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1

Probiotics: what are they? What are their effects on gut physiology?

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Probiotics can be defined as microbial cells that have a beneficial effect on the health and well-being of the host. Since the gastrointestinal mucosa is the surface of contact with probiotics, it seems evident that the first effects of probiotics relate to digestive function. A brief review of the literature indicates that probiotics have very few effects on the main physiological functions of the gastrointestinal tract, which are digestion, absorption and propulsion. The main action of probiotics can be summarised as a reinforcement of the intestinal mucosal barrier against deleterious agents. Experimental data indicate that some probiotics reduce pathological alterations in paracellular permeability to large molecules or bacteria, stimulate mucosal immunity, display a trophic action on the mucosa, reduce mucus degradation and interact with mediators of inflammation. Yoghurt may help lactose digestion, and some data needing confirmation indicate a stimulation of water absorption and an acceleration of intestinal transit by some bacteria.

Key words: colon; immunity; inflammation; intestine; mucosa; mucus; probiotic; permeability; transit; trophicity.

WHAT ARE PROBIOTICS?

Even if it is written in the Old Testament that 'Abraham owed his longevity to the consumption of sour milk'[1], the concept of probiotics probably evolved from a theory first proposed by Nobel Prize-winning Russian scientist Eli Metchnikoff, who suggested in 1908[2] that long the life of Bulgarian peasants resulted from their consumption of fermented milk products. The term 'probiotic' was first used by Lilly and Stillwell[3] in

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1965 to describe 'substances secreted by one microorganism which stimulate the growth of another'. A powerful evolution of this definition was coined by Parker in 1974[4], who proposed that probiotics are 'organisms and substances which contribute to intestinal microbial balance'. Fuller[5] then modified the definition in 1989 to 'a live microbial feed supplement which beneficially affects the host animal by improving its microbial balance'. This definition stresses the importance of viability and avoids the use of the too broad a term 'substances', which could even include antibiotics. Moreover, Fuller maintained and reinforced the concept of an action of probiotics on gut microflora. For him, probiotic treatments re-establish the natural condition that exists in the wild animal but has been disrupted by modern trends in the conditions used for rearing young animals, including human babies, and in modern approaches to nutrition and disease therapy.

In more modern definitions, the concept of an action on the gut microflora, and even that of live micro-organisms disappeared. Salminen et al[6], in 1998, defined probiotics as 'foods which contain live bacteria which are beneficial to health', whereas Marteau et al[7] in 2002 defined them as 'microbial cell preparations or components of microbial cells that have a beneficial effect on the health and well-being'. Some modern definitions include more precisely a preventive or therapeutic action of probiotics. Charteris et al[8], for example, defined probiotics as 'microorganisms, which, when ingested, may have a positive effect in the prevention and treatment of a specific pathologic condition'. Finally, since probiotics have been found to be effective in the treatment of some gastrointestinal diseases[9], they can be considered to be therapeutic agents.

Despite these numerous theoretical definitions, however, the practical question arises of whether or not a given micro-organism can be considered to be a probiotic. Some severe criteria have been proposed. Havenaar and Huis In't Veld[10], for example, proposed the following parameters to select a probiotic: total safety for the host, resistance to gastric acidity and pancreatic secretions, adhesion to epithelial cells, antimicrobial activity, inhibition of adhesion of pathogenic bacteria, resistance to antibiotics, tolerance to food additives and stability in the food matrix. The probiotics in use today have not been selected on the basis of all these criteria, but the most commonly used probiotics are strains of lactic acid bacteria such as *Lactobacillus*, *Bifidobacterium* and *Streptococcus*; the first two are known to resist gastric acid, bile salts and pancreatic enzymes, to adhere to colonic mucosa and readily to colonize the intestinal tract. Moreover, lactic acid bacteria have been demonstrated to inhibit the in vitro growth of many enteric pathogens, including *Salmonella typhimurium*, *Staphylococcus aureus*, *Escherichia coli*, *Clostridium perfringens* and *C. difficile*. *Saccharomyces boulardii*, a patented yeast preparation, also possesses properties that make it a probiotic agent. It inhibits the growth of several microbial pathogens, its temperature optimum is 37 °C, it survives transit through the gastrointestinal tract, and it is unaffected by antibiotic therapy. Table 1 gives examples of probiotics for which a significant therapeutic effect has been described.

PROBIOTICS AND GUT PHYSIOLOGY

Reinforcement of the gastrointestinal barrier

One task of the gut is to act as a barrier between the external and internal environments in order to prevent the entrance of potentially harmful compounds. A component of this barrier can be considered to be physical and consists of the epithelial

Table 1. Probiotics found in randomized controlled trials to have a significant therapeutic effect.

Disease	Probiotic
Antibiotic-associated diarrhoea	*Lactobacillus acidophilus* + *Lactobacillus bulgaricus* *Lactobacillus rhamnosus* GG *Enterococcus faecium* SF68 *Bifidobacterium longum* *Saccharomyces boulardii*
Acute gastroenteritis	*Lactobacillus rhamnosus* GC *Lactobacillus reuteri* *Lactobacillus casei* strain Shirota *Enterococcus faecium* SF68 *Saccharomyces boulardii*
Traveller's diarrhoea	*Lactobacillus acidophilus* *Lactobacillus acidophilus* + *Lactobacillus bulgaricus* *Lactobacillus fermentum* strain KLD *Lactobacillus rhamnosus* GG *Saccharomyces boulardii*

Reproduced from Marteau et al (2001).[9]

structure. The second component is functional and involves the abundant immune cells of the gut wall.

Physical barrier

Paracellular permeability

Among the numerous components that together form the intestinal barrier, paracellular tight junctional areas play a pivotal role in the control of intestinal permeability, which enables the passage of a solute by unmediated diffusion. Clinical studies have shown an altered intestinal permeability in a number of digestive disorders, including intestinal infections, Crohn's disease, celiac sprue, food intolerance and non-steroidal anti-inflammatory drug-induced enteropathy.[11] At colonic level, the commensal flora modulates the barrier function. The colonization of a rat's excluded colonic loop with *E. coli*, for example, increases paracellular permeability, whereas a reduction is observed after colonization with *Lactobacillus brevis*.[12] These changes in paracellular permeability may be associated with the adhesion of bacteria to the intestinal mucosa. In rats, dexamethasone treatment increased permeability and the counts of bacteria adhering to the mucosa.[13] Similarly, in vitro experiments have shown that the adherence of enteropathogenic *E. coli* to the intestinal epithelial cell monolayers disrupts the paracellular tight junction.[14] These relationships between intestinal permeability and bacteria suggest of a positive role of probiotics in preventing the alterations in paracellular permeability observed in several experimental models. A major side-effect of chemotherapeutic agents, such as methotrexate, is a severe gastroenteritis. In rats, intestinal paracellular permeability, assessed by Cr-EDTA clearance, has been found to be dramatically increased by methotrexate, this increased permeability being associated with a translocation of bacteria detected in the mesenteric lymph nodes, liver, spleen and blood. Both permeability and bacterial

translocation were strongly reduced when animals were orally treated with *L. reuteri* or *L. plantarum* for 3 days before and after methotrexate administration (Figure 1).[15]

The passage of large molecules through the mucosa does not, however, always correlate with alterations in mucosal permeability. In a neonatal rat model of necrotizing enterocolitis, severe lesions of the colonic wall were found in association with the passage of endotoxin into the plasma. A 3-day preventive treatment with *Bifidobacterium infantis* significantly reduced mortality, the number of colonic lesions and endotoxin passage but did not modify the lumen-to-blood permeability to dextran molecules.[16] In another study, intestinal permeability was evaluated by the passage of mannitol from the mucosal to the serosal side of rat small intestine placed in an Ussing chamber. The addition of *E. coli* to the mucosal part of the chamber strongly increased the passage of mannitol, the effect being abolished in rats previously treated with *L. plantarum* for 1 week.[17] Similarly, intestinal permeability assessed by the absorption of horseradish peroxidase (HRP) in an Ussing chamber has been found to be increased in cows milk suckling rats. The ingestion of *L. casei* associated with cows milk suppressed the increase in HRP absorption. However, intestinal total ionic conductance, an index of paracellular pathway, remained unchanged after the ingestion of cows milk, indicating the transcellular passage of HRP, confirmed by its accumulation in the cytoplasm of epithelial cells, as observed by electron microscopy.[18]

Probiotics could indeed protect against the passage of large molecules by mechanisms independent of paracellular permeability. Interleukin-10 (IL-10) gene-deficient mice develop a chronic colitis similar to human Crohn's disease. The colons of

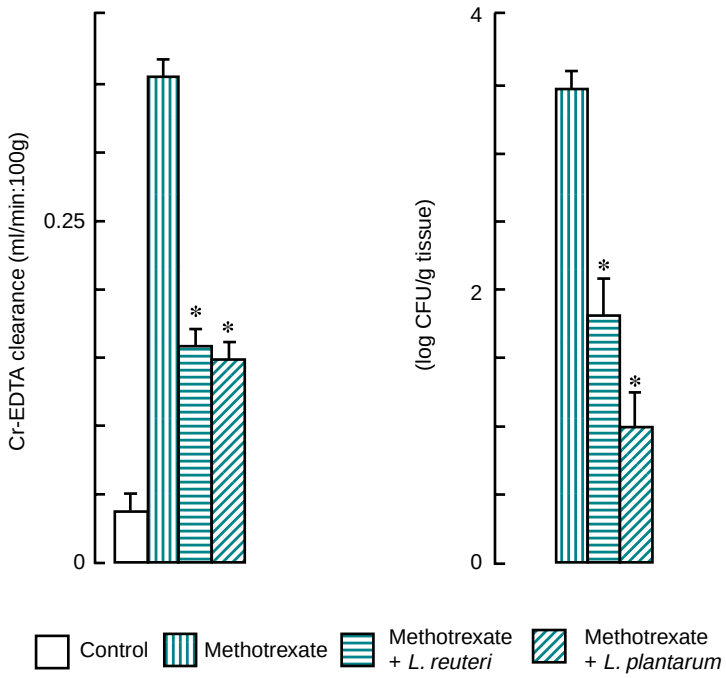

Figure 1. (Left) Reduction by *Lactobacillus reuteri* and *Lactobacillus plantarum* treatment of the increase in intestinal permeability, as assessed by Cr-EDTA clearance, induced by methotrexate in rats. (Right) Reduction by probiotic treatment of bacterial translocation in the mesenteric lymph nodes associated with an increase in intestinal permeability induced by methotrexate. Data from Mao et al (1996).[15]

IL-10 gene-deficient mice in an Ussing chamber are characterized by an increased permeability, as assessed by mannitol flux, and reduced electrical characteristics such as potential difference or short-circuit current, which indicate an alteration in electrolyte movements. These changes in mannitol flux and electrical values did not appear in IL-10 gene-deficient mice treated for 4 weeks with the probiotic compound VSL#3, which consists of a cocktail of *Bifidobacterium*, *Lactobacillus* and *Streptococcus* species. Moreover, VSL#3 directly applied to T84 monolayers in an Ussing chamber increased transepithelial resistance and decreased mannitol flux, suggesting an action on paracellular permeability. This effect is caused by a soluble proteinaceous factor secreted by the bacteria found in VSL#3 since it can be reproduced by the application of a VSL#3 culture medium and abolished when the culture media is treated by a proteinase.[19]

A protective action of probiotics has been also found at the level of the gastric mucosal barrier. In healthy volunteers, indomethacin increased gastric permeability, as assessed by urinary sucrose excretion, and small intestine permeability, assessed by urinary lactose/mannitol excretion. The gastric, but not intestinal, increase in permeability was prevented by treatment with *Lactobacillus* GG for 5 days before indomethacin administration. This effect was not observed after treatment with heat-killed bacteria.[20] A positive action of probiotic treatment has not, however, been found for severe alterations in gut barrier function. In humans, elective major abdominal surgery is often associated with bacterial translocation, gastric colonization with enteric organisms, and septic morbidity. All these surgical side-effects were not improved in patients receiving an oral preparation of *L. plantarum* 299v for 1 week before surgery.[21]

Mucosal trophic action

The positive effects of probiotics on gut function can in part be explained by a trophic action on the colonic mucosa. In the model of enterocolitis induced by methotrexate in the rat, the colon is characterized by a significant loss of the mucosal villous tips. This histological alteration has been found to be greatly improved in rats treated with *L. plantarum* or *L. reuteri*. This observation has been strongly reinforced by measurements of protein and DNA in the colonic mucosa. These parameters were reduced by about 80% in methotrexate-induced colitis but by only 40% when the animals received *L. plantarum* or *L. reuteri* for 3 days before and after methotrexate administration.[15] An elemental liquid diet is known to cause colonic mucosal atrophy in rats. The atrophy, assessed by the rate of crypt cell production (metaphase figures per hour) is strongly reduced when the liquid diet is associated with *L. casei* or *C. butyricum* ingestion (Figure 2).[22] These two studies clearly show a trophic action of some probiotics on colonic mucosa, but the mechanisms involved in this effect are speculative. Because short-chain fatty acids are known to stimulate the production of epithelial cells, Ichikawa et al[22] proposed that they mediate this effect, but this has still to be demonstrated.

Interactions with mucus

The luminal surface of the gastrointestinal tract is covered by a viscoelastic mucous gel that acts as an important protective barrier against the harsh luminal environment. Gut bacterial pathogens must traverse this mucus layer before they adhere to, colonize and subsequently invade the epithelial cells. Any change in mucus content and structure will compromise the mucosal defence barrier function of the mucous layer. An ability to degrade mucus is therefore considered to be one of the valuable indicators of

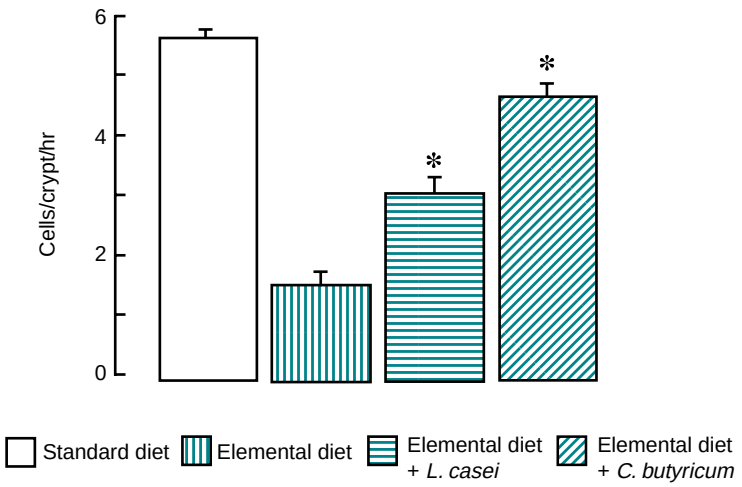

Figure 2. Influence of *Lactobacillus casei* and *Clostridium butyricum* treatment for 1 week on the reduced colonic crypt cell production rate associated with a liquid elemental diet in rats. Data from Ichikawa et al (1999).[22]

the pathogenicity and local toxicity of lumen bacteria.[23] Very strong interactions exist between mucus and colonic bacteria, and some studies indicate that an action on mucus is involved in the beneficial effects of probiotics. Probiotic bacteria have the ability to bind to intestinal mucus. For example, about 45% of *Lactobacillus* GG and 30% of *B. lactis* given orally to humans have been found adhering to stool mucus.[24] Moreover, it has been shown in pigs that *Lactobacillus* spp. inhabit the mucous layer of the small intestine and can grow and adhere to the ileal mucus.[25]

An interesting property of probiotics is that some at least are unable to degrade gastrointestinal mucus. Using different techniques to evaluate mucus degradation, this property has been shown for *L. casei* strain GG, *L. acidophilus* and *B. bifidum*[26], as well as for *L. rhamnosus* and *B. lactis*.[27] On the other hand, the normal human faecal flora is able to degrade mucin[28], and the production of mucin-degrading enzymes has been suggested as a determinant of virulence for a number of enteropathogens.[29] Another interesting property of probiotics is the inhibition of adhesion of enteropathogenic bacteria to mucus. *Enterococcus faecium*, for example, which is contained in many probiotic preparations, inhibits the adhesion of enterotoxigenic *E. coli* K88 to porcine small intestine mucus.[30]

Besides their mucus-degrading properties, bacteria are also involved in the control of the amount and nature of the mucus secreted. Goblet cells of germ-free rodents are fewer in number and smaller in size than those of conventionally raised mice.[31] In comparison with conventional rats, colonic sulphated mucins are decreased and sialomucins increased in germ-free rats.[32] It is, however, unknown whether these changes in mucus secretion are attributable to some specific bacteria. On the contrary, some particular bacteria are known to modify *MUC* gene expression. *Helicobacter pylori* for example, has been found to suppress *MUC1* and *MUC5A* gene expression in a human gastric cell line.[33] With regard to probiotics, it has been shown that *L. plantarum* 299v and *L. rhamnosus* GG increased the expression of both the *MUC2* and *MUC3* genes in HT29 cultures of colon cell. It was concluded that this property mediated the ability of these strains to inhibit the adherence of enteropathogenic *E. coli* to intestinal epithelial

cells since they do not inhibit adherence to non-intestinal HEp-2 cells, which express only minimal levels of *MUC2* and no *MUC3* mRNA.[34]

Functional barrier

Mucosal immunity

The action of probiotics on the immune response is relatively well documented (see Ref. [35] for a review). Many studies have, however, used in vitro cell cultures, so we have selected data obtained using intestinal tissues.

It is clearly established that intestinal micro-organisms are necessary for the development of the gut immune system, for example the intestinal epithelial lymphocytes and immunoglobulin (IgA) producing cells. There is, however, a question here: what kinds of bacterium are responsible for the development and activation of the mucosal immune system? A start has been made in answering this question, but some data indicate a positive role for probiotics. Secretory IgA actively produced by the intestine plays a central role in local immunity and has a significant function in creating a barrier against infection with pathogenic bacteria or viruses. In lactating mice treated with *B. lactis* Bb-12 for 12 days after delivery, significantly higher levels of faecal total IgA were found compared with controls. Moreover, anti-β-lactoglobulin IgA was found to be increased in the faeces, as well as in milk.[36] The significance of an increased intestinal IgA production by probiotics has been emphasized in several studies. Germ-free mice colonized with *Saccharomyces boulardii* display an increase in total and anti-*S. boulardii* IgA in comparison with non-colonized mice. In *S. boulardii*-monoassociated mice, the clearance of *E. coli* B41 was higher than in germ-free controls.[37] Mice treated by *B. lactis* HN019 and challenged with the enterohaemolytic pathogen *E. coli* O157:H7 display more intestinal tract IgA anti-*E. coli*, associated with a lower cumulative morbidity rate, than is seen in untreated mice.[38] Similar results have been obtained with another probiotic, *L. rhamnosus* HN001.[39] The resistance to parasitic infections provides an example of the consequence of the stimulation of intestinal immunity by probiotics. In mice treated by dead *L. casei* or supernatant from *L. casei* culture, the intestinal burden of adult worms is about 50% of that of control mice, 5 days after *Trichinella spiralis* infection.[40] In children (aged 15–31 months) immunized with polio vaccine and receiving a milk-based formula containing *B. lactis* Bb-12 for 21 days, faecal levels of total IgA and anti-poliovirus IgA were significantly higher than during the period preceding the intake (Figure 3).[41] A promotion of the IgA gut immune response has been also described in children with Crohn's disease treated with *Lactobacillus* GG.[42] Similarly, *Lactobacillus* GG stimulated the production of specific IgA against rotavirus in children with rotavirus diarrhoea.[43]

Some data indicate that probiotics are also able to modulate the production of IgE. Mice sensitized with ovalbumin, for example, showed a reduced production of IgE in serum when orally treated with heat-killed *L. casei* strain Shirota.[44] IgG production has also been found to be reduced in IL-10-deficient mice treated with *L. plantarum* 229v.[45] Such data cannot, however, be considered to be relevant enough to indicate any efficacy of probiotics in the treatment of allergy, which has been considered to be 'a very doubtful practice'.[46] With respect to intestinal epithelial lymphocytes, it has been shown that, in gnotobiotic rats colonized with *E. coli* O6:K13:H1, treatment with *L. plantarum* 299v significantly increased the density of CD25 + cells in the lamina propria.[47]

Probiotics have also been found able to modulate intestinal cytokine production. IL-10-deficient mice are characterized by a spontaneous colitis associated with high levels of colonic tumour necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) in basal

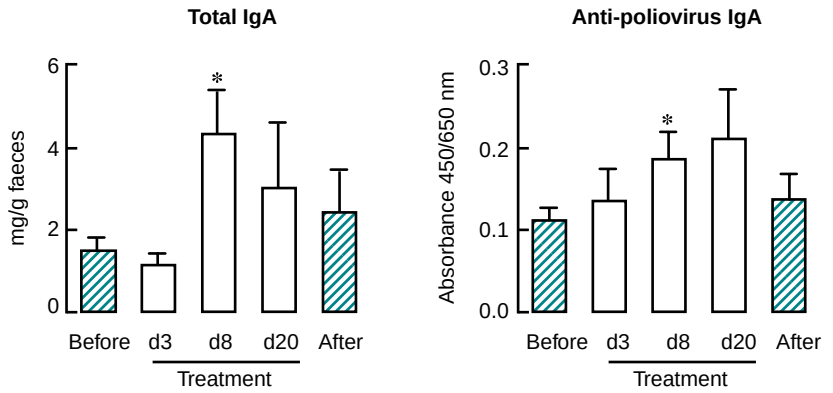

Figure 3. Faecal total and anti-poliovirus IgA in children before, during (days 3, 8 and 20) and after treatment with a cows milk-based formula containing *Bifidobacterium lactis*. Data from Fukushima et al (1998).[41]

conditions and after stimulation with lipopolysaccharide. A 4-week treatment with the probiotic cocktail VSL#3 restored normal basal and stimulated levels of these cytokines.[19] IL-10-deficient mice develop a colitis even under specific pathogen-free conditions or if kept germ-free. Treating specific pathogen-free IL-10-deficient mice with *L. plantarum* 299v significantly decreased the colonic mucosal production of IL-12 and IFN-γ, but this cytokine production remained unchanged in germ-free mice colonized with *L. plantarum*.[45] Conversely, yoghurt consumption in conventional rats induced an increase in IFN-γ production by the Peyer's patches, associated with an increase in the number of B-lymphocytes.[48] Such results underline the role of experimental models when establishing the properties of probiotics.

On the other hand, the effect of a given probiotic on the expression of a cytokine cannot be extrapolated to another probiotic. An in vitro study showed that three different bacteria (non-pathogenic *E. coli*, *L. sakei* and *L. johnsonii*) have very different effects on the secretion of some cytokines (IL-1β, IL-8, IL-10 and TNF-α) by CaCO-2 cells[49], effects that have been confirmed in vivo. In mice, oral treatment with *L. acidophilus* or *L. casei* stimulates IL-6 production by peritoneal cells, whereas *Bifidobacterium* attenuates it.[50] Similar data have been obtained using other experimental models. The peritoneal macrophages of conventional mice produced more IL-1 and IL-6 than those of germ-free mice. The colonization of germ-free mice by *E. coli* induced an IL-1 and IL-6 production similar to that seen with conventional mice, whereas colonization by *B. bifidum* did not increase IL-1 and IL-6 production in germ-free mice.[51]

A consequence of these actions of probiotics on immune functions, probably associated with other unknown mechanisms, is that some probiotics reduce mucosal neutrophil infiltration during experimental colonic inflammation. The increase in activity of myeloperoxidase, an enzyme found specifically in neutrophils, associated with an acetic acid-induced colitis in the rat has been shown to be reduced by a single intracolonic administration of *L. reuteri* R2LC.[52] This effect has been confirmed on the same experimental model of colitis by treating the animals orally for 7 days with *L. reuteri* R2LC.[53] The same study also indicated that another probiotic, *L. rhamnosus* GG, was not effective in the same experimental protocol. Myeloperoxidase activity in a methotrexate-induced enterocolitis is decreased by treatment with *L. reuteri* R2LC or *L. plantarum* DSM 9843.[15] The mouse strain SAMP1/Yit develops spontaneous

inflammation of the ileum and caecum except when reared under germ-free conditions;[54] the ileal myeloperoxidase activity associated with this spontaneous inflammation was strongly reduced by a 3-week treatment with milk fermented with *B. breve*, *B. bifidum* and *L. acidophilus*.[55]

Mediators of inflammation

Besides their action on immune function, probiotics can also modulate the production of inflammatory mediators in the intestinal epithelium, although this finding has been supported by only a few reports. In the model of necrotizing enterocolitis in the neonatal rat, a strong increase in phospholipase A2 has been observed in the intestinal wall. This increase was suppressed when newborn rat pups were given *B. infantis*.[16] On the other hand, antioxidative properties have been shown for some bacteria. *Bifidobacterium longum* ATCC 15708 and *L. acidophilus* ATCC 4356 are able to scavenge free radicals. This has been demonstrated by the scavenging of the α,α-diphenyl-β-picrylhydrazyl (DPPH) radical and leads to an inhibition of lipid peroxidation and a reduction of the cytotoxicity of a compound (4-nitroquinolin-N-oxide, 4NQO) inducing DNA oxidative damage on intestinal epithelial cells.[56] Nitric oxide is a ubiquitous mediator involved in inflammatory processes. *Lactobacillus rhamnosus* has been found to induce nitric oxide production in macrophages and a human colon epithelial cell line.[57] This nitric oxide production was mediated through the induction of inducible nitric oxide synthetase which is considered to be a pro-inflammatory event. This is in agreement with the absence of anti-inflammatory properties of *L. rhamnosus* mentioned above. However, unlike the endogenous nitric oxide produced by nitric oxide synthetase, exogenous nitric oxide in the digestive lumen exerts an anti-inflammatory effect.[58] Interestingly, some bacteria, for example *L. farciminis*, are able to reduce nitrite to nitric oxide, at least in vitro conditions.[59] If their nitrite-reducing properties persist in vivo, such bacteria are promising in terms of reducing colonic inflammation.

Gut function

The three main functions of the gut are to digest food, to absorb nutrients, water and electrolytes, and to propel the digestive material at a rate that allows optimal digestion and absorption. Except for the absorption of nutrients, for which no data have been found, the literature indicates that probiotics may improve or modify these functions.

Food digestion

The effects of probiotics on food digestion are mainly documented in farm animals, in which an improvement in digestion may explain the growth-promoting effect of probiotics. In humans, the most highly investigated aspect of probiotics in digestion is their compensation for lactase insufficiency. Numerous studies have shown that better lactose digestion occurs in lactose malabsorbers who consumed yoghurt rather than milk. This has been demonstrated by the lower hydrogen exhalation after ingesting the same amount of lactose in yoghurt in comparison with milk (see Ref. [60] for a review). Two hypotheses suggest that this effect does not correspond to a replacement of endogenous lactase by bacterial β-galactosidase. The gastric emptying of yoghurt has been found to be slower than that of milk[61], probably because of parameters such as viscosity or pH independent of the presence of bacteria. This delayed passage of lactose would give the residual endogenous lactase activity in the small intestine more time to hydrolyse the lactose. The second hypothesis is based on the fact that colonic

microflora contribute to lactose degradation in lactose maldigesters.[62] Since lactic acid bacteria can stimulate colonic bacterial activity[63], it has been suggested that the beneficial effects of yoghurt in lactose malabsorbers result from an improved digestion of lactose in the colon. Even if these two hypotheses cannot be excluded, bacterial β-galactosidase probably cleaves lactose into galactose and glucose in the small intestine. Rats fed yoghurt have an increased concentration of β-galactosidase of bacterial origin in the small intestine.[64] This absence of induction of endogenous β-galactosidase by yoghurt has also been confirmed in humans.[65]

Micro-organisms display an array of enzymes that may be useful for improving human digestion, but this has been poorly investigated. One example is that of patients with congenital sucrase-isomaltase deficiency, in whom the ingestion of a by product of the manufacture of baker's yeast (*Saccharomyces cervisiae*) containing sucrase activity reduces breath hydrogen excretion after a sucrose load.[66] Gastrointestinal protein digestion in the presence of fermented milk modifies the release of some amino acids and leads to the formation of new peptides.[67] These new peptides may have some biological activity, but this approach has also been poorly investigated. For example, casein hydrolysate produced by an extracellular proteinase from *L. helveticus* CP790 contains an antihypertensive peptide displaying an inhibitory action on angiotensin I-converting enzyme.[68] Finally, a promising usage of probiotics to improve digestion is the use of genetically modified bacteria expressing enzymes. A pioneering study in this field employed *Lactococcus lactis* expressing the lipase of *Staphylococcus hyicus* to enhance lipid digestion in pigs with an experimentally induced pancreatic insufficiency.[69]

Water absorption

Since an efficacy of some probiotics has been found in the treatment of diarrhoeal disease[9], we can suggest that they stimulate water and electrolyte absorption. As far as we know, however, there are no experimental data clearly indicating a pro-absorptive action of probiotics. Only electrical parameters such as short circuit current, which represent electrogenic chloride secretion and sodium absorption, have been used in few studies. As mentioned above, colonic sections of IL-10 gene-deficient mice placed in an Ussing chamber displayed low values of short circuit current in comparison with controls. These values were significantly ameliorated after a 1-month treatment with the probiotic cocktail VSL#3.[19] Another study mentions that increased intestinal permeability induced by cows milk suckling in rats was not associated with modifications of the short circuit current, which also remained unchanged after treatment with *L. casei*.[18]

Gastrointestinal transit

It is known that gastrointestinal transit is slower in germ-free than conventional animals.[70] Some studies performed in man indicate that a probiotic strain, *B. animalis* DN-173010, is able to modify gut transit. In elderly volunteers selected for a total gut transit greater than 40 hours, the regular consumption of milk fermented by this strain strongly accelerated transit.[71] In another study, the same strain accelerated colonic transit time after a 3-week treatment in healthy volunteers. Moreover, an acceleration specific to the sigmoid colon was only seen in women.[72] This effect in women has been confirmed in another study, in which it was also shown that the probiotic treatment did not affect faecal weight, pH, bacterial mass or bile acids.[7] However, these data concern only one bacterial strain and no mechanism of action has been proposed, which does

not allow any conclusion on the action of probiotics on intestinal transit or on any direct effect on the motor component.

SUMMARY

With the first definition proposed in 1965, probiotics can now be considered to be microbial cells that have a beneficial effect on the health and well-being of the host. The most commonly used probiotics are lactic acid bacteria such as *Lactobacillus*, *Bifidobacterium* and *Streptococcus*. The experimental data mentioned in the text indicate that a number of probiotics are able to modulate some characteristics of digestive physiology, such as mucosal immunity, mucosal trophicity and intestinal permeability. Other data tend to indicate a protective action on mucus and an interaction with mediators of inflammation, which seems a promising area for investigation. Even if all these are interesting mechanisms of action of probiotics, the data need to be confirmed using other experimental conditions before they can be viewed as having a predictive, preventive or therapeutic value in human beings. Many bacteria have strong potential enzyme activity, but the only route investigated has been the use of yoghurt, which produces β-galactosidase to improve lactose maldigestion. An action on water absorption has been indirectly shown, but this too must be confirmed. An effect on gastrointestinal transit cannot be concluded from the data available. Moreover, it is important to stress that each probiotic micro-organism displays its own properties so data obtained from one strain cannot be extrapolated to another. Moreover, the effects of probiotics on gastrointestinal function depend on the host's state of health or disease, and no extrapolation can be made from one disease to another or from a basal state to a specific disease.

Practice points

- the action of some probiotics on mucosal immunity may explain the anti-inflammatory properties identified in some clinical trials
- despite the promising effects of some probiotics in counteracting experimental alterations in intestinal permeability, no clinical trials have investigated any improvement in these alterations
- experimental data do not support the efficacy of some probiotics in diarrhoeal diseases

Research agenda

- probiotics must be considered as individual and well-characterized micro-organisms
- preclinical studies must be performed before clinical trials
- the mechanisms involved in the antidiarrhoeal effects of some probiotics need to be elucidated

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