Probiotics: Implications for Pediatric Health
PREFACE

It is no longer questioned that the interplay between our genes, nutrition, and our environment hold the keys to growth, development, and health. The foods that we use to nourish ourselves and our offspring come from our environment, and these not only provide nutrients for development, maintenance and repair of tissues and organs, but are now recognized to have functional components that directly affect organ function.

A major component of this interplay between our environment, the foods we consume and ourselves is the enormous biomass constituted by the microbes present in our surroundings. As with all living things around us, these relationships can be positive or can be detrimental. In our quest to rid ourselves from pathogens, during the short period of time of human existence, we have drastically changed our interaction with the microbial world. We’ve had some major victories over malnutrition, infection and many chronic diseases. However, the increasingly abundant and increasingly “sterile” food supply and environment appear to be at least in part responsible for the increase in other chronic diseases like obesity, and immune-related disorders, including asthma, allergies, arthritic disease, diabetes and inflammatory bowel diseases. Slowly, but increasingly, we are unraveling the fine mechanisms and interactions between our immune system and our microbial environment.

In no other place is this “crosstalk” between our immune system and microbes greater than in the gut lumen and gut mucosal surfaces. The gut, as a consequence, is our largest immune organ, and our intestinal microbiota (flora) the largest microbial ecological system with which we interact. This biomass is determined by many factors, and influenced by our early exposure to microbes, the way we are born, what we are fed in the first days of life, and what foods we eat. The nature of this interaction is clearly a factor in the “new and changed” immune response of “Western lifestyle” associated with the susceptibility to new pathogens and increase in chronic immune-related illnesses.

The idea that rather than completely avoiding microorganisms, or unsuccessfully attempting to completely sterilize our world, a healthy future likely involves the use of select bacteria to create a favorable interaction — one that may result in improved health for the human host. This has led to the concept of probiotics. This document is an attempt to summarize the explosive body of literature and knowledge on this topic, and the implications that they may already have today in the management of nutritional and overall human health, particularly that of our infants and children.
SUMMARY

The gastrointestinal tract is the primary and largest interface between the human body and its environment. As such, it is both the largest immune organ in the body and home to a complex microbial ecosystem. The intestinal microflora are a key factor influencing the health and wellbeing of the human host, particularly through modulation of its immune response. This microbial ecosystem is unique to each individual, changing throughout a person’s lifetime in response to modifications in health, diet and the environment.

Probiotics are defined as nonpathogenic organisms in the food supply that are capable of conferring a health benefit to the host by modifying gut microbial ecology. Lactic acid producing bacteria, including Bifidobacteria and Lactobacilli, are the most widely used probiotic organisms and hence some of the most widely studied. A growing body of literature reports various specific benefits and potential clinical applications for probiotics, and their use for digestive health and general wellbeing. More recently, their effect on various gut-associated immune mechanisms have become a focus of investigation, and these are helping to explain and further demonstrate these clinical observations. Research indicates that probiotics exert their effects both locally (by promoting the gut’s barrier function) and systemically (by modulating the immune system). The best-demonstrated potential clinical benefits of probiotic agents, specifically in the pediatric population, are in the management and prevention of acute diarrhea, antibiotic associated diarrhea, and evidence is mounting on their potential clinical benefits in atopic disease, inflammatory bowel conditions, and necrotizing enterocolitis. However, not all probiotic agents behave similarly, nor do they have similar documentation of safety and efficacy.

Bifidobacteria are nonpathogenic microbes uniquely appropriate for use in infants and children as probiotic agents. The intestinal flora of breastfed infants is dominated by Bifidobacteria, and this particular profile of intestinal microflora may be associated with some of the health benefits breast-feeding confers. Supplementation with Bifidobacteria has been increasingly studied in infants and children. Extensive use in clinical trials in infant feeding documents safety and increasingly, research indicates that incorporation of several of these microorganisms including Bifidobacterium lactis in the feeding of infants can exert a probiotic effect.
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1 INTRODUCTION

The mucosa of the gastrointestinal (GI) tract constitutes the largest area of contact between the human body and its environment. Via the intestinal mucosa, the host interacts with a large population of microorganisms present in the intestinal lumen (gut microbiota): both intestinal floral (resident bacteria) as well as all microorganisms ingested by the host. Since the GI tract functions as a barrier as well as a surface of interaction with the environment, it is not surprising that it contains the largest amount of lymphoid tissue in the body. Approximately 80% of all immunoglobulin-producing cells are also contained in the GI tract, making the gut associated lymphoid tissue (GALT) the major immune organ in the host.\(^1\,\!^2\)

The growing body of scientific evidence continues to elucidate the potential benefits of consumption of live microorganisms generally referred to as “probiotics”. By modifying the composition of the intestinal flora, and improving its “microbial balance”, probiotics confer a benefit to the host, particularly via modulation of GALT immune responses. Probiotics are thus generally defined as those microorganisms in the food supply that are nonpathogenic and capable of conferring a health benefit to the host by various mechanisms. “Pro-biotics” literally means “for life!” Common probiotic bacteria include species from the genera *Lactobacilli* and *Bifidobacteria*, the latter of which composes the predominant colonic flora of the breastfed infant. Although some yeasts (such as saccharomyces) have been described as having probiotic benefits, this document will focus on probiotic bacteria.

It is important to note that only strains of nonpathogenic bacteria that are shown to have a positive effect on the host can be considered probiotic agents. Not all non-pathogens are probiotics, nor do all probiotic strains have the same type of effect, or mechanism of action on the host. When referring to probiotic benefits, the specific microorganism and its particular effects need to be specified.

“Prebiotics” are generally defined as dietary substances that promote the growth of beneficial gut flora, particularly *Lactobacilli* and *Bifidobacteria* and may thus indirectly support the probiotic effects of these organisms. Breastmilk contains a number of components, including prebiotics such as milk oligosaccharides. These, as well as other types of oligosaccharides found in the diet of older children and adults, can favor the growth of *Bifidobacteria* and *Lactobacilli*. Sybiotics are various combinations of prebiotics and probiotics. An example of this would be a product that combines a live, active probiotic strain with oligosaccharides, shown to favor the growth or function of the specific probiotic bacteria.

This monograph reviews the relationship between the GI tract, its microflora, immunity and the effects of probiotics. The intent of this monograph is to assist healthcare professionals in understanding the concept of probiotics, and the implications they may have in daily practice, with a focus on infant and child health. This monograph is purely educational in purpose and is not intended as medical advice, nor is it intended to recommend any specific application or product.
2 THE INTESTINAL MICROFLORA AND HEALTH

Key points

- The intestinal microflora, a complex ecosystem comprising over 500 species of microorganisms, is vital to gut structure and function and is essential for human health.

- Nutritionally, the functions of the microflora include synthesis of micronutrients, fermentation of otherwise indigestible substances and metabolism of proteins, peptides, and bile acids.

- Bacterial colonization begins shortly after birth. In breastfed infants, *Bifidobacteria* species dominate in the GI tract.

- The composition of intestinal microflora changes with age and can be modified by diet, use of antibiotics, and environmental changes.

- Maintaining the natural bacterial equilibrium of the GI tract helps to minimize the presence of potentially pathogenic microorganisms and limit their negative effects.

- Alterations in intestinal microflora may predispose infants and children to some disease states including: allergy, inflammatory bowel disease and necrotizing enterocolitis.

- A balanced intestinal flora helps support a balanced immunologic response in the host by several mechanisms. These include an enhancement of humoral immunity (such as increased IgA secretion for protection from pathogens) and modulation of the T cell response (by decreasing the proinflammatory responses which can lead to allergic and inflammatory conditions).

2.1 Intestinal microflora and immunologic equilibrium in health and disease

The intestinal microflora is a complex ecosystem of utmost importance to the health and well being of its host. The differences between conventional and germ-free animals indicate the importance of intestinal bacterial colonization. For example, gnotobiotic animals (germ-free animals, born with sterile GI tracts) suffer from persistent enteritis, colitis, severe infections, and exhibit poor survival rates.

Among other functions, intestinal flora are responsible for the production of some micronutrients, such as vitamins, and for the process of fermentation (the metabolism of substrates, such as carbohydrates and dietary fibers not digested by human enzymes). Fermentation results in the production of short-chain fatty acids, predominantly acetate, propionate and butyrate, which are known to be trophic to the GI tract and can even provide small amounts of energy to the host. In addition, the gut microflora are responsible for the metabolism of some proteins and peptides and the transformation of sterols and bile acids. However, as will
be discussed below, the major effects of the intestinal microflora are mediated via their interaction with gut immune mechanisms.

The intestinal microflora includes both aerobic and anaerobic organisms. Approximately 500 different bacterial species exist in the adult colonic flora, totaling about $10^{12}$ prokariotic (bacterial) cells/mL intestinal contents – 10 times the total number of eukariotic (human) cells in the body. However, the vast majority of an individual’s microflora (99%) is made up of only 30 to 40 different species.\(^{(4)}\)

*In utero*, the GI tract is sterile. Shortly after birth, bacterial colonization of the human intestine begins. During the first 2 days of life an infant’s intestinal tract is rapidly colonized with bacteria consisting mainly of *Enterobacteria*. Diet also influences the development of the intestinal microflora. In breastfed infants the *Bifidobacteria* counts increase dramatically; 80% to 90% of the total flora belong to the *Bifidobacterium* species. Numbers of *Lactobacilli* and *Bacteroides* increase to a lesser extent, and *Enterobacteria* decrease.\(^{(5)}\)

![Figure 1. Bacterial flora in the stool of breastfed and formula-fed infants during the first days of life.](image)

Thus in breastfed infants the flora is predominantly *Bifidobacteria, Lactobacilli* and some *Staphylococci*. On the other hand, formula-fed babies of 1 to 4 months of age have a flora that is more complex, consisting mostly of coliforms and *Bacteroides* (Figure 1). With the introduction of new foods, the microflora begins to resemble that of adults. While the composition of the microflora varies among individuals, the composition within each individual remains stable over prolonged periods.\(^{(3,6)}\)
The colonization of the different segments of the GI tract also varies, both qualitatively and quantitatively (Figure 2). The mouth has a complex microflora consisting of facultative and strict anaerobes, including *Streptococci*, *Bacteroides*, *Lactobacilli* and yeasts. The microflora in the stomach consists predominantly of gram-positive aerobes at a relatively low concentration of less than $10^3$ colony-forming units/mL (CFU/mL). The small intestine is a transition between the barely colonized stomach and the rich microflora of the colon, with a bacterial concentration between $10^3$ and $10^4$ CFU/mL in the proximal intestine, increasing distally towards the colon. Past the ileocecal valve, the concentration of bacteria increases dramatically, and becomes even higher in the colon ($10^6 - 10^{12}$ CFU/mL). In reality, almost one third of the dry weight of fecal matter is bacterial cells. Cumulatively, it is estimated that bacteria account for 35% to 50% of the volume of human colonic contents.\(^{3,4,6,7}\)

**Figure 2. Microflora of the human GI tract.**

Maintaining the natural bacterial equilibrium within the GI tract is of significant importance, ideally keeping the presence of potentially pathogenic microorganisms to a minimum and achieving a predominance of nonpathogenic and desirable organisms. The balance between the components of the gut ecosystem is maintained by several control mechanisms:

- Gastric and biliary secretions create an environment inhospitable to the majority of pathogens ingested.
- Intestinal motility prevents stasis and bacterial overgrowth.
• Competition for nutrients by different bacterial species.
• Microbial-epithelial interactions (including competition for receptors and epithelial permeability factors).
• The host's immune system (including mucin and secretory IgA production).

In addition, several external factors can modify the intestinal microflora, such as diet, age, use of antibiotics or other medications, certain disease states, and environmental changes.

The GI tract mucosa and the gut associated lymphoid tissue (GALT) form an integrated protective gut barrier, which handles both luminal microorganisms and food antigens. The integrity of the mucosal barrier depends on a number of factors, including nutrition of the gut itself. However, it has been recently recognized that the gut flora are a major constituent of that barrier via its immunomodulation of the GALT.

Clinical and experimental studies suggest that the balance between nonpathogenic and potentially hostile GI bacteria is altered in a number of diseases and conditions affecting infants and young children. Deviations in intestinal microflora balance may predispose the infant to some disease states. Distinct patterns of neonatal gut microflora, such as elevated Clostridia and a reduced or atypical composition of Bifidobacteria, have been associated with subsequent development of atopic disease in infancy. Increased fecal Bacteroides and lower Bifidobacteria counts have been more often identified in infants presenting with atopic eczema than in healthy controls. Aberrations in microflora among patients with inflammatory bowel disease have been identified and implicated in its disease pathophysiology. Lactobacillus and Bifidobacteria counts are significantly reduced in feces of patients with Crohn's disease and relative ratios of Bifidobacteria to Bacteroides species appear to be associated with the severity of ulcerative colitis. Intestinal bacteria have also been implicated in the pathogenesis of necrotizing enterocolitis (NEC) and are essentially causative for conditions such as antibiotic associated diarrhea and diarrhea due to C. difficile. The gut microflora, through its effect on modulating both local and systemic immunologic and inflammatory responses, are thought to play a role in the gastrointestinal and overall health of the host. Changes in the balance of microflora at the onset of disease and during its course provide a rationale for interventions with probiotics in pediatric populations.

2.2 Gut flora and the immune system

The immune system is a complex network of specialized cells and organs responsible for host defense. Immune responses can be classified as innate or acquired (also called “adaptive”).

The innate branch of the immune system, what a human is born with, are basic immunologic defense mechanisms that do not require prior exposure to an antigen to mount a response and do not have “immunologic” memory. The innate immune system includes physical barriers such as the skin, as well as the mucosal membranes of the respiratory and GI tracts. The mucus layer of the intestinal mucosa is a mechanical line of defense against pathogens. Specific cells in the circulation and tissues, such as phagocytes
and natural killer cells, are also part of innate immunity, and provide the early phases of defense, in a non-specific way, while the responses of the adaptive immune system are set in motion.

The adaptive branch of the immune system, on the other hand, develops over time, requires exposure to specific antigens, is specific to these antigens, and has immunologic memory. Here, the immune system responds to antigens (such as food or bacterial proteins), and with repeated exposure to a particular antigen, the adaptive immune system becomes more efficient. Lymphocytes (both B and T cells) are key players in the adaptive immune system. B cells and antibodies are key components of the humoral immune response. T cells are a key component of the cellular immune response. T cells are classified according to the presence of membrane glycoproteins and their responses. One particular subset of T cells expressing the CD4 glycoprotein is referred to as T helper cells (Th).

T helper cells can be further categorized into a number of Th types: Th1, Th2 and TReg (regulatory), depending on the profile of cytokines (chemicals which attract other immune cells) they produce. Th1 cells promote a proinflammatory environment by releasing interleukin 2 (IL-2) and gamma interferon, which encourage T cell proliferation and macrophage activation, which are important in the inflammatory defense mechanisms of the host. However, when over expressed, it can lead to delayed-type hypersensitivity or inflammatory bowel disease. In contrast, Th2 cells promote a different type of reaction, releasing IL-4, IL-5 and IL-10, which among other things, promote eosinophil recruitment. Their over expression can result in an allergic response. TReg cells possess characteristics of both the Th1 and Th2 responses, and release cytokines such as TGF-β which can inhibit Th1 or Th2 over expression, playing a role in the development of tolerance to bacterial or food antigens.

The balance between these T lymphocyte and cytokine pathways thus plays a role in modulating the immune response between proinflammatory and tolerance promoting mechanisms. Food and bacterial antigens present in the gut lumen are key in activating the differentiation of T cells towards one pathway or another, as well as inducing generations of antibodies, particularly secretory IgA.
IBD = Inflammatory Bowel Disease, Th = T lymphocytes, Th0 (undifferentiated), TReg (Regulatory or Th3). Aside from enhancing secretory immune response, particularly IgA, food antigens and intestinal flora play a major role in T cell differentiation, and immuno-modulation. Activation of T cells and differentiation to Th1 and Th2 cells lead to secretion of different cytokines, responsible both for defense of the host as well as appropriate inflammatory responses. However, the over expression of either of these cell subsets can result in undesirable clinical conditions (allergy or Inflammatory bowel disease). Specific probiotic bacteria have been shown to enhance mucosal barrier, increasing expression of mucins, and limiting sensitization by antigens. Evidence also points to the fact they can inhibit proliferation of T cells, reducing Th1 and Th2 cytokines, while inducing development of T Regulatory cells which lead to anti-inflammatory cytokine production such as TGF. By this and other mechanisms, gut flora can exert an immuno-modulatory activity, balancing the T cell responses particularly in infancy, when immune regulatory aberrances can predispose to clinical disease such as atopy.

The production of antibodies to specific antigens is another important component of the adaptive branch of the immune system. B cells are responsible for antigen recognition and antibody production. One of the five functional classes of immunoglobulins produced by these cells is immunoglobulin A or IgA. The main function of IgA, when secreted into the gut lumen, is to protect from potential pathogens at the site of mucosal surfaces. Intestinal flora plays a major role in the maturation of immunoglobulin secretion. Establishment of a balanced intestinal flora can play a significant role in supporting both innate and adaptive immune responses. For example, microbes stimulate intestinal mucosa to secrete mucus and its mucins, which are a first line of defense against pathogens. They also stimulate maturation of IgA and IgM secretion. (17-19)
Certain bacteria may enhance secretory IgA function. On the other hand, specific gut microbes may exert an immunosuppressive function. By inhibiting proliferation of T cells producing both Th1 and Th2 cytokines, the inflammatory response to antigens is suppressed. In this way, the gut microflora can exert an immunomodulatory effect, protecting the host from infection while decreasing over expression of inflammatory GALT cells that can lead to conditions such as allergy or inflammatory bowel disease.\(^\text{20}\)

The “hygiene hypothesis” is based on the concept that lack of exposure to bacterial and other antigens in early childhood in industrialized societies is responsible for the growing epidemic of asthma, allergies, and other immune diseases. Atopic disease can result from an exaggerated Th2 type response to allergens and this leads to an increase in IgE production, which is a mediator of various allergic manifestations. On the other hand, Th1 activity inhibits IgE production.

Infants are born with a predominant Th2 type response, and at birth, their immune system is exposed to various allergens which further induce this pathway. However, exposure of the intestinal mucosa to microbes redirects immune T cell activity towards a Th1, away from a Th2 allergenic response, and balancing T cell responses decreasing the chances for developing allergy or other immune-related diseases. This mechanism supports the concept that the immune system requires exposure to a “balanced” intestinal flora and environmental microbes for adequate maturation and modulation of the immune response and is thought to partially explain the hygiene hypothesis.\(^\text{21-24}\)

While this hypothesis remains to be fully understood, it provides an additional basis for the introduction of bacterial antigens to the food supply – the concept of probiotics.

![Figure 4. Balance of T cell responses.](image)

In newborns, a Th2 response predominates, and gut permeability is high. Persistence of Th2 responses favor development of allergy in infancy. Factors favoring the Th1 response balance protective immunity and an allergic expression.
3 PROBIOTICS

Key points:
• Lactic acid producing bacteria (LAB) (such as Bifidobacteria and Lactobacilli) are the microorganisms widely used in food. Food and beverages containing LAB, including most fermented foods, constitute up to 40% of the global food supply.

• LAB, used in the food supply, are considered nonpathogenic, non-virulent, and non-toxic. Bifidobacteria in particular has not been associated with human disease or infection.

• Many strains of Lactobacilli and Bifidobacteria are traditional food-grade organisms generally recognized as safe (GRAS) and have a history of safe food use going back thousands of years.

• Probiotics are live organisms that, when ingested, are capable of conferring a health benefit to the host.

• A number of lactic acid producing bacteria have been shown to benefit the host’s health, and thus can be considered probiotics.

• Research with humans and animals indicates that probiotics may exert a clinical benefit, mainly via their effects on the immune system.

• Probiotics exert an effect on immunity in several ways including: locally (by maintaining the gut barrier function) and systemically (by modulating the gut immune response).

• Studies in infants, children, and adults have confirmed that administration of Bifidobacteria used as a probiotic is safe and well tolerated.

3.1 Probiotics: basic concept and definition

The definition of probiotics has evolved, as knowledge in this area has increased. Fuller proposed that probiotics are a “live microbial feed supplement that beneficially affects the host animal by improving its intestinal microbial balance.” Naidu suggested that a probiotic be defined as “a microbial dietary adjuvant that beneficially affects the host physiology, by modulating mucosal and systemic immunity, as well as improving nutritional and microbial balance in the intestinal tract.” In a report on health and nutritional properties of probiotics in food prepared jointly by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO), probiotics were referred to as “live microorganisms which, when consumed in adequate amounts as part of food, confer a health benefit on the host.” Saavedra proposed probiotics as a concept which “constitutes a purposeful attempt to modify the relation with our immediate microbial environment in ways that may benefit our health.”
Although the qualities identifying a probiotic continue to evolve, it is generally accepted that the basic characteristics include that it:
• Is a microbial organism.
• Remains viable and stable during use and storage prior to consumption.
• Is able to induce a host response as part of the intestinal ecosystem.
• Is beneficial to the host when consumed.

Additional criteria suggested by other authors include that the probiotic should be of natural or human origin and that it adheres to or colonizes the intestinal mucosa, at least temporarily.28,29

The consumption of nonpathogenic lactic acid-producing bacteria (LAB) has been part of dietary culture for centuries, mostly in the form of yogurt and other fermented milk products. Fermented milk products are a common component of diets in Asia, Europe and parts of Africa, and increasingly in North America. In early days, and until now, fermentation was an excellent way to preserve foods, particularly milk and cereals. The consumption of these products, particularly fermented milk (as yogurt or kefir) has significantly increased over the last decade. This is in part due to their increasing availability, as well as to the popular view of yogurt and other fermented products as “healthy foods”. Today, foods and beverages containing LAB constitute up to 40% of the food supply worldwide.30 Overall, a number of LAB, particularly members of the genera Lactobacilli, Bifidobacteria, and Streptococcus are the most widely used in the food supply. Research involving specific strains of these bacterial species also forms the vast majority of probiotic literature. However, it is important to note that any LAB used in food for fermentation, or other purposes does not indicate nor constitute a probiotic. A nonpathogenic bacteria is not considered to be a probiotic until it has been demonstrated to be of clinical benefit to the host.

Since the early published reports on probiotics, approximately 40 years ago, the scientific interest and literature have grown, with most recent years contributing approximately 600 scientific publications per year (Figure 5), and a similar trend in clinical trials. Amongst the more frequently studied probiotic agents are Lactobacillus species including: L. acidophilus, L. bulgaricus, L. johnsonii, L. reuteri, and L. rhamnosus. Lactobacillus GG (L. paracasei subspecies rhamnosus) is the better studied probiotic in humans. Throughout this document it is referred to as L. rhamnosus. Amongst Bifidobacteria, the better studied probiotic agents are B. breve, B. infantis, B. lactis, and B. longum. Over time, due to changes in nomenclature and taxonomy, B. animalis subspecies lactis has also been called B. bifidum, B. animalis or Bifidobacterium strain Bb12. In this document, it is referred to as B. lactis, another well studied probiotic, particularly in infants.
3.2 Effects on gut function and modification of gut microbial balance

The scientific interest in bacteria, as agents with the potential to support health, began with an observation by Metchnikoff. In 1907, he documented his observation of the long lives and good health of Bulgarian peasants and the potential association with their consumption of large amounts of milk soured with LAB. Subsequently, Tissier noted *Bifidobacteria* in the feces of breastfed babies and proposed a relationship between the presence of these bacteria and the fact that breastfed babies rarely experienced diarrhea. He hypothesized that the oral ingestion of *Bifidobacteria* could displace the bacteria responsible for diarrhea.

Modification of intestinal flora with probiotics

The infant microflora are susceptible to modification; however, colonization, even on a transient basis, must occur before health benefits of probiotics can be realized. Not all strains of probiotics are stable in acid and bile, or are able to adhere to the human epithelial cells necessary for their growth. However, a number of reports have indicated that various strains of *Bifidobacteria* and *Lactobacilli*, given orally, are successful in transiently colonizing in the gut of infants and young children. Langhendries and colleagues...
compared infant fecal flora of 1 month old infants who were breastfed to those fed a standard formula and those fed formula supplemented with *L. helveticus*, *Streptococcus thermophilus* (*S. thermophilus*), and *B. bifidum* (10⁴ CFU/g). After 1 month of feeding, those receiving probiotic-supplemented formula had *Bifidobacteria* stool counts similar to the breastfed babies, and significantly higher colonization than babies receiving standard formula. The formation of a *Bifidobacteria* predominant flora, similar to that found in breastfed infants, has been reported in other trials with *B. lactis* probiotics. Significant increases in fecal *Bifidobacteria* with *B. lactis* supplementation have been reported as early as 1 week after supplementation.

Other recent studies have confirmed that LAB probiotics can transiently colonize even the most vulnerable infants. *L. casei* was cultured from feces after supplementation to critically ill children and *L. rhamnosus* strains successfully colonized 80% of infants and children with infectious diarrhea after 5 days of treatment. A combination of *Bifidobacteria* strains provided to critically ill, preterm infants resulted in significantly earlier colonization of *Bifidobacteria* than controls. Similarly, *Bifidobacteria breve*, when provided several hours after birth to very low birth weight infants, was successful in promoting the colonization of *Bifidobacterium* and the formation of a normal intestinal flora. Preterm infants receiving formula with *Bifidobacteria* during the first month of life had similar bifidobacterial counts as age-matched breastfed infants. Finally, temporary colonization of an orally ingested probiotic mixture of *L. acidophilus*, *B. longum* (10⁷ CFU/g) and *B. breve* (10⁷ CFU/g) was confirmed when cultured from the stools of newborns receiving a variety of antibiotics.

As presented above, many species of *Lactobacilli* and *Bifidobacteria* have shown efficacy in transiently colonizing the infant, and in some cases with a resultant microflora similar to that of breastfed infants. It is clear, however, that once the intestinal flora of an infant is well established, the appearance and colonization in the intestine by probiotic bacteria is only temporary. In most well-documented cases, once the probiotic strain is discontinued from the diet, its numbers gradually disappear, with a return to the profile of organisms present prior to supplementation in the diet. This suggests that for probiotics to adequately stimulate GI immune function, continued ingestion is needed.

**Probiotic effects on immune function**

The specific quantities and ratios of comensal bacteria within the intestinal microflora that most positively influence the health and development of infants have yet to be determined. However, there is increasing evidence that resident *Lactobacilli* and *Bifidobacteria* can exert antimicrobial activities influencing both local and systemic immunity. 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 “normal” resident intestinal microflora or commensal bacteria may offer resistance to colonization by pathogens and thus function as an important constituent of the gut defense barrier. The presence of “healthy” microflora, such as *Bifidobacteria* and *Lactobacilli*, have been associated with the secretion of substrates that have antimicrobial properties and the expression of mRNA for glycoproteins (mucins MUC2 and MUC3) known to inhibit the adherence of pathogenic bacteria. As mentioned above, alterations in intestinal microflora may predispose infants and children to some disease states including: allergy, inflammatory bowel disease and necrotizing enterocolitis.

Some *Bifidobacteria* and *Lactobacilli* given orally may enhance the production of a balanced T helper cell response and stimulate production of IL-10 and transforming growth factor-β, both of which have a role in the development of allergic type immune responses. As mentioned above, this “healthy” microbial balance will favor a Th1 response. In newborns, where a Th2 response predominates and gut permeability is high, intestinal flora may decrease their propensity for development of atopic disease.

Of particular interest is the relationship between secretory immune function and intestinal flora. Secretory immunoglobulin A (sIgA), the most predominant immunoglobulin in mucosal surfaces, coats and protects mucosal surfaces against potential pathogens and neutralizes toxins and virulence factors from microbial pathogens. 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. Breastmilk contains significant levels of sIgA that are transferred to the infant, but in addition, *Bifidobacteria*, which predominates in breastfed infants, has been shown to stimulate the synthesis and secretion of IgA. On the other hand, during the neonatal period, sIgA in feces of formula-fed infants is essentially undetectable. Finally, as will be detailed below, *Bifidobacteria* and *Lactobacillus* given orally have been shown to influence sIgA in a number of animal trials and studies with infants that have investigated the effects of *B. lactis* supplementation on stimulating the mucosal immune response have reported positive and encouraging results.

In summary, the association of specific types of bacterial species (particularly *Lactobacilli* and *Bifidobacteria*), with maintenance of important gut barrier and immune mechanisms and the demonstration that microbial ecology can be modified by the consumption of these species, provides a basis to explain the benefits of probiotics, which will be discussed below.

**Effects documented for probiotics on gut barrier function and immune response**

*Innate immunity*

- Promotes mucin production
- Competes with, and inhibits growth of potential pathogens
• Decreases gut permeability
• Enhances natural killer cell activity, macrophage activation and phagocytosis

Adaptive immunity
• Increases IgA, IgG and IgM secreting cells
• Increases total and specific secretory IgA in serum and intestinal lumen
• Modulates Th1 and Th2 GALT responses

Mechanisms and benefits reported for probiotics

Mechanisms
• Increased ratio of Bifidobacteria & Lactobacilli to pathogens
• Maintain gut barrier function
• ↑ Mucin production
• ↓ Permeability
• Modulate gut immune response
  - ↑ Humoral immunity (secretory IgA production)
  - ↑ Cellular immunity
  - Modulate Th1/Th2 response

Clinical Benefits
• Balanced intestinal microflora
• ↓ Duration of acute diarrhea
• ↓ Incidence of acute diarrhea
• ↓ Antibiotic-associated diarrhea
• ↓ In severity & incidence of atopic disease
• ↓ NEC

Figure 6.

3.3 Safety of probiotics

In general, probiotics, including LAB, have had a long history of safe food use in human populations. Most LAB strains, used in the food supply are nonpathogenic, non-virulent, and non-toxigenic microorganisms. Many strains of Lactobacilli and Bifidobacteria are traditional food-grade organisms generally recognized as safe (GRAS) for use in the general food supply. Animal work has been conducted to investigate acute toxicity of probiotics, including L. rhamnosus and B. lactis, with no adverse effects observed on the animals’ general health, hematology, blood chemistry, gut mucosal histology or incidence of bacterial translocation.

In 40 years of study, data collected from over 140 adult clinical studies of probiotic LAB strains, involving nearly 8000 subjects, document that LAB are well tolerated. To date, there have been more than 70 clinical studies, involving more than 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. Available studies indicate no adverse impact at any age (including no growth problems and no infections) attributable to oral supplementation of Lactobacilli or Bifidobacteria in healthy infants. Safety
of probiotic use in infants has also been demonstrated by studies showing age-appropriate weight gain and
growth in the target population. No studies were found to report any negative effect of probiotic
administration on weight gain or growth. At least 10 studies of infants receiving probiotics demonstrate
either no difference or improved weight gain as a secondary outcome with probiotic supplementation.
Healthy newborns and infants consuming formula with *Bifidobacteria* have been shown to grow within the
norms of other infants consuming traditional infant formulas.\(^{94}\)

Probiotic bacteria are naturally susceptible to various antibiotics. They also have varied resistance
patterns, which may explain their potential for use in ameliorating antibiotic associated diarrhea, which is
discussed below.\(^{67-70}\)

No cases of infections from the consumption of commercial products containing *Bifidobacteria* have
been documented. There have been sporadic case reports of *L. rhamnosus* infections, possibly associated
with probiotic consumption.\(^{71,72}\) As stated in the Joint FAO/WHO Report on the Evaluation of Probiotics in
Food, “documented correlations between systemic infections and probiotic consumption are few, and all
occurred in patients with underlying medical conditions.”\(^{73}\)

The genera *Bifidobacteria* are of major relevance for use in infants, as they are the genera that predominate
in the gut of breastfed infants, and are not known to be pathogenic. Fermented foods, particularly yogurts
with *Bifidobacteria*, have been consumed for decades and increasingly used as weaning foods for infants.
*Bifidobacteria*, when used as probiotics, has not been documented to have any adverse effects.

**Use in vulnerable populations**

Data is still limited with respect to the administration of probiotics to groups potentially at risk, such as
the immunocompromised and severely ill. And there are various reports of Lactobacillimia in patients who are
immunocompromised or have indwelling central venous catheters.\(^{77,78}\) However, clinical trials have been
conducted with several groups of high risk infants and children, including infants born to HIV-positive
mothers\(^{74}\) and children with both chronic and acute diarrhea.\(^{79-85}\) Furthermore, premature infants
represent an at risk group, since they have underdeveloped gut and immune functions; several clinical studies
of probiotics have been performed in this population, and no probiotic related adverse side effects have been
reported.\(^{86-89}\) Data obtained on *Bifidobacteria* so far have not identified safety concerns, and it is anticipated
that research with *Bifidobacteria* will continue in these populations, given their safety record. Until more data
in such populations is available, it is expected that the consumption of probiotics, particularly *Lactobacilli*, by
severely immunocompromised infants would take place only under a doctor's supervision.
4 BENEFITS ASSOCIATED WITH THE USE OF PROBIOTICS

Key points:
- Various probiotic agents have been shown to improve gut barrier function and modulate GALT immune response. These proposed mechanisms explain the various clinical benefits which have been associated with the use of specific probiotic bacteria.

- The best described benefits of probiotics in infants and children are a reduction in duration of acute diarrhea and a decrease in incidence of acute rotavirus and antibiotic associated diarrhea.

- Probiotics have also been documented to decrease severity of atopic disease, and early intervention may decrease atopic sensitization.

- The benefits of probiotics in decreasing the incidence of necrotizing enterocolitis and ameliorating inflammatory bowel disease and other inflammatory conditions are beginning to be demonstrated.

<table>
<thead>
<tr>
<th>Areas of clinical investigation</th>
<th>Results reported with probiotic supplementation</th>
</tr>
</thead>
</table>
| Immunity                       | • Enhanced systemic and local humoral (antibody) immune response to rotavirus infection\(^{96}\)  
• Increase in total serum IgA,\(^{99,100}\) total fecal IgA,\(^{54}\) and antigen-specific serum IgA\(^{100,56,90,88}\)  
• Increase in IgA, IgG and IgM secreting cells\(^{99}\)  
• Enhancement of natural killer cell tumor-killing activity\(^{2,102}\)  
• Increased production of macrophages and activated phagocytosis\(^{2,48,102-104}\)  
• Enhanced mucosal resistance against GI infections\(^{24}\)  
• Beneficial increase in production of cytokines\(^{46,47,94,105}\)  
• Lower frequency of antibiotic use\(^{66,82}\)  
• Less febrile episodes, diarrheal episodes, clinic visits and day care absences\(^{82}\)  
| Modification of intestinal flora | • Improved ratio of anaerobic to potentially pathogenic bacteria\(^{30,83}\)  
• Improved fecal flora in preterm, low birth weight and/or healthy term infants by increasing Bifidobacteria intestinal colonization\(^{13,14,30,39}\)  
• Identification of supplemented strains in critically ill children,\(^{26}\) infants with acute infections,\(^{21}\) healthy infants,\(^{19}\) and newborns\(^{28}\)  
• Increases in fecal acetic acid and short-chain fatty acids, indicating fermentation\(^{46,83}\)  
• Stool short-chain fatty acids and lactate similar to breastfed babies\(^{50}\)  

<table>
<thead>
<tr>
<th>Diarrhea treatment</th>
<th>Prevention</th>
<th>Antibiotic associated diarrhea</th>
<th>Allergy</th>
<th>Necrotizing enterocolitis</th>
<th>Inflammatory bowel disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Decrease in frequency and/or duration of acute diarrhea</td>
<td>• Reduced incidence of diarrhea in chronically hospitalized and malnourished infants</td>
<td>• Reduced incidence of antibiotic associated diarrhea when given with antibiotics</td>
<td>• Lower incidence of atopic dermatitis</td>
<td>• Colonization of host-friendly bacteria in premature infants</td>
<td>• Successful colonization with <em>Lactobacilli</em> and <em>Bifidobacteria</em></td>
</tr>
<tr>
<td>• Decrease in frequency and duration of persistent diarrhea</td>
<td>• Reduced episodes of diarrhea in neonates and young infants</td>
<td>• Improvement in weight in young children on antibiotic therapy</td>
<td>• Improvement of atopic dermatitis</td>
<td>• Improved weight gain to lactose-containing formulas in neonates</td>
<td>• Improved cytokine profiles in ulcerative colitis</td>
</tr>
<tr>
<td>• Enhancement of IgG, IgA, and IgM secreting cells after treatment for acute diarrhea</td>
<td>• Less dehydration, medical consults, and use of oral rehydration solution</td>
<td>• Decrease in frequency of stools and more formed stools</td>
<td>• Improvement of symptomatological score, quality of life and/or cytokine profile in patients with allergic rhinitis</td>
<td>• Decreased incidence, severity, and mortality due to NEC in VLBW infants</td>
<td>• Improved symptoms and/or clinical activity index score of ulcerative colitis and/or Crohn’s disease</td>
</tr>
<tr>
<td>• Reduction in parental days lost from work</td>
<td>• Reduced number of days with diarrhea for children in residential and foster care settings.</td>
<td>• Decrease in frequency of stools and more formed stools</td>
<td>• Decreased serum eosinophil cationic protein levels</td>
<td>• Decreased incidence, severity, and mortality due to NEC in VLBW infants</td>
<td>• Decrease in endoscopic score of inflammation in ulcerative colitis</td>
</tr>
<tr>
<td>• Reduced length of hospitalization</td>
<td>• Day care settings and in poor urban areas</td>
<td>• Increased serum levels of anti-inflammatory IL-10, increased Th1 cytokine interferon gamma, all favoring a decreased allergic response</td>
<td>• Decreased GI symptoms, gut inflammation, intestinal permeability and protein loss in the stool</td>
<td>• Effective in maintaining remission in ulcerative colitis and Crohn’s disease</td>
<td>• Effective in maintaining remission in Crohn’s disease</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Decreased serum soluble CD4</td>
<td>• Decrease in number of days required to obtain remission in ulcerative colitis</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>• Increase in serum TGF-β</td>
<td>• Effective in maintaining remission in ulcerative colitis and Crohn’s disease</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Increased serum levels of anti-inflammatory IL-10, increased Th1 cytokine interferon gamma, all favoring a decreased allergic response</td>
<td>• Treatment and/or prevention of pouchitis</td>
</tr>
</tbody>
</table>
4.1 Probiotics and immune related benefits

There is increasing evidence that the use of probiotics can help support the immune system, by influencing both the innate and adaptive pathways by various mechanisms described above. The ability of probiotics to influence the gut associated lymphoid tissue (GALT) has lead to the development of prevention or treatment approaches for systemic diseases.\(^{(155)}\)

Animal work has provided some evidence of a beneficial probiotic effect on the immune system’s response to rotavirus infections. In mice and rhesus monkey animal models Bifidobacteria (B. breve, B. bifidum, and B. infantis) improved clinical outcomes, decreased rotaviral shedding, and increased rotavirus specific secretory IgA.\(^{(156-158)}\)

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.\(^{(6)}\) In infants and children with rotaviral diarrhea, L. rhamnosus increased nonspecific humoral response (increased numbers of IgA, IgG and IgM secreting cells) as well as anti-rotavirus specific secretory IgA.\(^{(98)}\) Healthy infants consuming formula supplemented with B. lactis increased their total fecal IgA and anti-poliovirus IgA following immunization.\(^{(54)}\)

In adults, consumption of L. acidophilus L1 and Bifidobacteria increased specific and total secretory IgA to salmonella following S. typhi oral vaccination to levels considered clinically relevant.\(^{(102)}\) These same probiotics, as well as B. lactis, have been shown to increase phagocytic activity against E. coli species as well as enhancing natural killer cell activity.\(^{(102,103)}\) Depending on the state of the host, probiotic bacteria has also been shown to exert beneficial effects on pro- and anti-inflammatory cytokine secretion.\(^{(6,54)}\) Increased levels of IL-1ß, TNF alpha, and interferon gamma were found by stimulating blood mononuclear cells \emph{in vitro} with a variety of species of probiotic bacteria isolated from yogurt which included Bifidobacteria and several other LAB.\(^{(159)}\) Yogurt consumption with specific live cultures was also shown to increase production of interferon gamma by isolated, stimulated T cells.\(^{(105)}\)

The influence of probiotics on cytokine secretion is related to cell-to-cell crosstalk, where the intestinal epithelial cells of the host play a pivotal role in processing signals given by bacteria, based on the continuously changing intestinal content. These signals then influence overall messages via expression of specific molecules or secretion of cytokines.\(^{(64)}\) Passage of probiotic organisms through the intestinal lumen may be sufficient to allow this cell-to-cell communication to occur. In addition, probiotic bacteria has been shown to modify the structure of potentially harmful antigens, altering their mode of immunogenicity, and to stimulate nonspecific host resistance.\(^{(106)}\) Changes in cytokine secretion could also be due to probiotic influence on the differentiation of T helper cells into Th1 or Th2 cells, which are responsible for secretion of distinct cytokines.\(^{(106)}\) 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.\(^{(29,160)}\)
4.2 Diarrhea

The immunologic mechanisms described above provide reasonable support for the study of probiotics in conditions that require adequate gut barrier and immune responses. The foremost example of this is acute infectious diarrhea. Diarrheal disease continues to be a major cause of infant and childhood hospitalizations and mortality worldwide. In developing countries, diarrheal disease ranks third among major causes of death in children less than five years of age, explaining nearly 20% of all mortalities in this group. In the United States, diarrhea is reported in 13% of all childhood hospitalizations and has an estimated cumulative incidence of 1 diarrhea hospitalization per 23 to 27 children by age 5. In addition, the annual proportion of rotavirus-associated hospitalizations for infants and children in the United States has increased from 1993 to 2002, with estimates exceeding 58,000 each year. The combined cost in the United States of inpatient and outpatient care for pediatric diarrhea is greater than $2 billion per year. Since diarrheal diseases are both a consequence, as well as a cause of major alterations in the intestinal flora, they have been the most logical, potential, clinical application for probiotics, and the main reason to focus on pediatric populations. As a consequence, the best-studied and demonstrated benefits of probiotics have been in the treatment and prevention of diarrheal disease in infants and children.

Treatment of diarrhea

At least 23 randomized, controlled trials have evaluated the efficacy of probiotics on the duration and frequency of acute diarrhea in infants and young children. Three meta-analyses, including results from up to 18 randomized, controlled trials of probiotics and acute diarrhea in infants and children have been published (Figure 7). Szajewska pooled results of 8 studies published prior to 2001. Compared to placebo, the relative risk of diarrhea lasting more than 3 days with probiotic supplementation was 0.43 (95% CI: 0.34-0.53). Duration of diarrhea was significantly reduced by 18.2 hours in the 733 children from these studies. L. rhamnosus was identified as a strain that was found particularly effective in decreasing the risk of diarrhea lasting more than three days. In a subgroup analysis of four studies, 297 children, primarily with rotavirus diarrhea, realized a significant reduction in diarrhea duration by 25 hours, compared to placebo (95% CI, -31.8 to -17.9 hours).

A second meta-analysis of studies with Lactobacilli combined results of 9 randomized, controlled trials (765 patients) in children less than 3 years of age with infectious diarrhea. Results indicated a reduction in duration of diarrhea by 16.8 hours and a reduction in diarrhea frequency of 1.6 stools per day after 1 day of probiotic treatment (95% CI: 0.7-2.6 fewer stools) compared with those who received placebo. Of the studies combined, at least 10^10 CFU of Lactobacillus per 48 hours seemed most effective. Another independently conducted meta-analysis collectively assessed the results of 18 randomized, controlled trials from children younger than 5 years (1917 patients). An estimated 19.2 hours less illness from diarrhea was found for children.
with acute diarrhea who were provided probiotics compared to controls. The probiotics studied included several strains of *Lactobacilli*, *Bifidobacteria*, and others. Subgroup analysis of trials that only provided *L. rhamnosus* showed a reduced duration of diarrhea by approximately 1.2 days (95% CI: -1.6 to -0.8 days); for studies that used probiotics other than *L. rhamnosus*, the pooled estimate was -0.6 days (95% CI: -0.9 to -0.3 days).  

**Figure 7.**

Given the results of these multiple, controlled clinical trials and meta-analyses, there appears to be adequate evidence of a statistically significant and potentially clinically important benefit of probiotic supplementation in the treatment of acute diarrhea in infants and young children. Beneficial effects tend to be both dose and strain dependent, more pronounced for watery diarrhea and rotavirus than for bacterial diarrhea, and more effective when provided soon after diagnosis.

**Prevention of diarrhea**

Preventive strategies might ultimately have the greatest potential for reducing the burden of diarrheal disease in infants and young children. A recent meta-analysis provided insight into the 34 randomized, clinical trials that evaluated the efficacy of probiotics in the prevention of acute diarrhea. Results from a subgroup

<table>
<thead>
<tr>
<th>Meta-analyses of randomized clinical trials (RCT) for treatment of acute diarrhea in infants and children</th>
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<tbody>
<tr>
<td><strong>Reduction in Duration of Diarrhea (hrs)</strong></td>
</tr>
<tr>
<td><strong>Szajewska 2001</strong></td>
</tr>
<tr>
<td>8 RCTs</td>
</tr>
<tr>
<td>-26.9 to -9.5 hrs</td>
</tr>
<tr>
<td>95% CI</td>
</tr>
<tr>
<td><strong>Van Niel 2002</strong></td>
</tr>
<tr>
<td>9 RCTs</td>
</tr>
<tr>
<td>-28.8 to -7.2 hrs</td>
</tr>
<tr>
<td>95% CI</td>
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<tr>
<td><strong>Huang 2002</strong></td>
</tr>
<tr>
<td>18 RCTs</td>
</tr>
<tr>
<td>-26.4 to -14.4 hrs</td>
</tr>
<tr>
<td>95% CI</td>
</tr>
</tbody>
</table>
analysis that included 12 studies with only infants and children are presented here. Probiotics significantly reduced the risk of developing diarrhea in infants and children by 57% (CI: 35 – 71%). The protective effect did not significantly vary among the probiotic strains used such as *B. lactis*, *L. rhamnosus*, *L. acidophilus*, and *L. bulgaricus*, *Saccharomyces boulardii* and other strains used alone or in combination with two or more strains.\(^{(170)}\)

Four randomized, clinical trials that have specifically addressed the effect of probiotics in the prevention of nosocomial diarrhea during hospitalization or stays in residential care centers have been identified (Figure 8). All included infants and children admitted for diagnoses other than diarrhea. One study of 81 infants that administered *L. rhamnosus* at a concentration of 6 x 10^9 CFU twice daily resulted in a significant reduction in risk of nosocomial diarrhea, compared with placebo (6.7 vs. 33.3%; RR = 0.2), as well as a reduction in the risk of rotaviral gastroenteritis in the probiotic supplemented children, compared to placebo (2.2% vs. 16.7%; RR = 0.13).\(^{(167)}\) In a second trial that provided a combination of *B. lactis* and *S. thermophilus* to infant formula, a significantly reduced prevalence of nosocomial diarrhea was reported. In this study of 55 infants, prevalence decreased from 31.0% in the placebo group to 7.0% in the probiotic group (RR = 0.2). The risk of rotaviral gastroenteritis was significantly reduced among the infants consuming the probiotic supplemented formula as well (RR = 0.3).\(^{(90)}\) Two additional randomized trials evaluating nosocomial diarrhea, one administering *L. rhamnosus*;\(^{(171)}\) and a second providing *B. lactis*\(^{(79)}\) reported decreased diarrhea prevalence in the probiotic treatment groups compared to controls. However, results did not meet statistical significance. Further support for the association between prevention of diarrheal disease and probiotic intake is found in results from 3 additional randomised, clinical trials, of non-hospitalized infants and children that reported significant decreases in diarrhea incidence with *B. lactis* or *L. rhamnosus*.\(^{(78,82,117)}\) It is clear that the protective effects seen will vary, depending on the population, geographic area, pathogen predominance and other environmental factors. No study has documented an increase (significant or not) in diarrheal disease with probiotic use.

The reduced incidence of diarrhea shown with various probiotic strains (similar to the observed benefits in treatment), appears to be greater for diarrhea of viral origin. In addition, decreased rotaviral shedding,\(^{(90)}\) reduction in duration of hospitalization\(^{(172)}\) and decreased hospitalization\(^{(173)}\) all suggest that the effect occurs on both the manifestations of the disease and on the course of the infection. These observations greatly bolster the arguments for finding ways to use probiotics in a long-term and prophylactic manner, particularly in infancy.
Antibiotic associated diarrhea

Antibiotic associated diarrhea is defined as an acute inflammation of the intestinal mucosa, caused by the administration of broad-spectrum antibiotics.\(^{(166)}\) The incidence of antibiotic associated diarrhea in children ranges from 8% to 30%.\(^{(174)}\) The use of probiotics in antibiotic associated diarrhea follows the logic that diarrhea results from an imbalance in normal, healthy, intestinal microflora caused by antibiotic therapy.

Probiotics are valuable in reducing the risk of antibiotic associated diarrhea in infants and children.\(^{(86,122,123,125,127,175)}\) Results from six 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 to placebo, reduced the risk of diarrhea from 28.5% to 11.9%.\(^{(166)}\) Beneficial effects were strongest for \(B.\) \(lactis\) and \(S.\) \(thermophilus\). Moderate reduction in diarrhea risk was associated with the use of \(L.\) \(rhamnosus\) and \(S.\) \(boulardii\).\(^{(166)}\)

\textit{Clostridium difficile} (\(C.\) \(difficile\)) causes approximately 25% of nosocomial antibiotic associated diarrhea in adults\(^{(166)}\) and \(C.\) \(difficile\) toxins can be detected in 10-25% of patients with antibiotic associated diarrhea,\(^{(175)}\) but is less common in infants and children. Although \(C.\) \(difficile\) has been reported as the most common bacterial agent associated with antibiotic associated diarrhea, in childhood, antibiotic associated diarrhea is often not due to \(C.\) \(difficile\).\(^{(166)}\) There are no randomized, controlled clinical trials which specifically evaluate the effect of probiotics on \(C.\) \(difficile\) diarrhea in infants or children. Reports on the use of \(L.\) \(plantarum\), \(B.\) \(longum\),

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**Figure 8.**

**Clinical trials on prevention of diarrheal disease in infants**

<table>
<thead>
<tr>
<th>Study</th>
<th>Reduction in incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chouraqui 2004</td>
<td>-5</td>
</tr>
<tr>
<td>Mastretta 2002</td>
<td>NS</td>
</tr>
<tr>
<td>Oberhelman 1999</td>
<td>-20</td>
</tr>
<tr>
<td>Saavedra 1994</td>
<td>NS</td>
</tr>
<tr>
<td>Szajewska 2001</td>
<td>-40</td>
</tr>
<tr>
<td>Weizman 2005</td>
<td>NS</td>
</tr>
<tr>
<td>Ziegler 2003</td>
<td>-60</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Reduction in incidence (%)</th>
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</thead>
<tbody>
<tr>
<td>-10</td>
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<tr>
<td>-20</td>
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<tr>
<td>-30</td>
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<tr>
<td>-80</td>
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<tr>
<td>-90</td>
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<tr>
<td>-100</td>
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</tbody>
</table>

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**Clinical trials on prevention of diarrheal disease in infants**

- **B. lactis**
- **L. rhamnosus**

---

**Clinical trials on prevention of diarrheal disease in infants**

- **NS**
Enterococcus faecium and L. rhamnosus in the effective treatment and diminished recurrence of disease in children with C. difficile colitis have been anecdotal, but encouraging.\(^{(179,180)}\)

4.3 Allergy

Multiple studies have investigated atopic dermatitis as a model for allergic and immunologic hypersensitivity to foods in infancy. L. rhamnosus and several strains of Bifidobacteria were used most often. Several possible mechanisms are attributable to potential benefit in atopic disease, including improved integrity of the gut barrier with decreased intestinal permeability. These possible mechanisms of action have been associated with positive immunologic responses in children with atopic dermatitis. These responses improve the integrity of the gut barrier by decreasing intestinal permeability, reducing both adherence of potential antigens and their systemic response, and modulating GALT immune response toward antigen tolerization.\(^{(29,49,98,128)}\) Lower counts of Bifidobacteria have been reported in atopic compared to non-atopic children preceding allergen sensitization. Bifidobacteria are hypothesized to more effectively promote tolerance to non-bacterial antigens, primarily by inhibiting the development of a Th2 type response.

In a randomized, double-blind study, infants with eczema and cow’s milk protein allergy, who received a hydrolyzed whey formula supplemented with L. rhamnosus, showed greater clinical improvement than those who received the hydrolyzed formula alone. The probiotic supplemented group excreted less TNF alpha and alpha-1-antitrypsin in their stool, suggesting that the probiotics decreased gut inflammation, resulting in decreased protein loss in the stool.\(^{(181)}\) Isolauri and colleagues randomized infants who had experienced eczema during exclusive breastfeeding, to receive either an extensively hydrolyzed whey-based formula with L. rhamnosus (10⁶ CFU/g) or B. lactis (10⁹ CFU/g), or the same formula without probiotics. After two months, infants who received either probiotic formula demonstrated a significant improvement in their eczema (as measured by SCORAD) compared to the non-supplemented formula group. 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 inflammatory response via IgA production and oral tolerance induction).\(^{(48)}\)

Impairment of the intestinal mucosal barrier appears to be involved in the pathogenesis of atopic dermatitis and eczema. In a double-blind, placebo-controlled, crossover study, Lactobacilli (L. rhamnosus and L. reuteri) were administered for 6 weeks to children with moderate to severe atopic dermatitis. During Lactobacilli supplementation, there was a significant decrease in the frequency of GI symptoms, and a positive association between tests for increased intestinal permeability and the severity of the eczema. After probiotic treatment, intestinal permeability decreased.\(^{(131)}\) In a similar double-blind, placebo-controlled, crossover study, 2 probiotic Lactobacillus strains were given together for 6 weeks to children with atopic dermatitis. After treatment, 56% of the patients experienced improvement in their eczema, versus only 15% with placebo.\(^{(130)}\)
These studies suggest that regular probiotic supplementation may stabilize intestinal barrier function, and play a role in modulating allergic responses leading to a decreased severity of atopic symptoms, particularly atopic dermatitis associated with cow’s milk protein.\textsuperscript{46,48,132}

4.4 Necrotizing enterocolitis (NEC)

The newborn gut microflora fosters integrity of the immune system, protects from infections with enteric pathogens, produces vitamins, and encourages mucosal maturation.\textsuperscript{182-184} The premature infant is exposed to a variety of stressors in the neonatal intensive care unit (NICU), which can suppress mucosal immunity and enhance the growth of enteric pathogens. In the preterm infant, bacterial colonization of the gut is delayed due to: the use of antibiotics, lack of exposure to normal maternal flora and breastmilk, and NICU infection control procedures (such as hand washing), making this a reasonable opportunity for intervention with probiotics.\textsuperscript{136}

The hypothetical benefits of probiotics in preterm infants include: reduction of enteric pathogens, improved enteral nutrition, reduced dependence upon TPN, increased gut mucosal barrier function, reduction in sepsis and antibiotic use, and prevention of NEC.\textsuperscript{183,185-187}

NEC is a condition seen primarily in premature infants, which can result in death or bowel resection, leading to short bowel syndrome, dependence upon parenteral nutrition, liver disease, and possibly liver and small bowel transplant. Aside from prematurity, other risk factors for the development of NEC include enteral feeding, an immature intestinal mucosa (seen with antenatal steroids), and abnormal bacterial colonization of the GI tract with bacterial translocation across the gut barrier to the bloodstream.\textsuperscript{183,186} The hypothesis that NEC may be due to a bacterial infection is supported by: 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.\textsuperscript{183}

Mechanisms by which probiotics could prevent NEC include: 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 enteral nutrition.\textsuperscript{184,186,188}

Using a tube-fed premature mirroring model of NEC, \textit{B. infantis} led to lower incidence of NEC, endotoxemia, and improved mucosal permeability and intestinal phospholipase A2 expression (a precursor of platelet activating factor) suggesting a clinical effect through modulation of the inflammatory cascade.\textsuperscript{189}
Figure 9. Possible pathophysiologic mechanisms in necrotizing enterocolitis:
Ischemia, combined with the lack of protective factors in formula, which are normally found in breastmilk, lead to mucosal injury. This is exacerbated by the colonization of pathogenic bacteria and by the release of inflammatory mediators. Bifidobacteria may interfere with the colonization of pathogenic bacteria and aid with the maturing of the mucosal immunity.

In humans, there are several published studies suggesting that probiotic supplementation can decrease the incidence of NEC. Hoyos evaluated the effect of orally administered *L. acidophilus* and *B. infantis* on the incidence of NEC in neonates in an NICU setting. Supplemented infants (n=1237) were compared to 1282 unsupplemented historical control patients hospitalized the previous year. There was a statistically significant decrease in the cases of NEC and NEC-associated mortality in the probiotic group, as compared with controls.(136) Small, uncontrolled studies in single NICUs have shown improved weight gain, better feeding tolerance, and lower serum endotoxin levels in infants supplemented with *Lactobacilli* and *Bifidobacteria* species.(39,97)

Several recent prospective studies have shown various degrees of reduction in relative risk of NEC with probiotics(190). Lin reported a large, prospective, randomized, blinded, controlled trial evaluating incidence and severity of NEC among very low birth weight (VLBW) infants.(95) *L. acidophilus* and *B. infantis* given twice daily with breastmilk significantly decreased the incidence of NEC compared to breastmilk alone (2 or 180 vs. 10 or 187). In another prospective, double-blind study of 585 premature infants in 12 NICUs, the group supplemented with *L. rhamnosus* was found to have a lower incidence of urinary tract infections and NEC than the control group; however, the difference was not statistically significant.(137) A recent study by Bin-Nun...
randomized VLBW neonates to receive either *B. infantis, S. thermophilus*, and *B. bifidus* at 10⁹ CFU/day, or no probiotic supplement. The incidence of NEC was reduced from 16.4% in the 73 control infants to 4% in the 72 supplemented infants. Three of the 15 babies who developed NEC died, and all of the deaths occurred in the non-supplemented group.

### 4.5 Inflammatory bowel disease (IBD)

The involvement of intestinal flora in IBD is being actively pursued to determine potential beneficial effects of probiotics in children and adults. It has been suggested that IBD is not simply the result of an alteration in the intestinal flora, but rather an overactive response of the immune system to a subset of native flora in genetically predisposed individuals. Clinical and experimental studies suggest that the relative balance of aggressive and protective bacterial species is altered in these disorders.

Animal models of colitis using various strains of *Lactobacillus* and *Bifidobacterium* have shown decreased inflammatory response or decreased markers of inflammation. Murine models of colitis were successfully treated with *Lactococcus lactis* secreting IL-10. Stimulation of isolated B cells and an increase in IgA antibody production resulted from *Bifidobacterium* treatment. Considering the interplay between the immune system and intestinal flora, the use of probiotics may be a reasonable approach to the management of IBD.

Studies with infants and young children with IBD and probiotics are limited. Two small trials suggest a potential for *L. rhamnosus* to decrease disease activity and increase specific IgA antibody secreting cells to various antigens.

Studies of IBD in adults are more numerous and have shown promising results with regards to probiotic supplementation on epithelial-related biological markers of inflammation, endoscopic assessment, and remission of ulcerative colitis (UC). A number of clinical trials using *B. longum, B. breve, B. bifidum, L. acidophilus*, and *B. lactis* have shown varying degrees of benefits in managing patients with UC. A combination of probiotics showed effective results in the maintenance of remission of pouchitis, a frequent complication after colonic resection in patients with UC where the remnant rectal pouch becomes inflamed. There appears to be a role for probiotic supplementation in decreasing inflammation and prolonging the time to relapse in adult patients with UC.
5 BIFIDOBACTERIA AS PROBIOTICS IN INFANTS AND CHILDREN

Key points:

- *Bifidobacteria* species are indigenous to the intestinal tract of both infants and adults.

- *Bifidobacteria* species are the most prevalent of all bacterial species that colonize the intestine of breastfed infants, compared to formula-fed infants, and are thought to be associated with some of the health benefits of breastfeeding.

- *Bifidobacteria* in general, and specifically *B. lactis*, are agents which survive GI digestion, reach the colon, populate the distal gut, produce short-chain fatty acids, and lower colonic pH; they are therefore uniquely suited as a probiotic agent for inclusion in the diets of infants.

- The safety of *Bifidobacteria* in general and *B. lactis* specifically has been well documented. Formulas supplemented with *B. lactis* have been marketed more than 15 years in more than 30 countries with an excellent safety record. To date, no serious adverse events have been reported in clinical trials.

- Multiple studies document various benefits with *B. lactis* in infants and children. These include:
  - Modification of intestinal flora to a more desirable profile
  - Improvement in gut barrier function and integrity
  - Positive modulation of the immune response.
  - Potential clinical protective effects from conditions such as diarrhea and atopic disease.

5.1 *Bifidobacteria*: relevance to pediatric nutrition

*Bifidobacteria* comprise as much as 25% of the cultivable intestinal microflora of adults and infants. In breastfed infants, *Bifidobacteria* are the dominant species, often outnumbering other bacteria by at least 100-fold. This dominance is seen as early as 4 days of age. A significant mechanism for this predominance is the number of "bifidogenic" factors, which have been identified in human breastmilk, and fosters the growth of these species above others. Amongst them are lactose and galacto-oligosaccharides. The lower buffering capacity of breastmilk (compared to infant formula), as well as its lower concentration of phosphate, may also contribute to its bifidogenic potential. The predominance of *Bifidobacteria* in their intestinal microflora is thought to be one of the mechanisms by which breastfed infants experience fewer episodes of acute diarrhea and intestinal infections than exclusively formula-fed infants. This has made *Bifidobacteria* excellent choice for probiotic study.

*Bifidobacteria* are a group of naturally occurring, gram-positive, non-motile, non-sporulating rods found in the intestinal tract of both humans and animals. The name is derived from their bifid or Y-shaped form (Figure 10). They ferment glucose, galactose and fructose, leading to production of both lactic and acetic acid. *Bifidobacteria* do not produce D-lactate. These by-products of fermentation
lower intestinal pH, are trophic to the gut, inhibit growth of pathogens and thus encourage a balanced bacterial environment in the host without raising concern that they may cause metabolic acidosis.\(^\text{295}\)

![Figure 10. *Bifidobacteria* at 15,600 x magnification.](image)

*Bifidobacteria* are obligate gram-positive anaerobes, with varied rod shapes. Growth in certain media gives them their characteristic Y shape. They readily ferment carbohydrates and produce short-chain fatty acids (acetate and lactate) and tolerate well low pH environments, which can be inhibitory to intestinal pathogens. They do not produce D-lactate, are non-pathogenic, and survive well gastric and biliary digestion. *Bifidobacteria* constitute the predominant genera in most breastfed infants.

*Bifidobacteria* meet the general criteria for a microorganism to be considered a probiotic. When given as a supplement, *Bifidobacteria* can remain viable in the stomach and continue to be viable into the small bowel and colon. As acid-tolerant microbes, some strains, such as *B. lactis* and *B. animalis*, can survive exposure at pH 3.5.\(^\text{206}\) Certain species of *Bifidobacteria* when given orally can transiently colonize the colon, increasing their presence even in premature infants, without undesirable GI symptoms.\(^\text{19,95,96}\) In addition, and unlike many other probiotic organisms, *Bifidobacteria* species tolerate a small amount of oxygen, making them more stable in supplemental food sources.\(^\text{209}\) *B. lactis* also has adaptability to industrial processes, stability during storage, and the ability to survive in the upper GI tract.\(^\text{208}\)

Molecular typing methods have now firmly established *Bifidobacterium* as a well-defined genus indigenous to the intestinal tract of infants and adults. Technological advances have also been made in identifying individual species of *Bifidobacterium*\(^\text{209}\) resulting in changes in taxonomy and nomenclature. Hence, the names *B. animalis*, *B. bifidum*, and *B. lactis* have been used over time in various studies to identify the specific microorganism, which is referred to in this document as *B. lactis*, whose metabolic and genetic characteristics have been fully described.\(^\text{209}\)

These advances in knowledge and desirable characteristics of *Bifidobacteria* have led to their successful worldwide use as a probiotic agent, particularly in pediatric populations. No serious adverse events associated with the consumption of *Bifidobacteria*-containing formulas or infant foods have been reported.
Most importantly, adequate tolerance in both healthy and premature infants and children has been extensively documented particularly for \textit{B. lactis}.\textsuperscript{[33,34,74-82,90,97]} Of the numerous \textit{Bifidobacteria} species that exist, \textit{B. lactis} has been the most extensively studied in pediatric populations and it is currently the only microorganism approved by the U.S. Food and Drug Administration (FDA) for inclusion in infant formulas. The first infant formula containing \textit{B. lactis} was introduced in 1991. Since that time, infant formula with \textit{B. lactis} has been marketed in over 30 countries worldwide and clinical research has increasingly demonstrated the beneficial effects seen in studies of \textit{Bifidobacteria} and \textit{B. lactis}, specifically in such areas as immune support, diarrheal disease, and allergy.

### 5.2 Clinical benefits of \textit{B. lactis} in infants and children

A summary of clinical trials with \textit{B. lactis} in infants and young children are presented in Table 2.

<table>
<thead>
<tr>
<th>Areas of clinical investigation</th>
<th>Positive results</th>
<th>References</th>
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<tr>
<td>Immunity</td>
<td>• Increase in gut barrier/decrease intestinal permeability\textsuperscript{[96]}</td>
<td>Stratiki, et al., 2006</td>
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<td></td>
<td>• Increased fecal IgA\textsuperscript{[210]}</td>
<td>Mohan, et al., 2006</td>
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<td>• Increase in number of specific IgA secreting cells\textsuperscript{[16]}</td>
<td>Rautava, et al., 2006</td>
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<td>• Increase in soluble CD14, a marker of immunologic maturation\textsuperscript{[96]}</td>
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<td></td>
<td>• Reduction in number of days with fever\textsuperscript{[81,82]}</td>
<td>Sazawal, et al., 2004</td>
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<td></td>
<td>• Reduction in number of febrile episodes\textsuperscript{[81,82]}</td>
<td>Weizman, et al., 2005</td>
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<td></td>
<td>• Reduction in antibiotic use\textsuperscript{[81]}</td>
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<td>• Reduction in number of severe illness days\textsuperscript{[81]}</td>
<td>Sazawal, et al., 2004</td>
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<td></td>
<td>• Reduction in otitis media infection\textsuperscript{[81]}</td>
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<td>• Decrease in soluble CD4\textsuperscript{[48]}</td>
<td>Isolauri, et al., 2000</td>
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<td></td>
<td>• Decrease in urinary EPX\textsuperscript{[48]}</td>
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<td></td>
<td>• Increase in serum TGF-\textit{β} \textsuperscript{1}</td>
<td>Phuapradit, et al., 1999</td>
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<td></td>
<td>• Antibody titers increased in control group, indicating higher subclinical infection than in supplemented group\textsuperscript{[20]}</td>
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<td>• Increase in total fecal IgA\textsuperscript{[24]}</td>
<td>Fukushima, et al., 1998</td>
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<td></td>
<td>• Increase in anti-poliovirus IgA\textsuperscript{[24]}</td>
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<td>Modification of intestinal flora</td>
<td>• Increase in fecal \textit{Bifidobacteria} and decrease in \textit{Clostridia} after 1 week\textsuperscript{[4,9]}</td>
<td>Mohan, et al., 2006</td>
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<td>• Decrease in \textit{Escherichia coli} and \textit{Bacteroides}\textsuperscript{[24]}</td>
<td>Kirjavainen, et al., 2002</td>
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<td></td>
<td>• Increase in fecal \textit{Bifidobacteria}\textsuperscript{[86,96]}</td>
<td>Mohan, et al., 2006</td>
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<td></td>
<td>• Increase in fecal acetic acid and short-chain fatty acids\textsuperscript{[86,96]}</td>
<td>Fukushima, et al., 1997</td>
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<td>• Stool pH similar to breastfed infants\textsuperscript{[86]}</td>
<td>Langhendries, et al., 1995</td>
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<td></td>
<td>• Stool \textit{Bifidobacteria} counts similar between breastfed and supplemented infants\textsuperscript{[21]}</td>
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<tr>
<td>Diarrhea, treatment</td>
<td>• Decrease in number of children with watery stools\textsuperscript{[36]}</td>
<td>Shamir, et al., 2005</td>
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<td></td>
<td>• Improvement in time to resolution of diarrhea\textsuperscript{[36]}</td>
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<td>Diarrhea, prevention</td>
<td>• Decrease in incidence of antibiotic associated diarrhea and dehydration&lt;sup&gt;86&lt;/sup&gt;</td>
<td>Correa, et al., 2005</td>
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<td>• Reduction in episodes of diarrhea&lt;sup&gt;86&lt;/sup&gt;</td>
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<td>• Reduction in number of days with diarrhea&lt;sup&gt;86&lt;/sup&gt;</td>
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<td>• Reduction in number of days with diarrhea&lt;sup&gt;79&lt;/sup&gt;</td>
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<td>• Lower relative risk of developing diarrhea&lt;sup&gt;79&lt;/sup&gt;</td>
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<td>• Decrease in incidence and prevalence of bloody diarrhea&lt;sup&gt;79&lt;/sup&gt;</td>
<td>Saavedra, et al., 1994</td>
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<tr>
<td></td>
<td>• Reduction in number of days and episodes of diarrhea&lt;sup&gt;79&lt;/sup&gt;</td>
<td>Sistek, et al., 2006</td>
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<td></td>
<td>• Reduction in incidence of diarrhea&lt;sup&gt;79&lt;/sup&gt;</td>
<td>Isolauri, et al., 2000</td>
</tr>
<tr>
<td></td>
<td>• Reduction in rotaviral shedding&lt;sup&gt;79&lt;/sup&gt;</td>
<td>Arvola, et al., 2002</td>
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| Allergy | • Improvement in SCORAD score among food-sensitized children<sup>129</sup> | Sistek, et al., 2006 |
|         | • Improvement in atopic eczema<sup>48,134</sup> | Mohan, et al., 2006 |
|         | • Reduced calprotectin, a marker for inflammation<sup>210</sup> | Kirjavainen, et al., 2002 |
|         | • Through modification of intestinal microflora, decrease in the microbial pattern associated with study atopic infants who were most intolerant<sup>212</sup> | Saavedra, et al., 2004 |

| Growth/ tolerance/ safety     | • Normal growth patterns<sup>33,66,76,78,82,118</sup> | Barclay, et al., 2006 |
|                              | • Improved weight gain/growth in preterm infants<sup>33,212</sup> | Mohan, et al., 2006 |
|                              | • Improved growth in malnourished infants and toddlers<sup>213</sup> | Stratiki, et al., 2006 |
|                              | • Improved growth in young children<sup>75</sup> | Nopchinda, et al., 2002 |
|                              | • Improved weight gain in full-term infants<sup>75</sup> | Cooper, et al., 2006 |
|                              | • Decreased frequency of reported colic or irritability<sup>90</sup> | Saavedra, et al., 2004 |
|                              | • Decreased frequency of spitting up<sup>90</sup> | Ziegler, et al., 2003 |
|                              | • Reduction in number of HGB values < 10 mg/dl and better overall iron status<sup>81</sup> | Sazawal, et al., 2004 |
|                              | • Reduced α-linolenic acid proportions in plasma neutral lipids in probiotic groups<sup>80</sup> | Kankaanpaa, et al., 2002 |
|                              | • Increase in proportion of α-linolenic acid phospholipids in B. lactis group<sup>80</sup> | Saavedra, et al., 1998 |
|                              | • No difference in health indicators<sup>80</sup> | Saavedra, et al., 2004 |
|                              | • More desirable stooling pattern<sup>90</sup> | Weizman, et al., 2005 |
|                              | • Decreased prevalence of diaper rash<sup>90</sup> | Huet, et al., 2006 |
|                              | • Acceptable tolerance with no adverse events<sup>33,66,76,78,82,92,93,210</sup> | Mohan, et al., 2006 |
|                              | • Reduced H<sub>9251</sub>-linolenic acid proportions in plasma neutral lipids in probiotic groups<sup>90</sup> | Mihatsch, et al., 2004 |
|                              | • Increase in proportion of H<sub>9251</sub>-linolenic acid phospholipids in B. lactis group<sup>90</sup> | Saavedra, et al., 2004 |
|                              | • No difference in health indicators<sup>90</sup> | Kullen, et al., 2005 |
|                              | • More desirable stooling pattern<sup>90</sup> | Saavedra, et al., 1998 |
|                              | • Decreased prevalence of diaper rash<sup>90</sup> | Langhendries, et al., 1995 |
Modification of intestinal microflora

Following ingestion of *B. lactis*, the microflora of formula-fed infants changes, reflecting an increase in *Bifidobacteria* counts. In some cases, counts reach similar levels as those found in breastfed infants. Increases in fecal *Bifidobacteria* have been recorded within seven days of *B. lactis* supplementation. In addition, beneficial shifts in stool pH and fecal short-chain fatty acids have confirmed fermentation by the ingested bacteria and their ability to survive through the upper GI tract. Such changes to the intestinal environment are also accompanied by decreased concentration of *Bacteroides* and *E. coli* in the GI tract of *B. lactis* supplemented infants. As with all probiotics, “true” colonization does not occur, and to maintain a desired balance of microbes, continued consumption appears to be necessary. *B. lactis* counts decrease after interruption of ingestion of formula containing this probiotic and are difficult to detect after seven days of formula discontinuation.

As mentioned earlier, not all probiotic bacteria, nor all *Bifidobacteria* have the same effects. The type and strain of bacteria transiently colonizing the intestine, and the timing, may determine its effect on the microflora at various locations of the intestine, as well as the various effects on gut function, integrity and GI-associated immunity.

**Effects on the immune system**

*B. lactis* supplementation in infants and young children has shown clinical benefits which are associated with an enhancement of immunity towards potential pathogens. A reduction in clinical outcomes, such as the number of febrile episodes, frequency of antibiotic use, rates of otitis media infections, overall number of severe illness days, and incidence and duration of rotaviral diarrhea have been shown. In addition, *B. lactis* has been shown efficacious in the prevention and treatment of other conditions that also have a strong immune component, such as atopic disease and NEC. This section describes clinical trials documenting various effects of *B. lactis* on markers of immune function, as well as related outcomes in various clinical conditions.

Mucosal humoral immunity is immature during the first few weeks after birth, but antimicrobial peptides, secretory IgA, and bifidogenic factors in breastmilk function to help protect the mucosa of neonates. Enhancement of local immunity, as measured by changes in specific IgA or number of IgA-secreting cells, has been associated with *B. lactis* supplementation. Fukushima provided *B. lactis* to healthy formula-fed children (15 to 31 months old), all of whom had completed oral polio vaccine. At least 200 mL of reconstituted formula containing 1 x 10⁹ CFU of *B. lactis* for 21 days was consumed by the seven children in the study. A significant increase in total fecal IgA and anti-poliovirus IgA, compared to the pre- and post-consumption level, was shown in this group of children. One hundred seventy-five hospitalized infants,
6-36 months of age, received either control formula or the same formula with \textit{B. lactis} \((10^8 \text{ CFU/g})\) or \textit{B. lactis} and \textit{S. thermophilus}. Salivary rotavirus-specific IgA antibodies were used to measure exposure to rotavirus infection. Infants receiving formula supplemented with either \textit{B. lactis} alone, or in combination with \textit{S. thermophilus}, had no significant change in antibody titers between pre- and post-study time points. However, greater than 30% of the control group had a fourfold increase in these antibodies, suggesting this group was protected from symptomatic rotavirus infection.\(^{(211)}\)

Rautava also recently evaluated the effects of approximately 11 months of \textit{B. lactis} and \textit{L. rhamnosus} supplementation on the mucosal immunologic maturation in young infants consuming formula with probiotics.\(^{(55)}\) Prior to 2 months of age, 81 infants were randomized to receive a milk-based formula containing both \textit{L. rhamnosus} and \textit{B. lactis}, or placebo. Although the number of total IgA secreting cells was not statistically different throughout the 12-month study period, the number of cow’s milk-specific IgA secreting cells was significantly higher in the probiotic group compared to control. In addition, serum sCD14, a marker of immunologic maturation, was significantly greater than placebo in infants provided probiotics.

The potential for \textit{B. lactis} to affect gut barrier function through enhanced maturation has recently been investigated in premature infants.\(^{(96)}\) After 4 weeks of \textit{B. lactis} feeding to preterm infants, lactulose absorption was significantly lower in the probiotic supplemented group compared to controls, suggesting improvement in intestinal permeability, which could help reduce bacterial translocation in this high-risk group. Another study with premature infants investigated the effects of supplemental \textit{B. lactis} \((4.8\times10^9 \text{ CFU for 21 days})\) on microbial colonization and its relationship to providing protection against infection.\(^{(206)}\) Results from this randomized, controlled study of 69 infants demonstrated that infants in the \textit{B. lactis} group, compared to control, had significantly higher fecal bifidobacterial counts and nearly a twofold increase in fecal IgA. In addition, fecal concentrations of calprotectin, a marker for inflammation, were significantly decreased in the \textit{B. lactis} group compared to infants without supplementation. Other trials have shown smaller differences in secretory IgA in stool with \textit{B. lactis}, within the range found in breastfed infants.\(^{(209)}\) Taken together, this evidence supports a role for \textit{B. lactis} either as a single probiotic, or in combination with \textit{L. rhamnosus} or \textit{S. thermophilus}, in positively influencing local and general mucosal immunity.

**Diarrheal disease**

As with other probiotics, the best documented benefits of \textit{B. lactis} have been on the incidence and duration of diarrhea in infants and young children, both in hospitalized and day care settings, and for infectious as well as antibiotic-associated diarrhea.\(^{(78,79,81,82,85,86,90,211)}\)
Saavedra and colleagues were among the first to document the efficacy of long-term consumption of *B. lactis* in infant formula for the prevention of rotaviral diarrhea.\(^{90}\) Sixty chronically hospitalized infants with no acute or chronic GI compromise received either control formula or formula with *B. lactis* \((10^8 \text{ CFU/g})\) and *S. thermophilus* throughout their hospital stay (mean 82 and 79 days, respectively). The infants fed *ad libitum* were observed for formula tolerance, incidence and severity of diarrhea, and stool microflora during diarrheal episodes. Infants supplemented with *B. lactis* had significantly fewer episodes of acute diarrhea than the controls. Most interestingly, only 10% of the infants receiving the probiotic formula shed rotavirus in their stool during the study compared to 38% of controls. These findings are consistent with others, such as of Phuapradit,\(^{211}\) who investigated 175 children (6-36 months old) living in a group residential facility. Among the unsupplemented children, greater than 30% had significant increases in rotavirus titers indicative of subclinical rotavirus infection, compared to nonsignificant findings among the infants that consumed formula with *Bifidobacteria* \((10^8 \text{ CFU/g})\) or *Bifidobacteria* and *S. thermophilus*.

Chouraqui and others also investigated the prophylactic effect of *B. lactis* on diarrhea in infants (0-8 months) living in residential day care or foster care centers.\(^{79}\) *B. lactis* was supplemented to an acidified formula at a minimum concentration of \(1.5 \times 10^8 \text{ CFU/L}\). Ninety infants were followed for an average of 4.5 months. The duration of diarrheal episodes was significantly reduced by 1.15 days in the *B. lactis* group compared to the nonsupplemented control. The authors concluded that feeding infants *B. lactis* reduced their risk of developing diarrhea by a factor of 1.9. Weizman also investigated the effect of *B. lactis* in preventing diarrhea in infants attending child care centers.\(^{82}\) In this large, well-controlled trial of 201 infants, infants consuming *B. lactis* formula also had significantly fewer days with diarrhea and episodes of diarrhea than controls.

Another recent study examined the efficacy of *B. lactis* supplementation on prevention of diarrhea in a group of infants and young children on antibiotic therapy for 15 days.\(^{86}\) One hundred fifty children, age 6-36 months, were randomized to receive formula with \(1 \times 10^7 \text{ CFU of } B. lactis\) and \(1 \times 10^6 \text{ CFU of } S. thermophilus\), or unsupplemented formula at the initiation of antibiotics. Thirteen infants (16%) of the 80 infants receiving the probiotic developed diarrhea, compared to a significantly greater number of children (24 of 77 infants; 31%) in the control group, yielding a reduction in incidence of nearly 48%.

In a double-blind, prospective study Shamir evaluated the effect of a combination of *B. lactis*, *L. acidophilus*, and *S. thermophilus* \((2 \times 10^9 \text{ CFU of each strain})\) along with 10 mg supplemental zinc and prebiotics in the treatment of acute gastroenteritis in children age 6-12 months.\(^{85}\) After 2 days of consuming the soy protein-based supplemented rice cereal, significantly fewer children in the supplemented group had watery stools compared to the control. Diarrhea resolution occurred after 1.4 days in the supplemented
group compared to 2 days in the control. Although results indicated that the duration and severity of acute gastroenteritis in young children were resolved significantly faster in the supplemented group, the independent effects of zinc and the probiotic combination have yet to be determined.

Currently, there appears to be adequate evidence to support that *B. lactis* may serve as an adjunct in the treatment and prevention of acute diarrhea in pediatric populations. In addition, more recent evidence provides encouraging results for a role of probiotics in antibiotic associated diarrhea in young children.

**Allergy**

There is good evidence to indicate that the type of intestinal flora in infants is associated with the risk of developing atopic disease. In particular, infants not colonized with *Bifidobacteria* appear to be at higher risk of developing atopy. This suggests that probiotics in general, as mentioned above, and *Bifidobacteria* in particular may modulate the gut immunologic response, not only enhancing activity against pathogens, but also down-regulating an exaggerated (allergic) response.

Work by Isolauri and colleagues showed favorable outcomes in atopic disease with *B. lactis* supplementation. Infants who had eczema during exclusive breastfeeding were weaned to either an extensively hydrolyzed whey-based formula with *L. rhamnosus* (10⁹ CFU/g) or *B. lactis* (10⁹ CFU/g), or the same formula without probiotic. After 6 months, skin condition and inflammatory markers improved in both of the probiotic groups. 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 inflammatory response via IgA production and oral tolerance induction). Arvola evaluated whether probiotic-supplemented formula could promote the gut barrier during weaning. A group of 56 infants with a mean age of 5 months, who had symptoms of atopic eczema during exclusive breastfeeding, were weaned to hydrolyzed formula without probiotics or the same formula supplemented with *B. lactis* or *L. rhamnosus*. The authors reported a reduction in severity of eczema with probiotics. Gut permeability was also studied, but no improvement was demonstrated. On the other hand, Stratiki showed improvement in gut permeability in premature infants receiving *B. lactis* supplementation.

Kirjavainen found a positive change in stool colonization with a decrease of *Bacteroides* and *E. coli* in a group of atopic infants receiving a hydrolyzed whey formula with *B. lactis* (10⁹ CFU/g) versus infants receiving the same formula without probiotic. Serum IgE correlated with *E. coli* counts, but only correlated with *Bacteroides* in the highly sensitized allergic group. In this study, probiotics appeared to influence the gut’s microbial environment in a way that decreased the GALT’s food allergen-related inflammatory response and to provide a barrier effect against antigens that might otherwise ultimately lead to systemic allergic symptoms (such as eczema). The highly sensitized group of atopic infants may have different colonization
patterns, and may respond differently to probiotic therapy than those without strong correlation to IgE. In a recent study, atopic children with dermatitis received $2 \times 10^{10}$ CFU/g of *B. lactis* and *L. rhamnosus* (n=29) or placebo (n=30). Among food-sensitized atopic children, there was a significant improvement in SCORAD scores after 12 weeks of the probiotic combination.(129)

5.3 Growth, tolerance, and safety of *Bifidobacteria*

Numerous clinical trials with *Bifidobacteria*, including a growing number with *B. Lactis* have demonstrated adequate growth and have not documented any adverse events. Thus far, the only probiotic as having GRAS status for use in infant formula is *B. Lactis*. Infant formulas with *B. lactis* have been marketed for more than 15 years, worldwide, with no reports of bacteremia nor infection. The most detailed controlled evaluation yet, assessing tolerance and safety of long-term probiotic use in infant formula, at more than one dose, has been documented by Saavedra. In this study 118 healthy infants, aged 3-24 months, attending day care centers, were randomized into three groups. A first group received formula with $1 \times 10^{6}$ CFU/g of added *B. lactis* and *S. thermophilus*, a second received the same probiotics added to formula at a higher concentration ($1 \times 10^{7}$ CFU/g of added *B. lactis* and *S. thermophilus*) while a third group served as the control with no added probiotic. There was no significant difference in quantity of formula consumed among the groups throughout the study period of approximately 7 months. All infants showed adequate growth and tolerance and there was no difference in any negative health indicators between the groups, according to weekly parental reports. This study documented the safety of a minimum daily intake of $10^{6}$ CFU/kg of body weight during a consumption period of up to 18 months.(66)

In this same patient cohort, stooling patterns of the *B. lactis*-supplemented infants were described as having more soft bowel movements and a lower prevalence of hard bowel movements with a decreased frequency of stooling which might be characterized as a more desirable stooling pattern. A decreased prevalence of diaper rash in the supplemented groups was associated with the decreased frequency of loose stool.(120)

Studies have documented safety and adequate growth with *B. Lactis* in infants from birth(82) and in vulnerable populations including preterm infants,(90-97,209) malnourished infants,(213) and infants born to mothers with HIV disease.(75)
6 CONCLUSIONS

The concept of a mutually beneficial interaction between humans and the vastly diverse microbial environment we live in emerged as soon as we learned about the existence of these organisms. At the turn of the century, at a time when infectious agents were increasingly found to be responsible for many human ailments; Eli Metchnikoff, while working at the Pasteur Institute realized that not all bacteria in our intestinal tract were pathogenic, and went even further to propose that consuming lactic acid bacteria could actually be beneficial to human health.

Since then we have come a long way. Probiotics have evolved from a concept to a tangible potential intervention that can help maintain health. The term “probiotics,” however, comprises a heterogeneous group of organisms with different biologic activities and effects. Although in this review they are mostly discussed generically, recommendations for use by practitioners should carefully take into account the specific microorganism reported to have desirable effects or benefits, as well as their demonstration of safety for human consumption.

While much remains to be learned, clinical benefits from specific probiotic organisms are increasingly better documented, and the mechanisms that explain their effects continue to be elucidated. This is making possible the emergence of options to better balance our interaction and relationships with these microbes, for the purposes of improving the health and well being of infants and children.

- Probiotics are organisms that, when consumed, have a positive effect on the health of the host.
- There is extensive literature supporting the functionality and various potential clinical benefits have been reported.
- Research indicates that probiotic effects occur locally via the promotion of the gut’s physical barrier, and systemically by modulation of the host’s immune system.
- *Bifidobacteria* are uniquely appropriate probiotics for infants who are formula-fed because *Bifidobacteria* are the most common bacteria found in the flora of breastfed infants.
- *B. lactis* is among one of the most widely studied *Bifidobacteria*. *B. lactis* has been safely fed to infants and shows a potential probiotic benefit.
7 REFERENCES


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