

Effect of Lactic Acid Bacteria on Diarrheal Diseases

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Microbial balance is an important factor in the maintenance of intestinal homeostasis, and yogurt or fermented milk supplementation has been proposed to control diarrheal diseases. A number of studies using animal models and clinical studies in humans have confirmed the beneficial effect of such fermented products in case of lactose intolerance, viral diarrhea or antibiotics-associated diarrhea. The mechanisms by which lactic acid bacteria exert their effects are multiple. Bacterial lactase improves the absorption of lactose, but fermented products slow down the intestinal transit facilitating the action of residual intestinal lactase. The transient passage of lactic acid bacteria in the digestive tract may represent a microbial barrier against the development of pathogenic bacteria, probably due to the release of compounds contributing to the maintenance of colonization resistance to pathogens. The beneficial effects are mainly described in the presence of live bacteria, but inactivated bacteria may also present preventive or curative capacities in diarrheal diseases. Moreover, lactic acid bacteria has been described as reinforcing the non-specific immune defence but also specific immunity, particularly the secretory immune system mediated by secretory IgA or IgM in response to particulate infectious antigens and perhaps to soluble food antigens. Other possible mechanisms include the trophic effect on the intestinal layer, and a down-regulatory activity in cow's milk allergy as well as anti-inflammatory effects have also been suggested.

Key teaching points:

- Lactic acid bacteria improve lactose intolerance.
- Secretory IgA immune response is enhanced by probiotics.

The intestinal microflora maintain a microbial barrier against the development of pathogenic bacteria in the digestive tract and are mandatory to the establishment of oral tolerance to food antigens [1]. Since the microbial balance is an important factor in the maintenance of intestinal homeostasis, live microbial supplementation (yogurt or fermented milks) have been proposed as healthy foods to control diarrheal disease. For the last few decades, an extensive work in that field has confirmed the beneficial effect of fermented products, in the cases of lactose intolerance, infectious diarrhea of viral origin (and less convincingly bacterial origin) or antibiotic-associated diarrhea. Although most of this work has been mainly descriptive, some insights into the mechanisms by which probiotics exert their beneficial effect on the intestinal function have recently emerged.

Various properties of lactic acid bacteria have been proposed as an explanation for their multiple beneficial effects. These properties may include the restoration of a normal intestinal micro

flora, the contribution to the elimination of pathogenic enteric bacteria, the reinforcement of the intestinal barrier capacity to exogenous antigens and the increase in humoral immunity and in the mucosal secretory IgA response. In addition to strengthening the specific immunity, lactic acid bacteria also seem to reinforce the non-specific mechanisms of defence such as phagocytosis and cytokine production. A trophic role on the intestinal epithelium has also been suggested, as well as the secretion by these probiotics of compounds having anti-inflammatory or antimicrobial effects.

All these *in vitro* or *in vivo* studies suggest that synergistic mechanisms are probably involved in the beneficial effects of fermented products in case of diarrheal diseases. A recent review on the treatment and prevention of intestinal infections with biotherapeutics agents has concluded that "there is now evidence that administration of selected microorganisms is beneficial in the prevention and treatment of certain intestinal infections" [2].

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MECHANISMS INVOLVED IN DIARRHEAL DISEASES

To better understand the mechanism by which lactic acid bacteria may interfere with the intestinal function and have a positive effect in diarrheal diseases, one has to take into account the driving forces involved in the dysregulation of electrolyte and water movements leading to diarrhea.

Diarrhea results from a disequilibrium in the water movements across the gut. Multiple factors can be involved in the regulation of water movements, mostly those interfering with the membrane transport systems in the intestinal epithelium. Water movements are mainly generated by the Na-solutes cotransport systems (Na-glucose) or the chloride (Cl^-) secretion through the apical membrane of the enterocytes, and these transporters or channels are highly regulated structures. Water movement always follows the ionic movements in the intestine: the Na^+ absorption generated by the Na-glucose cotransporter is associated with a net water absorption, whereas the Cl^- secretion by the enterocyte leads to water secretion in the intestinal lumen. In these conditions, any luminal or serosal factor acting on any element of this transport system is likely to modify the movements of electrolytes and water. In other words, a decreased Na-glucose cotransport or an increased Cl^- secretion will compromise the hydroelectrolytic homeostasis in the intestine. Factors acting either at the luminal side of the intestinal epithelium or at the serosal (blood) level are capable of affecting electrolyte and water movement, i.e. to induce diarrhea. Among the luminal factors, pathogenic bacteria can adhere to the brush border membrane of the enterocytes and alter the intestinal function by various mechanisms. They can induce attaching-effacing lesions of the brush border membrane or release enterotoxins stimulating chloride secretion or cytotoxin disrupting the epithelial integrity. A congenital defect in Na-glucose cotransport (glucose galactose malabsorption) is associated with a defect in Na and water absorption leading to diarrhea. Various serosal factors are also involved in the regulation of water movements: they compose the stimulation of the underlying immune system (mast cells, phagocytes, lymphocytes) and the release of immune mediators (cytokines), some of which are capable of directly or indirectly stimulate chloride secretion. The stimulation of the intrinsic nervous system in the intestinal mucosa leads to the release of neuro-mediators (Met-enkephalin, acetylcholine) known to activate directly the chloride secretion. Finally, complex interactions between the different cell types present in the submucosa are also involved in the regulation of intestinal secretion. 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, leading to water secretion and diarrhea. A typical case of osmotic diarrhea is that induced by lactose malabsorption due

to lactase deficiency or to the malabsorption of poorly absorbable saccharides (lactulose). Taken into account the multiple factors capable of regulating water and electrolyte absorption and secretion, it is probable that the beneficial effects, observed after administration of probiotics, on diarrheal diseases involve many different mechanisms.

EFFECT OF LACTIC ACID BACTERIA ON DIARRHEA OF VARIOUS ETIOLOGIES

The beneficial effect of fermented milk in children with diarrhea has been reviewed previously [3]. Although it is difficult to know whether the lactic bacteria by themselves or the fermented milk with all its constituents are responsible for such beneficial effects, diarrhea of various etiologies has been successfully treated with fermented milk or yogurt as described in the following paragraphs.

Diarrhea Due to Lactose Malabsorption

The most documented beneficial effect of yogurt on the intestinal function is observed in the case of lactose intolerance. Intestinal lactase deficiency is a frequent condition causing maldigestion and intolerance to lactose. Lactose digestion and absorption has been currently measured using breath hydrogen (H_2) tests. In 1984, Kolars [4] and Savaiano [5], using breath hydrogen tests in lactase deficient subjects, showed that there was a much better absorption of lactose from yogurt than from milk, probably due to intraintestinal digestion of lactose by lactase released from the yogurt microorganisms. In 1987, these results were confirmed by Dewit *et al.* [6], who showed in addition that the ingestion of yogurt or heated yogurt in lactose malabsorbers restored the secretion of insulin to control levels and that the ingestion of fresh, unheated yogurt increased the absorption of free fatty acids. It is not absolutely proven however, that the lactase activity (optimal activity at pH 6–8) persists *in vivo* at the duodenal level, since at pH 5.0 which is the pH observed in the duodenum, the beta-galactosidase activity in yogurt drops by 80%, probably preventing the bacterial beta-galactosidase from hydrolyzing lactose in the duodenum [7]. However, it was shown that in a lactase deficient population, lactose present in yoghurt was better tolerated than that in milk. One fifth of the lactase activity contained in yoghurt was still found in the terminal ileum, suggesting the persistence of the protein along the digestive tract. In addition, fresh yogurt was more efficient in facilitating lactose digestion than heated yoghurt [7].

In a study of lactose absorption in lactase deficient Gabonese children, a beneficial effect of a spray-drying of fermented milk was observed and ascribed to the persistence of lactase activity in the fermented milk [8]. A better efficiency of live *versus* pasteurized yogurt was suggested [9]. Altogether,

these results indicate that bacterial beta-galactosidase present in yogurt partly resists 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. The mechanisms by which the lactic acid bacteria improve the digestion of lactose are described in more detail below.

Treatment of Diarrhea

Acute Viral Diarrhea. Perhaps one of the most documented effects of bacteriotherapy concerns acute viral enteritis in children. The use of oral bacteria therapy in childhood during acute enteritis has early been described as beneficial in the treatment of diarrhea [10–12] and in restoring a normal intestinal ecosystem [13]. The immuno-potentiating effect of lactic acid bacteria was also early shown to have a favorable effect on infantile diarrhea by Perdigon *et al.* in Argentina [14]. Human isolates of *Lactobacillus casei* (Strain GG), now named *Lactobacillus rhamnosus*, has been extensively used in Finland to promote recovery from acute rotavirus diarrhea in children. *L. casei* GG in the form of fermented milk or freeze-dried powder was effective in shortening the course of acute diarrhea [15]. The same effect was recently observed in children with rotavirus diarrhea fed *Lactobacillus reuteri*, since the duration of watery diarrhea was 1.5 days in the treated infants versus 2.5 days in the matched control infants [16]. One of the beneficial effects of *L. casei* GG during the time course of rotavirus diarrhea was to reinforce the local immune defences through specific IgA response to rotavirus [17]. The enhancing effect on sIgA against rotavirus was significantly higher with the strain *L. casei* GG than with *L. casei subsp rhamnosus* (Lactophilus) or with a combination of *Streptococcus thermophilus* and *Lactobacillus delbrückii subsp. bulgaricus* (Yalacta) [18]. Moreover, the administration of viable *L. casei* GG was more efficient in promoting rotavirus specific IgA in serum than inactivated bacteria [19]. In Pakistan, the course of acute diarrhea in hospitalized children was improved in the group supplemented with *L. casei* GG since the frequency of vomiting and diarrhea was decreased in this group, as well as the percentage of children with persistent diarrhea at 48 hours (31% versus 75% in the treated and control group respectively, $p < 0.01$) [20]. In Karelian republic, *L. casei* GG was shown to decrease the duration of acute diarrhea (2.7 vs. 3.7 days) in children with viral acute diarrhea but not in those with bacterial diarrhea [21]. In Thailand, the mean duration of acute non-bloody diarrhea in children fed with freeze-dried preparation of *L. casei* GG was lower (1.9 days) than that observed in the placebo group (3.3 days, $p < 0.055$) [22].

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 five to 24 months was observed in the group receiving a milk formula supplemented with *Bifidobacterium bifidum* and *S. thermophilus* compared to infants receiving the control formula [23].

Bacterial Diarrhea. The data concerning a protective role of lactic acid bacteria in bacterial diarrhea are scanty in humans, and contradictory results are often reported in animals. Most studies have been done in animals (calves, piglets, mice, rabbit and horses) with various results. In a rabbit model of *E. coli* enterotoxin induced diarrhea, a *Lactobacillus*-containing preparation injected in infected ileal loops exhibited a significant anti-enterotoxin response [24]. The administration of killed *Lactobacillus acidophilus* in mice infected with a strain of enterotoxigenic *E. coli* extended their survival, but contradictory results were obtained later [25]; however a protective effect of probiotic fermented food mixture was found in a similar model of mice infected with *E. coli* [26]. A supplementation of calves with lactic-acid producing bacteria did not protect the animals against infection with the parasite *Cryptosporidium parvum* nor the fecal shedding of *C. parvum* oocysts [27]. A clinical trial of probiotic administration for prevention of Salmonella shedding in the postoperative period in horses with colics had no effect on Salmonella shedding and prevalence of diarrhea [28]. However, an *in vivo* study using conventional or germ-free mice orally infected with *Salmonella typhimurium* has shown that the human isolates *Lactobacillus acidophilus* strain LA1 had an antibacterial activity. The antibacterial activity was reproduced, *in vitro*, using Caco2 cells infected with *Salmonella typhimurium* and was shown to be linked to a factor insensitive to proteases and independent of lactic acid production, present in the culture supernatant of strain LA1 [29].

Besides the effect of probiotics observed on the time course of infection, a preventive effect has also been suggested in mice [30].

In humans, in case of recurrent *clostridium difficile* colitis, a successful treatment was obtained by using *L. rhamnosus* (strain GG) [31,32].

In Karelian republic, *L. casei* GG was shown to decrease the duration of acute diarrhea in children with viral acute diarrhea but not in those with bacterial diarrhea [21]. 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 (Lactinex), containing dried *Lactobacillus acidophilus* and *L. bulgaricus* [33,34].

Taken together, although beneficial effects have been reported in different animal or cell culture models; these results raise some doubt on the beneficial effect of a bacteriotherapy in case of bacterial diarrhea in humans.

Persistent or Chronic Diarrhea. Persistent diarrhea is defined as a diarrhea starting acutely but lasting at least two weeks. Interestingly, the beneficial effect of feeding fermented milk was also shown in Algerian children with persistent diarrhea. Those children had experienced a diarrhea for more than 13 days and were fed for five days with yogurt or milk. The clinical failure was observed in 14% in the children fed yogurt versus 42% in those fed milk, indicating a clinical advantage of feeding yogurt rather than milk in children with persistent diarrhea [35].

In a controlled randomized single blind clinical trial, the treatment of children with a specific chronic diarrhea with crystalline lactulose or with oral preparations of micro-organisms (Lactipan) promoted the complete remission of intestinal disorders. The authors suggested that the beneficial effect was due to the restoration of a balanced microflora [36].

Other non-controlled studies have reported a beneficial effect of fermented products on the time course of diarrhea. Feeding fermented milk to children (South America) with post-gastroenteritis syndrome eliminated the disease in 4.0 days and was even more beneficial in the patients with malnutrition [37]. Bhan *et al.*, in 1987, suggested that a beneficial effect of yogurt in comparison to milk was demonstrated in malnourished children with acute diarrhea [38]; however, a recent controlled study including 96 malnourished children indicated that routine substitution of yogurt in small frequent feedings to malnourished children with acute diarrhea as an addition to semisolid food did not achieve any significant clinical benefit *versus* milk [39].

In a study performed in Pakistan, the comparative use of a local fermented product containing yogurt and of a soya-based diet, in children under three months of age, showed that the local fermented diet allowed a better gain weight and a lower stool volume than the soya-based diet [40].

On the other hand, chronic diarrhea is often observed in children with a lactose intolerance secondary to a villus atrophy (for example in untreated coeliac disease) and leads to malnutrition. An Algerian study of nine children aged seven to 29 months presenting with chronic diarrhea has been done on the possible beneficial effect of fermented milk (lactose intake: 0.9 g/kg) compared to milk (lactose intake: 1.8 g/kg) in the lactose malabsorption and the diarrhea. The results have shown that yogurt significantly reduced the lactose intolerance as well as the volume and the occurrence of acidic feces [41].

Antibiotics-Associated Diarrhea or Radiotherapy-Induced Diarrhea. It is known that antibiotics used in various infectious pathologies may alter the intestinal microflora and the equilibrium in the bowel ecosystem.

Compared with children having received amoxicillin for only ten days, those who were given the antibiotics treatment, supplemented with lactobacilli, showed a lower incidence of diarrhea [42]. Similar beneficial effects of oral bacterial therapy with lactobacilli was reported in the prevention of diarrhea caused by amoxicillin administration in infancy [42]. However, Lactinex, a culture of *L. acidophilus* and *L. bulgaricus* was unable to prevent diarrhea in these patients [43]. By contrast, in adults, prophylactic administration of Lactinex in ampicillin-treated patients was effective in preventing the diarrhea [44]. In healthy volunteers, the erythromycin-associated diarrhea was decreased in those receiving *L. casei* GG but not in those taking pasteurized yogurt [45], indicating that the viability of lactic acid bacteria is mandatory to the beneficial effect. However, one study of antibiotic therapy in children suffering from

extraintestinal pathologies showed that a treatment with inactivated *Lactobacillus acidophilus* was efficient in preventing the intestinal dysmicrobism affections [46]. It has to be noted that complication of antibiotherapy can lead to *Clostridium difficile* colitis. A successful treatment of children with such a colitis was obtained with freeze-dried powder of *L. casei* GG containing 5×10^9 viable bacteria per gram [32].

In children with intractable diarrhea (having started after an antibiotherapy), the administration of Bifidobacterium or Bifidus yogurt dramatically improved the stool frequency within three to seven days [47].

Finally, in patients undergoing abdominal irradiation, the prevention of intestinal side effects (diarrhea) was obtained by the administration of live *Lactobacillus acidophilus* cultures [48].

Prevention of Diarrhea in Children

A few studies have been done on the potential preventive effect of fermented milks on the development of diarrhea. In 1989, Brunser *et al.* in Chile evaluated the effect on diarrheal disease of an acidified, modified powdered cow's milk infant formula (Pelargon) in 82 infants (Group I) for six months; 104 infants who received the same formula but non acidified served as controls (Group II). The incidence of diarrhea was lower in Group I ($p < 0.001$). The proportion of days during which the children suffered from acute diarrhea and the duration of the episodes were also lower in the children given the acidified milk ($p < 0.001$). These results indicated that acidified milk exerts a protective effect against diarrheal disease.

In 1994, Boudraa *et al.* in Algeria showed that feeding a dehydrated fermented milk containing *Bifidobacterium breve* and *S. thermophilus* (*Lactofidus*) to children at early weaning, significantly reduced the number of diarrheal episodes compared with feeding children an adapted milk formula during the three months of the study [49].

Importance of the Viability of Lactic Acid Bacteria on their Beneficial Effects

The viability of the lactic acid bacteria in the fermented milks used in the treatment of diarrhea is one of the factors possibly involved in their beneficial effect. In fresh yogurt or fermented milks, the bacterial counts normally reach 10^8 to 10^9 bacteria/mL. In some other fermented milks, such as *L. casei* GG enriched milk, the counts can reach 10^{10} to 10^{11} bacteria/mL. This is due to the fact that *L. casei* GG do not hydrolyze lactose and that β -galactosidase has to be added to the preparation to allow the milk fermentation. Therefore, *L. casei* GG is often used in the form of bacteria-enriched preparation, in which a defined number of bacteria are added. Other fermented products are commercially available as powder to be reconstituted in water. According to the method of dehydration (freeze-drying, evaporation) and the need to heat the product, the number of viable bacteria is greatly reduced to an extent which

is not always defined. It is obvious that all these factors can interfere with the final effect.

It seems that the viability of the lactic acid bacteria is important when considering certain diarrheal disease, whereas it is less important in other cases such as lactose intolerance.

In 1987, Dewit *et al.* [6] showed that the ingestion of yogurt or heated yogurt in lactose malabsorbers restored the secretion of insulin to control level and that the ingestion of fresh, unheated yogurt increased the absorption of free fatty acids. Indeed, it seems that the lactase activity (i.e., the intact β -galactosidase protein) can persist in the small intestine [7], explaining why heated yogurt is still efficient in lactose malabsorbers. However, in a lactase deficient population, fresh yogurt is more efficient in facilitating lactose digestion than heated yogurt [7]. In the same way, a study of lactose absorption in lactase deficient children showed a beneficial effect with a spray-drying fermented milk, and this effect was ascribed to the persistence of lactase activity in the fermented milk [8]. In another study of lactase deficient children, the efficiency of live yogurt was maximal and that of pasteurized yogurt was intermediate [9]. The lower effect of pasteurized product could be due both to the decrease of live bacteria and/or to the decrease of lactase content.

The use of live *versus* heated *L. casei* GG on the time course of rotavirus diarrhea has also been tested. It was shown that the fermented milk and the freeze-dried powder were both effective in shortening the course of acute diarrhea [15]. However, the administration of viable *L. casei* GG was more efficient in promoting rotavirus specific IgA in serum than heat inactivated bacteria [19].

In healthy volunteers, erythromycin-associated diarrhea was decreased in those receiving *L. casei* GG but not in those taking pasteurized yogurt [45], indicating that the viability of lactic acid bacteria is mandatory to the beneficial effect. Other studies tend to substantiate the efficiency of inactivated or freeze-dried products in case of antibiotherapy. In a study of antibiotic therapy in children suffering from intestinal or extraintestinal pathologies, a treatment with inactivated *Lactobacillus acidophilus* was efficient in preventing the intestinal dysmicrobism affections [46]. Also, a successful treatment of children with *Clostridium difficile* colitis was obtained with freeze-dried powder of *L. casei* GG containing 5×10^9 viable bacteria per gram [32].

MECHANISMS INVOLVED IN THE BENEFICIAL EFFECT OF LACTIC ACID BACTERIA

Effect on Lactose Malabsorption

The well-recognized beneficial effect of fermented products on lactose absorption in cases of lactase deficiency could be partly explained by the presence of bacterial lactase (β -galactosidase) in yogurt or fermented products, helping the lactose

cleavage and its subsequent absorption under the form of monosaccharides, as initially suggested [4,5]. Indeed, in yogurt, the lactose content is decreased by 30% compared with milk, probably owing to such hydrolysis. A better efficiency of live *versus* pasteurized yogurt was suggested [9], the lower effect of pasteurized product being due both to the decrease of live bacteria and/or to the decrease of lactase content. However, some observations suggest that bacterial lactase is probably not a unique explanation for the improvement of lactose absorption. Indeed, β -galactosidase activity is reduced by 80% at pH 5.0, which is the pH observed *in vivo* in the duodenum, owing to the buffering activity of yogurt. Also, a recent clinical study suggests that the bacterial lactase is perhaps not as important as suggested before [50]. The other explanation of improving lactose absorption could be the slowing down of the gastrointestinal transit of yogurt compared with milk [50], facilitating the contact between the residual lactase on enterocytes and the lactose in the lumen. Such a possibility was also suggested by others in children with lactose malabsorption [9] as well as in adults [51]. More likely, it is probable that both mechanisms are operating together.

Effect on the Resident Microflora and Pathogens

The development of an indigenous gut flora in neonates is a prerequisite to the protection of the intestinal function. During the neonatal period, the intestinal flora are enriched in bifidobacteria in breast-fed infants, while enterobacteria are predominant in case of bottle-fed infants [52] although most often a mixed population is found (R. Ducluzeau, personal communication). It is possible that the beneficial effect of fresh lactic acid bacteria is due to the transient proliferation of these bacteria in the digestive tract, *in vivo*. On the one hand, these bacteria need lactose to grow and therefore consume it and decrease its concentration in the intestinal lumen. On the other hand, the transient passage in the intestine of lactic acid bacteria may represent a microbial barrier against the development of pathogenic bacteria.

Some data have indicated in Peruvian children, that orally administered *L. casei* GG can survive to transit, although an efficient colonization was not demonstrated [53]. The fate of ingested lactic acid bacteria has also been studied extensively in normal volunteers. After ingestion of a fermented milk containing *L. acidophilus* and bifidobacterium sp, living bacteria were recovered in the ileum in quantities representing 1.5 and 37.5% of the ingested bacteria respectively [54]. Similar results were obtained in a study of six healthy adults indicating the survival of bifidobacterium species at the terminal ileum level [55].

In human neonates, the administration of a fermented whey-adapted infant formula containing viable bifidobacteria during the first two months of life allowed them to reach a prevalence of colonization with bifidobacteria similar to that of breast-fed infants [56].

The protective effect of the resident microflora is often weakened in case of antibiotherapy. There is an increasing amount of evidence that antibiotic treatment alters the protective flora and therefore predisposes to later infections. Although antibiotherapy has resolved many problems, it has also created a disequilibrium of the resident microflora and a decrease of colonization resistance to pathogens. The introduction of probiotic strains to reinforce human gut flora has therefore been tested. Lactobacilli are part of the normal anaerobic microflora, although they do not constitute the dominant microflora. By producing lactic and acetic acids, hydrogen peroxide and possibly antimicrobial substances, these microorganisms may contribute to the maintenance of colonization resistance. In view of these suspected beneficial effects, it has appeared important to maintain or increase the levels of lactobacilli in the intestinal microflora [57].

In addition, yogurt has been tested for its antibacterial properties [58]. This property has mostly been studied *in vitro*, by investigating the bactericidal and bacteriostatic effects of yogurt on three strains of *Escherichia coli* (two pathogenic and one nonpathogenic). It was concluded that both the live yogurt bacteria and an acidic pH around 4.5 are necessary for the bactericidal activity of the yogurt. However, the same bactericidal activity was observed for pathogenic or nonpathogenic strains of *E. coli*; this raises suspicion of the potential beneficial effect, *in vivo*. Another *in vitro* study using the Caco2 intestinal cell line [59] has suggested that the adherence of certain strains of lactobacillus to the intestinal epithelial cells, even when these are heat-killed lactobacilli, can significantly inhibit the binding of enteric pathogens. However, an *in vivo* study in rabbits infected with the enteroadherent pathogenic strain RDEC-1 and receiving either a milk- or yogurt-enriched diet showed that the duration of diarrhea and the fecal excretion of bacteria was the same in the milk- and yogurt-fed groups indicating that *in vivo*, yogurt did not interfere with the growth of the pathogenic *E. coli* [60].

Taken together, these studies indicate that although some bactericidal effects of yogurt have been found *in vitro*, it seems difficult to extrapolate to the *in vivo* situation, and some doubts can be raised on its efficiency in eliminating pathogenic bacteria from the intestinal tract.

Nonspecific Immune Defence

Nonspecific, anti-infective mechanisms of defence can be enhanced by ingestion of specific lactic acid bacteria strains. It has been postulated that these strains could be used as nutritional supplements to improve the weakened 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 fermented milks (with *Bifidobacterium bifidum* or *L. acidophilus* [strain LA1]) induced an increased phagocytosis of *E. coli*, *in*

vitro [61]. In animals, lactic acid bacteria also exhibit immunostimulating capacity: Perdigon *et al.*, in 1995 reported that different strains of Lactobacillus and *Streptococcus thermophilus* were capable of stimulating non-specific (macrophages) and specific (lymphocytes B and T) immunity in mice.

Effect on the Intestinal Barrier Function

The intestinal barrier function involves various factors capable of decreasing the absorption of potentially harmful microbial or soluble antigens. It includes various components contributing to the luminal degradation of antigens by digestive enzymes and a physical epithelial barrier comprising the mucus layer in which secretory IgA are trapped and the epithelial cells attached together through tight junctions. Therefore, lactic acid bacteria, by interfering with some of these components of the intestinal barrier can reinforce the host defenses. Different studies have suggested that *L. casei* GG was able to stabilize the intestinal permeability to macromolecules, particularly in the case of acute gastroenteritis in rats [62], but also to reverse the increase in intestinal permeability induced by cow's milk in suckling rats [63]. A recent study using a guinea-pig model of cow's milk allergy showed that feeding the guinea-pigs with a dried fermented milk led to a decrease in β -lactoglobulin absorption at the jejunal level, compared with feeding animals milk [64].

Specific Immunity. The effect of lactic acid bacteria on the secretory immune system has also been described. When antigens penetrate by the oral route, a secretory immune response is obtained which is mediated by secretory IgA or IgM. Various observations have reported that the administration of lactic acid bacteria improves the systemic and secretory immune response to particulate antigens and perhaps to soluble antigens.

In healthy volunteers, the oral administration of the *L. johnsonii* Strain LA1 was shown to increase the serum IgA concentration [65]; however, this result was not confirmed by others [66], who showed that the increase in serum IgA was small and that no modification of other Ig was detected.

In human volunteers ingesting an attenuated Salmonella typhi strain to mimic an enteropathogenic infection, the specific serum IgA titer was four-fold higher after three weeks in those who were supplemented with fermented milk (*Lactobacillus acidophilus* LA1 and *bifidobacteria*) than in those in the control group [65].

L. casei GG had an immunostimulating effect on oral rotavirus vaccination in infants. Infants who received *L. casei* GG showed an increased response with regard to rotavirus specific IgM secreting cells [67]. In addition, the same strain *L. casei* GG administered during acute rotavirus diarrhea in children has been shown to promote clinical recovery. This was associated with an augmentation of the local immune response to rotavirus since at convalescence 90% of the children supplemented with *L. casei* GG versus 46% in the control group developed an IgA specific antibody secreting cell response to rotavirus [17].

Viable *L. casei* GG were more efficient than heat inactivated *L. casei* GG in stimulating rotavirus-specific IgA secreting cells [19]. Although the hypothesis, that the beneficial effect of *L. casei* GG occurs through the increased secretory IgA response against rotavirus, seems to be attractive, one may wonder whether the IgA secretory response can be rapid enough (two to four days) to interfere with the infection, and other unknown mechanisms cannot be excluded.

Another effect of *L. casei* GG during rotavirus diarrhea could be the down-regulation of urease-producing bacteria overgrowth, thereby counteracting the disturbance of microbial balance observed during viral diarrhea [68].

The enhancement of the IgA secretory response against a soluble food antigen was also described in one study. The intestinal mucosal IgA response to β -lactoglobulin was compared in two groups of mice fed a whey protein diet with or without a culture condensate of *Bifidobacterium longum*. Both total IgA and anti- β -lactoglobulin IgA levels in tissue extracts of the small intestine were significantly higher in mice fed the *B. Longum*-containing diet than in control mice [69].

Other Possible Mechanisms

Trophic Effect. Recently, *in vitro* studies of the modulation of proliferation and morphotype expression of rat intestinal epithelial cell line IEC-6 by fermented milk were described. Different fermented milks (*Lactobacillus helveticus*, *Lactobacillus paracasei* or *bifidobacterium*) or yogurt, as well as non-fermented milk were shown to enhance trophic response of IEC cells in a dose-dependent manner. Fermented milk was more efficient than milk in stimulating mitochondrial dehydrogenase activity and DNA synthesis [70]. However, it is not known whether yogurt presents the same trophic properties *in vivo*.

Down Regulation of Cow's Milk Allergy? Several studies from E. Isolauri's group in Finland have suggested that probiotics (mainly *L. casei* GG) might be a novel approach in the management of food allergy. Indeed, in children allergic to cow's milk proteins and presenting with atopic eczema, feeding an extensively hydrolyzed whey formula supplemented with *L. casei* GG improved the clinical score of atopic dermatitis and decreased the intestinal excretion of α 1-antitrypsin and TNF α , compared with children fed the extensively hydrolyzed formula alone [71]. In addition, a possible mechanism was suggested since a mixture of bovine caseins hydrolyzed with *L. casei* GG-derived enzymes induced a suppression of lymphocyte proliferation [72] and a down regulation of anti-CD3 antibody-induced IL4 production [73], *in vitro*.

However, in the latter studies, it is not possible to analyze the respective role of casein-derived peptides and lactobacilli byproducts on these effects.

A short study of nine milk-hypersensitive adults, challenged with milk or *L. casei* GG-supplemented milk, suggested that the presence of the lactobacillus prevented the induction of phagocytosis through the complement receptor in monocytes [74].

Finally, in a guinea-pig model of cow's milk allergy, the administration of milk or fermented milks (with *Streptococcus thermophilus* and *bifidobacterium breve*) were equally able to induce β -lactoglobulin sensitization, suggesting that the ingestion of fermented milk instead of milk does not down-regulate the appearance of cow's milk allergy [64].

Anti-inflammatory Effect? Although results in that field are scanty, it has been reported that fermented milks may have some anti-inflammatory properties through their capacity to inhibit PAF. PAF is one of the most potent inflammatory phospholipid mediators secreted by proinflammatory cells. It is found in small amounts in dairy products. Recently, Antonopoulou *et al.* studied lipids with PAF and anti-PAF activity in cow's milk and yogurt and found that *Streptococcus thermophilus* and *Lactobacillus bulgaricus* biosynthesized important quantities of PAF inhibitors [75].

CONCLUSION

Yogurt and fermented milks have been considered healthy foods for centuries. The beneficial effects of such dairy foods have been extensively examined. The many variables involved in these beneficial effects make the global analysis and interpretation of the work difficult to analyze. Multiple variables are possibly involved in the final effect, including the population studied, the age of the patient, the microflora, the strains used, the doses and time-schedule of administration, the viability of the bacteria (heated, lyophilized or fresh products), and so on. In addition, studies are often non-controlled, making the interpretation difficult. Despite these drawbacks, and taking into account that no adverse effects have been described, but that many results underline the positive effects, it appears that a number of lactic acid bacteria are potentially useful in the treatment or prevention of diarrheal diseases.

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