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Two different approaches in the use of probiotics as therapeutics

PROUS SCIENCE

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PROBIOTICS: ISOLATED BACTERIA STRAIN OR MIXTURES OF DIFFERENT STRAINS?

Two different approaches in the use of probiotics as therapeutics

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Summary

Probiotics are cultures of beneficial bacteria from the healthy gut microflora that improve the balance of the intestinal milieu by modifying the intestinal microflora and suppressing enhanced inflammatory responses. Probiotics are currently the subject of intense and widespread research as functional foods since they are known to induce health benefits, may be used as pharmaceutical preparations, and have achieved a "generally recognized as safe" (GRAS) status. *Lactobacillus* strains can also be genetically engineered for use in oral immunotherapeutic applications, such as vaccination and delivery of immunoregulatory substances. In the present review we evaluate the two different approaches to the therapeutic use of probiotics. We also focus on recent findings in the field of molecular biology and genetics of the intestinal immune response related to the microflora and intestinal ecology, in order to understand the mechanisms of action of probiotics and their present indications in gastrointestinal diseases. Finally, with a view to future perspectives we provide some examples of probiotics that are being assessed and have great potential in improving the health of animals and man.

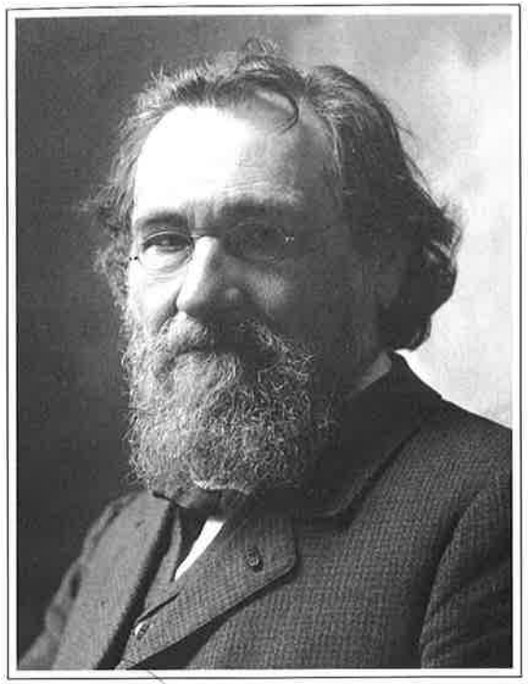

Fig. 1. Elie Metchnickoff (Ilya Ilyich Mechinikov, 1845-1916), the first scientist to propose the therapeutic use of lactic acid bacteria. © The Nobel Foundation.

History

Since the early observations by Elie Metchnikoff (Fig. 1), the first scientist who proposed the therapeutic use of lactic acid bacteria (1), a wealth of experiments have described the use of selected microorganisms, mainly belonging to the lactic acid bacteria family, for the prevention or treatment of several pathological conditions (2).

Lactic acid bacteria were first discovered by Pasteur in 1857. Their isolation from rancid milk was reported in 1878 by Lister, and later they were also isolated from the intestinal tract. In 1889 Tissier discovered *Bifidobacterium* spp., and in 1900 Moro discovered *Lactobacillus acidophilus* (3). According to G. Reid (4) probiotics were first promoted for therapeutic relief of intestinal disorders in 1906 by Tissier in a thesis present at the University of Paris (5).

The first stable cultures of *Lactobacillus casei* strain Shirota were made in 1930 by Dr. Minoru Shirota (Fig. 2) at the Microbial Laboratory of the Faculty of Medicine at Kyoto University, in Japan. This organism was isolated from the human intestine; it is resistant to gastric acid and bile acid and can therefore reach the lower intestine after oral administration. In 1935, Dr. Shirota developed "Yakult", a dairy product manufactured using *L. casei* strain Shirota. He hypothesized that daily intake of this organism might promote intestinal health and prevent diseases, thereby prolonging lifespan (3). Several studies have shown that *L. casei* strain Shirota and dairy products manufactured using this strain have various biological activities. This had led to beneficial strains of *L. casei* strain Shirota and their products have been termed "probiotics".

The exact mechanisms underlying the proposed actions of lactic acid bacteria remain vastly unknown, partly due to the complexity of the gastrointestinal ecosystem in which these biotherapeutic agents interact, and to the increasing variety of strains with potential probiotic characteristics. During the past decades, the beneficial effects of specific strains in preventing or treating intestinal disorders have been assessed in well-controlled clinical trials. Increasing evidence, including human studies, also supports the immunomodulatory role of given lactic acid bacterial strains. Having acquired GRAS status, they have entered the field of immunoregulation of chronic inflammatory diseases and vaccination.

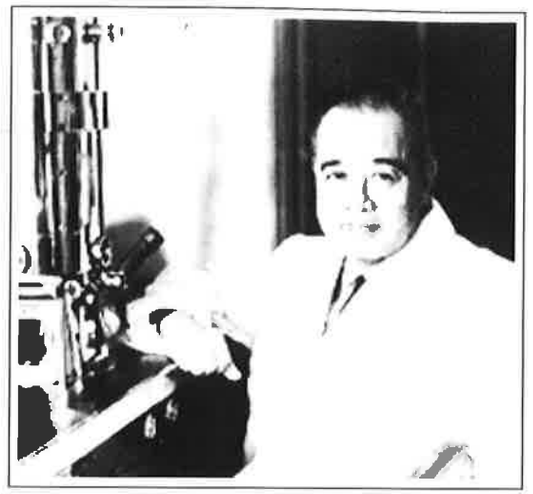

Fig. 2. Dr. Minoru Shirota (1899-1982), the first to make stable cultures of *Lactobacillus casei* strain Shirota. Reprinted from ref. 3 with permission from © Yakult Honscha Co., Ltd.

Introduction

Probiotics are "live microbial feed supplements which benefit the host by improving its intestinal microbial balance and which upon ingestion in certain numbers induce health benefits beyond inherent basic nutrition" (6). Probiotic bacteria belong to the natural flora with low or no pathogenicity and show functions that are important for the health and well-being of the host. Therefore, the maintenance of this ecological flora is important to prevent diseases, especially infections of the gastrointestinal tract (7). Since the last decade there has been an increased awareness of the beneficial effects of probiotics. Bengmark (7) elegantly described the reasons for this interest in microbial interference treatment (Table I).

Table I: Reasons for new interest in probiotics (7).

1)	Recognition that antibiotic therapy has not been successful to the extent one might have expected. Although it has no doubt solved some medical problems, it has also created some new ones.
2)	An increasing awareness of the fact that antibiotic treatment deranges the protective flora, and thereby predisposes to the alteration of infections.
3)	An increasing fear of antibiotic-resistant microbial strains, as a result of widespread overprescription and misuse of antibiotics.
4)	A fear that industry will no longer be able to develop effective antibiotics at a sufficient rate to compete with the development of microbial resistance to classic antibiotics.
5)	A widespread public interest in ecological methods.

Increasing evidence shows that probiotic bacteria modify the gastrointestinal microflora in such a way that the bacterial activitiy is advantageous to the health of the host without colonizing the gastrointestinal tract, as it supresses gastrointestinal inflammation, and modulates the inflammatory response (8). However, further validation of probiotic properties in humans and clarification of their mechanisms of action are needed to better understand the role of probiotics in promoting human health.

According to Salminen *et al.* (9), probiotic bacteria may be used to treat disturbed intestinal microflora and increased gut permeability, characteristics of many intestinal disorders. Interesting examples that have been investigated, but for which no definitive studies exist, include children with acute diarrhea (with the exception of acute diarrhea caused by rotaviruses) (10, 11), subjects with food allergy, colonic motility disorders, patients undergoing pelvic radiotherapy and prevention of colon cancer. There is some evidence in favor of the use of probiotics in adult infectious diarrhea, antibiotic-associated diarrhea and lactose intolerance (12). Furthermore, data supporting the benefits of probiotics in alcoholic liver disease, hepatic encephalopathy (13) and *Helicobacter pylori* infection (14, 15) have been published. In some of these disease states, altered intestinal microflora, impaired gut barrier and different types of intestinal inflammation are present. Since probiotic bacteria may survive the low gastric pH, the effects of the bile and the transient colonization of the intestine by adhering to the intestinal epithelium make these bacteria potential candidates for the treatment of these clinical conditions (9).

In addition to lactobacilli and bifidobacteria, probiotics might also be of yeast origin, such as *Saccharomyces*, *Aspergillus* and *Torulopsis*. It should be noted that these agents are rather dan-

gerous because in immunosuppressed subjects they can result in fungemia and fungal sepsis.

Although the mechanisms by which probiotics exert their effects *in vivo* have not yet been fully clarified, the luminal bacterial flora appears to play a major role in the initiation and perpetuation of chronic inflammatory bowel diseases in animal and human models. For example, colitis in the interleukin-10 (IL-10) gene-deficient mouse is associated with altered colonic microflora colonization, and may be modulated either by antibiotics or *Lactobacillus* subsp. treatment (16). In fact, most experimental animal models of intestinal inflammation require the presence of the microbial flora and do not develop disease in germ-free conditions. This was reported by Strober *et al.* (17): "in most if not all models the inflammation is driven by antigens in the normal mucosal microflora, which in effect means that it will be influenced by mitogens (*e.g.*, lipopolysaccharides [LPS], CpGs) and superantigens associated with these organisms that tend to induce IL-12 production and thus Th1 responses".

To isolate potentially effective probiotic bacteria, the microbial population adhering to surgically resected segments of the ileum was screened by Dunne *et al.* (18). From the 1500 bacterial strains obtained, a small number of *Lactobacillus* and *Bifidobacterium* were selected. Of these organisms, *Lactobacillus salivarius* subsp. *salivarius* strain UCC118 was shown to be acid and bile resistant. It adhered to epithelial cells, and *in vitro* it showed antagonism to pathogenic microorganisms (18). This specific strain is being widely investigated by several European groups led by a team of clinical scientists and food nutritionists in Cork, Ireland (18–20).

To date, the most common probiotics studied have been *Bifidobacterium* and *Lactobacillus*. Only a few strains have been characterized *in vitro*, in animal studies and in humans, and complete chromosome sequence has been obtained for *L. acidophilus* NCFM (21) and for the *L. casei* Shirota (personal communication).

One of the first *Lactobacillus* with therapeutic benefits isolated from human feces was *Lactobacillus rhamnosus* GG. It was administered during acute rotavirus diarrhea and promoted clinical recovery (22). *In vitro*, this *Lactobacillus* and *Lactobacillus plantarum* adhere to human mucosal cells (0.5–2.0 bacteria/cell) (23). *Lactobacillus* has a long history of safe use in the dairy industry and exists naturally in the human intestinal tract, which contains a myriad of microbes, collectively called microbiota. The microbial interference treatment is demonstrated by the use of lactobacilli, described by Sanders as promoters of human health (24). These bacteria were also effective in counteracting Gram-negative bacteria (7).

More recently members of the *Bifidobacterium* have been added to foods for probiotic purposes, probably encouraged by the discovery of their consistent presence as part of the normal microbiota of the human intestine. These bacteria are thought to be beneficial for the intestinal ecosystem and to provide protection against gastrointestinal infections and inflammatory bowel disease through their antagonistic activity against pathogens, their anti-allergic effects and other positive effects on the immune system (25).

This review focuses on the different mechanisms of action of probiotics to maintain the normal ecology of the intestine and analyzes data supporting the role of probiotics in the suppression of inflammatory responses.

Mechanism of action of probiotics: Two concepts

Two concepts have been proposed to understand the beneficial effects of probiotics (26). The first was based on the effects observed when only a particular strain was added as a food supplement (*e.g.*, *L. plantarum* and *L. casei* Shirota). The validity of this concept was demonstrated in several models and will be discussed below. A more recent concept has been derived from the use of a combination of lactic acid bacteria and bifidobacteria such as those present in VSL#3. This product contains 3×10^{11} cells/g of three strains of *Bifidobacterium (B. longum, B. infantis, B. breve)*, four strains of *Lactobacillus (L. acidophilus, L. casei, L. plantarum, L. delbrueckii* subsp. *bulgaricus)* and one strain of *Streptococcus (S. salivarius* subsp. *thermophilus)*. The stability and importance of this combination for regulating gastrointestinal inflammation has been demonstrated and will also be reviewed below (27).

The classical concept: Adherence, receptor competition and immunomodulation

The classical explanation of the effects of probiotics in the gastrointestinal tract is through the direct or indirect modulation of the endogenous flora or the immune system (28). In this explanation, direct contact with intestinal epithelial cells is a prerequisite. Evidence of cross-talk between

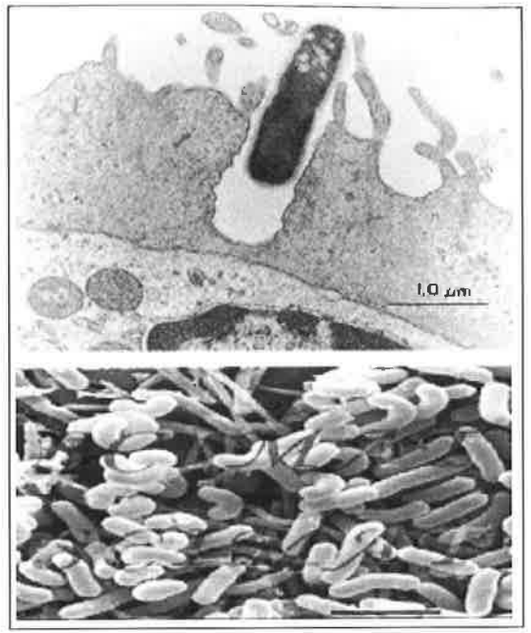

Fig. 3. A transmission electron micrograph of *Lactobacillus casei* strain Shirota in M-cells in rabbit ileum. A) Lactococci. B) Lactobacilli. Reprinted from ref. 3 with permission from © Yakult Honscha Co., Ltd.

lactobacilli and antigen-presenting cells of the intestine, such as M-cells, was observed in electron microscopic studies (Fig. 3). More recent microarray studies have shown that lactobacilli in contact with epithelial cells induce mRNA of several genes. The colonization of the intestine or the contact of probiotics with the intestinal wall might be achieved by different mechanisms, such as adherence, aggregation or high growth to avoid washing out, or continued consumption of probiotics (25).

Cross-talk between bacteria and between bacteria and epithelial cells

According to Bengmark (7), probiotics seem to be effective in controlling overgrowth of potentially pathogenic microorganisms with a bacterial, viral, and fungal origin (29). In addition to the effects of probiotics in antagonizing noxious or unwanted microorganisms, eliminating toxins and stimulating the intestinal immune defense, some data suggest that probiotics also participate in the regulation of intestinal function (23). Thus, the mechanisms by which the indigenous intestinal bacteria inhibit pathogens include competition for colonization sites and nutrients, production of toxic compounds, and stimulation of the immune system. These mechanisms are not mutually exclusive, and inhibition may include one, several, or all of these mechanisms (30). Probiotics counteracted various enteric pathogens in experimental models such as *Salmonella typhimurium* in mice (31), *Shigella* (32), *Clostridium difficile* (33), *Campylobacter jejuni* (34) and *Escherichia coli* (35). Studies currently aim to understand the cross-talk mechanisms between different bacteria and between bacteria and intestinal epithelial cells. The use of new technologies such as microarray provides a new view of the richness of these relationships (Kevin Collins, personal communication).

Modulation of the endogenous flora

The modulation of the endogenous flora is believed to occur through the antagonistic interaction between several bacterial strains that colonize the intestine. In order to colonize the intestine and exert their beneficial effects, probiotic bacteria must be able to adhere to mucosal epithelial cells. Since colonization is species-specific, the microorganisms should be of human origin, as mentioned above, and survive in the intestine while ingested (36).

Adherence and colonization

Adherence and colonization are crucial for the competitive exclusion of pathogens. Colonization also leads to immune modulation. Therefore, direct contact with intestinal epithelial cells is a prerequisite for some probiotic effects on the immune system. This helps to enhance leukocyte phagocytic activity against enterobacteria, an effect that was detected following administration of probiotic strains to healthy individuals. Lactic acid bacteria in food can transiently colonize the intestine and induce beneficial effects. Survival during intestinal transit and adhesion to the epithelium seem to be important for modifying the host's immune reactivity. Since *L. acidophilus* strain La1 adheres to enterocytes *in vitro*, it was hypothesized that contact with immune cells may also occur *in vivo*. *Bifidobacterium bifidum* strain Bb12, which shows high fecal colonization, is another potential immunomodulator (37). The most successful studies examining this idea involved the use of *Lactobacillus* GG at a dose of 1×10^{10} viable organisms per day and *Saccharomyces boulardii* yeast at a dose of 1 g/day (38).

Enhancement of the intestinal barrier

Another mechanism by which probiotic bacteria may induce protection is through the enhancement of intestinal barrier function (39). Isolauri *et al.* studied the effect of lactobacilli on the gut mucosal barrier. In an experimental model, rat pups were divided into three feeding groups at the age of 14 days. In addition to normal mother's milk the "milk" group received a daily gavage of cow's milk, the "milk-GG" group received *L. casei* strain GG with cow's milk, whereas controls received the same volume of water. At 21 days, the absorption of horseradish peroxidase across patch-free jejunal segments and segments containing Peyer's patches was studied in Ussing chambers. Results showed that the mean absorption of intact horseradish peroxidase was significantly different in the study groups both in patch-free segments and in segments containing Peyer's patches. There was a significant increase in the frequency of antibody secretor cells to β-lactoglobulin (enzyme-linked immunospot assay) in the milk-GG group. They concluded that prolonged cow's milk challenge in suckling rats increases gut permeability to intact proteins, whereas *Lactobacillus* GG counteracts this permeability disorder. These results suggest a link between the intensity of the antigen-specific immune response and the stabilization of the mucosal barrier (39).

Immunomodulatory effects

Although probiotics may promote nonspecific stimulation of the host immune system, such as immune cell proliferation, enhanced phagocytic activity and increased production of secretory immunoglobulin A (IgA), some experimental evidence suggest that *lactobacilli* decrease the host's immune response (6).

The presence of bacteria in the lumen and its epithelial cell adherence produce a variety of chemoattractants and cytokines that can pass on luminal signals to mucosal immune cells (40). On the humoral side, *Lactobacillus* GG significantly increases in IgG, IgA, and IgM secretion from circulating lymphocytes (22).

There is some evidence suggesting that probiotics nonspecifically modulate the host's immune system. The state of the immune system is characterized by the number of T- or B-cells, the levels of cytokines, the levels of antibodies and the phagocytic activity as a measure of cellular function. Activated T-cells can be divided into two major subsets based on their cytokine profile. In mice, T-helper 1 (Th1) cells produce principally IL-2, interferon-γ (IFN-γ) and lymphotoxin, now called tumor necrosis factor-β (TNF-β), whereas T-helper 2 (Th2) cells produce IL-4, IL-5, IL-6, IL-10 and IL-13. Human Th1 and Th2 cells not only produce a different set of cytokines but also present distinct functional properties and preferential expression of some activation markers (41) IL-1α and IL-1β has a prominent involvement in both systemic and local inflammatory reactions. IL-1β has numerous biological effects which are of relevance to inflammatory bowel disease such as induction of fever, arthralgia, hypotension, neutrophilia, increased acute phase response proteins, and hypoalbuminemia. A specific antagonist, the IL-1 receptor antagonist (IL-1RN) can block the effect of IL-1 by binding to IL-1 receptors without agonistic effects. It has been suggested that an imbalance in the IL-1β/IL-1ra ratio may lead to a dysregulated immune response. A mucosal imbalance of IL-1β/IL-1ra has been observed in inflammatory bowel disease (42). The molecular mechanisms underlying this imbalance are unknown, but evidence suggests that polymorphisms in these genes also affect the number of IL-1 and IL-1ra (43). In this respect, McGeehan *et al.* (44) described the capacity of a metalloproteinase inhibitor, GI 129471, to block TNF-α secretion both *in vitro* and *in vivo*. This inhibition occurs at a post-translational label. They suggest that TNF-α processing is mediated by a unique Zn^{2+} endopeptidase, which is inhibited by GI 129471 and represents a new target for therapeutic intervention in TNF-α associated pathologies. As described below, some probiotics help to control the inflammatory response and indirectly inhibit metalloproteinase activity. Fabia *et al.* (45) showed a significant decrease in lactobacilli concentrations in colonic biopsy specimens from patients with active ulcerative colitis. This may also be of importance in the host with a heightened abnormal immune response.

In patients with Crohn's disease there is a decrease in fecal *Bifidobacteria* concentration and the administration of *Lactobacillus GG* increased the intestinal IgA immune response and thereby promoted the gut immunological barrier (46). Similarly, in patients with active pouchitis there is a reduced fecal concentration of lactobacilli and bifidobacteria (47).

Ivanova *et al.* have examined the protective role of lyophilic dairy products (bulgaricum and biostim) with an active bacteria lactic acid strain. The protective effect was determined by the phagocytic activity and the activity of major immunoglobulins. These products were taken for one month in doses of 50 g/day. There were positive responses between the immune system and paraspecific resistance. The immunologic response was significantly higher after a lyophilic dairy diet (48).

Properties of probiotics as therapeutics

Lactic acid-producing microbes are the most common components of probiotic therapies and desirable members of the colonic microflora. The microorganisms should be, as stated above, of human origin and survive in the intestine although not for the long term (9, 49). Different strains of probiotic bacteria have very different and specialized functions (49). In their review, Campieri and Gionchetti (49) reported that most of the data on probiotics come from experimental conditions and create scepticism among researchers, because the mechanisms by which probiotic bacterial strains antagonize pathogenic gastrointestinal microorganisms or benefit the host *in vivo* have not yet been fully defined.

The exogenous administration of *Lactobacillus reuteri*, either as pure bacterial suspension or as fermented oatmeal soup, prevented the development of acetic acid-induced colitis or methotrexate-induced colitis in rats (49). *L. plantarum* might even more effectively attenuate the prevention of methotrexate-induced colitis.

It was very recently reported that nonvirulent *Salmonella* attenuated the synthesis of inflammatory effector molecules in response to diverse inflammatory stimuli in a model of human epithelial cells (50). This antiinflammatory action involved the inhibition of NFκB activation by blocking inhibitor κB-α ubiquitination and degradation (51). The NFκB signaling system was involved in regulating gene expression in cells such as B-cells (52). NFκB is a heterodimeric transcription factor that acts in both immune and nonimmune cells (52).

The signal detection by a cell surface receptor complex triggers a biochemical cascade in the cytoplasm, which frequently includes a Toll receptor family member and causes the degradation of a protein bound to the NFκB complex, IκB. The NFκB complex is then allowed to pass from the cytoplasm into the nucleus of the cell and, finally, bind and activate the transcription of one or more genes (52).

Nonpathogenic E. coli *strains as probiotics*

The proposed mechanism of action for a nonpathogenic strain such as *E. coli* is that they block the receptors to prevent the adhesion of bacteria. The antagonistic activity against pathogenic and nonpathogenic enterobacteria probably takes place through the production of antimicrobial substances and changes in the pH or chemical composition of the colonic lumen.

Probiotics seem the most promising option in the treatment of ulcerative colitis as they manipulate the normal intestinal flora (53). The possible mechanisms of action have been described by Farrell *et al.* (53). To test the effects of probiotic treatment, two groups were compared. The first group was given an oral preparation of nonpathogenic *E. coli* Nissle, two capsules a day and the second group 1.5 μg mesalazine daily. For this trial a total of 120 patients with inactive ulcerative colitis were included in a 12-week double-blind study to assess the efficacy of probiotics in preventing a relapse of the disease. The results showed no significant differences between the two groups. Consequently, probiotics offer another option for maintenance therapy of ulcerative colitis (54). Another study with ulcerative colitis patients was a double-blind 12-month trial. Two groups were compared one was given an oral *E. coli* preparation, two capsules twice a day and the other was given mesalazine 2.4 μg daily. The results suggested that treatment with a nonpathogenic *E. coli* had an equivalent effect to mesalazine in maintaining remission of ulcerative colitis (55). Although these randomized, controlled trials (54, 55) showed that an enteric-coated capsule containing nonpathogenic *E. coli* (Nissle 1917) is as effective as mesalazine for maintaining remission in ulcerative colitis, they have been criticized due to the low dose of mesalazine used. According to Faubion *et al.* (56) this study did have several flaws. The patient group was heterogeneous with regard to severity of illness (mild to severe), and was treated with several different corticosteroid formulations as well as the study medication. Furthermore, the doses of mesalazine used were relatively low and only a very small number of patients remained in remission at the end of the study (57).

Probiotics in experimental animal models

Several animal models such as pigs, transgenic mice and knockout mice, have been used to study the effects of probiotics. In this respect,

Ohashi *et al.* (58) described several studies in which they determined the effect of fermented milk prepared with *L. casei* strain Shirota on indigenous lactobacilli in pig large intestine for 2 weeks. A significant increase of acetate, propionate, and fecal organic acid concentration was observed. The fermented milk significantly reduced the fecal pH. Although the number of Shirota-strain bacteria in the intestinal contents (10^4) was much smaller than those of indigenous lactobacilli (10^8) CFU/g, the number of indigenous lactobacilli and bifidobacteria in pig intestine increased with fermented milk. Moreover, the phenotypic diversity of indigenous lactobacilli increased from three to eight with the fermented milk supplementation. The conclusion was that the fermented milk affected the indigenous *Lactobacillus* population and constitution (58).

In another study, Ohashi *et al.* (59) determined the effect of fermented milk prepared with *L. casei* strain Shirota on colonic motility by the strain gauge force transducer in a pig model. The contractions of the circular muscle layer of the cecum, upper colon, lower colon, and terminal colon in pigs were directly measured using this method in conscious animals. Feeding significantly stimulated the motility of the upper and lower colon and defecation significantly stimulated the motility of the upper and terminal colon. Feeding pigs with fermented milk for 2 weeks significantly activated the response to feeding in four portions of the large intestine. It increased motility of the terminal colon that did not promote defecation. The effects may be related to the stimulation of colonic fermentation as shown by a decrease in fecal pH (59).

In a 4-week ovalbumin-specific T-cell receptor transgenic mice food allergy model, Shida *et al.* (60) studied the ability of heat-killed *Lactobacillus* Shirota by using allergen-synthesized murine splenocyte cultures. The transgenic mice were fed a diet containing ovalbumin and injected with *Lactobacillus* Shirota intraperitoneally three times in the first week. Serum IgE and IgG1 responses as well as cytokine and antibody secretion by splenocytes were examined. *Lactobacillus* Shirota suppressed IgE production through promoting a dominant Th1-type response mediated by IL-12 induction. Furthermore, the inhibitory effect of *Lactobacillus* Shirota on systemic anaphylaxis induced by intravenous challenge of ovalbumin-fed ovalbumin-specific T-cell receptor transgenic mice with ovalbumin was tested. The results of this study suggested that intraperitoneal injection of *Lactobacillus* Shirota induced an IL-12 response in the serum of ovalbumin-specific T-cell receptor transgenic mice. In the food allergy model, *Lactobacillus* Shirota administration skewed the pattern of cytokine production by splenocytes toward Th1 dominance, and suppressed IgE and IgG1 secretion by splenocytes. On the other hand, anti-IL-12 antibody treatment seems to blocked the ability of *Lactobacillus* Shirota to modulate cytokine production. Moreover, *Lactobacillus* Shirota inhibited serum ovalbumin-specific IgE and IgG1 responses and also diminished systemic anaphylaxis. This suggests a possible use of this lactic acid bacterium in preventing food allergy.

In mouse feeding trials, milk-containing *L. salivarius* strain UCC118 was found to successfully colonize the murine gastrointestinal tract (18).

In another study, Kato *et al.* (61) described the marked antitumor activities and ability to modify immune responses of the lactic acid bacterium *L. casei* strain Shirota. In this study, they examined the possibility of *Lactobacillus* Shirota to induce the production of IL-12 as in Shida *et al.*, and also examined IFN-γ. These are important cytokines for antitumor and antimicrobial immunity, from murine splenocytes *in vitro* in order to clarify the mechanisms of its immune modification. Stimulation by *Lactobacillus* Shirota induced a marked production of IL-12 by X-ray-irradiated splenocytes. IFN-γ was produced by splenocytes by the stimulation with concanavalin A. *Lactobacillus* Shirota showed a synergistic stimulatory effect on the concanavalin A-induced production of IFN-γ, which was reduced by anti-IL-12 antibody treatment. IFN-γ production by splenocytes resulted from the production of IL-12 from X-ray-irradiated splenocytes stimulated by *Lactobacillus* Shirota. Furthermore, in BALB/c mice, the oral administration of viable *Lactobacillus* Shirota increased the production of IFN-γ but not that of IL-4 or IL-5 by splenocytes.

With regard to inflammatory bowel disease, it is interesting that in an experimental model, IL-10 deficient mice develop spontaneous colitis with features similar to the inflammation observed in patients with Crohn's disease. Histological damage begins within 4 weeks and reaches a plateau after 8 weeks. In these mice, within 2 weeks of age and before injury, there is alteration in bacterial colonization and an increase in translocated and mucosal adhered bacteria. These alterations reached a steady state 1 month before maximal histological

injury and persisted for months. It is of note that the concentration of *Lactobacillus* spp., a species that prevents adhesion of pathogens, was reduced within 2 weeks and reduced in a pattern paralleling mucosal injury (62).

L. plantarum attenuated an established colitis in the same knockout model (49). In a recent study, Konrad *et al.* (63) investigated the effect of soluble bacterial antigens extracted from *E. coli* (strain Laves) on the disease activity of murine colitis. C3H. IL-10 deficient and BALB/c mice with dextran sulfate sodium-induced colitis were treated with a bacterial lysate from *E. coli* or with placebo. Mice were monitored and inflammation was assessed by histological scoring, analysis of fecal IL-1β and measurement of cytokine production by ELISA. T-cell proliferation was quantified by ^{3}H-thymidine incorporation. The results showed that the use of *E. coli* lysate was effective in the improvement of murine colitis. This effect may be caused by a decreased Th1 reaction and by an induction of tolerance against bacterial antigens.

Probiotic treatment in vivo
in experimental animals and in man

To date only very few *in vivo* studies have been reported. In the experimental animal, treatment with *Helicobacter felis*-infected conventional mice with the spent culture supernatant of *L. acidophilus* strain LB inhibited the colonization of the stomach by the pathogen, although not for the long term (64). The application of the probiotic *L. casei* GG to conventional mice infected with *Streptococcus enterica serovar Typhimurium* significantly reduced the fecal cell counts of the pathogen adhesion of probiotic microorganisms to intestinal mucus of gnotobiotic mice (65).

Japanese investigators fed mice with bifidobacteria for 12 days and showed significantly high levels of fecal total IgA compared to the control group. Also, the levels of anti-β-lactoglobulin IgA in milk and fecal extracts were significantly higher in the bifidobacteria-fed group than in control group (66). These findings were believed to be due to protection against food antigens, since the intake of bifidobacteria can enhance local production of IgA in milk and the intestine, which may help to protect from exposure to food antigens (66).

The oral administration of *L. rhamnosus*, *L. acidophilus* and *B. lactis* to healthy mice results in an enhanced formation of serum antibodies. The degree of enhancement was increased by consuming *B. lactis* in an oligosaccharide-rich substrate (67), as well as a significant increase in the phagocytotic activity of peripheral blood leukocytes and peritoneal macrophages, compared with the control mice.

Dieleman *et al.* (68), have recently investigated the ability of *L. rhamnosus* GG in the prevention of colitis in transgenic rats monoassociated with *Bacteroides vulgatus* and investigated whether *Lactobacillus* GG or *L. plantarum* 299v may treat established colitis in specific pathogen-free transgenic rats and prevent recurrent disease after antibiotics were stopped. Germ-free B27 transgenic rats were monoassociated with *B. vulgatus* for 4 weeks following 2 weeks of colonization with *Lactobacillus* GG or no bacteria. Specific pathogen-free HLA-B27 transgenic rats received oral vancomycin and imipenem for 2 weeks, or water alone, followed by 4 weeks of treatment with oral *Lactobacillus* GG, *L. plantarum* 299v, or water only. Oral vancomycin and imipenem resulted in undetectable levels of *Bacteroides* spp., that may selectively induce colitis in HLA-B27 transgenic mice. Disease activity was quantified by blinded gross and histological scores, cecal myeloperoxidase activity, and levels of IL-1β, TNF, transforming growth factor-β (TGF-β) and IL-10. *Lactobacillus* GG did not prevent colitis in *B. vulgatus* co-associated transgenic rats or treat established disease in specific pathogen-free rats. *Lactobacillus* GG prevented colitis relapse in antibiotic-treated rats with significantly decreased gross cecal inflammation and histological scores, as well as cecal myeloperoxidase, IL-1β, and TNF. Cecal IL-10 was increased. *L. plantarum* 299v did not have any effect. This demonstrates the selective protective effects of two *Lactobacillus* species.

In man, a follow-up formula containing viable bifidobacteria was given to healthy Japanese children. Fecal levels of total IgA and antipoliovirus IgA during intake of the formula were significantly higher than those before intake (69). The consumption of formula containing viable *B. lactis* Bb12 for 20 days significantly increased the levels of total IgA and antipoliovirus IgA in feces of children aged 15-31 months (69).

Feeding children with a formula supplemented with bifidobacteria may provide protection against symptomatic rotavirus infection (11). The immune response to probiotic bacteria depends on the strain used, and they are only effective at a sufficiently high dosage.

More recently, Hideki Ishikawa *et al.* (70) studied the effect of *Bifidobacteria*-fermented milk on ulcerative colitis. Two groups were observed: a *Bifidobacteria*-fermented milk group, which was administred 100 ml *Bifidobacteria*-fermented milk per day for 1 year and a control group. Colonoscopies were performed, as were measures of general blood markers and examinations of the intestinal flora, including the analysis of fecal organic acids. Results showed an exacerbation ulcerative colitis symptoms in three out of 11 patients in the *Bifidobacteria*-fermented milk group, compared to nine out of 10 in the control group. This shows a significant reduction in exacerbations in the *Bifidobacteria*-fermented milk group. There were no differences in colonoscopic findings between the two groups at the end of the study. Furthermore, the analysis of microflora and the organic acids in the feces showed a significant reduction in the relative proportion of *B. vulgatus* in *Bacteroidaceae* and butyrate concentration, respectively, due to *Bifidobacteria*-fermented milk supplementation. It was concluded that that supplementation with *Bifidobacteria*-fermented milk was successful in maintaining remission in ulcerative colitis, and may have preventive effects on the relapse of this disease.

The new concept

A different explanation proposed by C. De Simone is based on the observations that particular strains are antagonistic against pathogenic bacteria through the production of antimicrobial substances, by promoting a reduced luminal colonic pH, and modulation of a variety of substances that are involved in healing of inflammation (such as interleukins, metalloproteinases and nitric oxide synthase [NOS]). Furthermore, it is based on the observation that the colon can be considered a "fermentation chamber", whose metabolic activity is profoundly altered by the bacterial composition and concentration. The colonization of the colon with beneficial bacteria modifies the intestinal content and this can exert an effect even on the activity and function of distant organs. These observations and the potential therapeutic benefits of manipulating the enteric microflora using probiotics has become an area of active investigation (7).

This new concept led to the formulation of the probiotic product VSL#3 (Table II) in high bacterial concentration (450 billion live bacteria per sachet). The probiotic preparation is formulated in sachets with a daily dose of 1 sachet b.i.d., depending on the severity of the disease and the number of stools per day (27).

Table II: Bacterial probiotics used in humans.

- *Streptococcus salivarius* subspecies *hermophilus*
- *Bifidobacterium* (*B. longum, B. infantis, B. brevis)*
- *Lactobacillus acidophilus*
- *Lactobacillus casei*
- *Lactobacillus delbruckei* subspecies *bulgarius*
- *Lactobacillus plantarum*

There is very important evidence that not all probiotic preparations are equivalent in terms of efficacy, since they include many different preparations (71) and differ also in the concentration of bacteria depending on the colony-forming units per gram. Furthermore, some probiotic preparations are made of a single bacterial strain and others with two or more strains. In addition to this, clinical studies with lactobacillus as a probiotic have shown variable results, which will be discussed below.

Total load of the intestinal bacteria

According to Madsen *et al.* (72), when IL-10 gene-deficient mice are raised under germ-free conditions the mucosal production of IFN-γ and TNF-α is within normal range, while both cytokines are up-regulated under normal conditions. This observation suggests that colitis in IL-10 gene-deficient mice occurs as a consequence of Th1-driven response to normal mucosal microflora. Treatment of both control and IL-10 gene-deficient mice with VSL#3 decreased IFN-γ and TNF-α secretion and substantially increased the total load of probiotics in the colon in both groups. The observed reduction in proinflammatory cytokine secretion indicates that the intestine is able to discriminate and define selective responses to different nonpathogenic strains of bacteria. These findings are similar to those by Haller *et al.* (73), who showed that intestinal epithelial cells are able to differentiate between commensal nonpathogenic bacteria and deliver distinct cytokine responses to underlying immune cells *in vitro*. In their study they co-cultured enterocyte-like CaCO-2 cells with human blood leukocytes in separate compartments of transwell cultures to determine whether nonpathogenic bacteria can modify the immune response of the intestinal epithelium. Haller *et al.* (73) stimulated CaCO-2 and peripheral blood mononuclear cells in

(PBMC) co-cultures with nonpathogenic bacteria and enteropathogenic *E. coli*. TNF-α, IL-1β, IL-8, monocyte chemoattracting protein 1 (MCP-1), and IL-10 were studied by enzyme-linked immunosorbent assays (cytokine secretion) and by semiquantitative reverse transcription-polymerase chain reaction. The results showed that the challenge of CaCO-2 cells with nonpathogenic *E. coli* and *Lactobacillus sakei* induced the expression of IL-8, monocyte chemoattractant protein-1 (MCP-1), IL-1β, and TNF-α mRNA in the presence of underlying leukocytes. Leukocyte sensitized CaCO-2 cells produced TNF-α and IL-1β, whereas IL-10 was exclusively secreted by human peripheral blood mononuclear cells. CaCO-2 cells alone remained hyporesponsive to the bacterial challenge. *Lactobacillus johnsonii*, an intestinal isolate, showed reduced potential to induce proinflammatory cytokines but increased TGF-β mRNA in leukocyte sensitized CaCO-2 cells. TNF-α was identified as one of the early mediators involved in cellular crosstalk. In the presence of leukocytes, discriminative activation of CaCO-2 cells was observed between enteropathogenic *E. coli* and nonpathogenic bacteria. It was concluded that the differential recognition of nonpathogenic bacteria by CaCO-2 cells required the presence of underlying leukocytes. These results strengthened the hypothesis that bacterial signaling at the mucosal surface depends on a network of cellular interactions (73).

Enhancement of the intestinal barrier

The lumen of the intestine contains bacteria, bacterial products, and dietary antigens capable of initiating and sustaining inflammation. The normal intestinal epithelium provides a relatively impermeable barrier to these luminal constituents. From the findings of a study done by Isolauri *et al.* (39), Madsen *et al.* (72) described an increase in the intestinal permeability in IL-10 gene-deficient mice. These models showed an increase in ileal and colonic permeability that is absent in mice raised under germ-free conditions. This suggests that the intestinal permeability defect in IL-10 gene-deficient mice is caused by a dysregulated immune response to normal enteric microflora and, furthermore, this permeability defect exists prior to the development of mucosal inflammation. VSL#3 treatment reduced colonic permeability in both IL-10 gene-deficient mice and control mice, suggesting that the type and quantity of bacterial species in the colon modulate intestinal permeability.

Adhesion and receptor competition

According to Madsen *et al.* (74), the intestinal permeability was reduced by VSL#3 treatment, partially as a result of probiotic bacterial-induced reduction in proinflammatory cytokine release. To determine the effect of VSL#3 on epithelial function, Madsen *et al.* exposed T_{84} monolayers to bacteria for different periods of time and placed them in Ussing chambers for measurement of Isc, PD, and mannitol permeability. *Staphylococcus dublin* invasion was reduced after exposure to the VSL#3. The results indicated that epithelial cells may also respond directly to certain probiotic bacteria or to a secreted bacterial factor. This supports the idea of a surface-acting proteinaceous component being released by a bacteria found in the VSL#3 mixture (74).

In this respect, Heinemann *et al.* (75) have demonstrated that a certain strain of *Lactobacillus* bacteria, *Lactobacillus fermentum* RC-14, releases surface-active components that may inhibit adhesion of bacteria. The results suggest that this *Lactobacillus* antiadhesive cell surface protein might protect against pathogens by preventing their adhesion. Another beneficial mechanism described by Madsen *et al.* (74) was the receptor competition. Probiotics compete with microbial pathogens for a limited number of receptors on the surface epithelium. Almost certainly this competition contributes to the beneficial effects of the VSL#3 probiotic compound *in vivo*, since their data showed a substantial increase in levels of adherent bacteria. However, the findings with T_{84} cells showed the following: i) that conditioned media had the same effect as live bacteria on monolayer permeability, and ii) that removal of VSL#3 bacteria before the invasion by *S. dublin* still resulted in protection from bacterial invasion. These observations indicated that some secreted factor is responsible for additional direct effects on epithelium.

Probiotics and the immune system

Gut-associated lymphoid tissue interacts with bacterial or dietary antigens inducing a humoral and a cellular immune response in the gut (8). Probiotics stimulate lymphocytes to produce cytokine IFN-γ, and prompt nonspecific phagocytic and lymphocytic activity. For example, subjects taking milk with *B. lactis* for 6 weeks significantly enhanced the levels of IFN-γ, and polymorphonuclear cell phagocytic capacity upon stimulation of their peripheral blood mononuclear cells in culture, in comparison to the placebo control group, which received milk alone (76).

Newberry *et al.* (77), observed in transgenic mice with a single lymphocyte population specific for dietary antigen, an obligatory input from the normal flora in the generation of regulatory lymphocytes that maintained oral tolerance, through the induction of macrophage prostaglandin E2 production via IL-10 and TGF-β. In this model, hen egg-white lysozyme is fed to mice expressing a transgenic T-cell receptor that recognizes hen egg-white lysozyme peptide 46–6. This did not result in intestinal pathology; however, the simultaneous administration of cyclooxygenase-2 inhibitors and dietary hen egg-white lysozyme increased the proliferation of lamina propria mononuclear cells and crypt epithelial cells, crypt expansion and villous blunting. Lamina propria mononuclear cells produce high levels of cyclooxygenase-2-dependent arachidonic acid metabolites, which act as immunomodulators in the immune response to dietary antigen. The results showed that cyclooxygenase-2-dependent arachidonic acid metabolites were essential in the development and maintenance of intestinal immune homeostasis (77).

Membrane metalloproteinases

Ulisse *et al.* (51) evaluated in a nonblinded study the tissue levels of pro- and antiinflammatory cytokines, NOS, and matrix metalloproteinases (MMP) both in control and inflamed pouches before and after antibiotic and probiotic treatment of patients with acute pouchitis. The results of this study demonstrated that VSL#3 is able to induce a significant increase in the expression of the antiinflammatory cytokine IL-10 in the mucosal pouch compared to inflamed and antibiotic-treated patients. Antibiotic treatment reduced the expression of the proinflammatory cytokines IFN-γ and TNF-α but not IL-1α. All three cytokines decreased further, in a statistically significant manner, after VSL#3 treatment.

Inflamed pouch tissue increased the levels of the inducible NOS (iNOS) and MMP activity. iNOS and MMP activity were reduced after antibiotic and probiotic treatment. The ability to increase IL-10 to a higher level than that observed in control pouches and to decrease proinflammatory cytokines and iNOS and MMP activity tissue levels to levels present in control pouches may suggest a mechanism of action that would explain the benefits of probiotics as maintenance treatment of patients with pouchitis.

iNOS plays an important role in modulating macrophage cellular function during inflammation and its level increases after stimulation with cytokines such as IL-1, TNF-α and IFN-γ. NOS activity levels were monitored in control and inflamed pouch tissues, and following antibiotic and probiotic activity an increase of iNOS activity was observed. Antibiotic treatment induced partial but significant reduction in iNOS activity level (1.0 ± 0.5 pmol/mg protein/min). This decrease was more pronounced, and statistically significant, after treatment with VSL#3.

MMP expression and activation play important roles in inflammatory processes and in the pathogenesis of inflammatory diseases. Macrophages and T-cells produce several MMPs and their expression is modulated by cytokines. MMPs degrade the basal membrane and extracellular matrix and are important to leukocyte migration and to the release of TNF-α from its membrane-bound form. TNF-α is a potent proinflammatory agent produced primarily by activated monocytes and macrophages. These studies demonstrated that probiotics may increase the pouch tissue level of IL-10 to a higher level than the observed in control pouches. On the other hand, they may reduce tissue levels of the proinflammatory cytokines IL-1α, IFN-γ and TNF-α to levels present in control pouches. This effect may be in turn responsible for the down-regulation of iNOS and MMP activity.

Probiotics and Toll-like receptors

The innate immune response is the first line of defense against infectious diseases. The main challenge for the host is to detect the pathogen and create a rapid defensive response (78). Sensing of bacterial products by the innate immune system is mediated by Toll-like receptors (TLRs). TLR2, TLR4, and TLR9, highly conserved through evolution and expressed on both enterocytes and immune cells, recognize specific microbial components, such as surface determinants and DNA sequences, and induce the production of Th1 cytokines through a process dependent on NFκB (54), which is activated as a response to pathogen-associated molecular patterns such as lipopolysaccharide (LPS). TLR4 is required for the recognition of LPS (79). Da Silva *et al.* (80) described that LPS initiates its biological activities through a heteromeric receptor complex containing CD14, TLR4, and at least one other protein, MD-2. LPS binds directly to CD14 but whether LPS then binds to TLR4 and/or MD-2 is unknown. The researchers used transient transfection to express human TLRs, MD-2, or CD14 alone or in different combinations in HEK 293 cells. Interactions

between LPS and these proteins were studied using a chemically modified, radio iodinated LPS containing a covalently linked, UV light-activated cross-linking group. They showed that LPS is cross-linked specifically to TLR4 and MD-2 only when co-expressed with CD14. These data support that LPS is in close proximity to the three known proteins of its membrane receptor complex. Thus, LPS binds directly to each of the members of the tripartite LPS receptor complex (80). Jiang *et al.* (81) found that CD14, a GPI-linked protein, plays a pivotal role in LPS-mediated signaling by potentiating leukocyte adherence, activation, and cytokine production. Recent studies have identified TLR4 as a membrane cofactor in LPS-mediated transmembrane signaling in cytokine induction, although the mechanism responsible for this cooperation is unknown. It was demonstrated that LPS triggers a physical association between CD14 and TLR4. Since LPS stimulation up-regulates CD14 and TLR4 expression, it was necessary to consider the possibility that these newly expressed molecules were associated with another independent of LPS stimulation. Although calcium ionophore A23187 increased the expression of CD14 and TLR4, there was no energy transfer. However, following A23187 treatment, LPS promoted physical proximity between CD14 and TLR4. Therefore, they suggested that a close interaction between CD14 and TLR4 participates in LPS signaling, leading to nuclear translocation of NFκB (81).

Abreu *et al.* (82) have described that intestinal epithelial cells express low levels of TLR4 and are unresponsive to LPS. The luminal surface of the colonic epithelium is continually exposed to Gram-negative commensal bacteria and LPS. The recognition of LPS by TLR4 results in proinflammatory gene expression in several cell types. Normally, commensal bacteria and their components do not elicit an inflammatory response from intestinal epithelial cells. In their study they explained the molecular mechanisms by which intestinal epithelial cells limit chronic activation in the presence of LPS. Three intestinal epithelial cell lines (Caco-2, T84, HT-29) were tested for their ability to activate an NFκB reporter gene in response to purified, protein-free LPS. No intestinal epithelial cell lines responded to LPS, whereas human dermal microvessel endothelial cells did respond to LPS. Intestinal epithelial cells responded vigorously to IL-1β demonstrating that the IL-1 receptor signaling pathway shared by TLRs was intact. Therefore, probiotics may interact with these sensors and contribute to their immunomodulation. *Lactobacillus* GG has been shown to induce such a NFκB-mediated interaction. Although this effect alone might suggest a direct link with decreased Th2-mediated allergy, it has become clear that the Th1/Th2 paradigm is inadequate to explain mucosal immune responses. Specific immunological unresponsiveness to dietary components (oral tolerance) and to commensal bacteria is critically dependent on inhibition of potential lymphocyte reactivity, and two recently recognized suppressor-cell populations are central in this process. In murine models Th3 and T-regulator 1 (Tr1) cells, that produce TGF-β and IL-10, respectively, down-regulate mucosal inflammatory responses through "bystander suppression" of nearby lymphocytes of various specificities: deficiency of either cytokine or cell type leads to mucosal inflammation as a consequence of unchecked response to the enteric flora (83).

Probiotics, genetics and the immune system

DNA from bacteria has stimulatory effects on mammalian immune cells, which depend on the presence of unmethylated CpG dinucleotides in the bacterial DNA. CpG DNA induces a strong T-helper-1-like inflammatory response (84). Data suggest that CpG-DNA initiates signaling via the TLR/IL-1R pathway in androgen-presenting cells (APCs) similar to LPS and to T helper cell-mediated CD40 ligation. Activation of the TLR/IL-1R signaling pathway by foreign bacterial DNA may be one way to initiate innate defense mechanisms against infectious pathogens *in vivo* (85).

Accumulating evidence has revealed the therapeutic potential of CpG DNA as adjuvants for vaccination strategies for cancer, allergy and infectious diseases. Hemmi *et al.* (84) showed in their study that cellular response to CpG DNA was mediated by a Toll-like receptor, TLR9. TLR9-deficient mice did not show any response to CpG DNA, including proliferation of splenocytes, inflammatory cytokine production from macrophages and maturation of dendritic cells. These experimental models showed resistance to the lethal effect of CpG DNA without any elevation of serum proinflammatory cytokine levels. The *in vivo* CpG-DNA-mediated T-helper type-1 response was also abolished in TLR9-deficient mice. Thus, vertebrate immune systems appear to have evolved a specific Toll-like receptor that distinguishes bacterial DNA from self-DNA.

In this regard, Chu *et al.* (86) have also described the synthetic oligodeoxynucleotides

(ODNs) that contain unmethylated CpG motifs (CpG-ODN) that induce macrophages to secrete IL-12, which in turn induce interferon IFN-γ secretion by NK cells. These cytokines may induce Th1 differentiation. Chu *et al.* (86) examined the effects of coadministered CpG ODN on the differentiation of Th responses to hen egg lysozyme (HEL). In both BALB/c (Th2-biased) and B10.D2 (Th1-biased) mice, immunization with HEL in incomplete Freund's adjuvant (IFA) resulted in Th2-dominated immune responses characterized by HEL-specific secretion of IL-5 but not IFN-γ. In contrast, immunization with IFA-HEL plus CpG-ODN switched the immune response to a Th1-dominated cytokine pattern, with high levels of HEL-specific IFN-γ secretion and decreased HEL-specific IL-5 production. IFA-HEL plus CpG ODN also induced anti-HEL IgG2a (a Th1-associated isotype), not induced by IFA-HEL alone. Control non-CpG ODN did not induce IFN-γ or IgG2a, excepting lesser increases in B10.D2 (Th1-biased) mice. Thus, CpG-ODN provide a signal to switch on Th1-dominated responses to coadministered antigen and are potential adjuvants for human vaccines to cause a protective Th1 immune response. Roman *et al.* (87) also observed that the Th1 responses are potently stimulated in gene-vaccinated animals by noncoding immunostimulatory DNA sequences (ISS) or ISS oligodeoxynucleotides. The mechanisms of action of ISS-DNAs, all of which foster Th1 responses and enhance cell-mediated immunity are listed in Table III (87).

Table III: The mechanisms of action of ISS-DNAs (63).

- Suppression of IgE synthesis
- Promoting IgE
- Production of IFN-γ
- Initiate production of IFN-γ, IFN-α, IFN-β, IL-12 and IL-18

According to Martin-Orozco *et al.* (88) bacterial genomic DNA, plasmid DNA and CpG containing ISS stimulate a Th1 response via the release of type-1 cytokines from macrophages, dendritic cells, NK cells and B-cells. They showed that ISS-enriched DNA up-regulates a distinct profile of cell surface molecules on macrophages and B-cells *in vitro* and *in vivo*. ISS-ODN and ISS-containing pDNA enhanced the expression of antigen presentation molecules (major histocompatibility complex [MHC] class I and II), co-stimulatory molecules (B7-1, B7-2 and CD40), cytokine receptors (IFN-γ receptor and IL-2 receptor), an adhesion molecule (ICAM-1) and an (Fc-γ receptor) on murine B-cells or bone marrow-derived macrophages. The increased expression of these surface molecules was found in purified cell populations and was largely independent of the effects of type-1 cytokines. Splenic antigen-presenting cells stimulated with ISS-ODN *in vivo* efficiently activated naive T-cells and bias their differentiation toward a Th1 phenotype *in vitro*. Thus, the induction of both type-1 cytokines and a distinct profile of cell surface molecules contribute to the powerful immunostimulatory effects of ISS-containing DNA on innate and adaptive immunity. These strong immunostimulatory effects explain mucosal immunity (89). Wagner *et al.* (90) also described the immunostimulatory effects as enhances of the host's defence against invading pathogens.

Rachmilewitz *et al.* (91) have hypothesized a role for ISS-DNA in experimental colitis. They described that the cell survival-promoting effects of ISS-DNA and its immunostimulatory properties on innate immunity could inhibit damage to the colonic mucosa and limit exposure of the involved mucosal tissue to commensal bacteria and their inflammatory products. These combined effects might thereby result in the inhibition of intestinal inflammation. Based on the observations described above they thought that the beneficial effects of probiotics might be derived from its DNA and, therefore, they evaluated the effect of DNA-free methylated and unmethylated genomic DNA (gDNA) isolated from the probiotic VSL#3 in experimental models of colitis. In this study groups of Balb/C mice were treated s.c. with ISS oligonuceotides (ODN, 10 μg) or with one of the genomic DNA preparations (50 μg) 2 hours prior to administration of 2.5% dextran sulfate sodium (DSS) to the drinking water, or to rectal installation of 0.5 mg trinitrobenzene sulfonic acid (TNBS) dissolved in 0.1 ml 50% ethanol. Mice were weighed and inspected for rectal bleeding and diarrhea and sacrificed after 1 week. The disease activity index (DAI) is the combined score of weight loss and rectal bleeding. The colon was weighed, sections obtained for histology and mucosal myeloperoxidase (MPO) activity was determined. The results of this study showed that the amelioration of DSS- and TNBS-induced colitis by VSL-gDNA reflected by DAI, MPO activity and the histological score was similar to that induced by ISS-ODN. VSL-M-gDNA, VSL-gDNA and control calf thymus gDNA did not affect the severity of DSS- or TNBS-induced colitis. Therefore, it was concluded that VSL#3 attenuation of experimental colitis was caused by the immunostimulatory and antiinflammatory properties of its genomic DNA sequences.

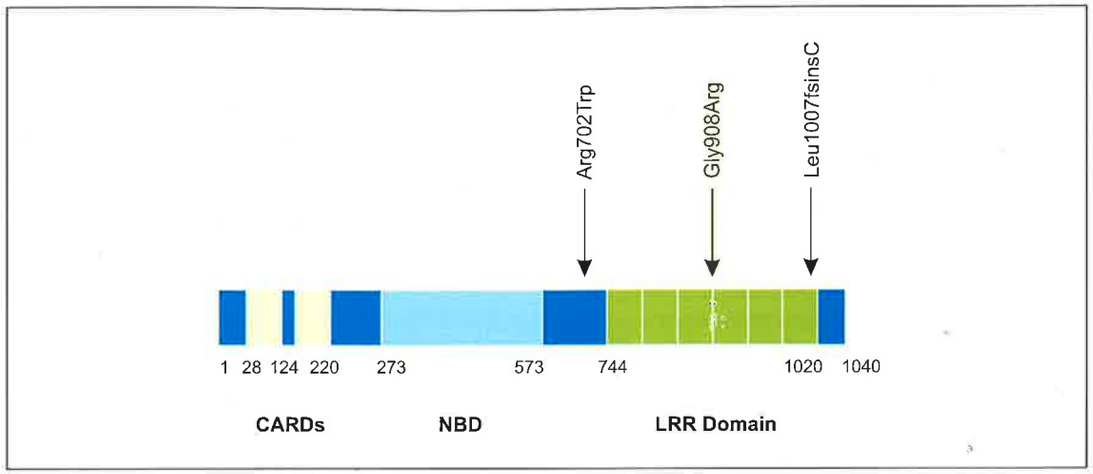

Fig. 4. Mutations in the LRR region of the NOD2/CARD15 gene associated with Crohn's disease.

The recent identification of NOD2/CARD15 as a susceptibility gene for Crohn's disease has provided another link between the immune response to enteric bacteria and the development of mucosal inflammation. NOD2/CARD15 is composed of two caspase recruitment domain (CARD), a nucleotide-binding domain and a leucin-rich-repeat region. The leucin-rich-repeat domain of NOD2/CARD15 has binding activity for bacterial peptidoglycans and its deletion stimulates the NFκB pathway. The most frequent variants of NOD2/CARD15 observed in Crohn's disease tend to cluster in the leucin-rich-repeat domain and its adjacent regions. This suggests that the leucin-rich-repeat domain of CD-associated variants is likely to be impaired in its recognition of microbial components. Continuing studies are investigating the pathophysiological mechanims induced by NOD2/CARD15 variants in the intestinal mucosa (68) (Figs. 4 and 5).

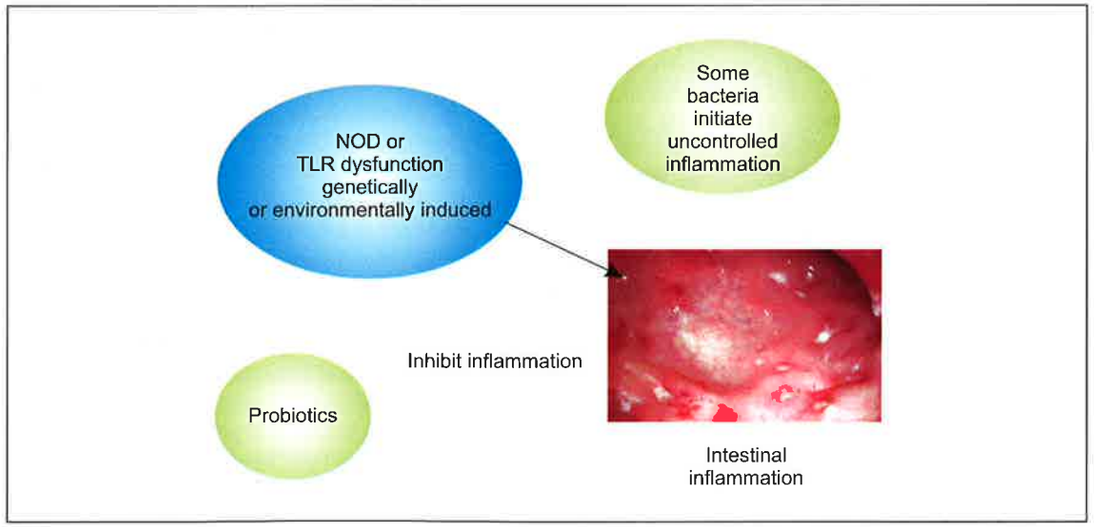

Fig. 5. Innate immunity and probiotics.

Properties of probiotics as therapeutics

It appears that the VSL#3 mixture acts directly on the epithelium to enhance barrier integrity, thus

demonstrating that other mechanisms of action different from the enhancement of IL-10 stimulation are likely to be involved in the antiinflammatory action of probiotics (62). The anti-inflammatory actions of probiotic bacteria on the intestinal epithelial cells occurred through inhibition of NFκB, since, as stated above, immune cells can distinguish prokaryotic DNA from vertebrate DNA by detecting methylated CpG dinucleotides. Bacterial DNA may therefore activate both innate and acquired immune responses. It is not known yet whether DNA from different bacterial species have differential immune responses. Intestinal epithelial cells are key components in the mucosal immune system, but it is still unknown whether TLR9, which participate in bacterial DNA recognition, are expressed in the epithelial cells. In their study they examined the effect of DNA from probiotic and pathogenic bacteria on cytokine release from epithelial cells and murine splenocytes. Madsen *et al.* (93) used DNA from VSL#3 or DNA from the pathogenic bacteria, *S. typhimurium* in a murine intestine model in the presence or absence of LPS. Live VSL#3 bacteria (10^3–10^7 CFU/ml) or isolated DNA (10 μg/ml) was applied to confluent HT-29 cells for 1 hour, followed by *S. typhimurium*, TNF-α or IL-1. NFκB activation and IL-8 release into the supernatant were measured by EMSA and ELISA, respectively. Splenocytes were isolated from IL-10 deficient mice and stimulated with fecal bacterial sonicates in the presence and absence of VSL#3 DNA or *E. coli* DNA. In intestinal tissue the presence of VSL#3 DNA, but not *S. typhimurium* DNA resulted in a 45% and 50% reduction in basal and LPS-stimulated IFN-γ secretion respectively. In HT-29 monolayers, VSL#3 and VSL#3 DNA resulted in a significant dose-dependent attenuation of IL-8 secretion and NFκB activation in response to *S. typhimurium,* TNF-α, and IL-1. In splenocytes, VSL#3 DNA, but not *E. coli* DNA, attenuated stimulated IFN-γ secretion by 98% and IL-12 by 64%. Thus, immune and epithelial cells may recognize and respond to DNA from probiotic bacteria with a down-regulation of proinflammatory cytokine secretion and, in intestinal epithelial cells, an attenuation of the NFκB signal pathway.

VSL#3 in animal models

The use of VSL#3 resulted in a complete normalization of physiological transport function and barrier integrity, in conjunction with a reduction in mucosal secretion of TNF-α and IFN-γ in the IL-10 gene-deficient mouse. Madsen *et al.* (74) described numerous studies that have characterized the ability of various strains of probiotics, especially the probiotic VSL#3, to modify the activity and cytokine expression of gut-associated lymphoid tissue and epithelial cells.

The rationale behind the use of a probiotic compound containing high levels of a mixture of different bacterial species was to attempt to influence the microbial composition of the colon through a synergistic action of the different strains in the mixture. One of the bacteria in this mixture, *Lactobacillus delbruekii* subsp. *bulgaricus* enhanced the mucus binding of *Bifidobacterium lactis in vitro*, suggesting that combinations of adherent probiotic strains can influence other strains' adhesion and activity in the human intestinal tract (74). In their study, Madsen *et al.* (74), showed that, compared with controls, IL-10 gene-deficient mice spontaneously secreted higher amounts of TNF-α from both the ileum and the colon. One of the observations showed that IL-10 gene-deficient mice developed spontaneous colitis similar to the inflammation observed in patients with Crohn's disease. Histological damage began within 4 weeks and reached a plateau after 8 weeks. In these mice within 2 weeks of age and before appearance of injury, there was an alteration in bacterial colonization and an increase in translocated and mucosal-adhered bacteria. These alterations reached a steady state 1-month before maximal histological injury and persisted for months. It was important that the concentration of *Lactobacillus* spp., a species that prevents adhesion of pathogens, was reduced within 2 weeks and reduced in a pattern paralleling mucosal injury (62). Colonic tissue from IL-10 gene-deficient mice responded to the presence of LPS with an enhanced release of TNF-α, thus suggesting the presence of monocytes and active inflammation. On the other hand, ileal tissue from IL-10 gene-deficient mice showed a twofold increase in the spontaneous basal TNF-α secretion, but compared to controls, there was no response to LPS with a significant increase in TNF-α secretion and no histological inflammation in this region. Control mice did not respond to the presence of LPS increase in TNF-α secretion in the ileum or in the colon.

In a study by Madsen *et al.* (74), IL-10 gene-deficient mice received VSL#3 for 4 weeks and showed, compared to untreated IL-10 mice, a significantly reduced TNF-α secretion in the ileum and colon. VSL#3 had no effects on cytokine secretion from the ileum of control mice. Compared to controls, IL-10 gene-deficient mice also showed an increase in spontaneous IFN-γ secretion both in ileum and colon, which was not stimulated by LPS. VSL#3 treatment normalized basal IFN-γ secretion in the ileum but not in the colon. These results suggested that the luminal microflora modulated the immune cytokine responses, both under normal and inflammatory conditions. It was also found that, even in the absence of the regulatory cytokine IL-10, the intestinal mucosa in IL-10 gene-deficient mice responded to the presence of the *Lactobacillus* and *Bifidobacterium* found in the VSL#3 mixture with a down-regulation of a proinflammatory immune response. This study showed that treatment of IL-10 gene-deficient mice with VSL#3 resulted in normalization of colonic physiologic function and barrier integrity in combination with reduction in mucosal levels of proinflammatory cytokines and a significant improvement in histological features.

Another study, carried out in alcohol-fed rodents, demonstrated that intestinal bacteria, bacterial endotoxin, and the endotoxin-inducible cytokine, TNF-α, modulated alcohol-induced liver damage (13). Treatments such as antibiotics and *Lactobacillus*, that inhibit endotoxin production by the intestinal flora, or treatments that inhibit the activity of TNF-α itself, significantly inhibited the development of steatohepatitis in animals ingesting large amounts of alcohol. In this respect, Li *et al.* (13) observed mice for 4 weeks and found that intestinal bacteria, endotoxin LPS- and LPS-induced cytokines such as TNF-α are important modulators of alcoholic fatty liver disease. Treatments that partially decontaminate intestinal flora and treatments that inhibit activity of TNF-α itself inhibited alcoholic fatty liver disease in experimental animals. In this regard, Li *et al.* (13) hypothesized that treatment with VSL#3 or anti-TNF-α antibodies would improve nonalcoholic fatty liver disease in these mice by down-regulating NFκB and JNK (13).

Probiotic treatment in vivo
in experimental animals and in man

There is some evidence suggesting that probiotics prevent infections caused by enteropathogens. About 47 different *Lactobacillus* strains have been tested *in vitro* for their ability to inhibit growth of a number of pathogens. Among the tested strains, *L. rhamnosus* 19070-2, *L. reuteri* DSM 12246, and *L. rhamnosus* LGG were identified most frequently in fecal samples; they were found in 10, eight, and seven of the 12 samples tested during the intervention period, respectively, whereas reisolations were less frequent in the washout period. Survival and reisolation of the bacteria *in vivo* appeared to be linked to pH tolerance, adhesion, and antimicrobial properties *in vitro* (94). The majority of the strains inhibited *Yersinia entero colitica* and *Bacillus cereus* and a few exhibited antimicrobial activities toward *Staphylococcus aureus* and *Salmonella enterica serovar typhimurium* (8).

Lactic acid bacteria in general may inhibit the growth of enteropathogens such as *E. coli, H. pylori, Listeria monocytogenes, Salmonella* spp. and others. This antagonistic activity is mediated either directly by the production of inhibitory substances such as lactic acid, hydrogen peroxide, bacteriocins, α-hydroxypropionaldehyde and other unidentified compounds or by competing with the pathogen for binding sites on the epithelial cell surface. As shown in Table III another criterion for effective probiotic organisms is the adhesion to epithelial cells, a characteristic of several lactobacilli and some bifidobacteria. The factors mediating cell adhesion are still unknown. It has been suggested that proteinaceous factors are involved. Lipoteichoic acid participates in the adhesion of *L. johnsonii La1* to epithelial Caco-2 cells (95).

A human feeding study conducted in 80 healthy volunteers showed that yogurt may be used as a vehicle for delivery of strain UCC118 to the human gastrointestinal tract with considerable efficacy in influencing gut flora and colonization. The investigators in Cork, Ireland, developed criteria for *in vitro* selection of probiotic bacteria that may reflect certain *in vivo* effects on the host, such as modulation of gastrointestinal tract microflora (18).

Probiotics in diarrhea

Antibiotic-associated diarrhea

Several reports exist on the benefits of probiotics in this common complication of the use of antibiotics. For example, in the prevention of antibiotic-associated diarrhea, 45 patients being treated with antibiotics were given, concurrently, one capsule of either *Enterococcus* SF68 or placebo for 7 days (10 centers) twice daily. *Enterococcus* SF 68 was effective in reducing the incidence of antibiotic-associated diarrhea compared to placebo (8.7% compared to 27.2%, respectively) (96). Important here is the evidence that *Enterococcus* SF68 has been withdrawn because of the risk of antibiotic resistance transfer. Not all reports are similarly en-

couraging but the type of probiotics used may be important in achieving results.

Probiotics in acute diarrhea

Multiple studies in children have shown that *Lactobacillus*, administered orally, may have antidiarrheal properties. To determine the effect of *Lactobacillus* GG on the course of acute diarrhea in hospitalized children, a prospective, placebo-controlled, triple-blind clinical trial was carried out in Pakistan. Forty children (mean age, 13 months) received either oral *Lactobacillus* GG ($n = 21$) or placebo ($n = 19$) twice daily for 2 days, after rehydration in addition to the usual diet. The clinical course of diarrhea was followed during the treatment period. The features for admission into the study groups were similar and were characterized by severe diarrhea, malnutrition and inappropriate management before presentation. Response was evident on day 2, when the frequency of both vomiting and diarrhea was less in the *Lactobacillus* group. In those patients with acute nonbloody diarrhea ($n = 32$), the percentage of children with persistent watery diarrhea at 48 hours was significantly lower in the *Lactobacillus* group (31% *versus* 75%). No significant difference was observed after 48 hours in those with bloody diarrhea (97).

Van Niel *et al.* (98) conducted a meta-analysis of randomized, controlled studies to assess whether treatment with *Lactobacillus* improved clinical outcome in children with acute infectious diarrhea. They conducted a search in bibliographic databases of traditional biomedical as well as complementary and alternative medicine literature published between 1966 and 2000. The original search yielded 26 studies, nine of which met the criteria. A reduction of 0.7 days in diarrhea duration and a reduction of 1.6 stools for diarrhea frequency was attained on day 2 of treatment in the participants who received *Lactobacillus* compared to those who received placebo. A preplanned subanalysis suggested a dose-effect relationship. The results of this meta-analysis suggested that *Lactobacillus* is safe and effective as a treatment for children with acute infectious diarrhea.

Probiotics in rotavirus diarrhea

Rotavirus was discovered in children with gastroenteritis by Bishop *et al.* in 1973 (99). This agent causes widespread morbidity and 870,000 deaths worldwide each year. As Bishop said, "after doing a lot of background reading, it became clear that there probably was an infectious agent but we could not get anything to grow in culture". Bishop *et al.* (99) participated in the development of vaccines against rotavirus, the first of which was licensed for use in the USA in 1998.

The effect of orally administered lactobacilli on acute rotavirus diarrhea was tested by Isolauri *et al.* (100) in 42 well-nourished children aged 5–28 months. After oral rehydration, the patients received human *L. casei* strain GG 10^{10} CFU twice daily for 5 days. The control group was not given lactobacilli. *Lactobacillus* GG was found in the feces of 83% of the group with *L. casei* strain GG. The diarrheal phase was shortened in that group. The dietary supplementation with lactobacilli significantly influenced the bacterial enzyme profile. Urease activity during diarrhea transiently increased in the control group but not in the group receiving *L. casei* strain GG. No intergroup differences were found in β-glucuronidase, β-glucosidase, and glycocholic acid hydrolase levels. Therefore, Isolauri *et al.* suggested that rotavirus infection gives rise to biphasic diarrhea, the first phase being an osmotic diarrhea and the second associated with overgrowth of specifically urease-producing bacteria. Oral bacteriotherapy appears to be a promising means to counteract the disturbed microbial balance.

To evaluate the ingested strain's adherent properties and ability to inhibit murine rotavirus infection, Duffy *et al.* (101) administered human *Bifidobacterium* sp. strain *bifidum* to BALB/c lactating mice ($n = 58$) and their litters ($n = 327$ pups). ELISA and anaerobic bacteriologic techniques were used to measure murine rotavirus shedding and colonization of *Bifidobacterium* in the small intestine. At 1316 days of gestation, pregnant dams (and their expected litters) were randomly assigned to one of four experimental groups as follows: normal controls; *B. bifidum*-treated only; murine rotavirus-infected only; and *B. bifidum*-treated plus murine rotavirus-infected dams and litters. During the acute phase of diarrhea, 80% of small-intestine cultures in *B. bifidum*-treated litters were positive for the ingested *B. bifidum* strain compared to 24% of fecal cultures. The examination of tissue cross sections under electron microscopy revealed the ingested *B. bifidum* strain survived passage through the upper gastrointestinal tract and adhered to the small-intestine epithelium. After the administration of the high dose of virus, diarrhea developed in all pups, but onset was significantly delayed in *B. bifidum*-treated plus murine rotavirus-infected litters compared to litters infected with murine rotavirus only. *B. bifidum*-treated plus murine rotavirus-infected pups demonstrated a significant reduction in murine rotavirus shedding compared with litters challenged with murine rotavirus only at day 2-10 after inoculation. More direct studies are needed to assess the mechanisms by which this anaerobe may modify the course of murine rotavirus infection at the level of gut epithelium.

Qiao *et al.* (102) evaluated the potential synergistic effects of *Bifidobacterium* spp. (*B. bifidum* and *B. infantis*), with or without prebiotic compounds (arabino-galactan, short-chain fructo-oligosaccharide, iso-malto-dextrins), on modulating the course of rhesus rotavirus infection, as well as their ability to mediate the associated mucosal and humoral immune responses. Therefore, they fed these species orally to Balb/c pups. Rotavirus-specific IgA and IgG in serum, rotavirus antigen, and specific IgA in feces were measured by ELISA. Mucosal total IgA and IgG levels were determined in Peyer's patches by flow cytometry. Significantly delayed onset and early resolution of diarrhea were observed in bifidobacteria-treated, rhesus rotavirus-infected mice compared with rhesus rotavirus-infected control mice. They saw that supplementation with prebiotic compounds did not shorten the clinical course of diarrhea more than that observed with bifidobacteria treatment alone. Rotavirus-specific IgA in feces was elevated 16-fold on day 5 postinfection in bifidobacteria-treated, rhesus rotavirus-infected mice compared with the rhesus rotavirus-infected only group. In addition, the level of rotavirus-specific IgA in serum was fourfold higher in bifidobacteria-treated, rhesus rotavirus-infected litters *versus* mice challenged with rhesus rotavirus alone on 28 and 42 days postinfection. They found no enhancement of the immune response in rhesus rotavirus-infected mice that were treated with both bifidobacteria and prebiotic compounds over those treated with bifidobacteria alone. These findings suggested that bifidobacteria may act as an adjuvant by modulating early mucosal and strong humoral rotavirus-specific immune responses, and mitigate the severity of rotavirus-induced diarrhea (102).

Probiotics in inflammatory bowel disease

The term chronic inflammatory bowel disease includes three disease types: ulcerative colitis, Crohn's disease, and an intermediate form (about 10%). Crohn's disease is defined as a chronic granulomatous inflammation of the digestive tract that most commonly involves the distal ileum, colon and anus. Less often, the disease affects the mouth, esophagus, stomach and duodenum. Occasionally, extraintestinal sites are affected and it is referred to as: "metastatic Crohn's disease". In ulcerative colitis, the colon is affected and the disease usually starts in the rectum and progresses proximally, although sometimes the first manifestation may be the involvement of the whole colon and rectum (panproctocolitis).

Both Crohn's disease and ulcerative colitis are more common in whites that in blacks. Both sexes are equally affected. The incidence is three- to sixfold higher in Jews compared to non-Jews.

Ulcerative colitis is slightly more common than Crohn's disease. In Western Europe and North of America, there are 3000-5000 new cases of Crohn's disease and 8000-10000 new cases of ulcerative colitis. The incidence and prevalence of Crohn's disease have been increasing five times faster than that of ulcerative colitis. Young people are more likely to be more affected by inflammatory bowel disease than older people, with a peak incidence at the age of 15-30 years.

The etiology of this disease is unknown. An infectious hypothesis has been considered for years, and *Mycobacterium paratuberculosis* has been mainly isolated from patients with Crohn's disease. However, some patients with ulcerative colitis and controls harbor this pathogen. Viruses have also been involved in the pathogenesis. Several factors other than infectious agents have been postulated as the cause of the disease. These different factors are immunologic, genetic and psychological. The chronic inflammatory nature of these diseases may indicate the presence of an infectious cause or the presence of a dysregulatory abnormality in the control of inflammation.

An increasing number of both clinical and laboratory observations support the importance of the ubiquitous luminal bacteria in the inflammatory responses of these disorders (103). Bacteria are present throughout the gastrointestinal tract but are not evenly distributed and their diversity and numerical importance vary in the different sections of the gastrointestinal tract (8, 103). In the stomach and duodenum there are facultative anaerobic bacteria (*Lactobacillus* spp. and *Enterobacteriaceae*), with a small number of bacteria that are predomi-

nantly Gram-positive and aerobic (103). In the lower distal part of the intestine there is a large variety of bacteria, mostly anaerobic bacteria belonging to *Bacteroides*, *Bifidobacterium*, *Clostridium*, *Fusobacterium*, *Peptostreptococcus* and *Ruminococcus* (8). There is a transition to higher concentrations of bacteria and increasing number of Gram-negative bacteria in the distal ileum. Across the ileocecal valve there is a dramatic increase in bacterial concentration and more anaerobes than aerobes (103).

Enteric bacteria have been detected in patients with Crohn's disease and in those with pouchitis. These patients may be effectively treated with antibiotics. Purified bacterial products may initiate and perpetuate experimental colitis. The inflammation is due to loss of normal tolerance to the commensal flora (103).

The onset of inflammation is associated with an imbalance in the intestinal microflora with relative predominance of "aggressive" bacteria and an insufficient concentration of "protective" species. Reconditioning the flora through either direct supplementation with protective bacteria or by indirect stimulation plays a protective role in inflammatory bowel disease (103).

Antioxidant properties, the ability to increase prostacyclin in endothelial cell cultures and the ability to modulate adhesion molecule expression on human lymphocytes are all effects which are relevant for the use of probiotics in the treatment of immunological disorders such as inflammatory bowel disease (27).

Probiotics in ulcerative colitis

Few data are available on the role of probiotics in human ulcerative colitis. Two studies have shown a significant decrease in lactobacilli concentration in colonic biopsies in patients with ulcerative colitis. Preventing or controlling the colitis is reported when the concentration of *Lactobacillus* was modulated through dietary supplementation with lactulose (prebiotic). This is a nondigestible food ingredient that affects the host by selectively stimulating the growth and activity of one or more "probiotic" bacteria, such as *Bifidobacterium* and *Lactobacillus* that have health-promoting properties (104).

Ulcerative colitis is a chronic inflammation of the rectal and colonic mucosa, with a poorly defined etiology. Its characteristics are bloody diarrhea and mucus associated with a negative stool culture for bacteria, ova, or parasites. There is also fecal stasis with bacterial overgrowth and mucosal ischemia. The therapeutic role of probiotics is shown through two studies; in one of these, oral administration of *Lactobacillus* GG caused an increase in intestinal IgA immune response in patients with Crohn's disease. In the other study, exogenous administration of *L. reuteri* (pure bacterial suspension or as fermented oatmeal soup) prevented acetic acid-induced colitis or methotrexate-induced colitis in rats.

These studies showed a significant decrease in lactobacilli concentration in patients with active ulcerative colitis. The results showed that *L. plantarum* was more effective in methotrexate-induced colitis, and *Lactobacillus* treatment prevented development of spontaneous colitis in IL-10 gene-deficient mice.

In an open label study with 20 patients, intolerant or allergic to 5-aminosalicylic acid (5-ASA), a treatment consisting of VSL#3 6 g (1800 billions bacteria)/day for 12 months was instituted. Clinical, endoscopic assessment and stool culture and fecal pH determination were recorded (105). Nineteen patients completed the trial and 15 were in remission for the whole year. Fecal concentrations of bifidobacteria, lactobacilli, and *S. salivarius* spp. *Thermophilus* were significantly increased in all patients and remained stable throughout the study. No changes were noted in the concentrations of total aerobic bacteria, suggesting that the beneficial effects of VSL#3 were not related to suppression of endogenous luminal flora. The treatment was well-tolerated with no reported significant side effects like those seen in the treatment with 5-ASA oral compounds. This shows that the probiotic preparation was able to colonize the intestine and suggested its possible usefulness in maintaining remission in ulcerative colitis patients intolerant or allergic to 5-ASA (105). The hypothesis from these studies is that the intestinal environment may contribute to the pathophysiology of ulcerative colitis.

Guslandi *et al.* (106) studied the efficacy of *S. boulardii* in ulcerative colitis patients. Twenty-five patients with a mild to moderate clinical flare-up of ulcerative colitis received additional treatment with *S. boulardii* 250 mg three times a day for 4 weeks during maintenance treatment with mesalazine. These patients were unsuitable for steroid therapy. Rachmilewitz's clinical activity index was calculated before and after the treatment. Of the 24 patients who completed the study, 17 attained clinical remission; this was endoscopically confirmed. The preliminary results suggested that *S. boulardii* may be effective in the treatment of ulcerative colitis.

Probiotics in pouchitis

Pouchitis is a nonspecific 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 extraintestinal manifestations, primary sclerosing cholangitis, positive serology for perinuclear antineutrophil cytoplasmic antibodies, and backwash ileitis (107).

Pouchitis is associated with bacterial overgrowth and dysbiosis, and antibiotics represent the first-choice treatment. 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 of lactobacilli and bifidobacteria concentrations has been found in ulcerative colitis, Crohn's disease 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 activity of the inflammation. 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.* (103), seven patients, after clinical, endoscopic, and histological diagnoses of inflammation of the ileal pouch anal anastomosis with a pouchitis disease activity index (PDAI) > 7, were treated with 2 g/day of rifaximin (a non absorbable antibiotic) and 1 g/day of ciprofloxacin for 1 month. All patients went into remission during this month, as judged by clinical, endoscopic and histological examination. After remission, all seven patients were treated with the highly concentrated probiotic mixture VSL#3 for nine months. No patient had a relapse in this period. All patients who received placebo had a relapse.

Probiotics in the maintenance of remission of chronic pouchitis

Gionchetti *et al.* (108) evaluated the efficacy of VSL#3 in the maintenance of remission of chronic pouchitis. Forty patients in clinical and endoscopic remission were randomized to receive either VSL#3 6 g/day, or an identical placebo for 9 months. The patients were assessed clinically every month and endoscopically and histologically every 2 months or in the event of relapse. Three patients (15%) in the VSL#3 group had relapses within the 9-month follow-up period, compared with 20 (100%) in the placebo group. In the VSL#3-treated group, the fecal concentration of lactobacilli, bifidobacteria, and *S. thermophilus* increased significantly from baseline levels. These results suggested that oral administration of this new probiotic preparation is effective in preventing flare-ups of chronic pouchitis (Fig. 6).

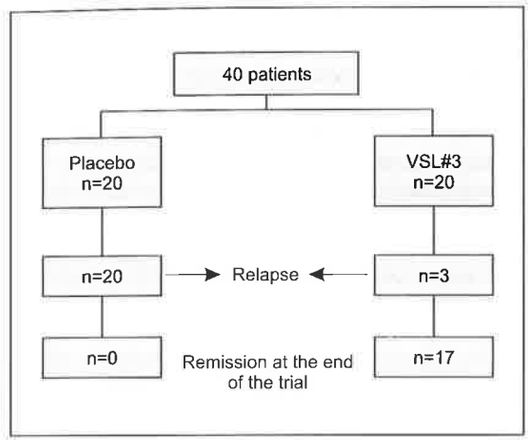

Fig. 6. Clinical outcome of patients according to treatment received. Reprinted from ref. 108 with permission from © American Gastroenterological Association.

Probiotics in the prevention of the onset of pouchitis

A positive effect of VSL#3 in the prevention of pouchitis has been reported by Gionchetti *et al.* (109), who compared probiotic therapy with VSL#3 *versus* placebo in the ability to prevent the onset of acute pouchitis during the first year after ileal pouch-anal anastomosis. Forty patients who underwent ileal pouch-anal anastomosis for ulcerative colitis were randomized to receive either VSL#3 or an identical placebo immediately after ileostomy closure for 1 year. Both groups consisted of 20 patients. The patients were assessed clinically, endoscopically, and histologically after 1, 3, 6, 9 and 12 months. Health-related quality of life was assessed using the Inflammatory Bowel Disease Questionnaire (IBDQ). Two of the 20 patients (10%) treated with VSL#3 had an episode of acute pouchitis compared with eight of the 20 patients (40%) treated with placebo. Treatment with VSL#3 determined a

significant improvement in IBDQ score, which was not the case with placebo. During treatment with VSL#3, fecal concentration of lactobacilli, bifidobacteria, and *S. salivarius* increased significantly. The fecal concentration of *Bacteroides*, clostridia, coliforms, and enterococci were not modified. This suggested that the beneficial effect was not mediated by the suppression of the endogenous flora. The treatment with VSL#3 was effective in the prevention of the onset of acute pouchitis and improved the quality of life of patients with ileal pouch anal anastomosis.

As mentioned above, probiotic bacteria do not survive for long, and rapidly disappear as soon as the treatment is stopped. Therefore, prophylactic probiotic therapy of pouchitis might require long-term treatment and might not be indicated for all patients. For this reason, VSL#3 would be highly beneficial for patients at high risk of chronic pouchitis. In these cases prophylactic probiotics may be administered, pouch function improved and their quality of life after ileal pouch anal anastomosis could be maintained. Katz *et al.* (107) suggested that probiotics should be used for maintaining remission in chronic pouchitis and as prophylaxis against pouch inflammation in high-risk patients. Although preliminary results suggest that high doses VSL#3 may improve active pouchitis, probiotic therapy seems to be more effective to prevent mucosal inflammation than to treat it.

Kuisma *et al.* (110) investigated the efficacy of *Lactobacillus* GG supplementation as primary therapy for pouchitis and its effect on the microbial flora. Twenty patients, with a previous history of pouchitis and endoscopic inflammation were recruited for a prospective, randomized, double-blind, placebo-controlled trial of *Lactobacillus* GG supplementation. Ten patients received *Lactobacillus* GG and 10 placebo, in two gelatin capsules b.i.d. for 3 months. Quantitative bacterial culture of fresh fecal samples and biopsies taken from the pouch and afferent limb was performed before and after supplementation. *Lactobacillus* GG supplementation was found to change the pouch intestinal flora by increasing the ratio of total fecal lactobacilli to total fecal anaerobes and enhancing the frequency of lactobacilli-positive cultures in the pouch and afferent limb mucosal biopsy samples. Only 40% of patients were colonized with *Lactobacillus* GG, and no differences were observed between the groups with regard to the mean PDAI or the total anaerobes or aerobes of fecal or tissue biopsy samples. Thus, a single-strain probiotic bacterium supplement of *Lactobacillus* GG changed the pouch intestinal bacterial flora, but was ineffective as primary therapy for a clinical or endoscopic response. More clinical trials are needed to evaluate the right placement and dosage of probiotics within a treatment regimen for pouchitis.

Probiotics in Crohn's disease

The therapeutic role of probiotics in the prevention of postoperative recurrence of Crohn's disease has been reported in some studies. Campieri *et al.* (111) studied the effects of VSL#3 in a randomized, investigator-blind trial. Forty patients with curative resection randomized within 1 week post surgery were divided into two groups of 20 patients. One group received mesalazine 4 g/day for 1 year and the other group received rifaximin 1.8 g/day for 3 months followed by VSL#3 6 g/day for 9 months. The endoscopic activity was assessed after 3 and 12 months. In the mesalazine group, eight patients had severe endoscopic recurrence after 3 months as well as after 12 months, whereas in the group with rifaximin and VSL#3, two patients had a severe recurrence after 3 months and two patients after 12 months. These results suggested the efficacy of the combination of a nonabsorbable antibiotic with a highly concentrated probiotic preparation in the prevention of severe endoscopic recurrence of Crohn's disease after surgical resection.

In a pilot study Guandalini *et al.* (112) investigated the possible effect of *Lactobacillus* GG in children with active Crohn's disease. Four male patients with a median age of 14.5 years (range 10–18) were enrolled. In terms of clinical outcome, the patients showed significant improvement. In three patients receiving *Lactobacillus* GG, it was possible to taper the dose of steroids.

In a third published study using *Lactobacillus* GG this effect could not be confirmed. Forty-five patients were randomized to receive *Lactobacillus* GG 12 billion CFU/day (23 patients) or placebo (22 patients). A clinical remission after 52 weeks was seen in 15 of the 23 patients with *Lactobacillus* GG (83.3%) and in 17 of the 22 patients with placebo (89.4%). Mild endoscopic activity was seen in nine of the 15 patients with remission in the *Lactobacillus* GG group (60%) and in six of the 17 patients with remission in the placebo group (35.3%). This study failed to show effectiveness in the postoperative prevention on Crohn's disease (113). More studies are therefore necessary. The limited experience indicates that different probiotics have different capacity to prevent intestinal inflammation.

Probiotics in irritable bowel syndrome

Irritable bowel syndrome is a widespread and multifactorial functional disorder of the digestive tract (114). 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 psychophysiological phenomena and colonic fermentation (115).

A large proportion of patients have periods characterized by sudden and unforeseeable changes in the two main symptoms, constipation and diarrhea, even within a few days (115). It is very likely that the syndrome represents different groups of patients with probably different pathogenesis. Irritable bowel syndrome may follow gastroenteritis and may be associated with an abnormal gut flora and with food intolerance (117). 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 bifidobacteria (115). 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 irritable bowel syndrome, 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 (115).

Some reports suggest that probiotics play a role in regulating the motility of the digestive tract (114). This may result in improvements in pain and flatulence in response to probiotic administration (115).

To assess whether preceding gastroenteritis or food intolerance were associated with colonic malfermentation, King *et al.* (117) conducted a crossover controlled trial with a standard diet and an exclusion diet matched for macronutrients in six female patients with irritable bowel syndrome and six female controls. In this study fecal excretion of fat, nitrogen, starch, and nonstarch polysaccharide was measured during the last 72 hours of each diet. The total excretion of hydrogen and methane were collected over 24 hours in purpose-built 1.4 m^3 whole body calorimeter. Breath hydrogen and methane excretion were measured for 3 hours 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 greater than in controls, whereas hydrogen production was higher. After lactulose, breath hydrogen was greater on the standard than on the exclusion diet. This means that colonic-gas production, particularly of hydrogen, is greater in patients with irritable bowel disease 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 (117).

Spiller *et al.* (118) studied the intestinal permeability (lactulose/mannitol ratio) and histological and immunological features in rectal biopsy specimens in 21 patients who had acute *Campylobacter* enteritis, 10 patients with postdysenteric irritable bowel syndrome and 12 asymptomatic controls. They found that the increased enteroendocrine cell counts, T lymphocytes, and gut permeability, which may survive for more than a year after *Campylobacter* enteritis, contribute to post-dysenteric irritable bowel syndrome (118), thus offering a rationale to use probiotics for several months after the infectious episode.

VSL#3 in patients with irritable bowel syndrome

The effect of the probiotics was studied by Brigidi *et al.* (119) in a clinical trial in which 10 patients suffering from this syndrome were administered the VSL#3 probiotic preparation. The results indicated that the administration of VSL#3 improved the clinical picture and changed the composition and biochemistry of fecal microbiota. The exact mechanisms of the positive effects are not known. The selection of patients may have had an important role in detecting the positive effects. Whether the induction of a significant increase in lactobacilli, bifidobacteria, and *S. thermophilus* contributed to the regulation of the motility disorders or the increase in fecal β-galactosidase with a decrease in urease content indicate that a good re-

sponse requires further study. The importance of this study is that it showed that the measurement of specific parameters and changes in the specific microflora was possible.

Kim *et al.* (120) investigated the effects of VSL#3 on gastrointestinal transit and symptoms of patients with Rome II-irritable bowel syndrome with predominant diarrhea. Twenty-five patients with diarrhea-predominant irritable bowel syndrome were randomly assigned to receive VSL#3 powder (450 billion lyophilized bacteria/day) or matching placebo twice daily for 8 weeks after a 2-week run-in period. Pre- and post-treatment gastrointestinal transit measurements were performed in all patients. The patients recorded their bowel function and symptoms daily in a diary during the 10-week study, which was powered to detect a 50% change in the primary colonic transit endpoint. There were no significant differences in mean gastrointestinal transit measurements, bowel function scores or satisfactory global symptom relief between the two treatment groups, pre- or post-therapy. The differences in abdominal bloating scores between treatments were borderline significant. Abdominal bloating was reduced with VSL#3, but not with placebo. Furthermore, VSL#3 had no effects on individual symptoms such as abdominal pain, gas and urgency. VSL#3 was well tolerated by all patients, and thus it seems to relieve the abdominal bloating in patients with diarrhea-predominant irritable bowel syndrome (120).

Future perspectives

Lactobacilli derived from the endogenous flora of normal donors are being increasingly used as probiotics in functional foods and as vaccine carriers. However, a variety of studies carried out with distinct strains of lactobacilli have suggested heterogeneous and strain-specific effects (Table IV).

To dissect this heterogeneity at the immunological level, Ibnou-Zekri *et al.* (137) selected two strains of lactobacilli that displayed similar properties *in vitro* and studied their impact on mucosal and systemic B-cell responses in monoxenic mice. Germ-free mice were colonized with *L. johnsonii* (NCC 533) or *L. paracasei* (NCC 2461). Bacterial loads were monitored for 30 days in intestinal tissues, and mucosal and systemic B-cell responses were measured. Although both *Lactobacillus* strains displayed similar growth, survival, and adherence properties *in vitro*, they colonized the intestinal lumen and translocated into mucosal lymphoid organs at different densities. *L. johnsonii* colonized the intestine very efficiently at high levels, whereas the number of *L. paracasei* decreased rapidly and it colonized at low levels. They determined whether this difference in colonization correlated with an induction of different types of immune responses, and observed that colonization with either strain induced similar germinal center formation and IgA-bearing lymphocytes in the mucosa, suggesting that both strains may activate mucosal B-cell responses. However, clear differences in the patterns of immunoglobulins were observed between the two strains in the mucosa and in the periphery. Therefore, despite similar *in vitro* probiotic properties, distinct *Lactobacillus* strains may colonize the gut differently and generate divergent immune responses.

In another study, Coakley *et al.* (138), assessed strains of *Lactobacillus*, *Lactococcus*, *Pediococcus* and *Bifidobacterium* for their ability to produce the health-promoting fatty acid conjugated linoleic acid from free linoleic acid. Strains of *Lactobacillus*, *Lactococcus*, *Pediococcus* and *Bifidobacterium* were grown in medium containing free linoleic acid. Growth of the bacteria in linoleic acid and conversion of the linoleic acid to conjugated linoleic acid was assessed.

Of the bacteria assessed, nine strains of *Bifidobacterium* produced the c9, t11 CLA isomer from free linoleic acid. The t9, t11 conjugated linoleic acid isomer was also produced by some strains, but at much lower concentrations. Thus, the production of conjugated linoleic acid by bifidobacteria showed considerable interspecies variation. *Bifidobacterium breve* and *B. dentium* were the most efficient producers of conjugated linoleic acid among the range of strains tested, with *B. breve* converting up to 65% linoleic acid to c9, t11 conjugated linoleic acid when grown in 0.55 mg/ml linoleic acid. Strains also varied considerably with respect to their sensitivity to linoleic acid. The production of conjugated linoleic acid by probiotic bifidobacteria offers a possible mechanism for some health-enhancing properties of bifidobacteria and provides novel opportunities for the development of functional foods.

Finally, it has been demonstrated that the therapeutic dose of IL-10 may be reduced by localized delivery of a probiotic genetically engineered to secrete the cytokine. Intragastric administration of IL-10-secreting *Lactococcus lactis* caused a 50% reduction in colitis in mice treated with dextran sulfate sodium and prevented the onset of colitis in

Table IV: Studies carried out with distinct strains of probiotics suggesting heterogeneous and strain-specific effects.

Indication	Probiotic	Result	Ref.
Rotavirus diarrhea in children	*L. casei* strain GG	42 children (5-28 months) Diarrheal phase shortened Significant influence on bacterial enzyme profile	(100)
in mice	*Bifidum*	58 lactating mice and 327 pups Significant reduction in murine rotavirus shedding	(101)
in mice	*Bifidum* and *infantis*	Significant delay in onset and early resolution of diarrhea Rotavirus-specific IgA in feces 16-fold elevated on day 5	(102)
in children	*Lactobacillus* GG every day of hospital stay	220 children, 21.4% breastfed and 78.8% non-breastfed received *Lactobacillus* GG. The attack rate of rotavirus infections among the patients who received probiotic was 25.4% (29 of 114 patients), while for the placebo group it was 30.2% (32 of 106 patients). *Lactobacillus* GG was not effective, breastfeeding was effective	(121)
Antibiotic-associated diarrhea	*Enterococcus* SF 68 vs. placebo	Reducing incidence of antibiotic-associated diarrhea: 8.7% in *Enterococcus* SF 68 group, and 27.2% in placebo group	(96)
Acute diarrhea, nonbloody	*Lactobacillus* GG vs. placebo	31% with *Lactobacillus* GG vs. 75% with placebo had persistent watery diarrhea at 48 h	(97)
Acute infectious diarrhea in children	*Lactobacillus*	Reduction in duration of diarrhea of 0.7 days Reduction in frequency of 1.6 stools on day 2 *Lactobacillus* safe and effective, possible dose effect	(98)
Acute-onset diarrhea of all causes	*Lactobacillus* GG in oral rehydration solution Multicenter trial	Diarrhea lasted >7 days in 10.7% of placebo group vs. 2.7% in *Lactobacillus* GG group. Hospital stay significantly shorter in *Lactobacillus* GG group	(122)
Atopic eczema and allergy to cow's milk	Viable and heat inactivated *Lactobacillus* GG	The treatment with heat-inactivated *Lactobacillus* GG was associated with adverse GI symptoms and diarrhea. SCORAD scores (interquartile range) decreased from 13 to 8 units in the placebo group, from 19 to 5 units in the viable *Lactobacillus* GG group, and from 15 to 7 units in the heat-inactivated *Lactobacillus* GG group. The decrease in the SCORAD scores within the viable *Lactobacillus* GG group tended to be greater than within the placebo group. Supplementation of infant formulas with viable but not heat-inactivated *Lactobacillus* GG is a potential approach for the management of atopic eczema and cow's milk allergy	(123)
Travellers' diarrhea	*L. fermentum* or *L. acidophilus*	No effect	(124)
Antibiotic-induced diarrhea	*Lactobacillus* GG	188 children, 25 placebo-treated, but only 7 LGG-treated patients had diarrhea (liquid stools≥2 per day). Effective in reduction of incidence of diarrhea.	(125)
	Saccharomyces boulardii	Helpful in prevention	(126)
		The efficacy for the prevention of antibiotic-associated diarrhea was 51%	(127)

Continued

Table IV: Studies carried out with distinct strains of probiotics suggesting heterogeneous and strain-specific effects (cont.).

Indication	Probiotic	Result	Ref.
Clostridium difficile-associated colitis	*S. boulardii*	Of 13 patients with recurring *C. difficile* cytotoxin-positive diarrhea, 11 had no further recurrence	(128)
	L. casei strain GG	Successful treatment in 5 adults (100%)	(129)
		Successful treatment in 4 children (100%) with multiple relapses of *C. difficile*	(130)
Crohn's disease	VSL#3 and rifaximin vs. mesalazine	In the mesalazine group 8 patients had recurrence after 3 and 12 months. In VSL#3 group, 2 patients had recurrence after 12 months	(111)
	Lactobacillus GG (in children)	4 male patients Significant improvement	(112)
	Lactobacillus GG	Clinical remission after 52 weeks in 15 of 23 patients with *Lactobacillus* GG and in 17 of 22 with placebo. Mild endoscopic activity in 9 of 15 patients in remission (60%) in *Lactobacillus* GG group and in 6 of 17(35.3%) patients with remission in the placebo group. This study failed to show effectiveness in the post-operative prevention of Crohn's disease	(131)
		Bowel tenderness appeared unaffected by *Lactobacillus* GG in 11 of 19 patients, decreased in 2 and worsened in 6 patients. *Lactobacillus* GG showed not to influence the number of daily liquid stools and hematochemical parameters. *Lactobacillus* GG not effective in reducing symptoms	(132)
Ulcerative colitis (UC)	VSL#3	Maintaining remission in 19 UC patients intolerant to mesalazine. 15 of these patients were in remission the whole year	(105)
	S. boulardii	17 of 24 patients in remission *S. boulardii* can be effective in treatment of UC	(106)
	E. coli strain Nissle compared with mesalazine (500 mg)	120 patients with inactive UC included in a double-blind study for 12 weeks. No significant differences between both groups.Use of higher dose of mesalazine (2.4 mg) confirmed previous results. Treatment with *E. coli* has equivalent effect to mesalazine	(54) (55)
	Bifidobacteria	21 patients. 11 received *Bifidobacteria*-fermented milk (BFM) and 10 were followed up for 1 year, in a randomized controlled trial. Exacerbation in 3 out of 11 subjects in the BFM group and in 9 out of 10 in the control group.	(70)
Pouchitis	VSL#3 after treatment with rifaximin and ciprofloxacin	7 patients were included and they all went into remission. There were no relapses, whereas all patients with placebo relapse dx	(103)
Maintenance of remission of pouchitis	VSL#3	Group of 40 patients (20 VSL#3 and 20 placebo), 15% had relapses in study group within 9 months and 100% had relapses in control group.VSL#3 is effective in preventing flare-ups.	(108)
Prevention of onset of pouchitis	VSL#3	Group of 40 patients (20 VSL#3 and 20 placebo), 10% of VSL#3 group had episode with acute pouchitis, compared with 40% of placebo group. VSL#3 is effective in prevention of onset of disease	(109)

Continued

Table IV: Studies carried out with distinct strains of probiotics suggesting heterogeneous and strain-specific effects (cont.).

Indication	Probiotic	Result	Ref.
Irritable bowel syndrome (IBS)	VSL#3	10 patients with a clinical improvement and change of composition and biochemistry of fecal microbiota	(119)
		25 patients with diarrhea-predominant IBS Reduction of abdominal bloating	(120)
Allergic disorders of the gastrointestinal tract		Significant improvement in infants	(133)
Control of allergic inflammation	*Bifidobacterium lactis* Bb-12 or *Lactobacillus* GG vs. placebo	After 2 months, there was a significant improvement in skin condition in patients given probiotic-supplemented formulas, as compared to the unsupplemented group	(134)
Prevention of atopic disease	*L. rhamnosus* GG	Perinatal administration of *Lactobacillus* GG decreases the prevention of atopic disease in at-risk infants	(135)
		107 children, 14 of 53 children receiving *Lactobacillus* developed atopic eczema, compared with 25 of 54 children receiving placebo. This suggests a preventive effect that extends beyond infancy	(136)

IL-10 gene-deficient mice (139). This observation has also opened the way for the use of probiotics as live vaccine delivery vectors (140).

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References

1. Morelli, L. *In vitro selection of probiotic lactobacilli: A critical appraisal.* Curr Issues Intest Microbiol 2000, 1: 59-67.
2. Mercenier, A., Pavan, S., Pot, B. *Probiotics as biotherapeutic agents: Present knowledge and future prospects.* Curr Pharm Des 2003, 9: 175-91.
3. Yokokura, T. *Lactobacillus casei strain shirota.* 1999.
4. Reid, G. *Testing the efficacy of probiotics.* En: Tannock, G.W., (Ed.). Probiotics: A critical review Horizon Scientific Press, Wymondham, UK; 1999; ?? 129-140.
5. Tissier, H. *Recherches sur la flore intestinale des nourissons (etat normal et pathologique).* Paris, University of Paris; 1906.
6. Berg, R.D. *Probiotics, prebiotics or "conbiotics"?* Trends Microbiol 1998, 6: 89-92.
7. Bengmark, S. *Ecological control of the gastrointestinal tract. The role of probiotic flora.* Gut 1998, 42: 2-7.
8. Simmering, R., Blaut, M. *Pro- and prebiotics – The tasty guardian angels?* Appl Microbiol Biotechnol 2001, 55: 19-28.
9. Salminen, S., Isolauri, E., Salminen, E. *Clinical uses of probiotics for stabilizing the gut mucosal barrier: Successful strains and future challenges.* Antonie Van Leeuwenhoek 1996,70: 347-58.
10. Yolken, R.H., Ojeh, C., Khatri, I.A., Sajjan, U., Forstner, J.F. *Intestinal mucins inhibit rotavirus replication in an oligosaccharide-dependent manner.* J Infect Dis 1994, 169: 1002-6.
11. Phuapradit, P., Varavithya, W., Vathanophas, K. et al. *Reduction of rotavirus infection in children receiving bifidobacteria-supplemented formula.* J Med Assoc Thai 1999, 82 (Suppl. 1): S43-8.
12. Marteau, P., Seksik, P., Jian, R. *Probiotics and intestinal health effects: A clinical perspective.* Br J Nutr 2002, 88 (Suppl. 1): S51-7.
13. Li, Z., Yang, S., Lin, H. et al. *Probiotics and antibodies to TNF inhibit inflammatory activity and improve nonalcoholic fatty liver disease.* Hepatology 2003, 37: 343-50.
14. Cremonini, F., Canducci, F., Di Caro, S. et al. *Helicobacter pylori treatment: A role for probiotics?* Dig Dis 2001, 19: 144-7.

15. Canducci, F., Cremonini, F., Armuzzi, A. et al. *Probiotics and Helicobacter pylori eradication.* Dig Liver Dis 2002, 34 (Suppl. 2): S81-3.
16. Madsen, K.L., Doyle, J.S., Tavernini, M.M., Jewell, L.D., Rennie, R.P., Fedorak, R.N. *Antibiotic therapy attenuates colitis in interleukin 10 gene-deficient mice.* Gastroenterology 2000, 118: 1094-105.
17. Strober, W., Fuss, I.J., Blumberg, R.S. *The immunology of mucosal models of inflammation.* Annu Rev Immunol 2002, 20: 495-549.
18. Dunne, C., O'Mahony, L., Murphy, L. et al. *In vitro selection criteria for probiotic bacteria of human origin: Correlation with in vivo findings.* Am J Clin Nutr 2001, 73: 386S-392S.
19. Dunne, C., Murphy, L., Flynn, S., O'Mahony, L. et al. *Probiotics: From myth to reality. Demonstration of functionality in animal models of disease and in human clinical trials.* Antonie Van Leeuwenhoek 1999, 76: 279-92.
20. Dunne, C. *Adaptation of bacteria to the intestinal niche: Probiotics and gut disorder.* Inflamm Bowel Dis 2001, 7: 136-45.
21. Sanders, M.E., Klaenhammer, T.R. *Invited review: The scientific basis of Lactobacillus acidophilus NCFM functionality as a probiotic.* J Dairy Sci 2001, 84: 319-31.
22. Kaila, M., Isolauri, E., Soppi, E., Virtanen, E., Laine, S., Arvilommi, H. *Enhancement of the circulating antibody secreting cell response in human diarrhea by a human Lactobacillus strain.* Pediatr Res 1992, 32: 141-4.
23. Bengmark, S., Gianotti, L. *Nutritional support to prevent and treat multiple organ failure.* World J Surg 1996, 20: 474-81.
24. Sanders, M.E. *Considerations for use of probiotic bacteria to modulate human health.* J Nutr 2000, 130: 384S-90S.
25. Vaughan, E.E., Mollet, B., deVos, W.M. *Functionality of probiotics and intestinal lactobacilli: Light in the intestinal tract tunnel.* Curr Opin Biotechnol 1999, 10: 505-10.
26. Karimi, O., Pena, A.S. *Clinical aspects of probiotics: News on the horizon.* Yakult 2003, in press.
27. De Simone, C. *VSL# 3 A probiotic preparation investigator brochure.* 2001.
28. Rambaud, J.C., Bouhnik, Y., Marteau, P., Pochart P. *Manipulation of the human gut microflora.* Proc Nutr Soc 1993, 52: 357-66.
29. O'Sullivan MGTGOSGCC, J.K. *Probiotic bacteria: Myth or reality?* Trends Food Sci Technol 1992, 3: 309-14.
30. Patterson, J.A., Burkholder, K.M. *Application of prebiotics and probiotics in poultry production.* Poult Sci 2003, 82: 627-31.
31. Perdigon, G., Nader de Macias, M.E., Álvarez, S., Oliver, G., Pesce de Ruiz Holgado, A.A. *Prevention of gastrointestinal infection using immunobiological methods with milk fermented with Lactobacillus casei and Lactobacillus acidophilus.* J Dairy Res 1990, 57: 255-64.
32. Rani, B., Khetarpaul, N. *Probiotic fermented food mixtures: Possible applications in clinical anti-diarrhoea usage.* Nutr Health 1998, 12: 97-105.
33. McFarland, L.V. *Re: Probiotics and C difficile diarrhea.* Am J Gastroenterol 2000, 95: 2128.
34. Sorokulova, I.B., Kirik, D.L., Pinchuk, II. *Probiotics against Campylobacter pathogens.* J Travel Med 1997, 4: 167-70.
35. Dembele, T., Obdrzalek, V., Votava, M. *Inhibition of bacterial pathogens by lactobacilli.* Zentralbl Bakteriol 1998, 288: 395-401.
36. Barrow, P.A., Brooker, B.E., Fuller, R., Newport, M.J. *The attachment of bacteria to the gastric epithelium of the pig and its importance in the microecology of the intestine.* J Appl Bacteriol 1980, 48: 147-54.
37. Schiffrin, E.J., Brassart, D., Servin, A.L., Rochat, F., Donnet-Hughes, A. *Immune modulation of blood leukocytes in humans by lactic acid bacteria: Criteria for strain selection.* Am J Clin Nutr 1997, 66: 515S-20S.
38. Madsen, K.L. *The use of probiotics in gastrointestinal disease.* Can J Gastroenterol 2001, 15: 817-22.
39. Isolauri, E., Majamaa, H., Arvola, T., Rantala, I., Virtanen, E., Arvilommi, H. *Lactobacillus casei strain GG reverses increased intestinal permeability induced by cow milk in suckling rats.* Gastroenterology 1993, 105: 1643-50.
40. Eckmann, L., Kagnoff, M.F., Fierer, J. *Intestinal epithelial cells as watchdogs for the natural immune system.* Trends Microbiol 1995, 3: 118-20.
41. Romagnani, S., Parronchi, P., D'Elios, M.M. et al. *An update on human Th1 and Th2 cells.* Int Arch Allergy Immunol 1997, 113: 153-6.
42. Casini-Raggi, V., Kam, L., Chong, Y.J., Fiocchi, C., Pizarro, T.T., Cominelli, F. *Mucosal imbalance of IL-1 and IL-1 receptor antagonist in inflammatory bowel disease. A novel mechanism of chronic intestinal inflammation.* J Immunol 1995, 154: 2434-40.
43. Peña, A.S. *Genetics of inflammatory bowel disease. The candidate gene approach: Susceptibility versus disease heterogeneity.* Dig Dis 1998, 16: 356-63.
44. McGeehan, G.M., Becherer, J.D., Bast, R.C. et al. *Regulation of tumour necrosis factor-alpha processing by a metalloproteinase inhibitor.* Nature 1994, 370: 558-61.
45. Fabia, R., Ar'Rajab, A., Johansson, M.L. et al. *Impairment of bacterial flora in human ulcerative colitis and experimental colitis in the rat.* Digestion 1993, 54: 248-55.
46. Malin, M., Suomalainen, H., Saxelin, M., Isolauri, E. *Promotion of IgA immune response in patients with Crohn's disease by oral bacteriotherapy with Lactobacillus GG.* Ann Nutr Metab 1996, 40: 137-45.
47. Ruseler-van Embden, J.G., Schouten, W.R., van Lieshout, L.M. *Pouchitis: Result of microbial imbalance?* Gut 1994, 35: 658-64.
48. Ivanova, T., Jekova, M. *Preventive role of lyophilic dairy products bulgaricum and biostim.* Cancer Lett 1997, 114: 93-5.
49. Campieri, M., Gionchetti, P. *Bacteria as the cause of ulcerative colitis.* Gut 2001, 48: 132-5.
50. Neish, A.S., Gewirtz, A.T., Zeng, H. et al. *Prokaryotic regulation of epithelial responses by inhibition of I-κ-B-α ubiquitination.* Science 2000, 289: 1560-3.
51. Ulisse, S., Gionchetti, P., D'Alo, S. et al. *Expression of cytokines, inducible nitric oxide synthase, and matrix metalloproteinases in pouchitis: Effects of probiotic treatment.* Am J Gastroenterol 2001, 96: 2691-9.
52. Friedman, R., Hughes, A.L. *Molecular evolution of the NF-κ-B signaling system.* Immunogenetics 2002, 53: 964-74.
53. Farrell, R.J., Peppercorn, M.A. *Ulcerative colitis.* Lancet 2002, 359: 331-40.
54. Kruis, W., Schutz, E., Fric, P., Fixa, B., Judmaier, G., Stolte, M. *Double-blind comparison of an oral Escherichia coli preparation and mesalazine in maintaining remission of ulcerative colitis.* Aliment Pharmacol Ther 1997, 11: 853-8.
55. Rembacken, B.J., Snelling, A.M., Hawkey, P.M., Chalmers, D.M., Axon, A.T. *Non-pathogenic Escherichia coli versus mesalazine for the treatment of ulcerative colitis: A randomised trial.* Lancet 1999, 354 :635-9.
56. Faubion, W.A., Sandborn, W.J. Probiotic therapy with E. coli for ulcerative colitis: take the good with the bad. Gastroenterology 2000; 118: 630-1.
57. Fedorak, R.N., Madsen, K. Application of probiotics for GI diseases. 2003 symposium mexico.
58. Ohashi, Y., Inoue, R., Tanaka, K., Matsuki, T., Umesaki, Y., Ushida, K. *Lactobacillus casei strain Shirota-fermented milk stimulates indigenous Lactobacilli in the pig intestine.* J Nutr Sci Vitaminol (Tokyo) 2001, 47: 172-6.
59. Ohashi, Y., Inoue, R., Tanaka, K., Umesaki, Y., Ushida, K. *Strain gauge force transducer and its application in a pig model to evaluate the effect of probiotic on colonic motility.* J Nutr Sci Vitaminol (Tokyo) 2001, 47: 351-6.
60. Shida, K., Takahashi, R., Iwadate, E. et al. *Lactobacillus casei strain Shirota suppresses serum immunoglobulin E and immunoglobulin G1 responses and systemic anaphylaxis in a food allergy model.* Clin Exp Allergy 2002, 32: 563-70.
61. Kato, I., Tanaka, K., Yokokura, T. *Lactic acid bacterium potently induces the production of interleukin-12 and interferon-γ by mouse splenocytes.* Int J Immunopharmacol 1999, 21: 121-31.
62. Madsen, K.L., Doyle, J.S., Jewell, L.D., Tavernini, M.M., Fedorak, R.N. *Lactobacillus species prevents colitis in interleukin 10 gene-deficient mice.* Gastroenterology 1999, 116: 1107-14.
63. Konrad, A., Mahler, M., Flogerzi, B. et al. *Amelioration of murine colitis by feeding a solution of lysed Escherichia coli.* Scand J Gastroenterol 2003, 38: 172-9.
64. Coconnier, M.H., Lievin, V., Hemery, E., Servin, A.L. *Antagonistic activity against Helicobacter infection in vitro and in vivo by the human Lactobacillus acidophilus strain LB.* Appl Environ Microbiol 1998, 64: 4573-80.
65. Hudault, S., Lievin, V., Bernet-Camard, M.F., Servin, A.L. *Antagonistic activity exerted in vitro and in vivo by Lactobacillus casei (strain GG) against Salmonella typhimurium C5 infection.* Appl Environ Microbiol 1997, 63: 513-8.
66. Fukushima, Y., Kawata, Y., Mizumachi, K., Kurisaki, J., Mitsuoka, T. *Effect of bifidobacteria feeding on fecal flora and production of immunoglobulins in lactating mouse.* Int J Food Microbiol 1999, 46: 193-7.
67. Chiang, B.L., Sheih, Y.H., Wang, L.H., Liao, C.K., Gill, H.S. *Enhancing immunity by dietary consumption of a probiotic lactic acid bacterium (Bifidobacterium lactis HN019): Optimization and definition of cellular immune responses.* Eur J Clin Nutr 2000, 54: 849-55.

68. Dieleman, L.A., Goerres, M.S., Arends, A. et al. *Lactobacillus GG prevents recurrence of colitis in HLA-B27 transgenic rats after antibiotic treatment.* Gut 2003, 52: 370-6.
69. Fukushima, Y., Kawata, Y., Hara, H., Terada, A., Mitsuoka, T. *Effect of a probiotic formula on intestinal immunoglobulin A production in healthy children.* Int J Food Microbiol 1998, 42: 39-44.
70. Ishikawa, H., Akedo, I., Umesaki, Y., Tanaka, R., Imaoka, A., Otani, T. *Randomized controlled trial of the effect of bifidobacteria-fermented milk on ulcerative colitis.* J Am Coll Nutr 2003; 22: 56-63.
71. Famularo, G., Trinchieri, V., De Simone, C. *Inflammatory bowel disease.* N Engl J Med 2002; 347: 1982-4; author reply 1982-4.
72. Madsen, K.L., Malfair, D., Gray, D., Doyle, J.S., Jewell, L.D., Fedorak, R.N. *Interleukin-10 gene-deficient mice develop a primary intestinal permeability defect in response to enteric microflora.* Inflamm Bowel Dis 1999, 5: 262-70.
73. Haller, D., Bode, C., Hammes, W.P., Pfeifer, A.M., Schiffrin, E.J., Blum, S. *Non-pathogenic bacteria elicit a differential cytokine response by intestinal epithelial cell/leucocyte co-cultures.* Gut 2000, 47: 79-87.
74. Madsen, K., Cornish, A., Soper, P. et al. *Probiotic bacteria enhance murine and human intestinal epithelial barrier function.* Gastroenterology 2001, 121: 580-91.
75. Heinemann, C., van Hylckama Vlieg, J.E., Janssen, D.B., Busscher, H.J., van der Mei, H.C., Reid, G. *Purification and characterization of a surface-binding protein from Lactobacillus fermentum RC-14 that inhibits adhesion of Enterococcus faecalis 1131.* FEMS Microbiol Lett 2000, 190: 177-80.
76. Arunachalam, K., Gill, H.S., Chandra, R.K. *Enhancement of natural immune function by dietary consumption of Bifidobacterium lactis (HN019).* Eur J Clin Nutr 2000, 54: 263-7.
77. Newberry, R.D., Stenson, W.F., Lorenz, R.G. *Cyclooxygenase-2-dependent arachidonic acid metabolites are essential modulators of the intestinal immune response to dietary antigen.* Nat Med 1999, 5: 900-6.
78. Aderem, A., Ulevitch, R.J. *Toll-like receptors in the induction of the innate immune response.* Nature 2000, 406: 782-7.
79. Abreu, M.T., Taylor, K.D., Lin, Y.C. et al. *Mutations in NOD2 are associated with fibrostenosing disease in patients with Crohn's disease.* Gastroenterology 2002, 123: 679-88.
80. da Silva Correia, J., Soldau, K., Christen, U., Tobias, P.S., Ulevitch, R.J. *Lipopolysaccharide is in close proximity to each of the proteins in its membrane receptor complex. Transfer from CD14 to TLR4 and MD-2.* J Biol Chem 2001, 276: 21129-35.
81. Jiang, Q., Akashi, S., Miyake, K., Petty, H.R. *Lipopolysaccharide induces physical proximity between CD14 and Toll-like receptor 4 (TLR4) prior to nuclear translocation of NF-κB.* J Immunol 2000, 165: 3541-4.
82. Abreu, M.T., Vora, P., Faure, E., Thomas, L.S., Arnold, E.T., Arditi, M. *Decreased expression of Toll-like receptor-4 and MD-2 correlates with intestinal epithelial cell protection against dysregulated proinflammatory gene expression in response to bacterial lipopolysaccharide.* J Immunol 2001, 167: 1609-16.
83. Murch, S.H. *The immunologic basis for intestinal food allergy.* Curr Opin Gastroenterol 2000, 16: 552-7.
84. Hemmi, H., Takeuchi, O., Kawai, T. et al. *A Toll-like receptor recognizes bacterial DNA.* Nature 2000, 408: 740-5.
85. Hacker, H., Vabulas, R.M., Takeuchi, O., Hoshino, K., Akira, S., Wagner, H. *Immune cell activation by bacterial CpG-DNA through myeloid differentiation marker 88 and tumor necrosis factor receptor-associated factor (TRAF) 6.* J Exp Med 2000, 192: 595-600.
86. Chu, R.S., Targoni, O.S., Krieg, A.M., Lehmann, P.V., Harding, C.V. *CpG oligodeoxynucleotides act as adjuvants that switch on T helper 1 (Th1) immunity.* J Exp Med 1997, 186: 1623-31.
87. Román, M., Martín-Orozco, E., Goodman, J.S. et al. *Immunostimulatory DNA sequences function as T helper-1-promoting adjuvants.* Nat Med 1997, 3: 849-54.
88. Martín-Orozco, E., Kobayashi, H., Van Uden, J., Nguyen, M.D., Kornbluth, R.S., Raz, E. *Enhancement of antigen-presenting cell surface molecules involved in cognate interactions by immunostimulatory DNA sequences.* Int Immunol 1999, 11: 1111-8.
89. Horner, A.A., Raz, E. *Immunostimulatory-sequence DNA is an effective mucosal adjuvant.* Curr Top Microbiol Immunol 2000, 247: 185-98.
90. Wagner, H. *Bacterial CpG DNA activates immune cells to signal infectious danger.* Adv Immunol 1999, 73: 329-68.

91. Rachmilewitz, D., Karmeli, F., Takabayashi, K. et al. *Immunostimulatory DNA ameliorates experimental and spontaneous murine colitis.* Gastroenterology 2002, 122: 1428-41.
92. Desreumaux, P., Colombel, J.F. *Intestinal flora and Crohn's disease.* Ann Pharm Fr 2003, 61: 276-81.
93. Madsen, K., Jijon, H., Yeung, H., McKaigney, C., De Simone, C. *DNA from probiotic bacteria exerts anti-inflammatory actions on intestinal epithelial cells by inhibition of NF-κB.* Gastroenterology 2002, 122 (Suppl. 1): A64.
94. Jacobsen, C.N., Rosenfeldt Nielsen, V., Hayford, A.E. et al. *Screening of probiotic activities of forty-seven strains of Lactobacillus spp. by in vitro techniques and evaluation of the colonization ability of five selected strains in humans.* Appl Environ Microbiol 1999, 65: 4949-56.
95. Granato, D., Perotti, F., Masserey, I. et al. *Cell surface-associated lipoteichoic acid acts as an adhesion factor for attachment of Lactobacillus johnsonii La1 to human enterocyte-like Caco-2 cells.* Appl Environ Microbiol 1999, 65: 1071-7.
96. Wunderlich, P.F., Braun, L., Fumagalli, I. et al. *Double-blind report on the efficacy of lactic acid- producing Enterococcus SF68 in the prevention of antibiotic-associated diarrhoea and in the treatment of acute diarrhoea.* J Int Med Res 1989, 17: 333-8.
97. Raza, S., Graham, S.M., Allen, S.J., Sultana, S., Cuevas, L., Hart, C.A. *Lactobacillus GG promotes recovery from acute nonbloody diarrhea in Pakistan.* Pediatr Infect Dis J 1995, 14: 107-11.
98. Van Niel, C.W., Feudtner, C., Garrison, M.M., Christakis, D.A. *Lactobacillus therapy for acute infectious diarrhea in children: A meta-analysis.* Pediatrics 2002, 109: 678-84.
99. Bishop, R. *Ruth Bishop: Rotaviruses and vaccines. Interview by Amanda Tattam.* Lancet 1999, 353: 1860.
100. Isolauri, E., Kaila, M., Mykkanen, H., Ling, W.H., Salminen, S. *Oral bacteriotherapy for viral gastroenteritis.* Dig Dis Sci 1994, 39: 2595-600.
101. Duffy, L.C., Zielezny, M.A., Riepenhoff-Talty, M. et al. *Effectiveness of Bifidobacterium bifidum in mediating the clinical course of murine rotavirus diarrhea.* Pediatr Res 1994, 35: 690-5.
102. Qiao, H., Duffy, L.C., Griffiths, E. et al. *Immune responses in rhesus rotavirus-challenged BALB/c mice treated with bifidobacteria and prebiotic supplements.* Pediatr Res 2002, 51: 750-5.
103. Campieri, M., Gionchetti, P. *Probiotics in inflammatory bowel disease: New insight to pathogenesis or a possible therapeutic alternative?* Gastroenterology 1999, 116: 1246-9.
104. Gibson, G.R., Roberfroid, M.B. *Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics.* J Nutr 1995, 125: 1401-12.
105. Venturi, A., Gionchetti, P., Rizzello, F. et al. *Impact on the composition of the faecal flora by a new probiotic preparation: Preliminary data on maintenance treatment of patients with ulcerative colitis.* Aliment Pharmacol Ther 1999, 13: 1103-8.
106. Guslandi, M., Giollo, P., Testoni, P.A. *A pilot trial of Saccharomyces boulardii in ulcerative colitis.* Eur J Gastroenterol Hepatol 2003, 15: 697-8.
107. Katz, J.A. *Prevention is the best defense: Probiotic prophylaxis of pouchitis.* Gastroenterology 2003, 124: 1535-8.
108. Gionchetti, P., Rizzello, F., Venturi, A. et al. *Oral bacteriotherapy as maintenance treatment in patients with chronic pouchitis: A double-blind, placebo-controlled trial.* Gastroenterology 2000, 119: 305-9.
109. Gionchetti, P., Rizzello, F., Helwig, U. et al. *Prophylaxis of pouchitis onset with probiotic therapy: A double-blind, placebo-controlled trial.* Gastroenterology 2003, 124: 1202-9.
110. Kuisma, J., Mentula, S., Jarvinen, H., Kahri, A., Saxelin, M., Farkkila, M. *Effect of Lactobacillus rhamnosus GG on ileal pouch inflammation and microbial flora.* Aliment Pharmacol Ther 2003, 17: 509-15.
111. Campieri, M., Rizzello, F., Venturi, A. et al. *Combination of antibiotic and probiotic treatment is efficacious in prophylaxis of post-operative recurrence of Crohn's disease: A randomized controlled study vs Mesalamine.* Gastroenterology 2000, 118: A781.
112. Guandalini, S. *Use of Lactobacillus-GG in paediatric Crohn's disease.* Dig Liver Dis 2002, 34 (Suppl. 2): S63-5.
113. Prantera, C., Scribano, M.L. *Probiotics and Crohn's disease.* Dig Liver Dis 2002, 34 (Suppl. 2): S66-7.

114. Niedzielin, K., Kordecki, H., Birkenfeld, B. *A controlled, double-blind, randomized study on the efficacy of Lactobacillus plantarum 299V in patients with irritable bowel syndrome.* Eur J Gastroenterol Hepatol 2001, 13: 1143-7.
115. Madden, J.A., Hunter, J.O. *A review of the role of the gut microflora in irritable bowel syndrome and the effects of probiotics.* Br J Nutr 2002, 88 (Suppl. 1): S67-72.
116. Bazzocchi, G., Gionchetti, P., Almerigi, P.F., Amadini, C., Campieri, M. *Intestinal microflora and oral bacteriotherapy in irritable bowel syndrome.* Dig Liver Dis 2002, 34 (Suppl. 2): S48-53.
117. King, T.S., Elia, M., Hunter, J.O. *Abnormal colonic fermentation in irritable bowel syndrome.* Lancet 1998, 352: 1187-9.
118. Spiller, R.C., Jenkins, D., Thornley, J.P. et al. *Increased rectal mucosal enteroendocrine cells, T lymphocytes, and increased gut permeability following acute Campylobacter enteritis and in post-dysenteric irritable bowel syndrome.* Gut 2000, 47: 804-11.
119. Brigidi, P., Vitali, B., Swennen, E., Bazzocchi, G., Matteuzzi, D. *Effects of probiotic administration upon the composition and enzymatic activity of human fecal microbiota in patients with irritable bowel syndrome or functional diarrhea.* Res Microbiol 2001, 152: 735-41.
120. Kim, H.J., Camilleri, M., McKinzie, S. et al. *A randomized controlled trial of a probiotic, VSL#3, on gut transit and symptoms in diarrhoea-predominant irritable bowel syndrome.* Aliment Pharmacol Ther 2003, 17: 895-904.
121. Mastretta, E., Longo, P., Laccisaglia, et al. *Effect of Lactobacillus GG and breast-feeding in the prevention of rotavirus nosocomial infection.* J Pediatr Gastroenterol Nutr 2002; 35: 527-31.
122. Guandalini, S., Pensabene, L., Zikri, et al. *Lactobacillus GG administered in oral rehydration solution to children with acute diarrhea: A multicenter European trial.* J Pediatr Gastroenterol Nutr 2000; 30: 54-60.
123. Kirjavainen, P.V., Salminen, S.J., Isolauri, E. *Probiotic bacteria in the management of atopic disease: Underscoring the importance of viability.* J Pediatr Gastroenterol Nutr 2003; 36: 223-7.
124. Katelaris, P.H., Salam, I., Farthing, M.J. *Lactobacilli to prevent traveler's diarrhea?* N Engl J Med 1995; 333: 1360-1.
125. Vanderhoof, J.A., Whitney, D.B., Antonson, D.L., Hanner, T.L., Lupo, J.V., Young, R.J. *Lactobacillus GG in the prevention of antibiotic-associated diarrhea in children.* J Pediatr 1999; 135: 564-8.
126. Surawicz, C.M., Elmer, G.W., Speelman, P., McFarland, L.V., Chinn, J., van Belle, G. *Prevention of antibiotic-associated diarrhea by Saccharomyces boulardii: A prospective study.* Gastroenterology 1989; 96: 981-8.
127. McFarland, L.V., Surawicz, C.M., et al. *Prevention of beta-lactam-associated diarrhea by Saccharomyces boulardii compared with placebo.* Am J Gastroenterol 1995; 90: 439-48.
128. Surawicz, C.M., McFarland, L.V., Elmer, G., Chinn, J. *Treatment of recurrent Clostridium difficile colitis with vancomycin and Saccharomyces boulardii.* Am J Gastroenterol 1989; 84: 1285-7.
129. Gorbach, S.L., Chang, TW, Goldin, B. *Successful treatment of relapsing Clostridium difficile colitis with Lactobacillus GG.* Lancet 1987; 2: 1519.
130. Biller, J.A., Katz, A.J., Flores, A.F., Buie, T.M., Gorbach, S.L. *Treatment of recurrent Clostridium difficile colitis with Lactobacillus GG.* J Pediatr Gastroenterol Nutr 1995; 21: 224-6.
131. Prantera, C., Scribano, M.L., Falasco, G., Andreoli, A., Luzi, C. *Ineffectiveness of probiotics in preventing recurrence after curative resection for Crohn's disease: A randomised controlled trial with Lactobacillus GG.* Gut 2002; 51: 405-9.
132. Biancone, L., De Nigris, F., Del Vecchio Blanco, et al. *Lactobacillus GG is not effective in subsiding bowel tenderness in inactive Crohn's disease.* Gastroenterology 2003: T1402A.
133. Vanderhoof, J.A., Young, R.J. *Role of probiotics in the management of patients with food allergy.* Ann Allergy Asthma Immunol 2003; 90: 99-103.
134. Isolauri, E., Arvola, T., Sutas, Y., Moilanen, E., Salminen, S. *Probiotics in the management of atopic eczema.* Clin Exp Allergy 2000; 30: 1604-10.

135. Kalliomäki, M., Salminen, S., Arvilommi, H., Kero, P., Koskinen, P., Isolauri, E. *Probiotics in primary prevention of atopic disease: A randomised placebo-controlled trial.* Lancet 2001; 357: 1076-9.
136. Kalliomaki, M., Salminen, S., Poussa, T., Arvilommi, H., Isolauri, E. *Probiotics and prevention of atopic disease: 4-year follow-up of a randomised placebo-controlled trial.* Lancet 2003; 361: 1869-71.
137. Ibnou-Zekri, N., Blum, S., Schiffrin, E.J., Von Der Weid, T. *Divergent patterns of colonization and immune response elicited from two intestinal Lactobacillus strains that display similar properties in vitro.* Infect Immun 2003, 71: 428-36.
138. Coakley, M., Ross, R.P., Nordgren, M., Fitzgerald, G., Devery, R., Stanton, C. *Conjugated linoleic acid biosynthesis by human-derived Bifidobacterium species.* J Appl Microbiol 2003, 94: 138-45.
139. Steidler, L., Hans, W., Schotte, L. et al. *Treatment of murine colitis by Lactococcus lactis secreting interleukin-10.* Science 2000, 289: 1352-5.
140. Seegers, J.F. *Lactobacilli as live vaccine delivery vectors: Progress and prospects.* Trends Biotechnol 2002, 20: 508-15.