CMLS, Cell. Mol. Life Sci. 59 (2002) 001–15
1420-682X/02/010001-15 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Review

Probiotic microorganisms: how they affect intestinal pathophysiology

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Received 30 January 2002; received after revision 8 March 2002; accepted 8 March 2002

Introduction

Probiotics attracted great interest throughout the last decade. A keyword search for 'probiotic or lactic acid bacteria' in the PubMed database reveals more than 3000 papers during the last 10 years and more than 400 papers in 2001. The aim of the present review is to update studies concerning the use of probiotics in treating gastrointestinal diseases as well as to highlight more recently understood effects of lactic acid bacteria, especially their participation in the maintenance of intestinal homeostasis and the regulation of adapted immune responses to environmental antigens.

One hundred years ago, Elie Metchnikoff was the first to stress the importance of intestinal lactobacilli in microflora in maintaining health and longevity [1]. The term 'probiotic' has been popularized by R. Fuller [2], and is defined as a live microbial feed supplement which beneficially affects the host by improving its intestinal microbial balance. This definition was later extended to include other beneficial effects such as immunomodulation.

Part of the beneficial effects of probiotic bacteria is likely related to the observation that a commensal microflora is necessary for the establishment of oral tolerance to food antigens [3] and participates in the maintenance of a microbial barrier facilitating the elimination of pathogenic organisms from the digestive tract. For a long time, based on empirical practice and later on scientific observations, live microbial supplementation (yogurt, fermented milk, bacterial lyophilizates, and more recently, infant formula, unfermented milk, juices or candy) have been proposed to control various digestive or extra-digestive diseases. In parallel, nutrient factors defined as prebiotics and favoring the development of a specific flora (such as growth-promoting bifidus factors consisting of various oligosaccharides) have also been used as an additional strategy. In breast-fed infants, oligosaccharides such as N-acetyl-glucosamine-containing oligosaccharides present in human milk represent the main source of carbon and energy for intestinal bacteria and promote the growth of bifidobacteria. Based on these observations, infant formulas containing a mixture of undigestible short-chain oligosaccharides conferring bifidogenic properties have been developed. A range of non-digestible dietary supplements have been identified that modify the balance of the intestinal microflora, stimulating the growth and/or activity of beneficial microorganisms and suppressing potentially deleterious bacteria. These supplements include lactulose, lactitol, a variety of oligosaccharides [trans-galactooligosaccharides (GOS) and fructooligosaccharides (FOS)] and inulin. Despite doubt in the scientific community as to the validity of pre/probiotic research, due to the weaknesses of scientific work, there are clinical studies which support and even advocate the probiotic concept, and European Union programs are allocating funds for the use of molecular biological tools in the analysis of the complex mechanisms governing health benefits. Within the fifth framework program of the European commission, research on probiotic, prebiotic and new functional foods has been an important focus of funding activities. The common objective of a better understanding of the interactions between resident or transient intestinal microflora and the human host is emerging.

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For several decades, studies have reported a proven beneficial effect of probiotic bacteria in connection with lactose intolerance, acute rotavirus diarrhea and antibiotic-associated diarrhea. Although this body of work has mainly been descriptive, some insights into the mechanisms involved in the beneficial effects of probiotics are now available. To better understand how probiotics can interact with intestinal physiology, the mechanisms involved in the maintenance of intestinal homeostasis have to be considered.

Mechanisms involved in intestinal homeostasis

Intestinal homeostasis relies upon the equilibrium between absorption (nutrients, ions), secretion (ions, IgA) and barrier capacity (to pathogens and macromolecules) of the digestive epithelium. These functions are controlled through multiple interactions with the endocrine, neurocrine, stromal and immune cells or with the resident bacterial microflora that regulate epithelial functions. When this homeostatic control is disturbed, chronic inflammation, diarrhea and disease may occur. To better understand the beneficial effects of lactic acid bacteria in digestive diseases, it is important to take into account the mechanisms involved in the dysregulation of epithelial functions.

A rupture in the hydroelectrolytic balance can be due either to a dysregulation of ion-coupled nutrient absorption or to an abnormal stimulation of ionic secretion, in turn driving water losses. Water movements are mainly generated by the Na-solutes co-transport systems (Na-glucose) or chloride (Cl^-) secretion across the apical membrane of intestinal epithelial cells, and such transporters or channels are highly regulated structures. Water movement follows ionic movements across the intestinal epithelium: Na^+ absorption is driven in part by Na-glucose co-transport, and leads to net water absorption, whereas Cl^- secretion drives water secretion in the intestinal lumen. Therefore, any luminal or serosal factor affecting these transport systems will also affect electrolyte and water movements. Among the luminal factors, pathogenic bacteria can adhere to the brush-border membrane of the enterocytes, inducing epithelial dysfunction. Attaching-effacing lesions of the brush-border membrane may ensure, or enterotoxins are released stimulating chloride secretion (diarrhea) or cytotoxins disrupting epithelial integrity. Besides the specific mechanisms involved in water movement in the gut, osmotic diarrhea can also be induced when a non-absorbable compound reaches the intestinal lumen and maintains an osmotic gradient between the intestinal lumen and the blood. A typical case of osmotic diarrhea is that induced by lactose malabsorption in the case of lactase deficiency. Serosal factors can also affect the regulation of water movements. Abnormal stimulation of the underlying immune system (mast cells, phagocytes, lymphocytes) leads to the release of inflammatory mediators capable of altering epithelial function. The enteric nervous system can also be involved through the abnormal release of neuromediators (Met-enkephalin, acetylcholine) known to activate chloride secretion directly.

Probiotic microorganisms: nature and presentation

To be a probiotic, a bacterial strain has to fulfill several criteria. It has to be healthy, to resist acid and bile, to adhere to intestinal epithelial cells, to be able to persist long enough in the digestive tract, to produce anti-microbials, to modulate immune responses and to resist technological processes. Probiotic microorganisms consist mostly of strains of *Lactobacillus*, *Bifidobacterium* and *Streptococcus*, bacterial types which have been used for centuries in the production of fermented dairy products.

Table 1 indicates the most widely used probiotic microorganisms. They mainly belong to the *Lactobacillus* and *Bifidobacterium* species (Gram+ bacteria), although some other species or other microorganisms (yeast) have also been a matter of interest.

Table 1. List of the most frequently used probiotic microorganisms.

Lactobacillus species	*Bifidobacterium* species	Others
L. acidophilus	*B. bifidum*	*Streptococcus thermophilus*
L. rhamnosus	*B. longum*	*Escherichia coli*
L. gasseri	*B. breve*	*Bacillus cereus*
L. reuteri	*B. infantis*	*Clostridium butyricum*
L. bulgaricus	*B. lactis*	*Enterococcus faecalis*
L. plantarum	*B. adolescentis*	*Enterococcus faecium*
L. johnsonii		VSL#3 (four strains of lactobacilli, three strains of bifidobacteria, one strain *Streptococcus salivarius* sp. *thermophilus*)
L. paracasei, L. casei		Yeast
L. salivarius		*Saccharomyces boulardii*
L. lactis		*Saccharomyces cerevisiae*

Lactobacillus and bifidobacteria are bacterial strains originating from human microflora. Lactobacilli are often part of the intestinal ecosystem but very variable amounts are found among people (ranging from 0 to 10^6 CFU/g feces). Bifidobacteria are also part of the human microflora, but species differ according to age: newborns are readily colonized by *Bifidobacterium breve* and *B. infantis* and colonization is favored in breast-fed compared to bottle-fed infants, whereas adults more often host *B. adolescentis*, *B. bifidum* and *B. longum.* However, ingested bifidobacteria, when administred as probiotics, do not persist permanently in the digestive tract when the oral bacterial load is stopped. This may be due to the fact that, on the one hand, host genotype contributes to the dominant microbial diversity suggesting specific interactions between microbes and humans [4, 5], and on the other hand, microflora establishment and maintenance is highly dependent on the food intake and style of diet. The resident intestinal microflora is complex and involves more than 400 bacterial species. It can be modified by dietary substances favoring the growth of certain bacterial species, for example oligosaccharides (prebiotics) used to promote the development of bifidobacteria. In other words, the composition of the indigenous microflora is specific to an individual host and exogenous bacteria are not easily established (fig. 1).

In addition, studies on probiotics in various animal experimental settings suggest an apparent variability in probiotic efficacy of a given microorganism in a given organism. In humans, where intestinal infections are heterogeneous and multifactorial, probiotic indications are likely to vary according to the disease or to its clinical course.

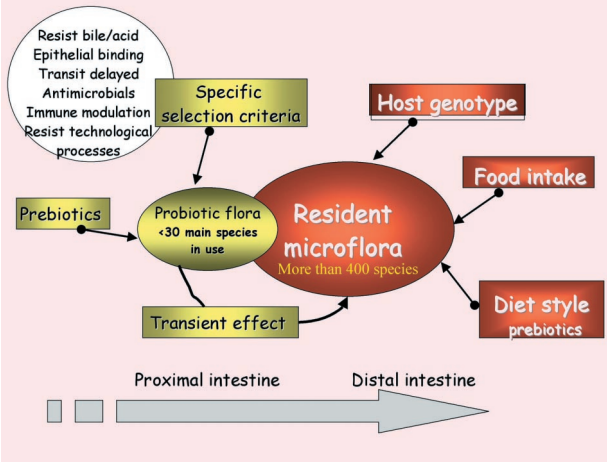

Figure 1. Relationship between resident and probiotic flora. The resident intestinal microflora is complex and involves more than 400 bacterial species. Host genotype contributes to the dominant microbial diversity suggesting specific interactions between microbes and humans, and microflora establishment and maintenance is highly dependent on food intake and style of diet. By contrast, ingested probiotic bacteria do not persist permanently in the digestive tract when the oral bacterial load is stopped. In fact, the composition of the indigenous microflora is specific to an individual host and exogenous bacteria are not easily established.

Effect of probiotic bacteria on gastrointestinal diseases

Probiotics alleviate diarrhea of various etiologies

Lactose intolerance

One of the most frequent digestive troubles is related to lactose malabsorption, due to epithelial lactase deficiency. Indeed, one of the most documented beneficial effects of yogurt is described for the case of lactose intolerance. In 1984, Kolars et al. [6] and Savaiano et al. [7] concomitantly showed in lactase-deficient subjects that lactose was absorbed much better from yogurt than from milk, probably due to the intraluminal digestion of lactose by the lactase released from yogurt microorganisms. These results were confirmed by Dewit et al. [8]. Not yet absolutely proven, however, is that the microbial lactase activity whose maximal activity is situated between pH 6–8 persists at the duodenal pH of 5.0. In fact, although β-galactosidase activity in yogurt drops by 80% in the duodenum, one-fifth of the yogurt lactase activity is still found in the terminal ileum, suggesting a relative persistence of the protein along the digestive tract. In addition, fresh yogurt is more efficient in facilitating lactose digestion than heated yogurt [9, 10]. Thus, bacterial β-galactosidase present in yogurt is partly resistant to luminal hydrolysis and can hydrolyze lactose, at least in the mid and distal part of the small intestine where the pH is compatible with its enzymatic activity.

Treatment of diarrhea

1) Acute viral diarrhea

For a long time, the use of oral bacteria therapy in childhood has been described as beneficial in the treatment of acute diarrhea [11–13]. Human isolates of *Lactobacillus rhamnosus* strain GG (*L.GG*) have been extensively used to promote recovery from acute rotavirus diarrhea in children. *L.GG,* when used as bacteria-supplemented milk or freeze-dried powder, is effective in shortening the course of acute diarrhea [14–16]. The same effect is observed in children with rotavirus diarrhea fed *L. reuteri* [14, 17]. A multicenter European trial has recently shown that administring oral rehydration solution containing *L.GG* to children with acute diarrhea is safe and results in a shorter duration of diarrhea and faster discharge from the hospital [18]. Recently, a randomized trial in young children showed that although infant formula or yogurt were equally effective in the treatment of acute watery diarrhea, yogurt feeding was associated with a clinically rel-

evant decrease in stool frequency and duration of diarrhea, especially in children with carbohydrate malabsorption [19].

2) Bacterial diarrhea

The data concerning a protective role of lactic acid bacteria in bacterial diarrhea are scanty in humans and contradictory results have been reported in animals. In a rabbit model of *Escherichia coli* enterotoxin-induced diarrhea, a *Lactobacillus*-containing preparation injected in infected ileal loops exhibited a significant anti-enterotoxin response [20]. The administration of killed *L. acidophilus* in mice infected with a strain of enterotoxigenic *E. coli* extended their survival and a protective effect of probiotic fermented food mixture was found in a similar model of mice infected with *E. coli* [21]. However, contradictory results were also reported [22]. Besides the effect of probiotics on the time course of infection, a preventive effect has also been suggested in mice [23]. However, a clinical trial of probiotic administration for the prevention of *Salmonella* shedding in horses had no effect on the prevalence of diarrhea [24]. In contrast, an in vivo study in mice orally infected with *Salmonella typhimurium* showed that the *L. acidophilus* strain LA1 had an antibacterial activity, which was reproduced, in vitro, using intestinal epithelial Caco2 cells infected with *S. typhimurium* and was shown to be linked to a factor present in the culture supernatant of the probiotic bacteria [25]. In neonatal rats, intestinal bifidobacterial colonization reduced the risk of necrotizing enterocolitis, by a mechanism which may involve a decreased bacterial translocation and inflammatory cascade [26].

In adult volunteers inoculated orally with *E. coli* strains producing heat-stable and heat-labile enterotoxins, the course of the diarrhea was not improved by a commercial preparation containing dried *L. acidophilus* and *L. bulgaricus* [27, 28]. In the case of chronic diarrhea associated with bacterial overgrowth in adults, a crossover randomized trial showed that a 7-day antibiotic treatment was effective in reducing daily stool frequency, whereas *Saccharomyces boulardii* had no effect on this parameter, at least during this short period of treatment [29].

Taken together, although beneficial effects have been reported in different animal or cell culture models, these results do not convincingly support a beneficial effect of probiotics in bacterial diarrhea.

3) Clostridium difficile colitis

In humans, in the case of recurrent *C. difficile* (CD) colitis, a successful treatment was obtained using *L.GG,* both in adults and children [30, 31]. Other probiotics such as *S. boulardii*, in combination with standard antibiotics were demonstrated as more effective than antibiotics alone in the treatment of recurrent CD colitis [32]. In a prospective, randomized, placebo-controlled trial of *L.GG* in combination with standard antibiotics for the treatment of *C. difficile* infection, *L.GG* seemed effective in reducing the 3-weeks recurrence rate of *C. difficile*, and patients felt better when taking *L.GG*, as compared with the placebo, with early disappearance of abdominal cramps and diarrhea [33]. Altogether, the use of probiotics for the treatment of primary and recurrent *C. difficile* diarrhea looks promising.

4) Persistent or chronic diarrhea

Persistent diarrhea is defined in children as a diarrhea starting acutely but lasting at least 2 weeks. A beneficial effect of feeding yogurt versus milk was shown in children with persistent diarrhea [34]. In a controlled randomized single-blind clinical trial, the treatment of children with chronic diarrhea with lactulose or preparation of probiotics (Lactipan) promoted complete remission of intestinal disorders [35]. Other studies have reported a beneficial effect of fermented products on the time course of diarrhea. Feeding fermented milk in children (South America) with postgastroenteritis syndrome eliminated the disease in 4.0 days, and was even more beneficial in the patients with malnutrition [36].

5) Antibiotics-associated diarrhea or radiotherapy-induced diarrhea

Antibiotics used in various infectious pathologies can alter the intestinal microflora and disrupt the equilibrium in the bowel ecosystem. Ingestion of probiotics has long been used to reduce the effect of such microbial alteration. The treatment and prevention of antibiotic-associated diarrhea has recently been reviewed [37]. Lactobacilli, especially *L.GG* were constantly reported as beneficial in antibiotic-associated diarrhea [38–41]. In adults, prophylactic administration of a culture of *L. acidophilus and L. bulgaricus* in ampicillin-treated patients was effective in preventing the diarrhea [42]. Finally, in patients undergoing abdominal irradiation, the prevention of intestinal side effect (diarrhea) was obtained by the administration of live *L. acidophilus* cultures [43] or by *L. rhamnosus* in comparison to placebo in a double-blind trial design [44].

Prevention of diarrhea in children

A few studies have been done on the potential role of probiotics or fermented milks in preventing the development of diarrhea. In 1989, Brunser et al. [45], in Chile, noted a decrease in the incidence of diarrhea in children fed an acidified modified powdered cow's milk infant formula (Pelargon) for 6 months compared to control children. Both the frequency and duration of acute diarrhea were improved. In 1994, Boudraa et al. [46], in Algeria, showed that feeding a dehydrated fermented milk containing *B. breve* and *Streptococcus thermophilus (Lactofidus)* to children at early weaning significantly reduced

the number of diarrhea episodes compared with children receiving an adapted milk formula during the 3 months of the study.

In a double-blind placebo-controlled trial, a preventive effect on the development of diarrhea and on the shedding of rotavirus in feces in infants aged 5–24 months was observed in the group receiving a milk formula supplemented with *B. bifidum* and *S. thermophilus* compared to infants receiving the control formula [47]. *L.GG* was also demonstrated as effective in the prevention of nosocomial diarrhea, particularly rotavirus gastroenteritis, in hospitalized infants [48].

A double-blind randomized trial on the effect of a long-term consumption of *L.GG*-supplemented milk on infections in children attending day-care centers recently concluded that *L.GG* may reduce respiratory infections and their severity, although the effect was modest [49]. The preventive role of *L.GG* toward diarrhea was also reported in undernourished children [50].

Role of bacterial viability

The question of bacterial viability is important because many of clinical studies suggest that live bacteria are mandatory to the beneficial effect of probiotic dietary supplements. In fresh dairy fermented products, the bacterial counts normally reach 10^8–10^9 bacteria/ml. In *L.GG*-supplemented milk, the counts can reach 10^{10}–10^{11} bacteria/ml. In dried fermented products, dehydration and heating greatly reduce the number of viable bacteria. The importance of viability of probiotic microorganisms may be different in different situations. In lactose intolerance, although lactase activity (i.e. the intact β-galactosidase protein) persists long enough to explain why heated yogurt is efficient in lactose malabsorbers [51], fresh yogurt is more efficient than heated yogurt in facilitating lactose digestion [9]. In diarrhea treatment, fresh fermented *L.GG* milk or the freeze-dried powder are both effective in shortening the course of acute rotavirus diarrhea in children [14] but the administration of viable *L.GG* is most efficient in promoting rotavirus-specific IgA in serum than heat-inactivated bacteria [52].

In antibiotic-associated diarrhea, the viability of lactic acid bacteria seems to be mandatory to the beneficial effect [38].

Prevention and treatment of *Helicobacter pylori* infection

H. pylori is among the few bacteria capable of colonizing the gastric mucosa, in some cases inducing the development of gastric inflammation or ulcers. In the latter case, bacterial eradication through anti-biotherapy is needed. Many additional therapeutic strategies have been made available to improve *H. pylori* eradication, including the use of probiotics. This biotherapeutics is often used as an adjunct to the traditional anti-biotherapy and clinical trials seem to confirm the improvement of eradication rate and the tolerability of multiple antibiotic regimens used for the infection. *L. acidophilus* strain LB secreting an anti-bacterial substance against *H. pylori* in vitro and in vivo was reported to decrease the adhesion and viability of *H. felis* in mice and of *H. pylori* in the cultured human mucosecreting HT29-MTX intestinal cell line [53].

In a gnotobiotic murine model of *H. pylori* infection, *L. salivarius* (but not *L. casei* or *L. acidophilus*) was shown to prevent infection or to suppress the infection by *H. pylori*, probably through the high amount of lactic acid produced [54, 55].

In a study of *H. pylori*-infected volunteers, an acidified milk LC1 containing *L. johsonnii* La1 was shown to decrease *H. pylori* density (but was not shown to improve eradication) and to reduce inflammation in the antrum [56]. After screening of more than 200 strains of lactobacilli for their capacities to adhere to gastric epithelial cells and resist gastric acidity, *L. gasseri* was shown to be able, in humans infected with *H. pylori*, to suppress the bacterium and to reduce mucosal inflammation [57]. Altogether, the lack of standardization in the type of probiotics used, and the dosage and timing of administration suggest that further trials are needed to clarify the role of probiotics as a treatment or adjunct of the treatment in *H. pylori* infections.

Probiotics in the treatment or maintenance therapy of inflammatory bowel diseases

The incidence of inflammatory bowel disease (IBD), ulcerative colitis and Crohn's disease, is increasing in Western Europe and the USA (80/100,000). In the absence of good knowledge of the causal factors, drugs that relieve symptoms such as mesalazine, antibiotics, corticoids, anti-diarrheal drugs and immunosuppressants have been used for treatment. This therapy leads to symptom-free periods with remission from acute episodes, but relapses frequently occur. The etiology of IBD is still not clear and numerous factors are involved in the damage to the mucosa, including microorganisms, psychological factors and nutritional habits. Some results indicate that IBD patients host an intestinal microflora containing few lactobacilli [58] and a decrease in bifidobacteria fecal concentration [59, 60]. In normal conditions, an immunologic tolerance is maintained toward the commensal enteric bacteria which prevents intestinal inflammation. This controlled homeostatic response is lost in susceptible individuals that develop chronic aggressive cellular immune response at the intestinal level. Because the intestinal microflora is essential for the development and perpetuation of colitis [61] and seems to be implicated in the pathogenesis of IBD, the rationale for using probiotics has recently emerged and probiotics have been proposed

as a therapeutic adjunct in IBD. There are increasing experimental and clinical data showing a reduction of inflammation and symptoms in enterocolitis treated with probiotics. Preliminary results with probiotics in animal models are encouraging: their efficacy in murine colitis, especially in interleukin (IL)-10–/– mice models [62, 63] has been suggested.

Few results however have been described in humans. Oral administration of *L.GG* in patients with Crohn's disease resulted in the promotion of the intestinal IgA immune response [64]. A preliminary study in four children with mild Crohn's disease suggested that *L.GG* may improve the gut barrier function and clinical status, but double-blind placebo-controlled trials have to confirm these results [65].

The pouch surgery of ulcerative colitis is often complicated by a non-specific inflammation of the ileal reservoir. Oral bacteriotherapy using a mixture of probiotic bacteria (VSL#3, see table 1) has recently been shown to be effective in preventing relapses of chronic pouchitis [66]. In addition, a double-blind comparison of an oral *E. coli* preparation (Nissle 1917) and mesalazine in maintaining remission of ulcerative colitis showed that the probiotic treatment was as effective as mesalazine in the maintainance therapy of ulcerative colitis [67]. Altogether, more convincing results are needed to confirm the advantage of using probiotics in IBD, but a trend to the beneficial effect of bacterial supplementation as an adjunct to treatment is emerging.

Use of recombinant probiotic bacteria in alleviating intestinal diseases

The concept of genetically engineered bacteria has been used by Steidler et al. [62] to treat murine colitis. The cytokine IL-10 was secreted in the intestinal lumen of mice by non-pathogenic genetically engineered bacteria (*Lactococcus lactis*) administered as a food supplement. Locally produced IL-10, after crossing the altered epithelial barrier, suppressed the inflammatory immune response by promoting the activity of regulatory T cells that counter-regulated Th1 cells. This approach was proposed as worth considering in the long-term management of IBD in humans.

In a recent study, Kruisselbrink et al. [68] showed that mucosal immunization of mice with a *Lactobacillus plantarum* recombinant expressing an immunodominant T cell epitope of Der p1 of house dust mites resulted in inhibition of IL-5 production in an antigen-specific manner whereas it also inhibited interferon (IFN)-γ production, as a non-specific effect. These results suggest that recombinant *L. plantarum* may be a potential candidate for the treatment of allergic disorders.

Gastrointestinal diseases can also be caused by pathogenic toxin-producing bacteria such as *Shigella*. Although *Shigella* infection can be diagnosed early in the course of the disease, no effective therapeutic intervention is possible. Also recently, recombinant bacteria (*E. coli*) that displayed a shiga toxin receptor mimic on their surface were shown to adsorb and neutralize shiga toxins very efficiently, and oral administration of these recombinant bacteria protected mice from a fatal shiga toxigenic *E. coli* infection [69]. All these examples illustrate the growing interest in recombinant probiotic bacteria in the treatment of various intestinal diseases (fig. 2).

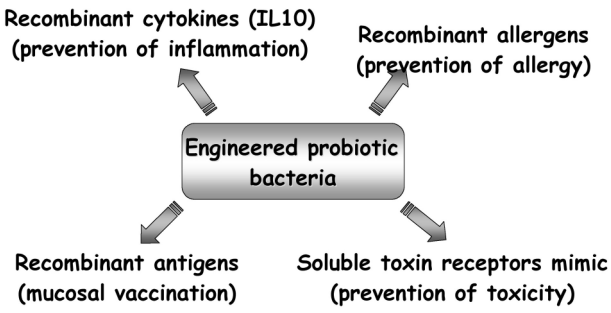

Figure 2. Use of engineered probiotic bacteria. The concept of genetically engineered bacteria has been used for various purposes. Immunosuppressive cytokines (IL-10) which are produced locally by engineered bacteria suppress the inflammatory immune response by activating regulatory T cells. Recombinant probiotic bacteria can also express T cell epitopes inhibiting allergic reactions [68]. Gastrointestinal diseases can also be caused by pathogenic toxin-producing bacteria such as *Shigella*. Recombinant bacteria (*E. coli*) that display a shiga toxin receptor mimic on their surface can adsorb and neutralize shiga toxins very efficiently, protecting mice from a fatal shiga toxigenic *E. coli* infection [69]. In addition, mucosal vaccination is presently being developed, using recombinant antigens. Novel high-efficiency *Lactobacillus* expression vectors have been designed to allow antigen expression either intracellularly, extracellularly or secreted and anchored to the surface. These expression vectors have been successfully used to construct different lactobacilli-expressing antigens (such as tetanus toxin fragment C) that after oral or nasal immunization in mice induce significant levels of circulating specific IgG [94, 95].

Probiotics in food allergy

The rationale for using probiotics as a possible treatment of allergic disorders is based on the fact that this immune disorder is due to the dysregulation of the Th1/Th2 balance in response to exogenous antigens. Various theories, including the 'hygiene hypothesis' have suggested that a modern style of life could be the basis of the increasing development of allergic diseases. Indeed, in most developed countries, the immune system is much less challenged as it used to be in the past by infectious diseases of various origins. The exposure to infectious agents generally leads to stimulation of type 1 helper (Th1) lymphocytes, and to the release of cytokines such as IFN-γ. T cells are classically classified as Th1, Th2 or Th3 types according to their profile of cytokine secretion. Th1 cells produce IFN-γ, IL-2 and tumor necrosis factor (TNF)-β,

which activate macrophages and are responsible for cell-mediated immunity. Type 2 (Th2) cells produce IL-4, IL-5, IL-10 and IL-13, which are responsible for antibody production (IgE mainly) and eosinophil activation. Th3 cells are regulatory cells mainly secreting suppressive cytokines such as transforming growth factor (TGF)-β. Th2 cells predominate in response to nematode infection or in allergic disorders. Theoretically, a Th1 response can protect against the development of allergic diseases, since Th1 and Th2 responses are considered mutually inhibitory. In the absence of stimulation of the immune system by infectious agents triggering Th1 responses, the main defense mechanism for parasitic infestation, i.e. Th2-type cytokine secretion and IgE antibodies, is still present, but it may be redirected against environmental substances such as food or respiratory antigens.

Several studies, mainly from E. Isolauri's group in Finland, have suggested that probiotics (*L.GG*) might be a novel approach in the management of food allergy. In children allergic to cow's milk proteins and presenting with atopic eczema, feeding an extensively hydrolyzed whey formula supplemented with *L.GG* improved the clinical score of atopic dermatitis and decreased the intestinal excretion of α1-anti-trypsin and TNF-α, compared with children fed the extensively hydrolyzed formula alone [70]. In addition, a possible mechanism was suggested, since a mixture of bovine caseins hydrolyzed with *L.*GG-derived enzymes induced a suppression of lymphocyte proliferation [71] and a down-regulation of anti-CD3 antibody-induced IL-4 production [72], in vitro. On the other hand, lactic acid bacteria have been shown to inhibit IgE production [73, 74]. By contrast, the administration of milk versus fermented milks (*S. thermophilus* and *B. breve)* were equally able to sensitize guinea pigs toward β-lactoglobulin, indicating that probiotics did not influence the development of cow's milk allergy [75].

A comparison of the intestinal flora of children in Sweden and Estonia suggested that the higher prevalence of allergy in Swedish children was associated with lower amounts of lactic acid bacteria in their intestinal flora [76, 77]. It was further reported that differences in the neonatal gut microflora preceded the development of atopy, suggesting that microflora imbalance in atopic children preceded the development of allergic manifestations [78].

In contrast to their propensity to counteract allergic development, lactic acid bacteria have also been shown to exert inhibitory effects on the development of Th1-mediated diseases, including colitis in mice [63], suggesting that they may modulate immunity by a mechanism which does not involve polarization of immune responses toward Th1. In a recent study, mucosal immunization of mice with *L. plantarum* recombinants resulted in inhibition of IL-5 production (Th2 cytokine) in an antigen-specific manner, whereas it also inhibited IFN-γ production (Th1 cytokine) as a non-specific effect [68]. This suggests that inhibition of Th2 cytokines (allergy) by lactobacilli is not necessarily accompanied by upregulated Th1 responses.

Probiotics in colon cancer

Colon cancer is a widely expressed cancer due to somatic mutations in colon cells occurring during the lifetime of an individual. The protective role of probiotics and prebiotics in colon cancer has recently been reviewed [79]. Genotoxic carcinogens including heterocyclic aromatic amines, which are formed during the cooking of meat, are a potential risk factor of colon cancer in high-meat consumers. In vitro studies have demonstrated that the cell wall of lactic acid bacteria can bind heterocyclic amines. Lactic acid bacteria or milk-fermented products have been shown to exhibit anti-mutagenic effects in the *S. typhimurium* mutagenicity assay [80], or in experimental animals [81]. The protective effect seems to be observed only at a high level of bacteria ($>5 \times 10^{12}$ colony forming units/l) [82]. Furthermore, butyrate, an organic acid produced at the colonic level by the gut flora, was recently shown as an inhibitor of the genotoxic activity of various compounds in human colon cells [83]. The carcinogenic effect of endogenous toxic and genotoxic compounds additionally believed to be influenced by the enzymatic activity of bacterial enzymes such as NADPH dehydrogenase, nitroreductase, β-glucosidase, β-glucuronidase or 7-α-dehydroxylase, which are more heavily expressed by enterobacteria, *Bacteroides* and clostridia than by lactobacilli and bifidobacteria. In fact, such enzymes are deleterious to the colon mucosa since they participate in the regeneration of toxic compounds that have been detoxified by the liver [84]. Despite promising studies in vitro and in vivo in experimental animals, in humans, epidemiological studies do not provide clear-cut results. Some studies have reported that individuals consuming fermented milks or yogurt had a lower incidence of colon cancer [85] or a lower propensity to develop large adenocarcinomas [86], but other studies did not find such beneficial effects [87]. Clearly, further studies are needed before the beneficial effects of probiotics in the prevention of human colon cancer can be confirmed.

Probiotics as vehicles of mucosal vaccines

Commensal bacteria such as lactic acid bacteria offer an attractive model of antigen delivery vehicles for mucosal vaccination, as recently reviewed [88–90]. Active vaccination as opposed to the induction of oral tolerance is crucially dependent on the particulate or soluble nature of the antigen delivered to the intestinal tract [91]. Enhanced immunogenicity of orally administred antigens is

achieved by presenting antigen to the immune system in association with bacteria or viral vectors (fig. 2). The recognized advantages of mucosal vaccination have led to the development of a new generation of vaccine that can be administred via various mucosal routes. Production of antigens by bacteria can occur in three different ways: intracellularly, extracellularly and surface bound. Novel high-efficiency *Lactobacillus* expression vectors have been designed to allow antigen expression either intracellularly, extracellularly or secreted and anchored to the surface. These expression vectors have been used successfully to construct different lactobacilli-expressing antigens such as tetanus toxin fragment C (TTFC), several rotavirus proteins or urease A and B subunits from *Helicobacter pylori* [92] Recombinant *L. casei* or *L. plantarum* expressing a high level of intracellular or cell wall-bound tetanus toxin fragment C [93] have been shown after oral and nasal immunization, in mice, to induce significant levels of circulating TTFC-specific IgG. Nasal administration induced in addition a secretory IgA response in bronchoalveolar fluids and antigen-specific B and T cell activation in draining lymph nodes [94]. Similar results were obtained after intranasal administration of recombinant *L. plantarum* expressing cytoplasmic TTFC, with a high level of specific IgG, bronchial mucosal IgA responses and specific T cell activation in cervical lymph nodes [95].

Mechanisms by which probiotic bacteria influence gastrointestinal physiology

Various properties of probiotic bacteria have been proposed as an explanation for their multiple beneficial effects. These properties include better lactose absorption in lactase-deficient subjects, restoration of a normal intestinal microflora, a contribution to the elimination of pathogenic enteric bacteria, reinforcement of the intestinal barrier capacity to exogenous antigens and an increase in humoral immunity and mucosal secretory IgA response. In addition to strengthening the specific immunity, lactic acid bacteria also seem to reinforce the non-specific mechanisms of defense such as phagocytosis and cytokine production. A trophic role on the intestinal epithelium has also been suggested, as well as the secretion of anti-inflammatory and anti-microbial molecules (bacteriocins). In vitro or in vivo studies suggest that synergistic mechanisms are probably involved in the beneficial effects of fermented products in case of intestinal diseases.

Lactose metabolism

The well-recognized beneficial effect of fermented products on lactose absorption in case of lactase deficiency can be partly explained by the presence of bacterial lactase (β-galactosidase) in yogurt or fermented products, helping lactose cleavage and its subsequent absorption under the form of monosaccharides, as initially suggested [6, 7]. Indeed, in yogurt, the lactose content 30% lower than in milk, probably due to such hydrolysis. However, some observations suggest that bacterial lactase is probably not a unique explanation for the improvement in lactose absorption, since β-galactosidase activity is reduced by 80% at pH 5.0, which is the pH observed in vivo in the duodenum. The other explanation of improving lactose absorption could be the slowing down of the gastrointestinal transit of yogurt compared with milk, facilitating prolonged contact between residual lactase on enterocytes and lactose in the lumen [10, 96, 97]. More likely, a synergistic effect of both mechanisms is probably involved in facilitating lactose digestion and absorption.

Effect of probiotics on the resident microflora and pathogens

The development of an indigenous gut flora in neonates and its maintenance in adults is prerequisite to the protection of intestinal function. During the neonatal period, the intestinal flora is enriched in bifidobacteria in breast-fed infants, while enterobacteria are predominant in the case of bottle-fed infants [98] although most often, a mixed population is found. The beneficial effect of viable lactic acid bacteria is possibly due to the transient proliferation of these bacteria in the digestive tract, in vivo, which represent a microbial barrier against the development of pathogenic bacteria.

The protective effect of the resident microflora is often weakened in the case of anti-biotherapy which creates a disequilibrium in the resident microflora and decreases the colonization resistance to pathogens. The introduction of lactic acid bacteria to reinforce human gut flora has been tested, since lactobacilli are part of the normal microflora, and, by producing lactic and acetic acids, hydrogen peroxide and anti-microbial substances, these microorganisms contribute to the maintenance of colonization resistance.

The persistence of ingested lactic acid bacteria in the gastrointestinal tract, at least for some days, is mandatory for their beneficial effect. Some data have indicated that orally administred *L.GG* can survive to transit, although an efficient colonization was not demonstrated [99]. In human neonates, the administration of a fermented whey-adapted infant formula containing viable bifidobacteria during the first 2 months of life, allowed them to reach a prevalence of colonization with bifidobacteria similar to that of breast-fed infants [100]. The fate of ingested lactic acid bacteria has been studied extensively in normal volunteers. After ingestion of a fermented milk containing *L. acidophilus* and *Bifidobacterium* sp., living bacte-

ria were recovered in the ileum in quantities representing 1 and 37% of the ingested bacteria, respectively [101]. Similar results confirmed the survival of *Bifidobacterium* sp. at the terminal ileum level [102].

The adherence of certain strains of *Lactobacillus* to the intestinal epithelial cells, which is a criterion of selection for probiotic strains, can significantly inhibit the binding of enteric pathogens, by competitive exclusion, as shown at least in vitro [53, 103, 104]. This inhibition of adhesion of enteropathogens could be due in part to the induction of intestinal mucin gene expression [105] and to the ability of probiotic bacteria to bind to human intestinal mucus [106, 107] and to human colonic cell lines [104, 108, 109]. However, in rabbits infected with the enteroadherent pathogenic strain RDEC-1, yogurt consumption did not interfere with the growth of the pathogenic *E. coli* [110].

Taken together, these studies indicate that although the bactericidal effect of yogurt has been demonstrated in vitro, it seems difficult to extrapolate to in vivo situations, and probiotic bacteria elimination of pathogenic microorganisms from the intestinal tract seems doubtful.

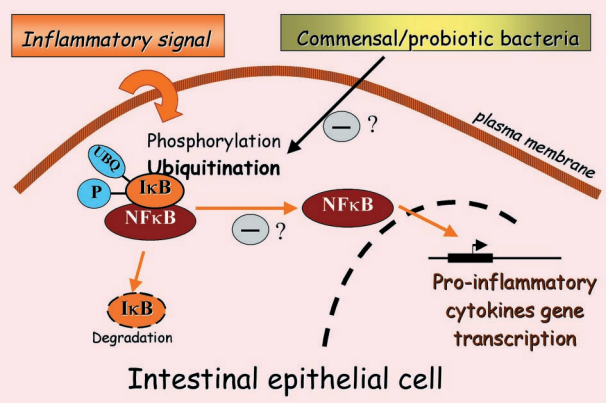

Figure 3. Hypothetical interactions between epithelial cells and probiotic bacteria in the inflammatory cascade. Recent data from Neish et al. [114] support the view that non-pathogenic bacteria may directly alleviate intestinal inflammation through mechanisms altering signal transduction pathways of pro-inflammatory cytokines, in particular the NF-κB transcription factors. In fact, in an epithelial cell line, a non-pathogenic *Salmonella* has been shown to inhibit ubiquitination and degradation of I-κB, an inhibitor of NFκB translocation in the nucleus. Whether or not this anti-inflammatory property could also be involved in the beneficial effects of probiotics remains to be determined.

Crosstalk between gut epithelium and commensal/probiotic bacteria and down-regulation of inflammation

Not only do bacteria interfere with intestinal physiology but, conversely, the intestinal environment is important in conditioning bacteria. The gut environment is susceptible to modifying ingested bacteria and to affecting their viability. Not only does the viability of bacteria in a probiotic food have to be taken into account but also their resistance to intestinal transit. In this respect, recent data suggest that some bacteria have a high rate of mutation in the digestive tract, initiating a faster adaptation to the hostile intestinal environment. Although initial mutations are favorable to the surviving of ingested bacteria, these mutations can turn out deleterious in secondary colonization [111], perhaps explaining why the individual host influences the outcome of a probiotic effect. Direct evidence for the importance of the commensal host-microbial relationship has recently been reported, showing that resident bacteria are capable of shaping our physiology. When germ-free mice are colonized with a bacterium of the dominant human microflora, *Bacteroides thetaiotaomicron*, an intestinal transcriptional response ensues, noticeably at the intestinal epithelial level. These alterations can interfere with several important functions including nutrient absorption, mucosal barrier function, intestinal maturation or xenobiotic metabolism [112]. Moreover, some other recent data support the view that non-pathogenic bacteria may directly alleviate intestinal inflammation through mechanisms altering signal transduction pathways of pro-inflammatory cytokines, in particular the NF-κB transcription factors [113, 114]. Other in vitro studies using the intestinal epithelial cell line CaCo-2 suggest that in the physiological inflammatory environment of the intestine, epithelial cells might be able to secrete more TGF-β, a tolerogenic cytokine, in the presence of lactobacilli [115]. Whether or not these anti-inflammatory properties are also involved in the beneficial effects of probiotics remains to be determined (fig. 3). Finally, in the changing environment of the human intestine, microorganisms seem to be capable of expressing an extensive array of distinct capsular polysaccharides, through phase variation dictated by invertible promoter regions upstream of the polysaccharide biosynthesis loci [5]. Such a property illustrates the important relationship existing between host and bacteria.

Reinforcement of the intestinal barrier function

The intestinal barrier function is composed of various factors susceptible of decreasing the absorption of potentially harmful microbial or soluble antigens. These factors include the capacity of digestive enzymes to degrade luminal antigens, the presence of an epithelial barrier composed of epithelial cells firmly connected via tight junctions and coated with a mucous layer with entrapped secretory IgA. The commensal flora can influence intestinal barrier function. In germ-free mice, the absence of intestinal microflora is associated with an increase in electrical resistance and a decrease in macromolecular transport, suggesting that commensal bacteria influence the epithelial physiology [116]. In fact, in rat colon, some

bacterial strains (*E. coli, Klebsiella pneumoniae, Streptococcus viridans*) can increase small-molecule absorption whereas others such as *Lactobacillus brevis* decrease this permeability, indicating that the composition of the commensal flora may play a role in digestive permeability to luminal substances [117]. Different studies have suggested that *L.GG* is able to stabilize the intestinal permeability to macromolecules, particularly in the case of acute gastroenteritis in rats [71], but also to reverse the increase in intestinal permeability induced by cow's milk in suckling rats [118]. A recent study using an animal model of cow's milk allergy has shown that feeding guinea pigs with dried fermented milk instead of milk led to a decrease in β-lactoglobulin absorption at the jejunal level [75]. Recently, the probiotic compound VSL#3 (see table 1) was shown to be effective in restoring the barrier function of IL-10-deficient mice and in reducing secretion of TNF-α and IFN-γ. It was also capable of increasing the electrical resistance of T84 epithelial monolayers by 20% within 6 h [119]. In healthy volunteers, indomethacin-induced gastric damage (but not intestinal damage) was prevented by a 5-day administration of live (but not killed) *L.GG* [120]. Altogether, these studies emphasize the capacity of some probiotic strains to stabilize the digestive mucosal barrier, a property that might be linked to the combined effects of probiotics on mucin expression, pathogenic bacterial growth, mucosal immunity and inflammation.

Strengthening of specific and non-specific immune responses

Secretory IgA immunity

The effect of lactic acid bacteria on the secretory immune system is well documented. When antigens, especially infectious antigens, are introduced via the oral route, secretory IgA or IgM are released as a defense mechanism. Lactic acid bacteria have been shown to improve the systemic and secretory immune response to luminal antigens.

One of the first beneficial effects described for *L.GG* was the reinforcement of local immune defense through an enhanced secretion of rotavirus-specific IgA [52, 121]. Whether these IgA antibodies have the capacity to counteract viral entry into epithelial cells remains to be established. *L.GG* also had an immunostimulating effect on oral rotavirus vaccination in infants since infants who received *L.GG* showed an increased response with regard to rotavirus-specific IgM-secreting cells [122].

In human volunteers, ingestion of an attenuated *Salmonella typhi* strain to mimic an enteropathogenic infection produced in a group supplemented with fermented milk (*L. acidophilus LA1* and bifidobacteria) a specific serum IgA response which was four times higher than in the control group [123]. However, this result was not confirmed by others [124] who found that the increase in serum IgA was small and that no modification of other Ig was detected.

The enhancement of the IgA secretory response against a soluble food antigen was also described in mice fed a whey protein diet with or without *B. longum,* since both total IgA and specific anti-β-lactoglobulin IgA were significantly higher in the small intestine of mice fed the *B. longum*-containing diet than in control mice [125].

Non-specific immune response

Non-specific, anti-infective mechanisms of defense can also be enhanced by ingestion of specific lactic acid bacteria strains. These strains have been postulated for use as nutritional supplements to improve the defective immune function of particular age groups such as neonates or elderly persons. In a study of the immunomodulation of human blood cells following lactic acid bacteria ingestion, the consumption of milks fermented with *B. bifidum* or *L. acidophilus* strain LA1 induced an increased phagocytosis of *E. coli*, in vitro [126]. In mice, different strains of lactobacillus and *S. thermophilus* were capable of stimulating non-specific (macrophages) and specific (lymphocytes B and T) immunity [127]. Recently, a comparison of cytokine release in the gut, after oral ingestion of various *Lactobacillus* strains in mice, showed that different *Lactobacillus* strains induce distinct mucosal cytokine profiles and possess differential intrinsic adjuvanticity. Most *Lactobacillus* strains induced TNF-α production by cytokine-producing cells in the gut villi, but non-cytokine-inducing strains were also identified. By contrast, in this study, no *Lactobacillus* strain emerged which induced a local production of TGF-β or IL-10, cytokines which have been described as permissive for oral tolerance induction [128]. Similarly, lactobacilli isolated from human intestine were potent stimulators of IL-12 production by human blood mononuclear cells or monocytes [129]. In addition, it was shown that *L. johnsonii* and *Lactobacillus sakei* strongly induced IFN-γ and IL-12 secretion (Th1 cytokines), but not IL-10 secretion, by human peripheral blood mononuclear cells in vitro [130]. However, in the intestinal epithelial cell line Caco-2 co-cultured on permeable filters in the presence of underlying leukocytes, *L. johnsonii* was shown to stimulate the secretion of TGF-β by epithelial cells although it was not capable of reducing the secretion of pro-inflammatory cytokines by leukocytes [115]. Clinical studies show that in children with atopic dermatitis, oral administration of *L.GG* during 8 weeks leads to an increase in IL-10 secretion by peripheral blood mononuclear cells and serum IL-10. Taking these studies into account, one must emphasize that the capacity of mucosa-associated lactobacilli or probiotic bacteria to stimulate a cytokine response by local mononuclear cells or lymphocytes depends in part on

their capacity to cross the gut epithelium before interacting with cells of the local immune system. Indeed, Gram– bacteria lipopolysaccharides as well as Gram+ bacteria cell walls are potent stimuli for activation of the monocytes/macrophages of the immune system [131] through the activation of CD14 and Toll-like receptors.

In addition, the cytoplasmic fraction of probiotic bacteria lysates seems able to inhibit lymphocyte proliferation [132], suggesting that soluble compounds susceptible to absorption by the intestinal epithelium may also play a role in immunoregulation. Surprisingly, such a property was initially described in certain strains of pathogenic *E. coli*, which were shown to produce a factor inhibiting mitogen-stimulated lymphokine mRNA expression in lymphocytes, without inhibiting expression of cytokines expressed in monocytes [133].

Alternatively, probiotic bacteria may act on underlying immune cells via the stimulation of intestinal epithelial cells as described above.

Conclusion

For centuries, yogurt and fermented milk have been thought to be foods with special benefits for health. More recently, a great deal of interest has developed concerning the many beneficial effects of probiotic microorganisms in a variety of pathological situations. However, the better we understand the molecular mechanisms of probiotic-host interactions, the better we can exploit their capacity to enhance mucosal immune responsiveness and to counteract pathogen colonization and the ensuing inflammatory processes. In addition to demonstrating the mechanisms underlying the known health benefits, the recent development of molecular biological techniques that permit construction of lactic acid bacteria which secrete cytokines or which present antigens in various ways to the immune system offers the chance to explore new potentials of these bacteria as immunomodulators.

Various other important points need to be emphasized. Resident microflora reside mainly at the distal end of the digestive tract. Sustained high doses of probiotic bacteria administered by the oral route may promote beneficial effects via the transient colonization of more proximal parts of the gut. Finally, as the gut translocation of probiotic microorganisms is probably negligible in physiological conditions, the effect of bacterial compounds released in the intestinal lumen and potentially absorbed by the gut epithelium, as well as the direct modifications of epithelial cell physiology due to bacterial contact (fig. 4), have to be considered as possible mechanisms by which probiotic microorganisms can be made to affect local or systemic immune function.

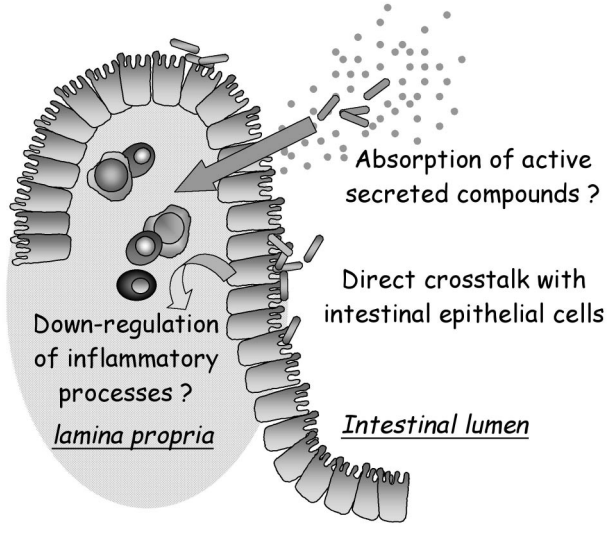

Figure 4. Probiotic bacteria: possible interactions at the intestinal epithelial level. High doses of probiotic bacteria administered by the oral route may allow beneficial effects through the transient colonization of more proximal parts of the gut. Finally, as the gut translocation of probiotic microorganisms is probably negligible, the effect of bacterial compounds released in the intestinal lumen and potentially absorbed by the gut epithelium, as well as the direct modulation of the epithelial cell in contact with bacteria have to be considered as possible mechanisms for the development of local or systemic effects of probiotic microorganisms.

1 Metchnikoff E. (1907) Lactic acid as inhibiting putrefaction. In: The Prolongation of Life: Optimistic Studies, pp. 161–183, Heinemann, London
2 Fuller R. (1989) Probiotics in man and animals. J. Appl. Bacteriol. **66:** 365–378
3 Gaboriau Routhiau V. and Moreau M. C. (1996) Gut flora allows recovery of oral tolerance to ovalbumin in mice after transient breakdown mediated by cholera toxin or *Escherichia coli* heat-labile enterotoxin. Pediatr. Res. **39:** 625–629
4 Vaughan E. E., Schut F., Heilig H. G., Zoetendal E. G., Vos W. M. de and Akkermans A. D. (2000) A molecular view of the intestinal ecosystem. Curr. Issues Intest. Microbiol. **1:** 1–12
5 Krinos C. M., Coyne M. J., Weinacht K. G., Tzianabos A. O., Kasper D. L. and Comstock L. E. (2001) Extensive surface diversity of a commensal microorganism by multiple DNA inversions. Nature **414:** 555–558
6 Kolars J. C., Lewitt M. D., Aouji M. and Savaiano D. A. (1984) Yogurt – an autodigesting source of lactose. N. Engl. J. Med. **310:** 1–3
7 Savaiano D. A., AbouElAnouar A., Smith D. E. and Levitt M. D. (1984) Lactose malabsorption from yogurt, pasteurized yogurt, sweet acidophilus milk, and cultured milk in lactase-deficient individuals. Am. J. Clin. Nutr. **40:** 1219–1223
8 Dewit O., Pochart P. and Desjeux J. F. (1988) Breath hydrogen concentration and plasma glucose, insulin and free fatty acid levels after lactose, milk, fresh or heated yogurt ingestion by healthy young adults with or without lactose malabsorption. Nutrition **4:** 131–136
9 Marteau P., Flourie B., Pochart P., Chastang C., Desjeux J. F. and Rambaud J. C. (1990) Effect of the microbial lactase (EC 3.2.1.23) activity in yoghurt on the intestinal absorption of lactose: an in vivo study in lactase-deficient humans. Br. J. Nutr. **64:** 71–79
10 Shermak M. A., Saavedra J. M., Jackson T. L., Huang S. S., Bayless T. M. and Perman J. A. (1995) Effect of yogurt on

symptoms and kinetics of hydrogen production in lactose-malabsorbing children. Am. J. Clin. Nutr. **62:** 1003–1006
11 Pene P., Linhard J. and Bernou J. C. (1966) The colibacillus-lactobacillus combination in the treatment of diarrhea in adults, children and infants. Sem. Hop. **42:** 241–244
12 Camatte R. (1966) Microbiologic compensation of oral antibiotherapy and treatment of acute infectious diarrhea with a new compound preparation based on lactic enzymes (in French). Gaz. Med. Fr. **73:** 138–141
13 Perdigon G., Nader de Macias M. E., Alvarez S., Medici M., Oliver G. and Pesce de Ruiz Holgado A. (1986) Immunopotentiating activity of lactic bacteria administered by oral route: favorable effect in infantile diarrheas. Medicina (B. Aires). **46:** 751–754
14 Isolauri E., Juntunen M., Rautanen T., Sillanaukee P. and Koivula T. (1991) A human *Lactobacillus* strain (*Lactobacillus casei* sp strain GG) promotes recovery from acute diarrhea in children. Pediatrics **88:** 90–97
15 Raza S., Graham S. M., Allen S. J., Sultana S., Cuevas L. and Hart C. A. (1995) *Lactobacillus* GG promotes recovery from acute nonbloody diarrhea in Pakistan. Pediatr. Infect. Dis. J. **14:** 107–111
16 Shornikova A. V., Isolauri E., Burkanova L., Lukovnikova S. and Vesikari T. (1997) A trial in the Karelian Republic of oral rehydration and *Lactobacillus* GG for treatment of acute diarrhoea. Acta Paediatr. **86:** 460–465
17 Shornikova A. V., Casas I. A., Mykkanen H., Salo E. and Vesikari T. (1997) Bacteriotherapy with *Lactobacillus reuteri* in rotavirus gastroenteritis. Pediatr. Infect. Dis. J. **16:** 1103–1107
18 Guandalini S., Pensabene L., Zikri M. A., Dias J. A., Casali L. G., Hoekstra H. et al. (2000) *Lactobacillus* GG administered in oral rehydration solution to children with acute diarrhea: a multicenter European trial. J. Pediatr. Gastroenterol. Nutr. **30:** 54–60
19 Boudraa G., Benbouabdellah M., Hachelaf W., Boisset M., Desjeux J. F. and Touhami M. (2001) Effect of feeding yogurt versus milk in children with acute diarrhea and carbohydrate malabsorption. J. Pediatr. Gastroenterol. Nutr. **33:** 307–313
20 Foster T. L., Winans L. Jr. and Carski T. R. (1980) Evaluation of lactobacillus preparation on eterotoxigenic *E. coli*-induced rabbit ileal loop reactions. Am. J. Gastroenterol. **73:** 238–243
21 Rani B. and Khetarpaul N. (1998) Probiotic fermented food mixtures: possible applications in clinical anti-diarrhoea usage. Nutr. Health **12:** 97–105
22 Fourniat J., Djaballi Z., Maccario J., Bourlioux P. and German A. (1986) Effect of the administration of killed *Lactobacillus acidophilus* on the survival of suckling mice infected with a strain of enterotoxigenic *Escherichia coli*. Ann. Rech. Vet. **17:** 401–407
23 Perdigon G., Nader de Macias M. E., Alvarez S., Oliver G. and Pesce de Ruiz Holgado A. A. (1990) Prevention of gastrointestinal infection using immunobiological methods with milk fermented with *Lactobacillus casei* and *Lactobacillus acidophilus*. J. Dairy Res. **57:** 255–264
24 Parraga M. E., Spier S. J., Thurmond M. and Hirsh D. (1997) A clinical trial of probiotic administration for prevention of *Salmonella* shedding in the postoperative period in horses with colic. J. Vet. Intern. Med. **11:** 36–41
25 Bernet-Camard M. F., Lievin V., Brassart D., Neeser J. R., Servin A. L. and Hudault S. (1997) The human *Lactobacillus acidophilus* strain LA1 secretes a nonbacteriocin antibacterial substance(s) active in vitro and in vivo. Appl. Environ. Microbiol. **63:** 2747–2753
26 Caplan M. S., Miller-Catchpole R., Kaup S., Russell T., Lickerman M., Amer M. et al. (1999) Bifidobacterial supplementation reduces the incidence of necrotizing enterocolitis in a neonatal rat model. Gastroenterology **117:** 577–583
27 Clements M. L., Levine M. M., Ristaino P. A., Daya V. E. and Hughes T. P. (1983) Exogenous lactobacilli fed to man – their fate and ability to prevent diarrheal disease. Prog. Food Nutr. Sci. **7:** 29–-37
28 Clements M. L., Levine M. M., Black R. E., Robins-Browne R. M., Cisneros L. A., Drusano G. L. et al. (1981) *Lactobacillus* prophylaxis for diarrhea due to enterotoxigenic *Escherichia coli*. Antimicrob. Agents Chemother. **20:** 104–108
29 Attar A., Flourie B., Rambaud J. C., Franchisseur C., Ruszniewski P. and Bouhnik Y. (1999) Antibiotic efficacy in small intestinal bacterial overgrowth-related chronic diarrhea: a crossover, randomized trial. Gastroenterology **117:** 794–797
30 Gorbach S. L., Chang T. W. and Goldin B. (1987) Successful treatment of relapsing *Clostridium difficile* colitis with *Lactobacillus* GG. Lancet **ii:** 1519
31 Biller J. A., Katz A. J., Flores A. F., Buie T. M. and Gorbach S. L. (1995) Treatment of recurrent *Clostridium difficile* colitis with *Lactobacillus* GG. J. Pediatr. Gastroenterol. Nutr. **21:** 224–226
32 McFarland L. V., Surawicz C. M., Greenberg R. N., Fekety R., Elmer G. W., Moyer K. A. et al. (1994) A randomized placebo-controlled trial of *Saccharomyces boulardii* in combination with standard antibiotics for *Clostridium difficile* disease. JAMA **271:** 1913–1918
33 Pochapin M. (2000) The effect of probiotics on *Clostridium difficile* diarrhea. Am. J. Gastroenterol. **95:** S11–S13
34 Boudraa G., Touhami M., Pochart P., Soltana R., Mary J. Y. and Desjeux J. F. (1990) Effect of feeding yogurt versus milk in children with persistent diarrhea. J. Pediatr. Gastroenterol. Nutr. **11:** 509–512
35 Roggero P., Volpe C., Ceccatelli M. P., Lambri A., Giuliani M. G., Donattini T. et al. (1990) Crystalline lactulose and oral preparations of micro-organisms for the treatment of chronic aspecific diarrhea in children: a controlled clinical study. Minerva Pediatr. **42:** 147–150
36 Gonzalez S. N., Cardozo R., Apella M. C. and Oliver G. (1994) Biotherapeutic role of fermented milk. Biotherapy **8:** 129–134
37 Bergogne-Berezin E. (2000) Treatment and prevention of antibiotic associated diarrhea. Int. J. Antimicrob. Agents **16:** 521–526
38 Siitonen S., Vapaatalo H., Salminen S., Gordin A., Saxelin M., Wikberg R. et al. (1990) Effect of *Lactobacillus* GG yoghurt in prevention of antibiotic associated diarrhoea. Ann. Med. **22:** 57–59
39 Contardi I. (1991) Oral bacterial therapy in prevention of antibiotic-induced diarrhea in childhood. Clin. Ter. **136:** 409–413
40 Vanderhoof J., Whitney D. and Antonson D. (2000) In children receiving antibiotics, does coadministration of lactobacillus GG reduce the incidence of diarrhea? West J. Med. **173:** 397
41 Arvola T., Laiho K., Torkkeli S., Mykkanen H., Salminen S., Maunula L. et al. (1999) Prophylactic *Lactobacillus* GG reduces antibiotic-associated diarrhea in children with respiratory infections: a randomized study. Pediatrics **104:** e64
42 Gotz V., Romankiewicz J. A., Moss J. and Murray H. W. (1979) Prophylaxis against ampicillin-associated diarrhea with a lactobacillus preparation. Am. J. Hosp. Pharm. **36:** 754–757
43 Salminen E., Elomaa I., Minkkinen J., Vapaatalo H. and Salminen S. (1988) Preservation of intestinal integrity during radiotherapy using live *Lactobacillus acidophilus* cultures. Clin. Radiol. **39:** 435–437
44 Urbancsek H., Kazar T., Mezes I. and Neumann K. (2001) Results of a double-blind, randomized study to evaluate the efficacy and safety of Antibiophilus in patients with radiation-induced diarrhoea. Eur. J. Gastroenterol. Hepatologica **13:** 391–396

45 Brunser O., Araya M., Espinoza J., Guesry P. R., Secretin M. C. and Pacheco I. (1989) Effect of an acidified milk on diarrhoea and the carrier state in infants of low socio-economic stratum. Acta Paediatr. Scand. **78:** 259–264

46 Boudraa G. (1994) Effect of fermented infant formula on incidence of diarrhea at early weaning. ESPGAN meeting 1994

47 Saavedra J. M., Bauman N. A., Oung I., Perman J. A. and Yolken R. H. (1994) Feeding of *Bifidobacterium bifidum* and *Streptococcus thermophilus* to infants in hospital for prevention of diarrhoea and shedding of rotavirus. Lancet **344:** 1046–1049

48 Szajewska H., Kotowska M., Mrukowicz J. Z., Armanska M. and Mikolajczyk W. (2001) Efficacy of *Lactobacillus* GG in prevention of nosocomial diarrhea in infants. J. Pediatr. **138:** 361–365

49 Hatakka K., Savilahti E., Ponka A., Meurman J. H., Poussa T., Nase L. et al. (2001) Effect of long term consumption of probiotic milk on infections in children attending day care centres: double blind, randomised trial. BMJ **322:** 1327

50 Oberhelman R. A., Gilman R. H., Sheen P., Taylor D. N., Black R. E., Cabrera L. et al. (1999) A placebo-controlled trial of *Lactobacillus* GG to prevent diarrhea in undernourished Peruvian children. J. Pediatr. **134**: 15–20

51 Gendrel D., Dupont C., Richard-Lenoble D., Gendrel C. and Chaussain M. (1990) Feeding lactose-intolerant children with a powdered fermented milk. J. Pediatr. Gastroenterol. Nutr. **10:** 44–46

52 Kaila M., Isolauri E., Saxelin M., Arvilommi H. and Vesikari T. (1995) Viable versus inactivated lactobacillus strain GG in acute rotavirus diarrhoea. Arch. Dis. Child **72:** 51–53

53 Coconnier M. H., Lievin V., Bernet-Camard M. F., Hudault S. and Servin A. L. (1997) Antibacterial effect of the adhering human *Lactobacillus acidophilus* strain LB. Antimicrob. Agents Chemother. **41:** 1046–1052

54 Aiba Y., Suzuki N., Kabir A. M., Takagi A. and Koga Y. (1998) Lactic acid-mediated suppression of *Helicobacter pylori* by the oral administration of *Lactobacillus salivarius* as a probiotic in a gnotobiotic murine model. Am. J. Gastroenterol. **93:** 2097–2101

55 Kabir A. M., Aiba Y., Takagi A., Kamiya S., Miwa T. and Koga Y. (1997) Prevention of *Helicobacter pylori* infection by lactobacilli in a gnotobiotic murine model. Gut **41:** 49–55

56 Felley C. P., Corthesy-Theulaz I., Rivero J. L., Sipponen P., Kaufmann M., Bauerfeind P. et al. (2001) Favourable effect of an acidified milk (LC-1) on *Helicobacter pylori* gastritis in man. Eur. J. Gastroenterol. Hepatol. **13:** 25–29

57 Sakamoto I., Igarashi M., Kimura K., Takagi A., Miwa T. and Koga Y. (2001) Suppressive effect of *Lactobacillus gasseri* OLL 2716 (LG21) on *Helicobacter pylori* infection in humans. J. Antimicrob. Chemother. **47:** 709–710

58 Fabia R., Ar'Rajab A., Johansson M. L., Andersson R., Willen R., Jeppsson B. et al. (1993) Impairment of bacterial flora in human ulcerative colitis and experimental colitis in the rat. Digestion **54:** 248–255

59 Favier C., Neut C., Mizon C., Cortot A., Colombel J. F. and Mizon J. (1997) Fecal beta-D-galactosidase production and *Bifidobacteria* are decreased in Crohn's disease. Dig. Dis. Sci. **42:** 817–822

60 Ruseler-van Embden J. G., Schouten W. R. and Lieshout L. M. van (1994) Pouchitis: result of microbial imbalance? Gut **35:** 658–664

61 Veltkamp C., Tonkonogy S. L., De Jong Y. P., Albright C., Grenther W. B., Balish E. et al. (2001) Continuous stimulation by normal luminal bacteria is essential for the development and perpetuation of colitis in Tg(epsilon26) mice. Gastroenterology **120:** 900–913

62 Steidler L., Hans W., Schotte L., Neirynck S., Obermeier F., Falk W. et al. (2000) Treatment of murine colitis by *Lactococcus lactis* secreting interleukin-10. Science. **289:** 1352–1355

63 Madsen K. L., Doyle J. S., Jewell L. D., Tavernini M. M. and Fedorak R. N. (1999) *Lactobacillus* species prevents colitis in interleukin 10 gene-deficient mice. Gastroenterology **116:** 1107–1114

64 Malin M., Suomalainen H., Saxelin M. and Isolauri E. (1996) Promotion of IgA immune response in patients with Crohn's disease by oral bacteriotherapy with *Lactobacillus* GG. Ann. Nutr. Metab **40:** 137–145

65 Gupta P., Andrew H., Kirschner B. S. and Guandalini S. (2000) Is lactobacillus GG helpful in children with Crohn's disease? Results of a preliminary, open-label study. J. Pediatr. Gastroenterol. Nutr. **31:** 453–457

66 Gionchetti P., Rizzello F., Venturi A., Brigidi P., Matteuzzi D., Bazzocchi G. et al. (2000) Oral bacteriotherapy as maintenance treatment in patients with chronic pouchitis: a double-blind, placebo-controlled trial. Gastroenterology **119:** 305–309

67 Kruis W., Schutz E., Fric P., Fixa B., Judmaier G. and Stolte M. (1997) Double-blind comparison of an oral *Escherichia coli* preparation and mesalazine in maintaining remission of ulcerative colitis. Aliment. Pharmacol. Ther. **11:** 853–858

68 Kruisselbrink A., Heijne Den Bak-Glashouwer M. J., Havenith C. E., Thole J. E. and Janssen R. (2001) Recombinant *Lactobacillus plantarum* inhibits house dust mite-specific T-cell responses. Clin. Exp. Immunol. **126:** 2–8

69 Paton J. C., Rogers T. J., Morona R. and Paton A. W. (2001) Oral administration of formaldehyde-killed recombinant bacteria expressing a mimic of the Shiga toxin receptor protects mice from fatal challenge with Shiga-toxigenic *Escherichia coli*. Infect. Immun. **69:** 1389–1393

70 Majamaa H. and Isolauri E. (1997) Probiotics: a novel approach in the management of food allergy. J. Allergy Clin. Immunol. **99:** 179–185

71 Isolauri E., Majamaa H., Arvola T., Rantala I., Virtanen E. and Arvilommi H. (1993) *Lactobacillus casei* strain GG reverses increased intestinal permeability induced by cow milk in suckling rats. Gastroenterology **105:** 1643–1650

72 Sutas Y., Hurme M. and Isolauri E. (1996) Down-regulation of anti-CD3 antibody-induced IL-4 production by bovine caseins hydrolysed with *Lactobacillus* GG-derived enzymes. Scand. J. Immunol. **43:** 687–689

73 Shida K., Makino K., Morishita A., Takamizawa K., Hachimura S., Ametani A. et al. (1998) *Lactobacillus casei* inhibits antigen-induced IgE secretion through regulation of cytokine production in murine splenocyte cultures. Int. Arch. Allergy Immunol. **115:** 278–287

74 Murosaki S., Yamamoto Y., Ito K., Inokuchi T., Kusaka H., Ikeda H. et al. (1998) Heat-killed *Lactobacillus plantarum* L-137 suppresses naturally fed antigen-specific IgE production by stimulation of IL-12 production in mice. J. Allergy Clin. Immunol. **102:** 57–64

75 Terpend K., Blaton MA., Candalh C., Wal JM., Pochart P. and Heyman M. (1998) Intestinal barrier function and cow's milk sensitization in guinea-pigs fed milk or fermented milk. J. Pediatr. Gastroenterol. Nutr. **28:** 191–198

76 Sepp E., Julge K., Vasar M., Naaber P., Bjorksten B. and Mikelsaar M. (1997) Intestinal microflora of Estonian and Swedish infants. Acta Paediatr. **86:** 956–961

77 Bjorksten B., Naaber P., Sepp E. and Mikelsaar M. (1999) The intestinal microflora in allergic Estonian and Swedish 2-year-old children. Clin. Exp. Allergy **29:** 342–346

78 Kalliomaki M., Kirjavainen P., Eerola E., Kero P., Salminen S. and Isolauri E. (2001) Distinct patterns of neonatal gut microflora in infants in whom atopy was and was not developing. J. Allergy Clin. Immunol. **107:** 129–134

79 Wollowski I., Rechkemmer G. and Pool-Zobel B. L. (2001) Protective role of probiotics and prebiotics in colon cancer. Am. J. Clin. Nutr. **73:** 451S–455S

80 Renner H. W. and Munzner R. (1991) The possible role of probiotics as dietary antimutagen. Mutat. Res. **262:** 239–245

81 Pool-Zobel B. L., Bertram B., Knoll M., Lambertz R., Neudecker C., Schillinger U. et al. (1993) Antigenotoxic properties of lactic acid bacteria in vivo in the gastrointestinal tract of rats. Nutr. Cancer **20:** 271–281

82 Bodana A. R. and Rao D. R. (1990) Antimutagenic activity of milk fermented by *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. J. Dairy Sci. **73:** 3379–3384

83 Abrahamse S. L., Pool-Zobel B. L. and Rechkemmer G. (1999) Potential of short chain fatty acids to modulate the induction of DNA damage and changes in the intracellular calcium concentration by oxidative stress in isolated rat distal colon cells. Carcinogenesis **20:** 629–634

84 Hawksworth G., Drasar B. S. and Hill M. J. (1971) Intestinal bacteria and the hydrolysis of glycosidic bonds. J. Med. Microbiol. **4:** 451–459

85 Young T. B. and Wolf D. A. (1988) Case-control study of proximal and distal colon cancer and diet in Wisconsin. Int. J. Cancer **42:** 167–175

86 Boutron M. C., Faivre J., Marteau P., Couillault C., Senesse P. and Quipourt V. (1996) Calcium, phosphorus, vitamin D, dairy products and colorectal carcinogenesis: a French case-control study. Br. J. Cancer **74:** 145–151

87 Kampman E., Goldbohm R. A., Brandt P. A. van den and Aken-Schneider P. van (1994) Fermented dairy products, calcium, and colorectal cancer in The Netherlands Cohort Study. Cancer Res. **54:** 3186–3190

88 Pouwels P. H., Vriesema A., Martinez B., Tielen F. J., Seegers J. F., Leer R. J. et al. (2001) Lactobacilli as vehicles for targeting antigens to mucosal tissues by surface exposition of foreign antigens. Methods Enzymol. **336:** 369–389

89 Thole J. E., Dalen P. J. van, Havenith C. E., Pouwels P. H., Seegers J. F., Tielen F. D. et al. (2000) Live bacterial delivery systems for development of mucosal vaccines. Curr. Opin. Mol. Ther. **2:** 94–99

90 Mercenier A., Muller-Alouf H. and Grangette C. (2000) Lactic acid bacteria as live vaccines. Curr. Issues Mol. Biol. **2:** 17–25

91 Brandtzaeg P. (1996) History of oral tolerance and mucosal immunity. Ann. N. Y. Acad. Sci. **778:** 1–27

92 Pouwels P. H., Leer R. J., Shaw M., Heijne Den Bak-Glashouwer M. J., Tielen F. D., Smit E. et al. (1998) Lactic acid bacteria as antigen delivery vehicles for oral immunization purposes. Int. J. Food Microbiol. **41:** 155–167

93 Maassen C. B., Laman J. D., Bak-Glashouwer M. J., Tielen F. J., Holten-Neelen J. C., Hoogteijling L. et al. (1999) Instruments for oral disease-intervention strategies: recombinant *Lactobacillus casei* expressing tetanus toxin fragment C for vaccination or myelin proteins for oral tolerance induction in multiple sclerosis. Vaccine **17:** 2117–2128

94 Shaw D. M., Gaerthe B., Leer R. J., Van Der Stap J. G., Smittenaar C., Heijne D. B.-G. et al. (2000) Engineering the microflora to vaccinate the mucosa: serum immunoglobulin G responses and activated draining cervical lymph nodes following mucosal application of tetanus toxin fragment C-expressing lactobacilli. Immunology **100:** 510–518

95 Grangette C., Muller-Alouf H., Goudercourt D., Geoffroy M. C., Turneer M. and Mercenier A. (2001) Mucosal immune responses and protection against tetanus toxin after intranasal immunization with recombinant *Lactobacillus plantarum*. Infect. Immun. **69:** 1547–1553

96 Gaon D., Doweck Y., Gomez Zavaglia A., Ruiz Holgado A. and Oliver G. (1995) Lactose digestion by milk fermented with *Lactobacillus acidophilus* and *Lactobacillus casei* of human origin. Medicina (B. Aires) **55:** 237–242

97 Vesa T. H., Marteau P., Zidi S., Briet F., Pochart P. and Rambaud J. C. (1996) Digestion and tolerance of lactose from yoghurt and different semi-solid fermented dairy products containing *Lactobacillus acidophilus* and bifidobacteria in lactose maldigesters – is bacterial lactase important? Eur. J. Clin. Nutr. **50:** 730–733

98 Rubaltelli F. F., Biadaioli R., Pecile P. and Nicoletti P. (1998) Intestinal flora in breast- and bottle-fed infants. J. Perinat. Med. **26:** 186–191

99 Sheen P., Oberhelman R. A., Gilman R. H., Cabrera L., Verastegui M. and Madico G. (1995) Short report: a placebo-controlled study of *Lactobacillus* GG colonization in one-to-three-year-old Peruvian children. Am. J. Trop. Med. Hyg. **52:** 389–392

100 Langhendries J. P., Detry J., Van Hees J., Lamboray J. M., Darimont J., Mozin M. J. et al. (1995) Effect of a fermented infant formula containing viable bifidobacteria on the fecal flora composition and pH of healthy full-term infants. J. Pediatr. Gastroenterol. Nutr. **21:** 177–181

101 Marteau P., Pochart P., Bouhnik Y., Zidi S., Goderel I. and Rambaud J. C. (1992) Survival of *Lactobacillus acidophilus* and *Bifidobacterium* sp. in the small intestine following ingestion in fermented milk: a rational basis for the use of probiotics in man. Gastroenterol. Clin. Biol. **16:** 25–28

102 Pochart P., Marteau P., Bouhnik Y., Goderel I., Bourlioux P. and Rambaud J. C. (1992) Survival of bifidobacteria ingested via fermented milk during their passage through the human small intestine: an in vivo study using intestinal perfusion. Am. J. Clin. Nutr. **55:** 78–80

103 Bernet M. F., Brassart D., Neeser J. R. and Servin A. L. (1994) *Lactobacillus acidophilus* LA 1 binds to cultured human intestinal cell lines and inhibits cell attachment and cell invasion by enterovirulent bacteria. Gut **35:** 483–489

104 Chauvière G., Coconnier M. H., Kerneis S., Darfeuille-Michaud A., Joly B. and Servin A. L. (1992) Competitive exclusion of diarrheagenic *Escherichia coli* (ETEC) from human enterocyte-like Caco-2 cells by heat- killed *Lactobacillus*. FEMS Microbiol. Lett. **70:** 213–217

105 Mack D. R., Michail S., Wei S., McDougall L. and Hollingsworth M. A. (1999) Probiotics inhibit enteropathogenic *E. coli* adherence in vitro by inducing intestinal mucin gene expression. Am. J. Physiol. **276:** G941–G950

106 Kirjavainen P. V., Ouwehand A. C., Isolauri E. and Salminen S. J. (1998) The ability of probiotic bacteria to bind to human intestinal mucus. FEMS Microbiol. Lett. **167:** 185–189

107 He F., Ouwehan A. C., Hashimoto H., Isolauri E., Benno Y. and Salminen S. (2001) Adhesion of *Bifidobacterium* spp. to human intestinal mucus. Microbiol. Immunol. **45:** 259–262

108 Adlerberth I., Ahrne S., Johansson M. L., Molin G., Hanson L. A. and Wold A. E. (1996) A mannose-specific adherence mechanism in *Lactobacillus plantarum* conferring binding to the human colonic cell line HT-29. Appl. Environ. Microbiol. **62:** 2244–2251

109 Granato D., Perotti F., Masserey I., Rouvet M., Golliard M., Servin A. et al. (1999) 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. **65:** 1071–1077

110 Gotteland M., Pochart P., Dabbech M., Bisetti N. and Desjeux J. F. (1992) In vivo effect of yogurt on excretion of enteropathogen *Escherichia coli* RDEC-1 during acute diarrhea in the just-weaned rabbit. J. Pediatr. Gastroenterol. Nutr. **14:** 264–267

111 Giraud A., Matic I., Tenaillon O., Clara A., Radman M., Fons M. et al. (2001) Costs and benefits of high mutation rates: adaptive evolution of bacteria in the mouse gut. Science **291:** 2606–2608

112 Hooper L. V., Wong M. H., Thelin A., Hansson L., Falk P. G. and Gordon J. I. (2001) Molecular analysis of commensal host-microbial relationships in the intestine. Science **291:** 881–884

113 Xavier R. J. and Podolsky D. K. (2000) Microbiology: how to get along – friendly microbes in a hostile world. Science **289:** 1483–1484

114 Neish A. S., Gewirtz A. T., Zeng H., Young A. N., Hobert M. E., Karmali V. et al. (2000) Prokaryotic regulation of epithelial responses by inhibition of IkappaB-alpha ubiquitination. Science **289:** 1560–1563

115 Haller D., Bode C., Hammes W. P., Pfeifer A. M., Schiffrin E. J. and Blum S. (2000) Non-pathogenic bacteria elicit a differential cytokine response by intestinal epithelial cell/leucocyte co-cultures. Gut **47:** 79–87

116 Heyman M., Crain-Denoyelle A. M., Corthier G., Morgat J. L. and Desjeux J. F. (1986) Postnatal development of protein absorption in conventional and germ-free mice. Am. J. Physiol. **251:** G326–G331

117 Garcia-Lafuente A., Antolin M., Guarner F., Crespo E. and Malagelada J. R. (2001) Modulation of colonic barrier function by the composition of the commensal flora in the rat. Gut **48:** 503–507

118 Isolauri E., Kaila M., Arvola T., Majamaa H., Rantala I., Virtanen E. et al. (1993) Diet during rotavirus enteritis affects jejunal permeability to macromolecules in suckling rats. Pediatr. Res. **33:** 548–553

119 Madsen K., Cornish A., Soper P., McKaigney C., Jijon H., Yachimec C. et al. (2001) Probiotic bacteria enhance murine and human intestinal epithelial barrier function. Gastroenterology **121:** 580–591

120 Gotteland M., Cruchet S. and Verbeke S. (2001) Effect of *Lactobacillus* ingestion on the gastrointestinal mucosal barrier alterations induced by indometacin in humans. Aliment. Pharmacol. Ther. **15:** 11–17

121 Kaila M., Isolauri E., Soppi E., Virtanen E., Laine S. and Arvilommi H. (1992) Enhancement of the circulating antibody secreting cell response in human diarrhea by a human *Lactobacillus* strain. Pediatr. Res. **32:** 141–144

122 Isolauri E., Joensuu J., Suomalainen H., Luomala M. and Vesikari T. (1995) Improved immunogenicity of oral D × RRV reassortant rotavirus vaccine by *Lactobacillus casei* GG. Vaccine **13:** 310–312

123 Link Amster H., Rochat F., Saudan K. Y., Mignot O. and Aeschlimann J. M. (1994) Modulation of a specific humoral immune response and changes in intestinal flora mediated through fermented milk intake. FEMS Immunol. Med. Microbiol. **10:** 55–63

124 Marteau P., Vaerman J. P., Dehennin J. P., Bord S., Brassart D., Pochart P. et al. (1997) Effects of intrajejunal perfusion and chronic ingestion of *Lactobacillus johnsonii* strain La1 on serum concentrations and jejunal secretions of immunoglobulins and serum proteins in healthy humans. Gastroenterol. Clin. Biol. **21:** 293–298

125 Takahashi T., Nakagawa E., Nara T., Yajima T. and Kuwata T. (1998) Effects of orally ingested *Bifidobacterium longum* on the mucosal IgA response of mice to dietary antigens. Biosci. Biotechnol. Biochem. **62:** 10–15

126 Schiffrin E. J., Rochat F., Link-Amster H., Aeschlimann J. M. and Donnet-Hughes A. (1995) Immunomodulation of human blood cells following the ingestion of lactic acid bacteria. J. Dairy Sci. **78:** 491–497

127 Perdigon G., Alvarez S., Rachid M., Aguero G. and Gobbato N. P. (1995) Immune system stimulation by probiotics. J. Dairy. Sci. **78:** 1597–606

128 Maassen C. B., Holten-Neelen C., Balk F., Bak-Glashouwer M. J., Leer R. J., Laman J. D. et al. (2000) Strain-dependent induction of cytokine profiles in the gut by orally administered *Lactobacillus* strains. Vaccine **18:** 2613–2623

129 Hessle C., Hanson L. A. and Wold A. E. (1999) Lactobacilli from human gastrointestinal mucosa are strong stimulators of IL-12 production. Clin. Exp. Immunol. **116:** 276–282

130 Haller D., Blum S., Bode C., Hammes W. P. and Schiffrin E. J. (2000) Activation of human peripheral blood mononuclear cells by nonpathogenic bacteria in vitro: evidence of NK cells as primary targets. Infect. Immun. **68:** 752–759

131 Miettinen M., Vuopio Varkila J. and Varkila K. (1996) Production of human tumor necrosis factor alpha, interleukin-6, and interleukin-10 is induced by lactic acid bacteria. Infect. Immun. **64:** 5403–5405

132 Pessi T., Sutas Y., Saxelin M., Kallioinen H. and Isolauri E. (1999) Antiproliferative effects of homogenates derived from five strains of candidate probiotic bacteria. Appl. Environ. Microbiol. **65:** 4725–4728

133 Klapproth J. M., Donnenberg M. S., Abraham J. M., Mobley H. L. and James S. P. (1995) Products of enteropathogenic Escherichia coli inhibit lymphocyte activation and lymphokine production. Infect. Immun. **63:** 2248–2254