Probiotics: defenders of gastrointestinal habitats

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Abstract

Intestinal microbiota play an important role in maintaining normal gastrointestinal (GI) function and ensuring that changes in the composition of the intestinal microbiota can promote GI function. The digestive tract is full of bacteria and many of these, including probiotics, are necessary for optimal digestive function. During bacterial gastroenteritis, harmful bacteria invade the digestive tract causing unpleasant symptoms and upsetting the balance between good and bad bacteria. Supplemental probiotics can help restore this balance. Studies have demonstrated that probiotics can often help reduce the severity of symptoms such as diarrhea and may help accelerate recovery. Probiotics are therapeutic preparations of live microorganisms administered in sufficient dosage to be beneficial to health. The therapeutic effects of these microorganisms appear to be strain specific. Primal Defense®, a unique, probiotic, bacterial compound, contains probiotics that support gut flora balance, promote consistent bowel function, control stomach acid levels to quickly eliminate burning sensation in the stomach and maintain immune system response. The probiotics in Primal Defense® maximize the benefits of a healthy diet by supporting normal absorption and assimilation of nutrients in the gut. Nearly 75% of our immune defenses are located in the digestive tract, so maintaining a favorable balance between good and bad gut bacteria is crucial to achieving and maintaining optimum health.

Introduction

The mucosa and the lumen of the mammalian gastrointestinal tract harbor complex communities of bacteria. These enteric microorganisms, often referred to as the indigenous or normal microbiota, belong to approximately 1000 species, the population size and distribution of which is variable along the gastrointestinal tract. Although the host has evolved various tolerogenic mechanisms to promote a stable and productive co-existence with its enteric microbiota, it remains highly responsive to enteropathogens. This ability of the intestine to discriminate between its indigenous microbiota represents a pivotal feature of efficient tolerance and homeostatic mechanisms.

Inflammatory bowel diseases (IBD), including ulcerative colitis (UC) and Crohn’s disease (CD), are spontaneously relapsing, immunologically mediated disorders of the intestinal tract. Homeostasis (tolerance) versus chronic intestinal inflammation is determined by either a regulated or an uncontrolled response of the host to the constant antigenic drive of enteric bacteria.4 In the genetically susceptible host, an ineffective mucosal barrier function, and the lack of appropriate mechanisms to terminate mucosal immune responses (loss of immunological tolerance) result in continuous stimulation of the mucosal immune system, leading to chronic inflammation.5

Although numerous studies have detailed the cell-mediated mucosal immune response in various animal models of chronic intestinal inflammation and in human IBD, very little is known about the molecular mechanisms of bacteria-specific crosstalk at the mucosal surfaces with respect to the development of chronic intestinal inflammation in the genetically susceptible host.6 In the present review, after describing the key players in innate and adaptive immune responses in the intestine, we focus on new insights into mechanisms underlying host-bacteria interaction in the context of intestinal inflammation.

Gastroenteritis

Gastric inflammation is a constant finding in patients infected with H. pylori and represents the host immune response to the organism. From a histological point of view, H. pylori-associated chronic gastritis is characterized by surface epithelial degeneration, infiltration of the mucosa by chronic inflammatory cells (lymphocytes, plasma cells, and occasional eosinophils), and a characteristic but variable active component consisting of neutrophils. Qualitative or quantitative differences in H. pylori-induced gastric mucosal inflammation may play a pivotal role in determining the varied clinical outcomes of infection. This review focuses on the interactions between the organism and host cells which lead to mucosal inflammation.

Colonization

The protective and immune barrier of the human gastrointestinal (GI) tract has various characteristics. It includes the epithelial layer, the mucous layer, the mechanics of peristalsis and desquamation, and secretory Immunoglobulin A (IgA) action, all of which have an impact on bacterial attachment. After attachment, colonic bacteria are prevented from mixing with the host’s eukaryotic cells by the epithelial layer, which acts as a vital barrier to invasion.7 The barrier’s healthy structure and proper functioning are essential for the health of the human host. In these complex systems, the delicate balance between the gastrointestinal tract and the microflora is cooperatively maintained (Figure 1).8

The GI tract is sterile until an infant ingests vaginal and fecal microflora during delivery. The population of microflora in the infant GI tract is further enhanced by feeding.9 The breast-fed infant contains a colon population of 90% Bifidobacteria with some Enterobacteriaceae and Enterococci present, but virtually no Bacteroides, Staphylococci, Lactobacilli, or Clostridia.10 In contrast, Bifidobacteria do not predominate in the bottle-fed infant. Breast-fed infants switched to cow’s milk or solid foods colonize Bifidobacteria, Clostridia, Lactobacilli, Bacteroides, Streptococci, and enterics.11 The type and number of indigenous microflora increase distally along the length of the gastrointestinal tract. The upper GI tract has relatively fewer bacteria secondary to saliva production and increased intestinal motility, which effectively move bacteria along the...
in the gastrointestinal barrier or in the composition of the microflora of the gut provide an opportunity for resultant malfunction and disease. For instance, overgrowth of one bacterial species can upset the ecosystem of the gut and result in derangement of beneficial characteristics (Figure 2).19

Overgrowth of one bacterial species or imbalances in microflora resulting from a disturbed mucosal layer can alter digestive function, intestinal products, and/or immunological function.20 In addition, a defective epithelial layer can allow bacteria to gain entry into the human host. This breach can arouse an inflammatory response in the host that has the potential to further alter normal function.21

Colonization of the gut with appropriate microflora contributes to its ability to function normally. Commensal microflora byproducts contribute to the health of the intestinal tract and include short-chain fatty acids (SCFAs), polyamines, vitamins, antioxidants, and amino acids.22 For example, the SCFA butyric acid, derived from carbohydrate fermentation, provides the main fuel for colonocytes in the large intestine. In addition, Lactobacillus species can prevent food decay, preserve antioxidants and vitamins, remove toxic food components, and prevent pathogenesis of Enterobacteriaceae, *S. aureus*, and Enterococci found in fermented foods.23

Probiotics modulate not only the endogenous flora of the GI tract but also the immune system. Lactobacilli augment both cellular and humoral immunity.24 Lactic acid-producing bacteria stimulate various aspects of the immune system, including phagocytic function of macrophages, natural killer cells, monocytes, and neutrophils. Clearly, interaction of commensal gastrointestinal flora with the gut-associated immune system is an important key to maintaining normal immune function.25

**Enzymatic digestion**

All metabolic processes in the body rely on enzymes for detoxification and energy production. Our bodies initially evolved to function on raw enzyme-rich foods that assist the digestive process and use little of our body’s natural enzyme supply and energy.26 The average diet of mostly cooked food, over time, depletes the body’s ability to produce enough enzymes to do all of the important functions they are designed to do, including fully digesting a meal. This results in partially digested fats and proteins being absorbed into the bloodstream, creating Floating Immune Complexes (FIC).27 These FICs overwhelm the immune system’s ability to dispose of them, so the body stores these harmful toxins in body tissues.28 Toxins in the bloodstream cause inflammation, uric acid crystals in joints (gout), allergic reactions, and may result in arterial plaque.29 Through time, this continued toxic condition can cause disease. Supplementing our meals with digestive enzymes supports the body’s ability to fully digest and utilize food and nutritional supplements.30 They also remove digestive remnants and waste products, leaving our pancreas and the other endocrine organs with the resources to produce the metabolic and systemic enzymes needed for such mechanisms as tissue repair and toxin removal.31 Friendly flora such as *Lactobacillus Acidophillus* and *bifidobacterium* are important to the intestinal tract for maintaining proper pH and also for controlling the population of potential pathogenic organisms like...
Clostridium and Candida. Plant flora enzymes have now been proven to be very effective in promoting the role of good bacteria to control pathogens. Another role of good bacteria is the actual synthesis of highly favorable natural chemicals in the colon through the fermentation process. These fermentive products include such molecular species as natural antibiotics and, very importantly, digestive enzymes. These enzymes can play an extremely important role in the digestion of otherwise incompletely digested food substances, especially proteins.33

Lactose maldigestion occurs frequently, especially in adults (primary lactose maldigestion) and in individuals with bowel resection or enteritis (secondary lactose maldigestion). It is well established that those with lactose maldigestion experience better digestion and tolerance of the lactose contained in yogurt than of that contained in milk.35 The mechanisms involved have been extensively investigated. The importance of the viability of lactic acid bacteria was only speculated because pasteurization reduced the observed digestibility.35 At least 2 mechanisms, which do not exclude each other, have been shown: i) digestion of lactose in the gut lumen by the lactase contained in the yogurt bacteria (the yogurt bacteria deliver lactase when lyzed by bile acids); and ii) slower intestinal delivery or transit time of yogurt compared with milk.36 In clinical practice, the replacement of milk with yogurt or fermented dairy products allows for better digestion, and decreases diarrhea and other symptoms of intolerance in subjects with lactose intolerance, in children with diarrhea, and in subjects with short-bowel syndrome.37 An enhanced digestion of a sucrose load was shown in infants with sucrase deficiency when they consumed Saccharomyces cerevisiae, i.e. a yeast that contains the enzyme sucrase. This is yet another example of a direct effect of a probiotic; however, its relevance in the treatment of sucrase deficient subjects has not been established.38

Probiotics

Effect on immunity

The gastrointestinal tract functions as a barrier against antigens from microorganisms and food. The generation of immunophysiological regulation in the gut depends on the establishment of indigenous microflora.39 This has led to the introduction of novel therapeutic interventions based on the consumption of cultures of beneficial live microorganisms that act as probiotics.40 Among the possible mechanisms of probiotic therapy is promotion of a non-immunological gut defense barrier which includes the normalization of increased intestinal permeability and altered gut microecology.41 Another possible mechanism of probiotic therapy is improvement of the intestine’s immunological barrier, particularly through intestinal IgA responses and alleviation of intestinal inflammatory responses, which produce a gut-stabilizing effect.42 Many probiotic effects are mediated through immune regulation, particularly through controlling the balance of proinflammatory and anti-inflammatory cytokines. Data show that probiotics can be used as innovative tools to alleviate intestinal inflammation, normalize gut mucosal dysfunction, and down-regulate reactions of hypersensitivity.43

Mechanism

The exact mechanism by which probiotics function in the GI tract can be defined as: i) competitive exclusion of enteric pathogens; ii) neutralization of dietary carcinogens; iii) production of antimicrobial metabolites; and iv) modulation of mucosal immune responses (Figure 3).44 The proper balance between good and bad bacteria largely determines the health of the gut and, as we are learning, the organism as a whole. Probiotics may help prevent an imbalance in which too many bad or harmful bacteria reside in the digestive tract.45 A growing body of evidence has emerged confirming the positive effect and potential of probiotics in humans. Recent research has implicated probiotics in the treatment of other diseases, including atopic eczema, autism, cancer, and food allergies.46 However, to date the vast majority of studies have focused on the defense and integrity of the intestinal flora and the immune system.47

Probiotics may take up residence in the body and neutralize the effects of offending bacteria. They colonize the exterior surface of cells in the GI tract and prevent potentially detrimental pathogenic organisms from proliferating. Probiotics also produce components shown to hinder the growth of certain types of harmful bacteria, as well as lower the risk for altered metabolic activity.48

The use of probiotics to prevent and treat a wide variety of conditions has gained favor in the past decade. This is in part due to a need to find alternatives to traditional therapies, such as antibiotics, as well as to the lack of efficient treatments for GI ailments.49 While there is an increasing number of reports of the efficacy of probiotics in the treatment of diseases such as pouchitis, diarrhea, and IBS, the scientific basis for the use of probiotics is just beginning to be understood. We will focus on the potential applications for probiotics in the treatment of diarrheal disease. Several examples will highlight how probiotics may be selected for and utilized against pathogens causing gastrointestinal disease.50

Gut defense

The role of the normal intestinal flora as an extremely important host defense mechanism is now beginning to be appreciated. In normal situations, an individual’s intestinal flora is highly effective in resisting colonization by potentially pathogenic invaders.31 The indigenous flora produce a variety of antimicrobial substances, including colicins and short chain fatty acids, which are potentially bacterial and bacteriostatic and are, therefore, consid—

Figure 3. Probiotic and beneficial effect in the intestine. Description of beneficial bacteria. Their serrated factors pathogen and intestinal mucosa.
erred to inhibit the growth of invading organisms. The intestinal immune system must be able to discriminate between potentially pathogenic microbial antigens and the non-pathogenic dietary and indigenous microbial antigens in order to avoid both invasive infections and chronic inflammatory conditions. The lack of response to dietary proteins and indigenous microbiota, known as oral tolerance, is the result of several distinct processes. Furthermore, there is growing evidence to suggest that resident microbiota provide the intestinal immune system with endogenous stimuli which are essential for its normal maturation and function.

The surface of mucosal membranes is protected by a local adaptive immune system. The gut-associated lymphoid tissue represents the largest mass of lymphoid tissue in the human body. Consequently, it constitutes an important element of the total immunological capacity of the host. The regulatory events of the intestinal immune response occur in different physiological compartments: aggregated in follicles and Peyer’s patches and distributed within the mucosa, the intestinal epithelium, and secretory sites. The intraepithelial T lymphocytes mainly exhibit a suppressor and cytotoxic phenotype, whereas the lamina propria cells exhibit a helper and inducer phenotype. The lamina propria is endowed with lymphocytes belonging to the cell lineage. IgA antibody production is abundant at mucosal surfaces. In contrast with IgA in serum, secretory IgA is present in dimeric or polymeric form. Secretory IgA is resistant to intraluminal proteolysis and does not activate complement or inflammatory responses, which makes secretory IgA ideal for protecting mucosal surfaces. There are differences between the upper and lower parts of the human gut-associated immune system in the isotype distribution of immunoglobulin-producing cells. IgA1 immunocytes predominate in the small intestine, whereas IgA2-producing cells are most frequent in the colon, the latter being more resistant to bacterial proteases.

Lymphocytes

The lymphocyte maturation cycle involves antigen transport across Peyer’s patches and the presentation of antigens to T lymphocytes of a helper and inducer phenotype, which proliferate and induce cell response. The specific antibody-secreting lymphocytes appear in peripheral blood 2-4 days after antigen exposure, reach a maximum concentration after 6-8 days, and persist in the blood for 2-3 weeks. Studies show that these cells can reside in the gut. Homing receptors on lymphocytes, which interact with ligands on endothelial cells, target the migration of lymphocytes into tissues. Antigen-specific systemic suppression after oral antigen introduction can be seen after 1-2 days and oral tolerance to systemic challenge becomes established within 5-7 days. Lymphocytes, particularly those of B-cell lineage, can induce enterocytes into M-cell-like cells, a unique epithelium that comprises cuboidal epithelial cells, very few goblet cells, and specialized antigen sampling cells, which are typical to Peyer’s patches. These cells effectively transfer particles and microbes from the gut lumen into underlying follicles. The induction of gut-secreting B cells, i.e., by probiotics, may influence mucosal immunity beyond the secretion of IgA. In addition, intraepithelial and lamina propria sites are activated to produce protective cytokines, and mesenteric lymph nodes secrete polymeric IgA (pIgA). It has recently been demonstrated that dendritic cells in the lamina propria can extend their appendices between epithelial cells and, via TLR-2 and TLR-4 on their surface, they can sample commensal bacterial molecular patterns. The interaction leads to maturation of the dendritic cells and to the release of cytokines, which orchestrate the conversion of precursor T-helper cells (Th0) into the mature, balanced response of the four effector T-helper cell types (Th1, Th2, and Th3/Th1), an important component in the prevention of disease. Furthermore, commensal bacteria can cross microfold cells and interact with antigen-presenting cells in mesenteric lymph nodes to activate naive plasma cells into becoming pIgA-producing B cells. pIgA, in turn, coats the mucosal surface to control subsequent microbial and antigen penetration.

There is an intimate interplay between different subsets of T cells and antigen-presenting cells, such as dendritic cells, in the intestine. In murine models, high basa levels of IL-4, IL-10 and IFN-α expression have been detected in the intestinal mucosa and this cytokine milieu may be crucial for the induction of Th2 and Th3 type responsiveness. However, as recently pointed out, there may be major differences between species in mucosal immune responses. In fact, reported data indicate a Th1-skewed cytokine profile as a constant finding in the human intestine. A transient induction of IFN-γ producing Th1 cells has been detected in the early phases of oral tolerance formation. Even though both Th1 and Th2 cytokines regulate the function of Th3 cells, neither is essential for the induction of peripheral tolerance in a murine model. Therefore, the individual role of each functional subset of T cells in the inductive phase of oral tolerance remains to be clarified, but it is evident that Th3 cells provide tolerogenic suppression both in the intestine and in other target organs. The small intestine is challenged by a myriad of antigens encountered by way of the enteric route. Furthermore, the small intestine is exposed to rapid and constant changes in the composition of the antigen load. Most antigens are excluded by a well-functioning mucosal barrier in the gut. In addition to the first line of gut defense, immune exclusion and specialized antigen transport mechanisms are to be found in the villous epithelium. Antigens are absorbed across the epithelial layer by transcytosis; here, the main degradative pathway entails lysosomal processing of the antigen. This second line of defense, immune elimination, is directed toward the removal of antigens that have penetrated the mucosa. A minor pathway allows for the transport of unprocessed antigens. Peyer’s patches, crucial in determining the subsequent immune responses to the presence of the antigen, are covered by the M cells. In general, antigen transport across this epithelium is characterized by rapid uptake and reduced degradation. Antigens are presented to subjacent T cells; these differentiate into various effector cells that mediate active immune suppression and promote the differentiation of IgA-secreting B cells. As a result of the absorption process across the intestinal mucosa, dietary antigens are altered into a tolerogenic form (Figure 3). Consequently, hyporesponsiveness to antigens, e.g., food proteins, and oral tolerance are hallmarks of the intestinal immune system.

Immune response

There is strong scientific evidence to support the effects of probiotics on the immune system, providing irrefutable proof that certain probiotic strains play a role in modulating both non-specific and specific host immune responses. Non-specific, or innate, immune responses are a host’s first line of defense. Natural killer cells and phagocytes, residing in the peripheral blood and tissues, are the major cellular effectors of non-specific immunity. Natural killer cells effectively fight off viruses, whereas phagocytic cells protect against microbial infections. Both produce a variety of compounds that can destroy both invasive materials as well as normal tissues. The enormous implications of this are discussed below.

Specific immune responses can be separated into two categories: humoral immunity, and cell-mediated immunity. In the humoral immune response, B lymphocytes synthesize specific immunoglobulin molecules, or antibodies, that are excreted from the cell and bind to the invading substance. In the cellular
immune response, T lymphocytes, bearing immunoglobulin-like molecules on their surfaces, recognize and kill foreign or aberrant cells. T cells can be divided into 2 subtypes according to their cytokine profile: Th1 and Th2. Th1 cells are essential to cell-mediated immunity and produce IL-2, IFN-γ and tumor necrosis factor α (TNF-α). The main products of Th2 are IL-4, IL-5, and IL-10 and are associated with humoral immunity and allergic responses (Figure 3).

Dietary consumption of certain probiotic strains have been shown to enhance non-specific immunity, including phagocytosis and lymphocyte proliferation demonstrating the effectiveness of probiotics in stimulating cellular immune responses. Healthy middle-aged and elderly men and women have been shown to experience a significant enhancement of cell phagocytosis and natural killer cell tumor killing activity following twice daily consumption of *B. lactis*. The authors suggest that the enhanced immunity observed in relation to the *B. lactis* may be largely related to the secretion of pro-inflammatory cytokines, such as interleukin-12 and interleukin-18, which stimulate natural killer cell activity and interferon production. These results support that another study in which consumption of *B. lactis* was positively associated with increases in the total proportions of T lymphocytes and natural killer cells.

Probiotics play an essential role in the intestinal mucosal barrier, including modulating intestinal immune response and competitively inhibiting the adhesion of pathogenic bacteria to the epithelial wall of the intestine. Intestinal epithelium plays an important role in innate immunity. When stimulated by cytokines, such as TNF-α, the intestinal epithelia release pro-inflammatory cytokines, including IL-8 and IL-10. However, in some gastrointestinal diseases, such as IBD and acute gastroenteritis, cytokines are activated in innate immunity. When stimulated by *E. coli* infection of the gut mucosa, cytokines are released which stimulate the gut immune system. As a result, the gut immune system experiences a significant enhancement of immunity observed in relation to the *B. lactis*.

**Specific immunity**

*Lactobacillus rhamnosus* on cytokine secretion and T-lymphocyte activation, thus demonstrating the positive immunomodulating effects of oral administration of lactic acid bacteria. Healthy participants taking a daily oral dose of 2 billion colony forming units (cfu) of *L. rhamnosus* experienced a reduced secretion of pro-inflammatory TNF-α and increased IL-10 and IL-4 activity. TNF-α is key to the pathogenesis of altered mucosal immunity. A critical factor in the pathogenesis of Crohn’s disease (CD) is the secretion of TNF-α by T lymphocytes. Co-cultures of inflamed tissue with various probiotic strains have been proven to significantly reduce TNF-α secretion. Since transcriptional control of IL-8 is mediated by transcription factor NF-κb, it has been hypothesized that the normal intestinal microflora down-regulates inflammation by inhibiting NF-κb activation. This hints at a possible probiotic genomceutical intervention.

As we have seen, therapeutic administration of probiotics is often advocated for their immunomodulatory properties and anti-inflammatory activities at mucosal barrier sites. However, only recently have the molecular mechanisms by which probiotics modulate immune responses been clarified (Table 1). Immunostimulating DNA sequences have been shown to effectively reduce or prevent symptoms of colitis in animal studies. Furthermore, administration of irradiated probiotics significantly improve experimental colitis in murine models, as do viable probiotic strains, suggesting that the anti-inflammatory activities associated with probiotics are mediated by their own DNA, rather than products of their metabolism or intestinal colonization. This theory is further supported by data suggesting that genomic DNA released by exogenous bifidobacteria provide a stimulus for mucosal IL-10 production in human peripheral blood mononuclear cells. The interesting conclusion that may be made in the light of this research is that dead bacteria ingested during probiotic administration have a therapeutic effect in addition to the viable cells.

The ability of probiotics to confer enhanced humoral and cell-mediated resistance against pathogens has been well-documented. For example, it was demonstrated that a significant increase in lymphocyte proliferative responses, phagocytic capacities, and localized antibody production occurs in response to oral administration of lactic acid bacteria in mice infected with *Salmonella typhimurium*. *Lactobacillus casei* has been associated with increases in specific mucosal and serum antibody responses in children with acute rotavirus diarrhea.

The inhibitive effect of probiotics on pathogens is generally dependent on the reduction of pathogen viability or through interference with adhesion and/or invasion of the pathogen. However, in a study in which Lactobacillus strains were tested in an *in vitro* model of enterohemorrhagic *Escherichia coli* infection of a human colon epithelial cell line, the protective effect was due to the presence of viable *L. rhamnosus* cells. In this model, killed *L. rhamnosus* and other *Lactobacillus* strains did not have any impact on the inhibitory effect. Because the positive effect of *L. rhamnosus* was not dose-dependent, it was...
postulated that an intimate interaction between the host cell responses occurred, thereby minimizing the internalizing reaction.\textsuperscript{113}

Ingestion of specific probiotics has been shown to have immunomodulatory effects on many aspects of humoral and cell-mediated immunity. In one study, designed to examine the relationship between oral administration of probiotics and immunity in mice, the results indicate strain-dependent variation in the ability of probiotics to influence T-cell activation.\textsuperscript{114}

The type of T-cell response, whether it be a Th1, Th2, or Th3/Tr1 response, is controlled predominantly by interactions between DC and T cells. In humans, induced IL-10 by DC and co-culture of naïve T cells with probiotic-treated DC led to a decrease in Th1 polarized cells. In a different experimental system in which monocye-derive DC were cultured with the probiotic \textit{L. rhamnosus} and the subsequent effect on T cells was assessed, decreased T-cell proliferation and T-cell cytokine production, particularly IL-2, IL-4, and IL-10, was demonstrated.\textsuperscript{115} This \textit{in vitro} effect of \textit{L. rhamnosus} on DC and subsequent T-cell hyperresponsiveness was reflected in \textit{in vivo} studies in which healthy controls and patients with CD were fed \textit{L. rhamnosus} for two weeks.\textsuperscript{116} Ingestion of \textit{L. rhamnosus} reduced IFN-γ and IL-2 production by peripheral T cells in CD patients and also reduced IL-4 production in healthy controls.\textsuperscript{117} Probiotic bacteria influence the generation of regulatory T cells in a murine model of contact dermatitis. Daily oral administration of fermented milk containing the probiotic \textit{L. casei} DN-114 001 reduced antigen-specific skin inflammation by controlling the antigen-specific T-cell response in hapten 2,4-dinitrofluorobenzene, a model of allergic contact dermatitis mediated by CD8+ CTL and controlled by CD4+ regulatory T cells.\textsuperscript{118} The alleviation of contact hypersensitivity by prior feeding with \textit{L. casei} was due to downregulation of the hapten-specific CD8+ T-cell response as indicated by a decrease in expansion of hapten-specific IFN-γ-producing CD8+ effectors. Furthermore,

<table>
<thead>
<tr>
<th>Probiotic</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{B. longum}</td>
<td>Improvement of clinical appearance of chronic inflammation in patients, decreases in TNF-α and IL-1α s.\textsuperscript{200}</td>
</tr>
<tr>
<td>\textbf{BIFICO} (3 bifidobacteria species)</td>
<td>Prevention of flare-ups of chronic ulcerative colitis, inactivation of NF-κB, decreased expressions of TNF-α and IL-1β and elevated expression of IL-10.\textsuperscript{201}</td>
</tr>
<tr>
<td>\textit{L. salivarius} ssp. \textit{salivarius} CECT5713</td>
<td>Recovery of inflamed tissue in TNBS model of rat colitis, increase in TNF-α and iNOS (inducible NO synthase) expression.\textsuperscript{202}</td>
</tr>
<tr>
<td>\textit{L. fermentum}, \textit{L. casei}</td>
<td>Improvement of histology in a TNBS model of rat colitis, decreased levels of TNF-α and i-NOS expression.\textsuperscript{203}</td>
</tr>
<tr>
<td>\textit{L. casei} \textit{Shirota}</td>
<td>Improvement in murine chronic inflammatory bowel disease, downregulation of proinflammatory cytokines such as IL-6 and IFN-γ.\textsuperscript{204}</td>
</tr>
<tr>
<td>\textit{L. casei} DN-114 001</td>
<td>Reduction in numbers of activated T lymphocytes in the lamina propria of Crohn’s disease mucosa, decrease of IL-6 and TNF-α.\textsuperscript{205}</td>
</tr>
<tr>
<td>\textit{L. plantarum} 299v</td>
<td>Decreased IL-12, IFN-γ and IgG2a at the mucosal level of specific pathogen free IL-10 KO mice. Decreased mesenteric lymph node IL-12 and IFN-γ production as well as histological colitis scores in the pre-treatment of GF mice that were exposed to normal flora.\textsuperscript{206}</td>
</tr>
<tr>
<td>\textit{L. rhamnosus} GG</td>
<td>Allowing intestinal inflammation, decrease in TNF-α. Specific inhibition of macrophages TNF-α production by a contact independent mechanism.\textsuperscript{207}</td>
</tr>
<tr>
<td>\textit{L. salivarius} UCC118</td>
<td>Reduced production of proinflammatory cytokines in IL-10 KO mice injected subcutaneously with the probiotic strain.\textsuperscript{208}</td>
</tr>
<tr>
<td>\textit{L. salivarius} UCC118</td>
<td>Reduction of C. perfringens, coliforms and enterococcus levels in IL-10 KO mice. Production of a peptide that inhibits a broad range of pathogens such as \textit{Bacillus}, \textit{Staphylococcus}, \textit{Enterococcus}, \textit{Listeria} and \textit{Salmonella} species.\textsuperscript{209}</td>
</tr>
<tr>
<td>\textit{L. reuteri}</td>
<td>Decreased concentration of colonic \textit{Lactobacillus} species and increased concentration of mucosal adherent bacteria associated with colitis attenuation.\textsuperscript{210} Delayed relapse into pouchitis after surgical resection in human patients.\textsuperscript{211} \textit{L. rhamnosus} \textit{GG}, \textit{L. rhamnosus} \textit{Lc705}, \textit{P. freudenreichii} ssp. \textit{shermanii} \textit{JS} and \textit{B. breve} \textit{Bb99}.\textsuperscript{212} Alleviating IBS symptoms.\textsuperscript{213}</td>
</tr>
<tr>
<td>\textit{B. and L. plantarum}</td>
<td>Improvement of the disease activity index in an induced rat colitis model.\textsuperscript{214}</td>
</tr>
<tr>
<td>\textit{L. rhamnosus} GG</td>
<td>Improvement in the clinical status in children with mildly to moderately active stable Crohn’s disease.\textsuperscript{215}</td>
</tr>
<tr>
<td>\textit{L. casei} \textit{Shirota}</td>
<td>Improvement in the clinical condition of murin DSS model of ulcerative colitis.\textsuperscript{216}</td>
</tr>
<tr>
<td>\textit{L. plantarum} \textit{NCIMB8826}AD1t</td>
<td>Reduction in secretion of proinflammatory cytokines by peripheral blood mononuclear cells and monocytes and increase in IL-10 production in a murine colitis model.</td>
</tr>
<tr>
<td>\textit{Lc. lactis} IL-10</td>
<td>Reduction in colitis in mice treated with DSS and prevention of the onset of colitis in IL-10 knockout mice.</td>
</tr>
<tr>
<td>\textit{L. casei} BL23 MinKat</td>
<td>Reduction in cecal and colonic inflammatory scores in a DSS-induced colitis model. Significant reduction of physiological damage in a TNBS-induced colitis model.\textsuperscript{218} Slight increase in catalase activity in the intestines and prevention of colon cancer in mice administered the cancer-inducing drug DMH.</td>
</tr>
<tr>
<td>\textit{Lc. lactis} N29811 and \textit{L. plantarum} NCIMB8826+pNZ804 sodA</td>
<td>Reduction in macroscopic damage in rats administered TNBS to induce colitis.\textsuperscript{19,30,10}</td>
</tr>
<tr>
<td>\textit{L. gasseri} NC1501 (sod overexpression)</td>
<td>Reduction in inflammation in IL-10 deficient mice.\textsuperscript{210}</td>
</tr>
<tr>
<td>\textit{L. casei} BL23 + sodA</td>
<td>Attenuation of colonic histological damage scores in a DSS-induced colitis model. Significant reduction in physiological damage in a TNBS-induced colitis model.\textsuperscript{12}</td>
</tr>
</tbody>
</table>
experiments in mice deficient in CD4+ cells indicated that these cells are mandatory for the effect of L. casei on contact hypersensitivity. It was proposed that L. casei reduced contact hypersensitivity by direct or indirect activation of regulatory CD4+ T cells. IL-10-dependent regulatory CD4+ T cells bear surface TGF-β. These cells appear to be similar to CD25+ regulatory T cells that inhibit cell-transfer colitis by a TGFβ-dependent mechanism.

Clinical applications

The effect of probiotics on bacteria

Probiotics reduce plasma levels of bacterial endotoxin concentrations, at least in part, by inhibiting translocation of bacteria across the GI lumen into the bloodstream. Lactobacillus colonization in germ-free rats has been shown to decrease gut permeability to mannitol. In addition, administration of Lactobacillus to IL-10 knockout mice decreased translocation of bacteria to extraintestinal sites and reduced myeloperoxidase concentrations, often associated with inflammation in the bowel. Decreases in translocation of bacteria may occur as a result of the ability of probiotics to tighten the mucosal barrier. Although very little is known about specific molecular mechanisms by which indigenous flora tighten the mucosal barrier, this may be accomplished by bacterial-epithelial crosstalk and upregulation of growth factors and receptor sites. Whatever the method of barrier to bacterial entry, the net effect is to modulate systemic intestinal allergy and inflammation. Allergy-induced intestinal inflammation mediated by focal tumor necrosis factor-alpha is decreased by Lactobacillus. Lactobacillus also increases mucosal regeneration and reduces fecal urease production, a correlate of inflammation associated with chronic arthritis. There are several ways probiotic microflora can prevent pathogenic bacteria from adhering and colonizing the gut mucosa (Table 2). Probiotics disallow colonization by disease-provoking bacteria through competition for nutrients, immune system upregulation, production of antitoxins, and upregulation of intestinal mucin genes. Increased mucous production prevents adherence and colonization by competing microflora, thereby preventing any imbalance. Therefore, the practice of combining probiotics needs to include beneficial bacteria that do not inhibit other included strains.

Adherence of normal, beneficial flora competitively inhibits colonization of the mucosa by pathogenic bacteria and reduces overstimulation of the immune system. A healthy colon with adequate mucus production and appropriate bacterial colonization prevents the adherence of pathogenic bacteria, modulates inflammatory processes, and prevents widespread inflammatory disorders.

Probiotics used to treat intestinal ailments such as irritable bowel syndrome are antagonistic within a specific spectrum by inhibiting other strains of Lactobacilli. Therefore, the practice of combining probiotics needs to include beneficial bacteria that do not inhibit other included strains.

Potential benefits of probiotics for preventing antimicrobial-associated diarrhea have yielded mixed results. One prevention study reported a reduction in incidence of antimicrobial-associated diarrhea in infants by 48%. Probiotics may also be incorporated in treatment regimens for infectious gastroenteritis. Several meta-analyses of numerous clinical trials with different probiotics documented reductions in the length of the disease course of gastroenteritis that ranged from 17 to 30 h. Examined in another way, meta-analyses of probiotics used in clinical trials of gastroenteritis noted significantly reduced incidence of diarrhea lasting longer than three days (i.e. prolonged diarrhea). The incidence of prolonged diarrhea was diminished by 30% or 60%, respectively, depending on the study. The probiotic agent, LGG, contributed to a significant reduction in rotavirus diarrhea after three days of treatment administered to children as part of oral rehydration therapy. In addition, probiotics are promising agents for preventing and treating antimicrobial-associated diarrhea, although intention-to-treat analyses have not demonstrated any benefit.

Antibiotic-associated diarrhea

One of the most well-recognized uses of probiotics is for diarrheal diseases. The prevention and management of acute viral and bacterial diarrhea, as well as the control of antibiotic-associated diarrhea, are areas of significant potential benefit. A number of specific strains, including Lactobacillus GG, L. reuteri, Saccharomyces boulardii, Bifidobacteria species, and others, have been shown to have significant benefit for diarrhea. In the pediatric population, probiotics appear to benefit viral diarrhea, possibly by increasing secretory IgA and decreasing viral shedding, suggesting an immunological mechanism. Although numerous different probiotic strains, doses, and populations in these studies make it difficult to generalize, it is clear probiotic agents are becoming an important tool in the treatment of gastrointestinal problems in infants and children.

Although gastroenteritis as the cause of acute diarrhea may resolve spontaneously within a few days, it can be associated with morbidity, and increased direct and indirect medical costs. Acute diarrheal episodes can be related to viral, bacterial, or parasitic pathogens. Several studies have demonstrated improvement when acute diarrheal disorders, including rotavirus infection, traveler’s diarrhea, and more serious bacterial infections such as Clostridium difficile, are treated with probiotics. Importantly, studies using Lactobacillus species or Saccharomyces boulardii suggest a beneficial role during C. difficile-related infections. In populations with
small bowel bacterial overgrowth, and in particular those with short bowel syndrome, Lactobacillus species were shown to be efficacious in ameliorating the symptom complex. However, in 10 patients during a 7-day, double-blind, randomized trial comparing antibiotic therapy to Saccharomyces boulardii, S. boulardii was ineffective in eliminating overgrowth of small bowel intestinal bacteria once it was established.

In vitro studies demonstrate probiotic agents inhibit adherence of dysbiotic organisms to intestinal epithelial cells. This inhibition is hypothesized to be mediated through the ability to increase expression of MUC2 and MUC3 intestinal mucins. Bacterial-to-epithelial cell binding is a multi-stage process, the first stage of which is characterized by an initial interaction of bacteria with the enterocyte layer. Probiotics increase intestinal mucin production, which prevents the attachment of enteropathogens. The attachment could be prevented by steric hindrance (a slight structural difference in the bacterial ligand interfering with proper attachment to the receptor) or through competitive inhibition for attachment sites on mucins mimicking epithelial cell bacterial attachment sites. Enhancement of innate defense mechanisms in the gastrointestinal tract, such as mucin production, might be preventive or therapeutic, but this still remains to be clarified.

**Probiotics in irritable bowel syndrome**

Probiotics exhibit a direct effect in the gut in the treatment of inflammatory and functional bowel disorders. In one of the most common functional bowel disorders, IBS, Lactobacillus strains were shown in clinical trials to reduce abdominal pain, bloating, flatulence and constipation. It was also observed that Saccharomyces boulardii decreased diarrhea in IBS, but was not effective in alleviating other symptoms of the syndrome.

IBS is a widespread and multifactorial functional disorder of the digestive tract. It affects 8-22% of the population with a higher prevalence in women. It accounts for 20-50% of referrals to gastroenterology clinics and is characterized by abdominal pain, excessive flatus, variable bowel habit and abdominal bloating for which there is no evidence of detectable organic disease. Suggested etiologies include gut motility and psychological disorders as well as psycho-physiological phenomena and colonic fermentation. A large proportion of patients have periods of time characterized by sudden and unforeseeable changes in the two main symptoms, constipation and diarrhea, even within a few days. It is very likely that the syndrome represents different groups of patients probably with a different pathogenesis. IBS may follow gastroenteritis and may be associated with an abnormal gut flora and with food intolerance. The fecal microflora in some of these patients has been shown to be abnormal with higher numbers of facultative organisms and low numbers of lactobacilli and bifid bacteria.

Bacteria are the major component of formed stools and are influenced by substrates arriving with the ileal affluent. Stool production is related to quantitative and qualitative aspects of the colonic microflora and nearly 80% of the fecal dry weight consists of bacteria, 50% of which are viable.

Although there is no evidence of food allergy in IBS, food intolerance has been identified and exclusion diets are beneficial to many of these patients. Food intolerance may be caused by an abnormal fermentation of food residues in the colon as a result of disruption of the normal flora.

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**Table 2. Clinical application of probiotics.**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Probiotic</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD</td>
<td>S. boulardii</td>
<td>Relapse in 6% of patients supplemented with probiotic strain versus 38% with conventional treatment only. Median pediatric CD activity index scores at four weeks were 73% lower than baseline and intestinal.</td>
</tr>
<tr>
<td>CD</td>
<td>Lactobacillus</td>
<td>The number of specific antibody secreting cells in the IgA class to b-lactoglobulin increased significantly from 0.2 (0.04-1.3) to 1.4 (0.3-6.0)/10⁶ cells and to casein from 0.3 (0.1-1.4) to 1.0 (0.2-4.8)/10⁶ cells.</td>
</tr>
<tr>
<td>IBS</td>
<td>B. infantis 35624</td>
<td>Alleviation of IBS symptoms and normalization of the antinflammatory-proinflammatory ratio.</td>
</tr>
<tr>
<td>IBS</td>
<td>BIFICO (3 bifidobacteria species)</td>
<td>Relapse in 20% of patients in probiotic group versus 93% in the placebo group. The probiotic impeded the activation of NF-κb, decreased the expressions of TNF-α and IL-1β and increased the expression of IL-10.</td>
</tr>
<tr>
<td>IBS</td>
<td>L. rhamnosus GG, L. rhamnosus Lc785, P. freudenreichii spp shermanii IS, B. breve Rb99</td>
<td>The total symptom score (abdominal pain+distension+flatulence+borborygmi) was reduced 42% in the probiotic group compared with 6% in the placebo group.</td>
</tr>
<tr>
<td>IBS</td>
<td>Prescript-Assist (probiotic+prebiotic complex containing 29 soil-based, pH-resistant microflora)</td>
<td>The probiotic + prebiotic treatment showed short-term and long-term reductions in IBS symptoms.</td>
</tr>
<tr>
<td>IBS</td>
<td>Prescript-Assist</td>
<td>The probiotic+prebiotic treatment was associated with significant reductions in 3 sub-syndromic factors of IBS: 1) general ill feelings; 2) nausea and indigestion; 3) flatulence and colitis.</td>
</tr>
<tr>
<td>PCH</td>
<td>VSL#3 (probiotic preparation containing 3 B, 4 L and 1 S strains)</td>
<td>The probiotic mixture was effective in maintaining antibiotic-induced remission for at least one year in patients with recurrent or refractory pouchitis (85%) versus 6% in the placebo group.</td>
</tr>
<tr>
<td>PCH</td>
<td>VSL#3</td>
<td>10% of patients treated with probiotics had an episode of acute pouchitis compared with 40% treated with placebo. Treatment with probiotic improved IBS Questionnaire score versus placebo.</td>
</tr>
<tr>
<td>UC</td>
<td>E. coli Nissle 1917</td>
<td>The probiotic treatment was just as effective as the conventional treatment (mesalazine) in maintaining remission.</td>
</tr>
<tr>
<td>UC</td>
<td>VSL#3</td>
<td>Probiotic supplementation improved remission compared to conventional treatment (balsalazide) alone.</td>
</tr>
<tr>
<td>UC</td>
<td>VSL#3</td>
<td>Probiotic preparation maintains remission (75%) and there was no relapse (0%) of intestinal disease while on probiotics.</td>
</tr>
</tbody>
</table>
To assess whether preceding gastroenteritis or food intolerance were associated with colonic malfermentation, Bamford et al. conducted a crossover controlled trial with a standard diet and an exclusion diet matched for macronutrients in 6 female patients with IBS and 6 female controls. In this study, fecal excretion of fat, nitrogen, starch, and non-starch polysaccharide was measured during the last 72 h of each diet. The total excretion of hydrogen and methane were collected over 24 h in a purpose-built 1.4 m³ whole body calorimeter. Breath hydrogen and methane excretion were measured for 3 h after 20 g oral lactulose. The maximum rate of gas excretion was significantly greater in patients than in controls. The total gas production in patients was not higher than in controls, whereas hydrogen production was higher on the standard than on the exclusion diet. This means that colonic-gas production, particularly of hydrogen, is greater in patients with IBS than in controls, and both symptoms and gas production are reduced by an exclusion diet. This reduction may be associated with alterations in the activity of hydrogen-consuming bacteria. It was, therefore, concluded that fermentation may be an important factor in the pathogenesis of this syndrome.

Studies were carried out on the intestinal permeability (Lactulose/mannitol ratio) and histological and immunological features in rectal biopsy specimens from 21 patients with acute Campylobacter enteritis, 10 patients with post-dysenteric IBS, and 12 asymptomatic controls. They found that the increased enteroeocrine cell counts, T lymphocytes, and gut permeability, which may survive for more than a year after Campylobacter enteritis, contribute to post-dysenteric IBS, thus offering a rationale to use probiotics for several months after the infectious episode.

**Improved lactose digestion and other direct enzymatic effects**

Lactose malabsorption occurs frequently, especially in adults (primary lactose malabsorption) and in individuals with bowel resection or enteritis (secondary lactose malabsorption). It is well-established that persons with lactose malabsorption experience better digestion and tolerance of the lactose contained in yogurt than of that contained in milk. The mechanisms involved have been extensively investigated. There has been speculation concerning the importance of the viability of lactic acid bacteria since pasteurization reduced the observed digestibility. At least 2 mechanisms, which do not exclude each other, have been shown: i) digestion of lactose in the gut lumen by the lactase contained in the yogurt bacteria (the yogurt bacteria deliver lactase when lyzed by bile acids); and ii) slower intestinal delivery or transit time of yogurt compared with milk. In clinical practice, the replacement of milk with yogurt or fermented dairy products allows for better digestion, and decreases diarrhea and other symptoms of intolerance in subjects with lactose intolerance, in children with diarrhea, and in subjects with short-bowel syndrome. An enhanced digestion of a sucrose load was shown in infants with sucrase deficiency when they consumed Saccharomyces cerevisiae, i.e. a yeast that contains the enzyme sucrase.

This is yet another example of a direct effect of a probiotic; however, its relevance in the treatment of sucrase deficient subjects has not been established.

**Ulcerative colitis**

Patients with mild to moderate active colitis, who had been unresponsive or intolerant to standard therapy, received 20-30 g of a prebiotic fermented barley drink in a non-randomized, open-label fashion. At four weeks, this treatment resulted in significant clinical and endoscopic improvement. Previous studies with this prebiotic demonstrated positive effects on epithelial cell restitution, suppression of nuclear factor-B binding activity, increased short chain fatty acid production, and enhanced growth of probiotic bacterial strains. Fujimori et al. conducted a randomized trial of the use of Bifidobacterium-fermented milk in the treatment of ulcerative colitis. Eleven subjects received the Bifidobacterium-fermented milk for one year, whereas the control group did not. Exacerbation of symptoms was seen in 3 of the 11 subjects in the group treated with Bifidobacterium-fermented milk and in 9 of 10 in the control group (P=0.01). Analysis of the microflora and the organic acids of the feces demonstrated a significant reduction in the relative proportion of B. vulgatus and in Bacteroidaceae. In an open-label pilot trial with S. bouardi, a group of 25 patients with mild to moderate ulcerative colitis received S. bouardi for four weeks in addition to mesalamine. Of the 24 patients who completed the study, 17 achieved clinical remissions.

Finally, human fecal rectal infusions in 6 selected patients with ulcerative colitis were carried out in a novel protocol. Fecal flora donors were healthy adults. Fecal suspensions were administered as a retention enema daily for five days. Full clinical remission and cessation of ulcerative colitis medication were achieved in all patients. Interestingly, at 1-3 years after human fecal infusion, all patients were free of endoscopic and histological evidence of ulcerative colitis.

**Crohn’s disease**

In keeping with the concept of an altered probiotic profile in patients with CD, studies using molecular methodology to examine RNA demonstrated that enterobacteria were significantly increased in active quiescent CD and significantly lower in healthy controls. Interestingly, 30% of the dominant flora belonged to as yet unidentified phylogenetic groups.

Different probiotic strains may have differential effects in patients with CD. Ileal specimens from patients with CD were cultured with various probiotic agents. Release of TNF-α by inflamed mucosa was significantly reduced by co-culture with L. casei or Lactobacillus bulgaricus but not with Lactobacillus crispatus or E. coli.

Clinical trials with probiotics have shown inconsistent results in treating adult CD (Table 1). A small pediatric non-randomized pilot study suggested that Lactobacillus GG may improve gut barrier function and clinical status in children with mildly to moderately active stable CD. However, in a larger controlled double-blind pediatric study, Lactobacillus GG did not prolong time to relapse in children with CD.

**Pouchitis**

Pouchitis is a non-specific inflammation of the ileal reservoir that may appear after surgery for ulcerative colitis, and results in various clinical symptoms. It is a well-recognized long-term complication of restorative proctocolectomy.

The risk of pouchitis increases in patients with a history of extra-intestinal manifestations, primary sclerosing cholangitis, positive serology for perinuclear anti-neutrophil cytoplasmic antibodies, and backwash ileitis. Pouchitis is associated with bacterial overgrowth and dysbiosis, and antibiotics represent the treatment of choice. The distal ileum and the large bowel, the sites with the highest bacterial concentration, are the most frequently affected by inflammation. Enteric bacteria or their products have been detected within the inflamed mucosa.

A significant decrease in lactobacilli and bifidobacteria concentrations has been found in ulcerative colitis, CD and pouchitis. Lactobacilli as maintenance showed less frequent relapses of pouchitis than those using placebo. Diversion of the fecal stream in the small and large intestine reduces the inflammatory action. The luminal contents and purified bacterial products added to isolated intestinal loops trigger systemic and local signs of inflammation.
In a study by Campieri et al., 179 7 patients, after clinical, endoscopic, and histological diagnoses of inflammation of the ileal pouch anal anastomosis with a pouchitis disease activity index of more than 7, were treated with 2 g/day of rifaximin (a non-absorbable antibiotic) and 1 g/day of ciprofloxacin for one month. 198

All patients went into remission during this month, as judged by clinical, endoscopic and histological examination. After remission, all 7 patients were treated with the highly concentrated probiotic mixture VSL3 for nine months. No patient relapsed in this period. All patients who received placebo relapsed. 199

Intestinal microbiota play an important role in maintaining normal GI function and ensuring that changes in the composition of the intestinal microbiota can contribute to the development of GI function (Table 2). 200-219

Probiotics maximize the benefits of a healthy diet by supporting normal absorption and assimilation of nutrients in the gut. Maintaining a favorable bacterial balance in the intestines is crucial to achieving and maintaining optimum health.

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