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Effects of probiotics - new prospects

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Président de la Mission Scientifique

For several years now, knowledge on the subject of probiotics and their implications for human health has been steadily converging. The most classic effects have been corroborated by a wealth of similar *in vivo* data to which more recent works, also the result of clinical research, have added new possibilities for their action. At the same time, the use of cell or animal models, in particular with controlled flora have made it possible to gradually decipher some of the action mechanisms and equally to confirm the reality of the effects they underpin.

The functional complementation provided by probiotics to individuals lacking enterocyte lactase is, therefore, now solidly established. The appearance of substitute enzyme activity, linked to probiotic ingestion has even been shown *in situ* by ileal intubation, providing the beginnings of an explanation. For a long time it was thought that to act, the enzyme needed to be released into the lumen by an unknown process that more or less completely destroyed the bacteria. In reality, it has become clear that microbial permease activity is essential for lactose hydrolysis, which suggests that its metabolism by bacteria transiting through the intestinal lumen is, on the contrary, a proof of their functional integrity. This underlines the extreme importance of the physiological status of probiotics, here lactose operon induction, on the result observed.

The treatment of digestive disorders in babies and young children gives another example of such convergence. Numerous publications have reported the beneficial effect of probiotics in treating acute infant diarrhoea. Despite major differences in study design and in the nature and doses of the probiotics administered, almost all result in a remission of the digestive disorder earlier in the infants receiving probiotics than in those who received none. An analysis of all the results highlighted a link between the intensity of the benefit obtained and the dose of probiotics administered. It therefore seems that the effect is at its maximum on diarrhoea of viral origin and low to non-existent on bacterial gastroenteritis, a result in concordance with the fact that administering probiotics reduces rotavirus in the faeces of infected infants in spectacular fashion. These observations shed further light on the results obtained using polarized cell cultures (HT29), used as models of human enterocytes. After incubation in the presence of certain bacterial species, the glycosylation of surface molecules is modified. As a result the rotavirus adhesion sites are hidden and consequently the cells resist its action. Therefore, the direct interaction between bacteria and intestinal cells could modify some of the physiological properties of the host decisively.

The relationship between bacterial flora and intestinal cells is therefore not just a simple coexistence but undoubtedly represents a dynamic balance that is constantly adjusted thanks to an information exchange. In fact, some bacteria are able to act on the genetic expression of enterocytes, affecting notably the genes involved in the absorption of macro and micronutrients or the constitution of desmosomes. Numerous arguments show that some of the effects of probiotics most recently identified, in particular the modulation of the intestinal inflammatory response described in this newsletter, depend on this type of direct interaction. On the other hand, the properties of enterocytes, for example their surface glycosylation, could affect bacteria on contact, in that sugars represent both a source of substrates and an adhesion site.

Listing probiotics according simply to their taxonomic criteria appears now to be of little relevance. The real challenge is now to understand the different action mechanisms that they implement during the real conditions of their transit. Identifying the factors involved requires characterising the physiological status of probiotics at various points in the digestive tube and depending on the food intake, i.e. to finalize methods that - at the very least - analyse the expression of their genome *in situ*. Animals with controlled flora will then provide a powerful tool for studying the reciprocal influence of the physiological properties of bacteria and those of their host, but are unlikely to receive final validation in human beings.

Probiotics prolonging remission in cases of pouchitis

The efficacy of some strains of probiotics has been shown in the domain of preventing relapses in inflammatory intestinal diseases, i.e. pouchitis, ulcerative colitis and Crohn's disease.

The efficacy of a combination of 8 strains of VSL#3 lactic bacteria was observed in cases of pouchitis during a controlled, double-blind clinical study (2). During the study, no patients who received a mixture of probiotics suffered a relapse during 9 months as opposed to 15 % in the control group. More recently, the same team (3) renewed the study over a longer time period.

The study was carried out of 36 patients suffering from refractory or recurrent pouchitis that was in remission after a

4-week course of antibiotics. They were randomised into 2 groups, one received 6 g of VSL#3 in one daily dose for one year (or until relapse if it occurred) and the other a placebo. To monitor the patients, a symptomatic, endoscopic and histological evaluation was performed before the treatment, and after 2 months and then 12 months.

Remission was observed in 85 % of the patients treated as opposed to 1 % of the control group ($p < 0.0001$). This result indicates that treatment with a probiotic mix is effective in maintaining the remission obtained by a high dosage course of antibiotics in patients suffering from refractory or recurrent pouchitis. Alongside the positive clinical, endoscopic and histological criteria, the patients

also declared they felt a real improvement in their quality of life.

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3• Mimura T, Rizzello F, Helwig U, Poggioli G, Schreiber S, Talbot IC, Nicholls RJ, Gionchetti P, Campieri M, Kamm MA (2004). Once daily high dose probiotic therapy (VSL#3) for maintaining remission in recurrent or refractory pouchitis. *Gut*. 53(1):108-14.

L. plantarum 299 modulating the intestinal inflammatory response

Intestinal inflammatory diseases are characterised by excessive inflammation of the mucosa. Recent studies see the cause as a break in tolerance vis-à-vis the normal intestinal flora in the genesis of this disease (4). This supports the theory by which hyperactivity against the antigens of the normal flora is involved in the inflammatory process with the T lymphocytes failing to regulate the inflammatory response (5).

An English team examined *in vitro* the pattern of cytokine secretion involved in the inflammatory process (6). Mononuclear cellules isolated from the mucous membranes of patients who were either healthy or suffering from ulcerative colitis were used in the study. Cytokine induction was evaluated in the presence of *Lactobacillus plantarum* 299 or pathogenic bacteria (*E. coli*, enteropathogenic *E. coli* and *Salmonella dublin*).

The production of IL-1 β interleukin by lymphocytes coming from the normal mucous membrane is significantly higher

after stimulation with *E. coli* and enteropathogenic *E. coli* compared to non stimulated lymphocytes ($p < 0.05$ et $p < 0.01$ respectively). In the ulcerative colitis mucosa, production was stimulated significantly when the lymphocytes were incubated with one of the 3 strains of pathogenic bacteria.

As for the production of TNF α and IFN γ , an increase in the production of these cytokines was particularly observed in the presence of *E. coli* in the normal mucosa ($p < 0.05$) and ulcerative mucosa ($p < 0.05$).

No significant increase in production of these three cytokines (IL-1 β , TNF α and IFN γ) was seen when the lymphocytes, from either a normal or pathogenic mucosa, were incubated with *L. plantarum* 299. However, the incubation of this probiotic with lymphocytes from the pathogenic mucosa stimulated the production of IL-10 interleukin ($p < 0.05$). Intracellular labelling confirmed that the increase in IL-10 levels comes from an

increase in production by T lymphocytes and macrophages.

Therefore, *L. plantarum* 299 would appear to stimulate the production of anti-inflammatory interleukin. In other words, *Lactobacillus plantarum* provides beneficial immuno-modulating activity for mucosa affected by ulcerative colitis. This result, from an *in vitro* study attempts to explain the mechanism action of some probiotics in improving inflammatory conditions of the intestinal mucosa observed during certain diseases.

4• Duchmann R, Kaiser I, Hermann E, Mayet W, Ewe K, Meyer zum Buschenfelde KH (1995). Tolerance exists towards resident intestinal flora but is broken in active inflammatory bowel disease (IBD) *Clin Exp Immunol*. 102(3):448-55.

5• Groux H, Powrie F (1999). Regulatory T cells and inflammatory bowel disease. *Immunol Today*. 20(10):442-5.

6• Pathmakanthan S, Li CK, Cowie J, Hawkey CJ (2004). *Lactobacillus plantarum* 299: beneficial *in vitro* immunomodulation in cells extracted from inflamed human colon. *J Gastroenterol Hepatol*. 19(2):166-73.

Fermented milk in the prevention of antibiotic induced diarrhoea

Intake of antibiotics exposes the patient to an increased risk of upsetting the intestinal flora that may lead, in the least serious cases to abdominal pain and diarrhoea. In the last few years, controlled clinical studies have shown that some probiotics significantly reduce the effects resulting from consumption of antibiotics. In the United Kingdom, a team has studied the efficacy of probiotics to treat diarrhoea induced by antibiotics.

A group of 202 patients (average age 70), hospitalised for different disorders and given a course of antibiotics was split into two groups (9). One was given 454 g per day of fermented milk containing *Lactobacillus acidophilus*, *Streptococcus*

thermophilus and *Lactobacillus bulgaricus*; the second group received a placebo under the same conditions. Treatment lasted 8 days.

In the patients who consumed the fermented milk, a reduction in the frequency of the diarrhoea was noticed (12 % vs. 24 %, $p=0.004$) and a significantly shorter duration (23 days in total vs. 60). The accumulated number of patients not suffering from diarrhoea was significantly different between the two groups ($p=0.002$).

This study would appear to confirm the efficacy of *L. acidophilus* fermented milk and yoghurt ferments in preventing diarrhoea caused by taking antibiotics.

The result, although seemingly convincing, should be interpreted with caution since the study was not carried out double blind.

7• Wistrom J, Norrby SR, Myhre EB, Eriksson S, Granstrom G, Lagergren L, Englund G, Nord CE, Svenungsson B (2001). Frequency of antibiotic associated diarrhea in 2462 antibiotic-treated hospitalized patients: a prospective study. *J Antimicrob Chemother* 47(1):43-50.

8• Marteau Ph, Seksik P, Jian R (2002). Probiotics and intestinal health effects: a clinical perspective. *Brit J Nutr* 88(suppl 1):S51-S57.

9• Beniwal RS, Arena VC, Thomas L, Narla S, Imperiale TF, Chaudhry RA, Ahmad UA (2003). A randomized trial of yogurt for prevention of antibiotic-associated diarrhea. *Dig Dis Sci*. 48(10):2077-82.

Prevention of enteric infections by probiotics in undernourished mice

Under-nourishment is frequently associated with a deregulation of the immune response and an increase in susceptibility to infection (10). Gabriela Perdigon's team questioned the possibility of using probiotics as an adjuvant in a re-nutrition protocol for under-nourished mice and the possibility that these probiotics may give protection against enteropathogenic bacteria (11).

The undernourished mice were subjected to a milk-based diet before either yoghurt or *Lactobacillus casei* fermented milk was introduced. After 5 days of consuming fermented milk products, the mice were infected with *Salmonella typhimurium* or entero-invasive *E. coli*. Seven days after infection, the mice were sacrificed and the spleen, liver and small intestine removed. The organs underwent various analyses - an evaluation of pathogen transit and an analysis of the immunoglobulins secreted into the intestinal fluids.

Compared to normally nourished mice, the liver of the under-nourished mice were significantly infected with *S. typhimurium*. In the mice who had received the fermented milk there was no

S. typhimurium or *E. coli* colonisation observed in the liver or spleen.

When a comparison was made with normally nourished control animals, the under-nourishment resulted in a reduction in the number of IgA and IgG secreting cells in the small intestine ($p<0.01$). The re-nutrition caused an increase in these parameters ($p<0.01$). The introduction of fermented milk had no influence on the parameters.

The other consequence of under-nourishment was the number of CD3+, CD4+ et CD8+ lymphocytes in the small intestine ($p<0.01$). A return to normal feeding stimulated an increase in the T lymphocyte population and the consumption of fermented milk caused a greater increase in CD3+ and CD4+.

In comparison with normally nourished mice, under-nourishment also caused a reduction ($p<0.01$) in the number of ab and gd receptors in the T cells (TCR). The re-nutrition protocol caused an increase in each of these receptor types and the fermented milk caused an increase in the period ($p<0.01$).

The IgA rates specific to pathogenic bacteria were specifically ($p<0.05$) lower in the intestinal fluids in under-nourished mice than in those nourished normally. Re-nutrition helped increase these levels but in significant fashion only for *E. coli*. On the other hand, each of the fermented milk products caused an increase in the *S. typhimurium* specific and *E. coli* specific IgA ($p<0.05$).

These results show that the consumption of probiotics avoids the translocation of pathogenic bacteria toward the liver and the spleen. They suggest a significant influence of probiotics on CD3+, CD4+ et CD8+ lymphocyte expression as well as lymphocyte antigen receptors. The positive modulation of these immune parameters in the intestine tends to increased resistance to enteric infections.

10• Chandra RK (1997). Nutrition and the immune system : an introduction. *Am J Clin Nutr* 66:460S-463S.

11• Cano PG, Perdigon G (2003). Probiotics induce resistance to enteropathogens in a re-nourished mouse model. *J Dairy Res*. 70(4):433-40.

Probiotics and colorectal cancerogenesis

Cancer of the colon is the most common cancer in France, affecting about 4 % of the population. The intestinal flora and the immune system both play a role in colon cancerogenesis. Several studies have shown that some probiotics may reduce the activity of enzymes, mutagens, or secondary biliary acids in the faeces and may be involved in the colon cancerogenesis process (12).

Researchers have tested the preventative effect of a combination of probiotics, *Lactobacillus acidophilus*, *Lactobacillus helveticus* and *Bifidobacterium* sp., on cancerogenesis induced in rats by exposure to azoxymethane (13). This chemical agent is well known as a colon-specific carcinogen.

The 105 rats included in the test were split into 3 groups: Group A (control) received neither probiotics nor azoxymethane; Group B was given azoxymethane and group C received a probiotic preparation in its feeding before and during exposure to azoxymethane. The appearance of aberrant intestinal crypt foci was analysed at 20 weeks; the appearance of aberrant crypt foci was considered significant of preneoplastic lesions and the number of foci was correlated to the degree of malignity.

In group B, there was an increase in the number of cells blocked in metaphase both in the aberrant crypt foci and

crypts of normal appearance (vs. control $p < 0.01$). The number of lymphocytes in the mesenteric glands was significantly reduced compared to groups A and C ($p < 0.01$). In group C, the number of aberrant crypt foci was smaller than that observed in group B ($p < 0.05$). In total, 90 % of the group B rats and 50 % of the group C rats developed cancer of the colon.

The results indicate that the probiotics used were able to exert a significant antimutagenic effect. However, these results are at least partially contradicted by the results of a second study (14). In this one, the carcinogen used was 1,2-dimethylhydrazine (DMH) and the probiotic was *Lactococcus lactis*.

Sixty-five rats were divided into different groups. The probiotic was introduced into the feeding at the end of the period of exposure to DMH. Twenty-two weeks after the first injection of DMH, results showed that there was no difference between the rats receiving the probiotic and those without it as regards the incidence, number and size of the colic tumours. At 52 weeks, the survival rate of the rats that had received the lactic bacteria with the DMH was lower than for those that only received the DMH.

This second study shows that *Lactococcus lactis* has no inhibiting

effect on the spread of tumours induced with DMH in rats.

The results of these studies, contradictory in appearance, emphasize that the ability of probiotics to exert an antimutagenic effect is probably dependent on different parameters - the type of probiotic used, the carcinogen administered and the period over which the probiotic was given (before or after exposure to the carcinogen). It thus appears dangerous to conclude that probiotics have positive action as anticancer agents in general without taking care to compare each of these parameters.

12• Marteau PH, Daniel F, Morales E, Seksik Ph, Jian R (2003). Protection contre les maladies intestinales par des probiotiques. Cah Nutr Diet 38(6):363-368.

13• Marotta F, Naito Y, Minelli E, Tajiri H, Bertuccelli J, Wu CC, Min CH, Hotten P, Fesce E (2003). Chemopreventive effect of a probiotic preparation on the development of preneoplastic and neoplastic colonic lesions: an experimental study. Hepatogastroenterology. 50(54):1914-8.

14• Li W, Li CB (2003). Lack of inhibitory effects of lactic acid bacteria on 1,2-dimethylhydrazine-induced colon tumors in rats. World J Gastroenterol 9(11):2469-2473.

This scientific letter "Yoghurts & fermented milks" is also available on the following website:
www.maison-du-lait.com

Tolerance and safety of probiotics - a long term study

Consumption over one year of infant formula supplemented with *Bifidobacterium lactis* Bb12 and *Streptococcus thermophilus* is perfectly tolerated and provides a guarantee of total safety at both doses used (1×10^6 and 1×10^7 cfu of *B. Lactis* Bb12/g of formula). This

clinical study, conducted double blind with placebo, involved a group of 118 infants with an average age of seven months when they were enrolled in the study. One of the benefits identified by adding probiotics was a less frequent use of antibiotics in these infants,

revealing a better resistance to certain infections.

15• Saavedra JM, Abi-Hanna A, Moore N, Yolken RH (2004). Long-term consumption of infant formulas containing live probiotic bacteria: tolerance and safety. *Am J Clin Nutr.* 79(2):261-7.

Lactococcus lactis a potential candidate for the status of probiotic

By definition, one of the major properties of probiotics is their ability to survive in the digestive tract. *Lactococcus lactis* is a lactic bacterium used in cheese making. This bacterium does not have probiotic status. However, the sur-

vival of some strains of *Lactococcus* has been shown *in vitro* and *in vivo* in mice. This observation is a preliminary to examining the effects on health of this bacterium.

16• Kimoto H, Nomura M, Kobayashi M, Mizumachi K, Okamoto T (2003). Survival of lactococci during passage through mouse digestive tract. *Can J Microbiol.* 2003 Nov;49(11):707-11.

Effects on health of probiotics - a magazine dedicated to this theme

"Best Practice & Research Clinical Gastroenterology" has chosen to dedicate volume 17 to a number of articles providing a synthesis of the latest breakthroughs regarding the effects on human health of probiotics. Twelve articles have been published including the following four:

➤ Probiotics essentially exert their effects by helping to strengthen the barrier function of the gastro-intestinal mucous membrane (action on trophism, interaction with the mucus, etc.). Their effects on the functions of digestion, absorption and propulsion of the gastro-intestinal tract are today less well documented.

17• Jean Fioramonti, Vassilia Theodorou and Lionel Bueno (2003). Probiotics: what are they? What are their effects on gut physiology? *Best Pract Res Clin Gastroenterol* 17(5): 711-724.

➤ Several clinical studies show the prophylactic or therapeutic efficacy of certain probiotics on gastro-intestinal infections (gastroenteritis of viral origin,

diarrhoea, *Helicobacter pylori* infections, etc.). This article discusses the mechanisms, both immune and non-immune, stimulated by probiotics and playing a role in the host's defence against these infections.

18• Harsharnjit S. Gill (2003). Probiotics to enhance anti-infective defences in the gastrointestinal tract. *Best Pract Res Clin Gastroenterol* 17(5):755-773.

➤ A host of arguments support certain probiotics in their positive role fighting intestinal inflammatory diseases (pouchitis, Crohn's disease and ulcerative rectocolitis) in particular in preventing relapses. The data from rigorously conducted clinical studies are still insufficient to draw conclusions on the role of probiotics on this type of pathology but encouraging enough to promote further studies in this area.

19• Cyrus P. Tamboli, Christel Caucheteux, Antoine Cortot, Jean-Frédéric Colombel, Pierre Desreumaux (2003). Probiotics in inflammatory bowel disease: a critical review. *Best Pract Res Clin Gastroenterol* 17(5):805-820.

➤ A large amount of evidence has been provided by the studies conducted on animals targeting the efficacy of probiotics in fighting cancer. In humans, we do not yet have direct experimental evidence but strong presumptions exist. This synthesis article lists the types of studies available on the anti-cancer effects of probiotics (*in vitro*, in animals, epidemiological studies and intervention studies) and analyses the potential underlying mechanisms.

20• Joseph Rafter (2003). Probiotics and colon cancer. *Best Pract Res Clin Gastroenterol* 17(5):849-859.

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- 23• Brovko LY, Vandenende C, Chu B, Ng KY, Brooks A, Griffiths MW (2003).**
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- 26• Mukai T, Kaneko S, Matsumoto M, Ohori H (2004).**
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Lactobacillus bacteremia, clinical significance, and patient outcome, with special focus on probiotic *L. rhamnosus* GG. *Clin Infect Dis*. 38(1):62-9.

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