limited to adults, with limited and inconsistent results.

Acute Diarrhea

By far, the best-studied clinical outcome with use of probiotic bacteria has been that of acute diarrheal disease. The larger number of trials documents therapeutic use of probiotics as supplements early in the course of the disease. The majority of studies have included various species of *Lactobacilli*, and by far the most used has been *L rhamnosus (GG)*. Four meta-analyses have recently been reviewed.[73] *L rhamnosus (GG)*, in particular, has shown efficacy when given as a supplement early in the course of rotaviral diarrhea. The most consistent effect reported is a reduction in duration of illness (by ½ to 1½ days). While for the individual infant the effect may be modest, the larger effect on the population may be significant. Cost-benefit analyses and compliance assessment have not been done.

Another body of literature examines the reduction in incidence (prevention) of acute diarrheal disease. Several studies, with various levels of significance, document a reduction in incidence or severity of acute diarrhea with *Bifidobacteria*, mainly *B lactis*,[75–78] and with *Lactobacilli*, mainly *L rhamnosus* (*GG*),[79,80] though protection is not always significant.[81] In addition, both *L rhamnosus* (*GG*) and *L reuteri* (during treatment)[82] and *B lactis* (used prophylactically)[75] have documented reduced rotaviral shedding. A recent meta-analysis reviewed 34 randomized, clinical trials that evaluated the efficacy of probiotics in the prevention of acute

Table 1
Probiotic clinical trials in infants and children: immune protective marker outcomes

Reference	Patient population	No. subjects	Probiotics used in intervention/dosage	Source	Protective immunity
Kaila, 1992[69]	Infants with rotaviral diarrhea	44	*L rhamnosus* (*GG*); 1×10^{10} to 1×10^{11} cfu, twice daily	Supplemented yogurt	↑ Total IgG, IgA, and IgM during acute phase ↑ Rotavirus-specific IgA after convalescence
Saavedra, 1994[75]	Healthy infants	55	*B lactis*; 1.9×10^{8} cfu/g formula *S thermophilus*; 0.14×10^{8} cfu/g formula	Supplemented formula	↓ Rotavirus shedding
Isolauri, 1995[68]	Healthy infants	46	*L casei* strain GG (LGG); 5×10^{10} cfu, twice daily	Powder supplement in water	↑ Rotavirus IgA seroconversion and rotavirus-specific IgM after oral vaccination
Fukushima, 1997[55]	Healthy infants	9	*B lactis*; 1×10^{9} cfu/d	Supplemented formula	↑ Fecal bifidobacteria ↓ Fecal clostridia ↓ Fecal ammonia and indole ↑ Acetic acid
Fukushima, 1998[31]	Healthy infants	7	*B lactis*; 1×10^{9} cfu/d	Supplemented formula	↑ Total fecal IgA and anti-poliovirus IgA after oral vaccination
Gupta, 2000[62]	Children with Crohn's	4	*L rhamnosus* (*GG*); 1×10^{10} cfu, twice daily	Tablets	↓ Intestinal permeability
Cukrowska, 2002[113]	Preterm infants	61	*E coli* Nissle 1917; 1×10^{8} cfu daily for 5 days, followed by 3 times weekly for 3 wks	Oral suspension applied to infants	↑ Anti-*E coli* Nissle 1917 IgA and total IgM
Kirjavainen, 2002[56]	Atopic infants	21	*B lactis*; 8×10^{10} cfu/kg body weight	Supplemented formula	↓ Fecal bacteroides and *E coli* ↑ Fecal *Lactobacilli/Enterococci* in highly sensitized group compared to less sensitized group
Mullie, 2004[33]	Healthy infants	30	*B breve, S thermophilus*; dosage not specified	Supplemented formula	↑ Antipolio fecal IgA after immunization
Rinne, 2005[66]	Healthy breastfed infants with risk of atopy	96	*L rhamnosus* (*GG*); 1×10^{10} cfu/d by mothers during last month of pregnancy, and same probiotic and dose to infants after the neonatal period	Powder supplement in water	↑ Total IgM, IgA, and IgG secreting cells
Viljanen, 2005[67]	Atopic infants	230	*L rhamnosus* (*GG*); 5×10^{9} cfu, twice daily OR mixture of: *L rhamnosus* (*GG*); 5×10^{9} cfu, *L rhamnosus*; 5×10^{9} cfu, *B breve*; 2×10^{8} cfu, and *P freudenreichii shermanii*; 2×10^{9} cfu, twice daily	Capsules in which contents were mixed with food	*L rhamnosus* (GG): ↑ Fecal IgA Mixture of probiotics: nonsignificant increase in fecal IgA
Bakker-Zierikzee, 2006[34]	Healthy infants	57	*B lactis*; 6×10^{9} cfu/100 mL formula or prebiotic	Supplemented formula	↑ Fecal sIgA in prebiotic group Nonsignificant increase in fecal sIgA in probiotic group

(continued)

Table 1
(Continued)

Reference	Patient population	No. subjects	Probiotics used in intervention/dosage	Source	Protective immunity
Kukkonen, 2006[114]	Healthy infants with risk of atopy	74	*L rhamnosus* (*GG*); 5×10^9 cfu *L rhamnosus*; 5×10^9 cfu *B breve*; 2×10^8 cfu *P freudenreichii shermanii*; 2×10^9 cfu, twice daily, by mothers during last month of pregnancy. Infants received same probiotics, once daily, after birth.	Capsules; contents added to sugar syrup for infants	↑ Number of infants with high anti-Hib IgG after vaccination Nonsignificant change in diphtheria and tetanus IgG titers
Mohan, 2006[59]	Preterm infants	69	*B lactis*; 1.6×10^9 cfu/d for 3 days, and 4.8×10^9 cfu for 18 days	Supplemented formula	↑ Fecal bifidobacteria ↓ Fecal enterobacteriaceae and clostridium
Rautava, 2006[32]	Healthy infants	72	*B lactis*; 1×10^{10} cfu/d *L rhamnosus* (*GG*); 1×10^{10} cfu/d	Supplemented formula	↑ Milk specific IgA secreting cells
Stratiki, 2007[60]	Preterm infants	41	*B lactis*; 2×10^7 cfu/g dry product	Supplemented formula	Improved intestinal permeability ↑ Bifidobacterial count

*Unless noted, results shown are those reported as significant in the individual studies.
cfu, colony-forming units; Hib, Haemophilus influenza type b; Ig, immunoglobulin; sIgA, surface immunoglobulin A.

diarrhea. Probiotics significantly reduced the risk of diarrhea developing in infants and children by 57% (confidence interval, 35%–71%). The protective effect did not significantly vary among the probiotic strains used, including *B lactis*, *L rhamnosus* (*GG*), *L acidophilus*, *S boulardii*, and other agents used alone or in combination with 2 or more strains.[83] These findings, in addition to reduced duration of hospitalization[84] and decreased hospitalization,[85] all suggest that the effect occurs on both the manifestations of the disease and on the course of the infection. No study to date has documented an increase (significant or not) in diarrheal disease with probiotic use. These observations greatly bolster the arguments for finding ways to use specific probiotics in a long-term and prophylactic manner, particularly in infancy.

Antibiotic-Associated Diarrhea

Several probiotic bacteria appear to be valuable in reducing the risk of antibiotic-associated diarrhea in infants and children.[86–91] Results from 6 randomized, controlled trials that collectively assessed 766 children for the efficacy of probiotics in the prevention of antibiotic-associated diarrhea indicated that concomitant treatment with probiotics, compared with placebo, reduced the risk of diarrhea from 28.5% to 11.9%.[92] Beneficial effects were strongest for *B lactis* and *S thermophilus* given in infant formula and *L rhamnosus (GG)* as a supplement. There are no randomized, controlled, clinical trials that specifically evaluate the effect of probiotics on *C difficile* diarrhea in infants or children.

Allergy

As mentioned above, microbial-gut interactions can improve the integrity of the gut barrier by decreasing intestinal permeability, reducing both adherence of potential antigens and their systemic effect, and by modulating GALT immune response toward antigen tolerance.[27,39,69,93] Lower counts of *Bifidobacteria* have been reported in atopic *vs* nonatopic children preceding allergen sensitization. *Bifidobacteria* are hypothesized to more effectively promote tolerance to nonbacterial antigens, primarily by inhibiting the development of a Th2-type (proallergic) response.

Infants with atopic dermatitis who received hydrolyzed whey formula supplemented with *L rhamnosus (GG)* showed greater clinical improvement than those who received the hydrolyzed formula alone. They also excreted less TNF-α and α-1-antitrypsin in their stool, suggesting that the probiotics decreased gut inflammation.[94] Atopic infants treated with extensively hydrolyzed whey-based formula with *L rhamnosus (GG)* or *B lactis* showed greater improvement in severity of skin manifestations than with hydrolysate formula alone. The probiotic-supplemented group also demonstrated a reduction in serum soluble CD4 (a marker of T-cell activation) and an increase in serum TGF-β1 (involved in suppressing the inflam-

Table 2
Probiotic clinical trials in infants and children: reported immune modulatory outcomes

Reference by first author	Patient population	No. subjects	Probiotics used in intervention/dosage	Source	Immune modulatory and anti-inflammatory markers
Majamaa, 1997[93]	Atopic infants	37	*L rhamnosus* (*GG*); 5×10^8 cfu/g formula	Supplemented formula	↓ Fecal α-1 antitrypsin ↓ Fecal TNF-α
Isolauri, 2000[26]	Breastfed atopic infants	27	*B lactis*; 1×10^9 cfu/g formula OR *L rhamnosus* (*GG*); 3×10^8 cfu/g formula	Supplemented formula	*B lactis*: ↓ Serum sCD4, IL-2, TGF-β1 and urinary eosinophil protein X *L rhamnosus* (*GG*): ↓ Serum sCD4 and urinary eosinophil protein X Nonsignificant increase in TGF-β1
Pessi, 2000[28]	Atopic infants	9	*L rhamnosus* (*GG*); 1×10^{10} cfu, twice daily	Supplemented formula	↑ IL-10 Nonsignificant change in TNF-α, IL-2, or IFN-γ
Arvola, 2002[115]	Atopic infants	56	*B lactis*; (dosage not specified) OR *L rhamnosus* (*GG*); (dosage not specified)	Supplemented formula	Both probiotics: ↓ Fecal α-1 antitrypsin ↓ Urinary eosinophil protein X No effect on gut permeability
Kirjavainen, 2002[56]	Atopic infants	21	*B lactis*; 8×10^{10} cfu/kg body weight	Supplemented formula	Positive correlation between serum IgE and *E coli* or *Bacteroides*
Rosenfeldt, 2003[116]	Atopic children	43	*L rhamnosus* (*GG*); 1×10^{10} cfu, twice daily *L reuteri*; 1×10^{10} cfu, twice daily	Supplemented in water	↓ Serum eosinophil cationic protein Nonsignificant change in IL-2, IL-4, IL-10 or IFN-γ
Pohjavuori, 2004[95]	Atopic infants	119	*L rhamnosus* (*GG*); 5×10^9 cfu, twice daily OR mixture of: *L rhamnosus* (*GG*); 5×10^9 cfu, *L rhamnosus*; 5×10^9 cfu, *B breve*; 2×10^8 cfu, and *P freudenreichii shermanii*; 2×10^9 cfu, twice daily	Capsule in which contents were mixed with food	*L rhamnosus* (*GG*): ↑ IFN-γ in cow's milk allergic and IgE-associated dermatitis group Mixture of probiotics: ↑ IL-4 in cow's milk allergic group
Viljanen, 2005[67]	Atopic infants	230	*L rhamnosus* (*GG*); 5×10^9 cfu, twice daily OR mixture of: *L rhamnosus* (*GG*); 5×10^9 cfu, *L rhamnosus*; 5×10^9 cfu, *B breve*; 2×10^8 cfu, and *P freudenreichii shermanii*; 2×10^9 cfu, twice daily	Capsules in which contents were mixed with food	*L rhamnosus* (*GG*): ↓ Fecal α-1 antitrypsin Mixture of probiotics: Nonsignificant ↓ Fecal α-1 antitrypsin
Fujii, 2006[117]	Preterm infants	19	*B breve*; 1×10^9 cfu, twice daily	Powder supplement in glucose solution, provided *via* nasogastric tube	↑ Serum TGF-β1
Rautava, 2006[32]	Healthy infants	72	*B lactis*; 1×10^{10} cfu/d *L rhamnosus* (*GG*); 1×10^{10} cfu/d	Supplemented formula	↑ Serum sCD14 cells

*Unless noted, results shown are those reported as significant in the individual studies.
cfu, colony-forming unit; IFN, interferon; IL, interleukin; TGF, transforming growth factor.

matory response *via* IgA production and oral tolerance induction).[26] Tables 1 and 3 summarize the studies done on treatment and management of atopy, as well as the inflammatory markers associated with these positive responses. These studies suggest that regular probiotic supplementation may stabilize intestinal barrier function and play a role in modulating allergic responses leading to a

Table 3
Probiotic clinical trials in infants and children: Reported clinical outcomes

Topic	Clinical benefit	Reference by first author
Diarrhea treatment	Decreased stool frequency	Szymanski, 2006[118] Canani, 2001[119] Gaon, 2003[120] Sarker, 2005[121]* Pashapour, 2006[122] Boudraa, 2001[123] Sudarmo, 2003[124] Kurugol, 2005[125] Lee, 2001[126] Kaila, 1992[69]
	Decreased duration	Rosenfeldt, 2002[127]† Sarker, 2005[121]* Gaon, 2003[120] Guandalini, 2000[85] Boudraa, 2001[123] Shamir, 2005[128] Kurugol, 2005[125] Sudarmo, 2005[124] Kaila, 1992[69] Lee, 2001[126]
	Reduced length of hospitalization or severity during hospitalization	Rosenfeldt, 2002[82]† Kurugol, 2005[125] Pashapour, 2006[122] Sudarmo, 2003[124] Guadalini, 2000[85]
Diarrhea prevention	Reduced incidence	Ziegler, 2003[76] Saavedra, 1994[75] Oberhelman, 1999[80] Sazawal, 2004[129] Weizman, 2005[77] Szajewska, 2001[130] Pedone, 2000[131] Hatakka, 2001[132]
	Reduced severity	Chouraqui, 2004[78] Weizman, 2005[77] Thibault, 2004[133] Saran, 2002[134] Ziegler, 2003[76]
Antibiotic-associated diarrhea	Reduction in incidence or severity	Arvola 1999[86] Correa, 2005[87] Jirapinyo, 2002[88] Kotowska, 2005[89] Tankanow, 1990[90] Vanderhoof, 1999[91]
Necrotizing enterocolitis	Reduced incidence and severity	Bin Nun, 2005[104] Hoyos, 1999[101] Lin, 2005[103]
Atopic dermatitis prevention	Reduced incidence	Kalliomaki, 2001[39] Kalliomaki, 2003[27]
Atopic dermatitis treatment	Reduced severity	Isolauri, 2000[26] Majamaa, 1997[93] Sistek, 2006[135]‡ Rosenfeldt, 2003[116] Rosenfeldt, 2004[136] Pohjavuori, 2004[95] Weston, 2005[137] Arvola, 2002[115] Kirjavainen, 2003[138] Viljanen, 2005b[139]
Allergic rhinitis	Reduced severity	Peng, 2005[140] Wang, 2004[141]

*Within nonrotavirus diarrhea group.
†Within early intervention group.
‡Within food-sensitized group.

decreased severity of atopic symptoms, particularly atopic dermatitis associated with cow's milk protein.[24,26,95]

In a recent study, a positive change in stool colonization in atopic infants supplemented with *B lactis* has been shown with a decrease of *Bacteroides* and *E coli* in the stool. Most interestingly, serum IgE correlated with *E coli* counts, and in highly sensitized infants, IgE correlated with *Bacteroides* counts. Thus, certain probiotics seem to influence the gut's allergen-stimulated inflammatory response and provide a barrier effect against antigens that might otherwise ultimately lead to systemic allergic symptoms (such as eczema).[56]

NEC

The newborn gut microflora foster integrity of the immune system, protect from infections with enteric pathogens, produce vitamins, and encourage mucosal maturation.[42,96,97] The premature infant is exposed to a variety of factors that negatively affect their possibilities of attaining an adequate or appropriate colonization. These factors include increasing exposure to potential delayed colonization, colonization with "neonatal intensive care unit (NICU) environmental microbes," use of antibiotics, lack of exposure to normal maternal flora and breast milk, invasive procedures, immature mucosa, and increased chances for bacterial and antigenic translocation. Cases of NEC cluster in time and place; germ-free animals do not get NEC, and changes in bacterial metabolic activity (hydrogen gas production) precede the development of NEC.[42] These observations strongly support the idea that microflora establishment and composition in premature infants is a major determinant in the pathophysiology of NEC.

The theoretical benefits of probiotics in preterm infants include reduction of enteric pathogens, improved gut structure and function, facilitation of enteral nutrition, reduced dependence on parenteral nutrition (PN), increased gut mucosal barrier function, reduction in sepsis and antibiotic use, and ultimately prevention of NEC.[42,44,98,99]

Mechanisms by which probiotics could prevent NEC include increase in favorable type microflora with reduced colonization by pathogens, increased intestinal barrier to translocation of bacteria into the bloodstream, modification of the host response to microbial products by sensitization and immunization, and enhanced tolerance and advancement of enteral nutrition.[44,98,100]

Initial studies are very encouraging. A recent clinical trial demonstrated a significant improvement in balance of gut flora with increased bifidobacterial counts associated with lower intestinal permeability in preterm infants.[60] A first retrospective study of orally administered *L acidophilus* and *B infantis* on the incidence of NEC in more than 12,000 premature infants compared with a similar number of historical controls showed a significant reduction in NEC incidence and NEC-associated mortality in the probiotic group.[101] In a prospective, double-blind study of 585 premature infants in 12 NICUs, the group supplemented with *L rhamnosus* (*GG*) was found to have a lower incidence of urinary tract infections and lower, but not statistically significant, incidence of NEC.[102] Two more recent trials have shown various degrees of reduction in relative risk of NEC with probiotics. A study in very-low-birth-weight (VLBW) infants using *L acidophilus* and *B infantis* given twice daily with breast milk significantly decreased NEC incidence compared with the incidence in infants given breast milk alone (2 or 180 *vs* 10 or 187).[103] A second trial of neonates receiving *B infantis*, *S thermophilus*, and *B bifidus* at 10^9 cfu/day *vs* no probiotic supplement[104] showed a NEC incidence reduction from 16.4% in the 73 control infants to 4% in the 72 supplemented infants and deaths occurring in the nonsupplemented group.

In summary, there is a clear clinical rationale for attempting to exert an effect on intestinal microflora in certain populations, particularly young infants and children. Specific clinical benefits are increasingly demonstrated for specific bacteria, which determine their probiotic capability. The protective and immune modulatory mechanisms that explain these effects are increasingly being documented. Figure 1 summarizes the mechanisms and clinical effects of probiotics in a general way, noting that each different probiotic bacterium may provide different benefits *via* different mechanisms.

Safety, Dose, and Regulatory Aspects of Probiotics Use

Most LAB strains used in the food supply are nonpathogenic, nonvirulent, and nontoxigenic microorganisms.[105–107] Many strains of *Lactobacilli* and *Bifidobacteria* are traditional food-grade organisms generally recognized as safe for use in the food supply. To date, there have been >70 clinical studies involving >4000 children and infants (both term and preterm) consuming infant formula or foods containing microbial ingredients, with no reports of adverse probiotic-related side effects. A Joint FAO/WHO Report on the Evaluation of Probiotics in Food stated that "documented correlations between systemic infections and probiotic consumption are few, and all occurred in patients with underlying medical conditions."[108]

Sporadic lactobacillemia from environmental, dietary, or fecal lactobacilli has been very rarely reported. Case reports of *L rhamnosus* (*GG*) infections possibly associated with probiotic consumption, in immunocompromised patients have been even less common.[109,110] The mechanisms for these infections and route of contamination are unclear. Newborn infants can develop infection from many species of resident microflora. However, despite the

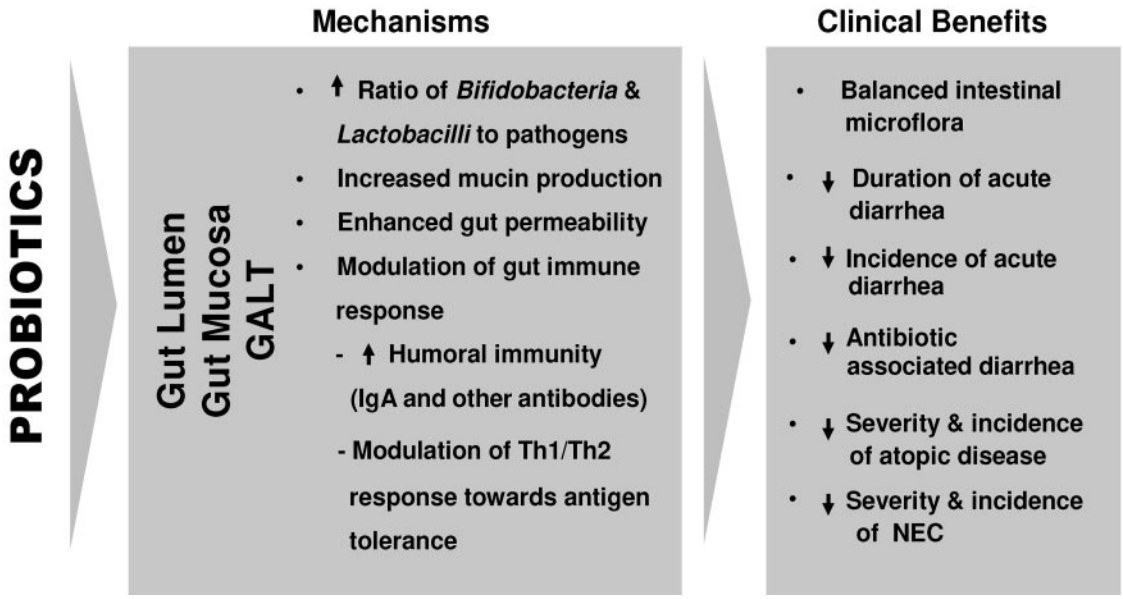

Figure 1. Summary of reported mechanisms and related clinical benefits of probiotics in pediatrics. Probiotics can act on the luminal microbial environment, the mucosal epithelium, and gut-associated lymphoid tissue (GALT). The physiologic effects reported with specific probiotics on gut barrier and immune function explain the various clinical benefits reported in pediatric populations. Individual microbes have specific effects and benefits.
NEC, necrotizing enterocolitis.

fact that *Bifidobacteria* are the most abundant in newborns (particularly in breastfed infants), no clear pathogenicity of resident *Bifidobacteria* has been reported. *Bifidobacteria* are also present in many foods, particularly yogurts, including yogurts often used in infant weaning. Hundreds of thousands of metric tons of dairy products containing *Bifidobacteria* are consumed worldwide annually, increasingly by weaning infants. As opposed to the rare reported episodes of lactobacillemia (some associated to ingested *Lactobacilli*), bifidobacteremia has not been sporadically reported, whether associated with consumption of commercial products containing *Bifidobacteria* or not. *Bifidobacteria* have also been consumed in infant formulas for >15 years worldwide and have not been associated with any pathologic or adverse event.

In particular, studies have documented safety and adequate growth with *B lactis* in infants from birth[77] and in vulnerable populations, including preterm infants,[59,60] malnourished infants,[111] and infants born to mothers with HIV disease.[112] From the safety point of view, according to current available information, *Bifidobacteria*, particularly *B lactis*, has a uniquely strong safety profile, making it a good probiotic candidate for newborns and young infants. *Lactobacilli*, particularly *L rhamnosus (GG)*, also seems generally safe and may be a probiotic appropriate for older infants and children. Until adequate data are available for each specific probiotic bacterium, use of probiotics in general cannot be recommended in immunocompromised populations. However, as safety is better documented for specific bacteria, we may be able to use them in certain populations (such as premature infants) that may stand to benefit the most from probiotic use.

Although "dosing" studies have not been done, doses vary greatly between trials, and viability in many clinical trials is not always well documented; no studies reporting efficacy for several outcomes have been done with $<10^7$ to 10^{10} colony forming units (cfu) per "serving" or per dose. Total daily intake (depending on the method and vehicle used to deliver the probiotic) fluctuates in most of these studies between 10^8 and 10^{10} cfu/day.

It is also important to recognize that "overdosing" with probiotic bacteria is not only difficult but improbable if not impossible. Most products with viable organisms that have been studied contain 10^7 to 10^{10} cfu per serving, assuming there is good quality control of viability through their shelf life. Bacterial counts in the distal gut can fluctuate up to 10^{12} cfu per mL of luminal content. So, for example, if a product contains 10^9 cfu per serving, 100 servings would need to be consumed together to begin approaching bacterial concentrations in the distal gut lumen. It is likely that a more profound change in gut ecology and immune response to probiotics is occurring at the proximal bowel level, particularly the small bowel (which in "modern society" regularly "sees" sterile food), than in the distal gut, which is significantly more exposed to bacterial stimuli.

Last, there is the issue of mode of delivery. It is reasonable that if a probiotic is to be used therapeutically, it could be reasonably provided as a "dose" in a tablet or capsule form. However, when it comes to pediatric use, where preventive approaches are always preferred (such as for allergy, antibiotic-

associated diarrhea, or acute viral diarrhea), the long-term delivery of these agents is more practical if select probiotic bacteria are incorporated into the diet, such as in children's yogurts or beverages, infant formulas, or weaning foods. This approach can also facilitate compliance and decrease costs compared with a daily "supplement."

Regulatory oversight in general has not been adequate, particularly for probiotic products commercialized as supplements. In North America, specification of strain and amounts has been better documented for some probiotic supplements like *L rhamnosus (GG)* or for *L casei* and *L reuterii* in beverages or formulas; however, oversight varies greatly from country to country. While infant formulas and foods containing probiotic bacteria are widely available worldwide, *B lactis* is the only probiotic bacterium that has undergone FDA evaluation for use in infant formulas from birth and can be commercialized for this application. Much work still remains to better regulate the use and commercialization of probiotic products in general, and for pediatric populations in particular.

Conclusion

The concept of probiotics has greatly evolved in a relatively short time from a concept to documentation of specific organisms that have adequate documentation of safety and that, in specific amounts in specific pediatric populations, yield specific benefits. Although there is still much to be learned, our increasing appreciation of the mechanisms that underlie our interaction with our microbial environment also supports these benefits. These mechanisms, particularly those associated with the immune response of the host, operate early in life, with potential long-term consequences. The most effective application of probiotic microbes may lie in the appropriate and safe incorporation of these organisms into the diet as functional components for disease prevention and general health maintenance of infants and children.

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