Interactions between human genes and non pathogenic bacteria: a new period of discoveries

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We are just aware of how nonpathogenic bacteria, amongst them probiotics, are able to interact with human intestinal physiology and how this action may improve the functions of this organ which plays a pivotal role in human health.

The fact that the expression of human genes may be modulated by prokaryotes is now a well accepted idea. Additionally, it appears that this modulation seems to be a key step in intestinal development, and in protection of the intestinal mucosa.

Bacteria inhabit earth since 2.5 milliards years. Genesis of eukaryotes's evolution occurred in a world elaborated by micro-organisms, inducing multiple interactions. For example, the Human Genome Project showed that several human genes related to the synthesis of 223 proteins are shared with prokaryotes (Salzberg et al 2001).

The presence of numerous species of bacteria and an estimated amount of 10^{12} bacteria/gram lead us to consider the intestine as the organ where interactions between bacteria and human cells are the more, this frequent leading to important impacts on human physiology.

Now, it is well established that epithelial cells development is stimulated by enteric bacteria. A team of researchers from USA recently demonstrated that colonization of germ free mice by Bacteroides thetaiotaomicron (a prominent component of the normal mouse and human intestinal microflora) modulates expression of genes involved in several important intestinal functions, including nutrient absorption, mucosal barrier fortification, xenobiotic metabolism, detoxication, motricity, and postnatal intestinal maturation (Hooper et al 2001).

Probiotics may also influence other protective functions of the intestinal mucosa including synthesis and secretion of antibacterial peptides, mucins...At the present time, Syndifrais supports a project in this field.

Pathogenic bacteria may also stimulate intestinal responses of the epithelium via cytokines responses. Effects of non pathogenic bacteria on regulating factors of the immune system are less studied than the effect of enteropathogenic bacteria. Nevertheless, two years ago, it was demonstrated that nonvirulent strains of Salmonella attenuate synthesis of inflammatory effector molecules elicited by diverse proinflammatory stimuli. This immunosuppressive effect involves inhibition of the IkappaB/NF-kappaB pathway (Neish et al 2000).

These results indicate that nonvirulent bacteria are able to influence deeply the intestinal biology by acting on numerous cellular effectors. Many of these responses may be beneficial for human health. We are just at the beginning of a new period of discoveries and new developments.
Managing diarrhoea by probiotics

These last years, numerous studies related to the effects of dairy starters on the incidence of diarrhoea have been published (1, 2, 3). The current bibliographic data allowed two teams to carry two meta-analysis which results are presented below.

Diarrhoea is a common adverse effect of antibiotic treatments. As antibiotic diarrhoea mostly results from disequilibrium of the normal intestinal microflora, research has focused on the benefit of administrating living organisms (probiotics) to restore the normal flora.

The first meta-analysis, carried by a team from the Imperial College School of Medicine at London, assessed the efficacy of probiotics in prevention and treatment of diarrhoea associated with the use of antibiotics (4).

This meta-analysis included 9 papers that looked at prevention of antibiotic associated diarrhoea and was performed as randomized, double blind, placebo controlled trials.

Four trials used Saccharomyces boulardii, four used lactobacilli (Lactobacillus GG ; L. acidophilus and L. bulgaricus (2 studies) ; L. acidophilus et B. longum) and one used a strain of Enterococcus faecium that produced lactic acid.

In all nine trials, the probiotics were given in combination with antibiotics and the control groups received placebo and the antibiotic.

The odds ratio in favour of active treatment over placebo in preventing diarrhoea associated with antibiotics was 0.39 (p<0.001) for the yeast and 0.34 (p<0.01) for lactobacilli. The combined odds ratio was 0.37 (p<0.001) in favour of active treatment over placebo.

This meta-analysis suggests that S. boulardii and lactobacilli have the potential to prevent antibiotic associated diarrhoea and that probiotics may be used in this situation. The efficacy of probiotics in treating antibiotic associated diarrhoea is another question that remains open.

The second meta-analysis concerned existing randomized, controlled studies to test the hypothesis that treatment with Lactobacillus improves clinical outcomes in children with infectious diarrhoea (5).

The original search yielded 26 studies but only 9 met the following criteria : adequately randomized studies, blinded, controlled trials in which the treatment group received Lactobacillus and the control group received an adequate placebo and reported clinical outcome measures of diarrhoea intensity.

In these 9 clinical trials, age of subjects was between 3 and 37 months, the pathogen was not always identified nevertheless rotavirus was detected in seven studies.

The strains of probiotics were L. acidophilus (1 study), L. reuteri (2 studies), Lactobacillus GG (4 studies) and a mixture of L. acidophilus and L. bulgaricus (2 studies). Summary point estimates indicate a reduction in diarrhoea duration of 0.7 days and a reduction in diarrhoea frequency of stools on day 2 of treatment in the participants who received placebo.

In regard of this analysis, it seems that Lactobacillus intake is effective as a treatment for children with acute infectious diarrhoea. This conclusion is sustained by the results of a recent study which is not included in the meta-analysis.

This prospective placebo-controlled double blind study was conducted in India and enrolled 112 newborn infants (6). The subjects were randomized to receive a daily oral dose of Lactobacillus sporogenes or placebo for one year.

Diarrhoea due to rotavirus infection was experienced by 84% of the subjects. The group fed probiotic had fewer episodes of diarrhoea than the placebo group (3.4 ± 1 vs. 8.6 ± 1.7, p<0.02) and less number of days of illness (13 ± 3 days vs. 35 ± 5, p<0.01).

These observations suggest that prophylactic feeding of Lactobacillus has a preventive effect on the incidence and duration of acute rotavirus diarrhoea.

Results of these meta-analyses suggest that several probiotics, and particularly lactobacilli, are efficient in preventing adult diarrhoea induced by antibiotic intake, and in preventing and treating children infectious diarrhoea. Actually the challenge is less to demonstrate the efficiency of probiotics against diarrhoea than to identify the more efficient strains, to determine the vehicle of administration and to assess the relevant amounts of probiotics that may induce a beneficial effect for the host.

Probiotics as antimutagenic agents:

Heterocyclic aromatic amines (HAA) are initiating agents of colon carcinogenesis in animals and are suspected in the aetiology of human colon cancer. These compounds are produced during the cooking of several foods such as muscle meats and fish. In the context of prevention, the protective effects of diet factors, as fermented milks, are of interest. A research supported by funds from Danone aimed to evaluate the level of action of fermented milks using early and intermediate end point markers of the carcinogenesis process (7).

For 15 experimental weeks, male rats groups received one of the four diets : control, supplemented with milk, supplemented with Bifidobacterium...
animalis DN-173 010 or Streptococcus thermophilus DN-001 158. After one week of acclimatization, HAA were administered for a 7 week induction period. Faecal mutagenicity was quantified during the induction period. Tumourigenesis biomarkers were assessed : DNA lesions and enzymatic cytochrom P450 activity were measured in the colon and in the liver at week 8 of the trial and aberrant crypts were scored at week 15.

The results showed that dairy products decreased the incidence of aberrant crypts in rats by 66%, 96% and 93% respectively to diets supplemented with milk, *B. animalis* fermented milk and *S. thermophilus* fermented milk. Intermediate biomarkers showed that there was a decrease in HAA metabolism, faecal mutagenicity and colon DNA lesions. The biomarkers used in this study showed that dairy products may act on the early steps of the carcinogenesis process. Therefore, dairy products may help to prevent colon tumourigenesis induced by food carcinogens. This effect being more pronounced in the case of milk fermented with lactic acid bacteria.

### Beneficial influence of probiotics on intestinal permeability

The intestinal mucosa plays a fundamental role in sustaining a physical barrier against translocation of exogenous substances (food, antigens, bacteria...) from the luminal content of the bowel. The adequate functioning of this barrier is warranted by the control of intestinal permeability which alteration may lead to pathogenesis in several conditions. For example, several studies have demonstrated that pathogenic bacteria such as *Escherichia coli* may disrupt the intestinal barrier and enhance permeability in the gastrointestinal tract. Based on the consideration that probiotics have potentially beneficial role in the gastrointestinal tract, a team from Lund University in Sweden examined if the administration of *Lactobacillus plantarum* 299v may inhibit *E. coli*-induced increase in permeability in rat intestine (8). For this purpose researchers analyzed the passage of manitol in the Ussing chamber model. One week before the experiment, the rats had a free access to an oatmeal drink containing the probiotic. Direct exposure of intestinal segments (mounted in the Ussing chambers) to *E. coli* induced a 53% increase in manitol passage across the intestinal wall (p<0.05) whereas pre-treatment of the rats with the probiotic abolished this increase. Notably, neither administration of *L. plantarum* 299v in the using chambers nor administration of the probiotic to non-infected rats had any effect on mannitol passage.

If mannitol may be considered as a good marker of intestinal permeability, these data demonstrate that *L. plantarum* 299v protects against *E. coli*-induced increase in permeability in a rat model and consequently exerts a beneficial effect on the gastrointestinal tract.

### Probiotics enhance colonic transit time

Colonic transit disturbances as constipation are frequent. Probiotics are believed to influence intestinal transit. However, the evidence is poor as are lacking (9).

The results obtained by a randomised, double-blinded and controlled study attempted to confirm this beneficial effect of probiotics (10). Two test products were used : yoghurt and the Danone ‘BIO’ product which is a milk fermented by yoghurt starters and *Bifidobacterium animalis* DN-173 010.

The trial comprised 4 consecutive periods of 10 days : 10-day run-in period and two 10-day ingestion periods separated by an interval of 10 days. Healthy women were asked to take one of the two products for the first ingestion period and the other product for the second ingestion period. Subjects also ingested a marker which distribution (visible after abdominal X-ray) assessed the colonic transit time. The total and sigmoid transit times were shorter during *B. animalis* intake compared to yoghurt period. Left and right colonic transit times, pH, faecal weight, bacterial mass and bile acids were not affected. Thus, compared to yoghurt product, milk fermented by yoghurt starters and *B. animalis* was able to shorten the colonic transit time in healthy women. The authors conclude that consumption of the fermented milk ‘BIO’ influenced the colonic motor function. This effect seems to be specific to the probiotic strain *B. animalis* DN-173 010. This conclusion is strengthened by the results of two other clinical trials carried with the same probiotic strain. In fact, it was demonstrated for healthy adults (11) and for the elderly (12), intestinal transit times were shortened after *B. animalis* DN-173 010 consumption. **

Probiotics seems to be able to lower cholesterol in rats...

Contradictory results are published on the effects of fermented dairy products on cholesterol concentrations. Several studies reported that fermented dairy products lower serum concentrations in animals and humans and other trial did not report any modification.

What about the effects of Lactobacillus gasseri SBT0270 administration on serum lipids in hypercholesterolemic rats?

An effective dose of 109 viable cells/day given in milk for 14 days exerted its hypercholesterolemic effect in hypercholesterolemic rats fed with a cholesterol-enriched diet (13).

The total cholesterol, LDL cholesterol and triglyceride were respectively 39%, 25% and 43% respectively lower in the group that received the probiotic than the control group.

This trial showed that consumption of probiotic was effective in lowering cholesterol in rats and that this effect is dose-dependant.

... but not in humans

Canadian researchers asked the question about the effect of Kefir on serum lipids concentrations (14).

The trial was conducted in thirteen healthy mildly hypercholesterolemic male subjects. They were asked to maintain their habitual diet and to consume 500 ml/day of milk or Kefir supplement in a randomized crossover trial for 2 periods of 4 weeks. They were blinded to the dairy supplement consumed.

After 4 weeks of supplementation, Kefir had no effect on total cholesterol, LDL cholesterol, HDL cholesterol or triglyceride concentrations nor on cholesterol fractional synthesis rates.

Under the condition of this trial, which is not designed to check the alimentary intakes, the results of this study do not support Kefir consumption as a cholesterol lowering agent in mildly hypercholesterolemic men.

Tripeptides from milk as hypotensive agents in a rat model

Several milk proteins are precursors of peptides which possess various biochemical and physiological properties, including antithrombotic, immunomodulatory and antihypertensive effects (15, 16). These active peptides can be released from milk proteins after enzymatic proteolysis either during gastrointestinal digestion or during fermentation of milk lactic acid bacteria.

In a previous study, it was reported that two tripeptides IPP and VPP had an antihypertensive effect in hypertensive rats (17) after a single intake.

In a recent study, researchers from Helsinki University investigated the effect of long-term (12 weeks) oral administration of these tripeptides, or a sour milk product containing these peptides on development of hypertension in spontaneously young hypertensive rats (18).

The trial showed that development of hypertension was attenuated in the groups receiving tripeptides or sour milk as compared to control group. At the end of treatment period, systolic blood pressure was 176±1 mmHg in sour milk group, 181±2 mmHg in peptide group and 193±1 mmHg in control group (p<0.001).

After treatment withdrawal, systolic blood pressure rose gradually reaching the level of control group within four weeks follow-up.

In conclusion long term intake of the tripeptides or sour milk containing these peptides attenuated the development of hypertension in young rats.

This scientific letter “Yoghurts & fermented milks” is also available on the following websites:
www.maison-du-lait.com and www.syndifrais.org
Molecular characterisation of a bacteriocin

Intestinal microflora is believed to promote health directly through its metabolic activity and its physical presence, providing benefits for the host. One of the desirable properties of probiotics is the ability to produce antimicrobial substances such as bacteriocins. Bacteriocins are bioactive peptides or peptide complexes that have a bactericidal or bacteriostatic effect on other species.

It was shown that the probiotic Lactobacillus salivarius UCC118, isolated from human gastrointestinal tract, produces a bacteriocin called ABP-118 which was characterized at the genetic level (19).

As adhesion to host mucosal surfaces is considered as the initial step in infection by any micro-organism, it is hypothesized that probiotics may compete with the pathogens for the same adhesion sites and consequently may prevent infection (21).

A gene from Lactobacillus reuteri 1063 encoding a cell surface protein that adheres to mucus components in vitro has been cloned and sequenced (22).

This 358 kDa protein, called Mub, shows features typical for other cell-surface proteins of Gram+ bacteria. Mub adhered in vitro to pig mucus components in the pH range 3-7.4, with maximum binding at pH4-5. As showed by using antibodies, Mub is present on cell surface of L. reuteri and it is detected in the growth medium.

As adhesion with host mucosal surfaces is also considered as the initial step in infection by any micro-organism, it is hypothesized that adherent probiotics may prevent infection by inhibiting pathogens adhesion.

The authors planned to construct Mub mutants in order to evaluate the role of Mub in colonization and in control of pathogenic micro-organisms.

A protein involved in probiotics adhesion to intestinal mucus

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Immunomodulating effects of probiotics: a hypothesis on the underlined mechanism

The effects of milks fermented by Lactobacillus helveticus and its non-proteolytic variant on mucosal and tumoral immunity were studied in mice (23).

The immune response was assessed by analysing the activity of the peritoneal macrophages, the number of cells secreting IgA associated with the gut- and bronchial-associated lymphoid tissue.

After feeding of milk fermented with L. helveticus wild type, the number of sigA increased at both the intestinal and bronchial levels. This effect was not noticeable when milk was fermented with protease-lacking L. helveticus variant.

This result suggests that lactic acid bacteria have implications in modulation of the host’s immune response and that biopeptides released by the probiotic contribute to this effect.

We are sad to hear that Professor Bernard Beafrère dead on August. Bernard Beafrère, paediatrician, teacher at the Clermont-Ferrand university of medicine and researcher particularly involved in the field of proteins and nutrition/health relationship, was also director of the Centre de Recherche en Nutrition Humaine in the same town. At the beginning of this year, he was nominated manager of the department ‘Nutrition Alimentation et Sécurité Alimentaire’ of the AFSSA agency. He was also president of the ‘Nutrition Committee’ of this agency. With him, one of the prominent experts in the ‘nutrition world’ disappeared. We offer to his family and to his collaborators our condolences.
The data base LAB-DOC organised by SYNDIFRAIS, brought together the bibliographic references of the international scientific publications accompanied by the authors’ summaries.


