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SCIENTIFIC SURVEY . LACTIC ACID BACTERIA . PROBIOTICS

edito A new role for commensal flora - regulating carbohydrate/lipid metabolism

Nadine Cerf-Bensussan
INSERM

Recent work by J. Gordon's team has shed new light on the interactions between the host and its intestinal flora, and the remarkable way these have adapted during their co-evolution over several millions of years.

Simple commensals able to take advantage of metabolites available in the host, our bacteria have now acquired the status of fully-fledged partners. With our bodies, they are a remarkable new example of the symbioses developed between various organisms during evolution, similar, for example, to that linking trees and their mycorrhiza.

Besides their function as a barrier against pathogens, established many years ago, the intestinal bacteria have a major impact on the post-natal maturation of the intestine and immune system. Thanks to their thousands of genes, different to those available in the host's genome, they are able to offer the host a whole new range of metabolic activities. In particular, these make it possible to digest a wide range of vegetable carbohydrates that would otherwise not be digestible.

This latter function would appear to contribute to the major impact the flora has on the host's carbohydrate/lipid metabolism. J. Gordon's team has shown this recently on a murine model. Exposing a germ-free mouse to conventional flora causes an increase of 50 -80 % in adipose mass and a reduction in food intake of 20 %. These changes also include an increase in glycaemia and insulinaemia and a doubling of the hepatic synthesis of the triglycerides attributed to the activation of the ChREB transcription factor and the induction of two of this factor's enzyme targets - acetyl-CoA carboxylase and fatty acid synthetase.

The impact of the flora on glycaemia can be explained by a better use of food carbohydrates, but also by its role in the induction of the Na⁺/glucose cotransporter and intestinal angiogenesis. Besides its effects on the liver, the flora promotes greater storage of triglycerides in the adipocytes and therefore a hypertrophy of these cells, linked to increased activity of the lipoprotein lipase. This enzyme is subjected to negative retrocontrol by the *fiaf* protein secreted by the adipose tissue, the liver and the intestine. The authors show that the flora inhibits the production of *fiaf* in the intestine and suggest that this molecule is the relay for the effects of the flora on the modifications of the adipose tissue, since these effects are not observed in animals whose *fiaf* has been inactivated.

Several comments can be made as a result. It is first of all tempting to suggest that throughout evolution, the flora has played a complementary role to that of our thrifty genotype, optimising the body's use of a low diet dominated by products of vegetable origin. In the current context of the world obesity epidemic, this function can obviously be seen as negative. Is it possible to hope, through improved knowledge of the flora and its metabolic capacities, that this function could be manipulated to adapt it to current needs? In relation to this, a second comment must be made. Although the impact of the flora on numerous functions of the host has now been established, the bacterial molecules and the host receptors involved in these interactions remain largely unknown. Better knowledge of these factors would obviously enable them to be manipulated to the host's benefit. Gordon suggests, but does not show, the possible role of PPAR γ in *fiaf* modulation. This hypothesis, if confirmed, would support the already established links between the flora and PPAR γ , and inflammation and lipid metabolism. It would also make it possible to design or improve intervention strategies, thanks to the numerous agonists (or antagonists) already identified for this molecule.

Backhed F, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, Semenkovich CF, Gordon JI (2004). The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci U S A. 101(44):15718-23.

Probiotics prevent *Clostridium difficile* diarrhoea in the elderly

Antibiotics can cause different side effects including damage to the endogenous intestinal flora. This imbalance promotes infection by *Clostridium difficile*, a pathogenic agent responsible for serious intestinal disorders. The impact of the consumption of probiotics on the balance of the intestinal flora, and even, in some cases, its restoration, is an avenue that has been widely researched today. One of the expected benefits is the prevention of "opportunistic" infections like those caused by *C. difficile*.

A clinical study, conducted double blind and placebo controlled, analyses the effect of the joint administration of probiotics and antibiotics on 138 elderly hospitalised patients (1). During a 20-day treatment with antibiotics (the type of antibiotic used is not named), the patients received a daily capsule of either 2×10^{10} cfu of *Lactobacillus acidophilus* and *Bifidobacterium bifidum*, or a placebo. The parameters observed were

the frequency of the diarrhoea attacks and the presence of *C. difficile* and its toxins in the faeces.

The number of patients affected by *C. difficile* was comparable in the control and test groups. However, of all the patients that suffered from diarrhoea, the proportion that had the *C. difficile* toxin was significantly lower in the group receiving probiotics (2.9 % vs. 7.25 %) than in the group receiving the placebo. In the same way, of the 138 subjects, the number in which the toxin was found was smaller in the group receiving the probiotics (46 % vs. 78 %) compared to the control group.

These results suggest that the consumption of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* probiotics at the same time as treatment with antibiotics prevents the apparition of *C. difficile* related diarrhoea in the elderly. The patients consuming probio-

tics appeared to be asymptomatic carriers of *C. difficile*, the pathogen was present but the toxins were not detected and/or no diarrhoea attacks occur. The authors therefore concluded that probiotics may act by neutralising toxins and not by preventing intestinal colonisation by the pathogen.

The authors emphasise what is at stake economically with *C. difficile* infections - probiotic supplementation could reduce the cost of looking after these patients by 50 %.

1• Plummer S, Weaver MA, Harris JC, Dee P, Hunter J. (2004). *Clostridium difficile* pilot study: effects of probiotic supplementation on the incidence of *C. difficile* diarrhoea. *Int Microbiol.* 7(1):59-62.

A synbiotic optimises weight gain in sick children

In seriously ill children, the risks of malnutrition are increased by treatment with antibiotics. Maintaining suitable nutrition is therefore of great importance if the patients' growth is not to be affected. The administration of nutritional supplements during a course of antibiotics may be helpful in maximising the intake of energy and reducing the risk of weight loss and dehydration. Since the gastro-intestinal benefits of probiotics are promising, a team of researchers has analysed the influence of consuming probiotics along with nutritional supplements. During a phase III clinical study, the researchers tested the impact of the consumption of synbiotics* on children receiving antibiotics (2).

The protocol included 140 children, aged between 1 and 6, receiving antibiotics for ENT infections. During the course of antibiotics (7.5 to 9 days), the children received 360 or 480 ml per day of one of the following three drinks : fruit juice, nutritional supplement (lipids, carbohydrates and proteins), the same supplement associated with a symbiotic. The synbiotic provides a daily dose of 1.2 to 1.68 g of FOS and 3.6×10^8 cfu to 4.8×10^8 cfu of *Lactobacillus acidophilus* and *Bifidobacteria*. The energy consumption, weight gain, appetite, activity level, gastro-intestinal tolerance - diarrhoea, vomiting, etc. and the lac-

tobacillus and bifidobacillus faecal loads were all studied.

The total energy consumption was greater in the group receiving the nutritional supplement and the synbiotic, compared to the other two groups ($p < 0.05$). The weight gain at the end of the treatment period along with the increased lactobacillus faecal charge in the group receiving both the nutritional supplement and the synbiotics were significantly greater to those observed in the group receiving the fruit juice ($p = 0.011$ and $p = 0.045$ respectively). The other parameters were not modified significantly.

The results show the innocuous nature of the preparations administered that caused no gastro-intestinal intolerance. They confirm that the administration of synbiotics during a course of antibiotics enables faster lactobacillus recolonisation in as much as the lactobacillus faecal load is increased. It should be noted that a similar effect exists for bifidobacteria, although it is not statistically significant. The small magnitude of this effect can, however, be explained by the small size of the sample (parameter analysed on a sub-group of 49 patients).

The synbiotic administered also causes an increase in energy consumption and weight gain in children taking antibio-

tics. According to the authors, these increases could be due to an improvement in the gastro-intestinal state or other parameters influencing food intake such as sleep, mood and circadian rhythm.

If these results were to be confirmed by other clinical studies, arguments could be made in favour of the use of nutritional supplements associated with pre-probiotics in sick children treated with antibiotics, in order to improve energy consumption and prevent weight loss.

* A synbiotic is a combination of probiotic(s) and prebiotic(s). Prebiotics, e.g. fructo-oligosaccharides (FOS), are substrates that stimulate the growth of certain bacteria in the intestine.

2• Schrezenmeir J, Heller K, McCue M, Llamas C, Lam W, Burow H, Kindling-Rohracker M, Fischer W, Sengespeik HC, Comer GM, Alarcon P. (2004). Benefits of oral supplementation with and without synbiotics in young children with acute bacterial infections. *Clin Pediatr (Phila).* 43(3):239-49.

Probiotics fighting allergy

Clinical and epidemiologic studies have suggested that probiotics play a role in the primary prevention of atopic manifestations (3-6). More precisely, some strains of probiotic may be capable of causing the production of IL-12 and IFN γ . These cytokines are capable of modulating the Th1/Th2 response by polarising it to a type Th1 (anti-inflammatory) response. Furthermore, the increase in the circulation of hematopoietic progenitors in the peripheral blood (7-9) along with their infiltration into the mucous membranes and the skin (10) in patients suffering from allergic asthma, rhinitis and atopic dermatitis indicates that the hematopoietic processes play a role in the manifestation of the allergy.

Italian researchers have tested the potential effect of a mixture of probiotics sold on the market on the circulation of immature CD34+* cells in patients suffering from allergies (11). Fourteen patients (aged between 6 and 48) suffering from allergic manifestations (asthma and/or conjunctivitis, rhinitis, urticaria, atopic dermatitis and food allergies) were enrolled for this pilot study. Allergen-specific serum IgE were found in twelve patients. The probiotic mixture used is sold in dried form

in sachets (ENDOLAC®). Each sachet contains a mixture of *Lactobacillus acidophilus* (1×10^7 cfu), *Lactobacillus delbrueckii* (1×10^6 cfu) and *Streptococcus thermophilus* (1×10^9 cfu). The patients received one sachet per day for a month. The clinical symptoms and the levels of CD34+ cells were determined for each patient before and after the treatment.

Consumption over one month of the probiotic mixture caused a significant reduction in the CD34+ cells ($p=0.001$) along with a reduction in the severity of the allergy's symptoms. This reduction was observed in all 14 patients, in particular those in whom allergen-specific serum IgE were found. The treatment was well tolerated by all the patients.

This study shows that, on the one hand, probiotics are capable of preventing clinical manifestations of the allergy and, on the other hand, there is a correlation between the preventive effect and the reduction in immature CD34+ cells. The innocuous nature of the treatment opens up the perspective of using probiotics as an adjuvant to classic allergy treatments. However, the efficacy of such a treatment must first be confirmed by clinical studies conducted dou-

ble-blind and placebo-controlled and on a wider scale. It would also be important to show that a reduction in the number of hematopoietic precursors only occurs in allergic subjects by restoring a normal number of these cells. Reducing the number of CD34+ cells that are precursors to all the hematopoietic cells could be harmful.

* The CD34 surface antigen is expressed for a sub-population of stem cells and for progenitors already engaged in one or more type(s) of cell differentiation. The CD34+ fraction of primitive hematopoietic cells contains the vast majority of hematopoietic progenitors.

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This scientific letter "Yoghurts & fermented milks" is also available on the following website:

www.maison-du-lait.com

Stimulation of intestinal immunity by probiotics found in cheese

Gabriela Perdigon's research team specialises in the immunomodulating ability of probiotics in mice models. This team recently tested the effects of fresh cheese high in probiotics on the intestinal immunity of the mouse (12).

The mice were put into three groups. One group received fresh cheese A containing the following strains - *Streptococcus thermophilus*, *Lactococcus lactis*, *Bifidobacterium bifidum*, *Lactobacillus acidophilus* and *Lactobacillus paracasei*. For the other two groups (control), one was subjected to a classic diet and the other was given fresh cheese B only containing *Streptococcus thermophilus* and *Lactococcus lactis*. The mice were fed in this way for 2, 5 or 7 days. The phagocytic activity of the peritoneal macrophages, the number of IgA secreting cells in the colon and small intestine and the ratio of CD4+/CD8+ lymphocytes in the small intestine were observed. The presence and number of each of the cheese bacteria were determined in the Peyer's patches, immune cells in the villi and the colon nodules and crypt foci.

In the small intestine, the phagocyte activity of the macrophages was increased significantly in the mice given cheese A for 5 or 7 days, compared to the mice in the control group. A return to normal was observed 8 days after consumption was stopped. Equally, the number of cells secreting IgA and the ratio of CD4+/CD8+ lymphocytes were significantly increased in the small intestine of the mice fed on cheese A for 5 or 7 days whereas no difference was seen in the mice given cheese B at either 2, 5 or 7 days. However, in the colon, no difference was noted between the 3 groups of mice.

The cytological images showed that in the mice given cheese A, the Peyer's patches and crypt foci were more massively occupied than those of the mice receiving cheese B. *B. bifidum* and *L. paracasei* were mainly located in the Peyer's patches whereas *L. acidophilus* was found essentially in the colon nodules.

These results show that the probiotics *B. bifidum*, *L. acidophilus* and *L. paracasei* have a strong affinity with the parts of the intestine where the lymph tissue is

concentrated (Peyer's patches, crypts and nodules). Besides, these probiotics are able to stimulate the key parameters of the intestinal immune system - the peritoneal macrophages in their role as key elements in specific and non-specific immunity, IgA as the predominant immunoglobulins of the mucosa and T CD4+/CD8+ lymphocytes, responsible for B lymphocytes maturing into IgA producing cells.

This study shows that the probiotics *B. bifidum*, *L. acidophilus* and *L. paracasei* can influence the intestinal immune response of mice. This modulation of the immune system, positive from a health standpoint, can also be expected in humans.

12• Medici M, Vinderola CG, Perdigon G (2004). Gut mucosal immunomodulation by probiotic fresh cheese. Int Dairy J. 14:611-618.

Highlighting the anti-inflammatory activity of probiotics

The results of different studies converge to suggest that probiotics could exert anti-inflammatory activity. This ability is particularly important, for example, in the context of the prevention of allergies and inflammatory bowel diseases. The goal of a team of researchers from the INSERM was to characterise the action mechanisms behind the anti-inflammatory activity of probiotics, to determine the bacterial metabolites that may be involved and their ability to cross the intestinal barrier (13).

Five bacteria were studied : *Bifidobacterium breve*, *Streptococcus thermophilus* (for probiotics), *Bifidobacterium bifidum*, *Ruminococcus gnavus* and a strain of streptococcus (for commensal bacteria). Two types of immune cells were tested : peripheral blood mononuclear cells and a monocyte precursor line (THP-1). Lipopolysaccharide (LPS) was used as an inflammation inducing agent. The production of TNF α and IL-10, the binding of LPS to the CD14 receptors of the immune cells as well as the translocation of the NF κ B factor were analysed.

The immune cells were subjected to the inflammatory agent in the presence or the absence of the culture medium of each of the bacteria being tested with or without protein hydrolysis of this medium. To evaluate the effect of probiotics the other side of the intestinal barrier, a monolayer of HT29 human intestinal epithelial cells

in either a normal or inflammatory state, was used to model the intestinal mucous. The supernatant fluids of the bacteria were placed in the apical* compartment. After incubation, the medium of the basal* compartment was analysed in order to search for metabolites able to modulate the inflammatory response.

The supernatant fluids of all the strains of bacteria tested inhibit the production of TNF α , nevertheless the effect of the probiotics was significantly greater to that of the commensal bacteria (p<0.04). The inhibition exerted by the probiotics was dose-dependant and not affected by the hydrolysis of the culture medium proteins. Beyond the cell layer that models the intestinal barrier and in a normal situation, only *B. breve* has an inhibiting effect (p<0.0012). In a situation of inflammation, the inhibiting effect of the two probiotics was significantly greater than in a normal situation and significant for both strains (p<0.001). These tests did not highlight any significant modifications in IL-10 production.

Both probiotics also inhibit the nuclear translocation of the NF κ B factor caused by the LPS (p<0.05) as well as LPS binding to the immune cell receptors (p<0.0001).

The experiment shows that the *B. breve* and *S. thermophilus* metabolites inhibit the *in vitro* production of TNF α caused by the LPS. This impact of probiotics is greater

than the one exerted by commensal bacteria. The inhibiting effect of the probiotics is exerted via metabolites able to cross the epithelial layer simulating the intestinal barrier. In this way, these two probiotics may play a role in reducing the inflammatory reaction by exerting a systemic action.

The results also enable the authors to propose two possible action paths to explain the inhibition they observed : regulation of the related inflammatory signal through the NF κ B and limiting LPS access to the monocytes.

Compared to the action of commensal bacteria, the authors state that regular intake of probiotics could be advantageous for the upper parts of the gastrointestinal tract that are not massively colonised by the endogenous flora.

* In this model, the apical compartment represents the intestinal lumen and the basal compartment the internal medium.

13• Menard S, Candalh C, Bambou JC, Terpend K, Cerf-Bennus N, Heyman M. (2004). Lactic acid bacteria secrete metabolites retaining anti-inflammatory properties after intestinal transport. Gut. 53(6):821-8.

Probiotics preventing nosocomial infections

In humans, bacterial translocation is a major cause of the nosocomial infections observed after surgery on the abdomen. In rats, liver resection and/or colonic anastomosis lead to an increase in the bacterial concentration in the caecum and mesenteric lymph glands in the rat's liver. In the rat, it is shown that consumption of a mixture of 4 probiotics (*Pediococcus pentoseceus*,

Lactococcus raffinolactis, *Lactobacillus paracasei*, *Lactobacillus plantarum*) along with fibres (beta-glucan, inulin, pectin, starch), before and after the surgery, significantly reduces the bacterial load in the lymph glands. This result, if validated in humans, would make it possible to see probiotics as a potential tool for preventing infection during surgery.

14• Seehofer D, Rayes N, Schiller R, Stockmann M, Muller AR, Schirmeier A, Schaeper F, Tullius SG, Bengmark S, Neuhaus P (2004). Probiotics partly reverse increased bacterial translocation after simultaneous liver resection and colonic anastomosis in rats. *J Surg Res.* 117(2):262-71.

L. rhamnosus GG interacts with the prostaglandin pathway

Lactobacillus rhamnosus GG is one of the probiotics most studied today. Clinical studies show the efficacy of this probiotic in treating certain intestinal disorders and in preventing atopic diseases. The underlying mechanisms are still not well known. An *in vitro* study of a model of human colon cells shows

that *L. rhamnosus* GG causes the expression of type 2 cyclo-oxygenase, an enzyme involved in the prostaglandin pathway. The effect is dose-dependant. This result reveals one of the cell mechanisms involved in the interaction between *L. rhamnosus* GG and the intestinal cells.

15• Korhonen R, Kosonen O, Korpela R, Moilanen E (2004). The expression of COX2 protein induced by *Lactobacillus rhamnosus* GG, endotoxin and lipoteichoic acid in T84 epithelial cells. *Lett Appl Microbiol.* 39(1):19-24.

Fermented milk fighting osteoporosis - a new avenue of research

The consumption of milk fermented with *Lactobacillus helveticus* has a positive effect on the calcium and bone metabolism of post-menopausal women. This result was obtained by a Finnish team during a randomised clinical study that was controlled a placebo (unfermented milk) and performed double blind on

20 postmenopausal women. The researchers showed that the consumption of fermented milk via this probiotic caused an increase in calcium levels in the blood and its binding to the bones. This new result poses the question of the potential use of probiotics to prevent osteoporosis.

16• Narva M, Nevala R, Poussa T, Korpela R (2004). The effect of *Lactobacillus helveticus* fermented milk on acute changes in calcium metabolism in postmenopausal women. *Eur J Nutr.* 43(2):61-8.

Probiotics as a potential tool for the prevention of cancer of the colon

The results obtained in animals and *in vitro* tend to show that consuming probiotics reduces the risk of cancer of the colon. However, similar data in humans is as yet insufficient to substantiate this. A German team has shown that consuming milk fermented with *Lactobacillus acidophilus* and *Bifidobacterium bifidum* reduces the geno-

toxic potential of the subjects' faeces. The genotoxicity was evaluated from the damage done to the DNA of colon cells in cultures where they were in contact with human faecal water. This effect observed *ex vivo* may reflect a protective effect exerted by probiotics against the risk of cancer *in vivo* in humans.

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