The gut epithelium is in contact with a unique environment that groups together different species of commensal and pathogenic micro-organisms. Its surface is covered with a layer of mucus that not only forms a physical barrier but that, through the presence of glycoconjugates, interferes with the adhesion of pathogenic micro-organisms to the intestinal epithelium or the binding of toxins to their receptors.

Over the last ten years, numerous works have shown that bacteria - cell dialogue exists within the intestinal ecosystem. The dialogue is based on an exchange of molecular signals able to modulate the expression of certain genes and/or the activity of certain enzymes. Very interesting results have been obtained, in particular concerning the ability of the commensal flora and transiting bacteria to modify the structure of the glycoconjugates.

By comparing the profiles of glycosylation of mouse with flora that was either conventional, axenic or monocontaminated with a bacteria of the dominant endogenous intestinal flora (Bacteroides thetaiotaomicron), Jeff Gordon’s team has shown that the total flora and also B. thetaiotaomicron were able to modify the glucidic profile of the cells, in particular the fucose operon operates (Bry et al 1996).

We have shown, by using similar murine models, that the modifications caused by B. thetaiotaomicron were not limited just to fucose but also affected other sugars. The level of expression and the distribution of glycoconjugates throughout the digestive tract were thus seen to be modified (Freitas et al 2002). Dialogue can take place without direct contact between the cells and the micro-organisms, thanks to factors excreted by bacteria. In fact, a simpler model (cell lines cultivated in vitro) enabled us to identify in the supernatant fluids of Bacteroides thetaiotaomicron, one or more soluble "moduline" factors of low molecular weight and thermosensitive able to increase the quantity of galactoconjugates on the surface of the cells (Freitas et al 2001). On the same cellular model, a comparable effect was obtained for a Lactobacillus casei thermosensitive able to increase the quantity of galactoconjugates on the surface of the cells (Freitas et al 2003). The moduline produced by these two bacteria not only causes a modification of the profile of the glycosylation, but also protects against infection from rotavirus (Freitas et al 2003). Our hypothesis is that an increase in the amount of galactose residue could hide some of the rotavirus binding sites and thus reduce some of its power to infect.
To all evidence, consuming probiotics has a beneficial effect on the gastro-intestinal tract. How? Which probiotics? At what doses? For what kind of disorders? These are the questions still being asked today.

Three double-blind, randomized and controlled studies have posed the question of the effectiveness of probiotics to counter the side effects of antibiotics - infant diarrhoea, digestive discomfort and a gut flora imbalance in adults infected by the pathogenic bacterium, *H. pylori*.

These three studies underline the advantages of consuming probiotics while taking antibiotics.

### Probiotics reducing the risk of an onset of antibiotic-associated diarrhoea

A Brazilian study tested the ability of probiotics to prevent the onset of diarrhoea caused by antibiotics in infants (1).

The study protocol (double blind and randomized) included 157 children aged between 6 and 36 that were to be treated with antibiotics. For thirty days, the patients were given either a commercial, non-fermented milk formula containing a probiotic mixture (*Bifidobacterium lactis*, $10^7$ cfu/g of powdered milk and *Streptococcus thermophilus*, $10^6$ cfu/g of powdered milk) or the same product without probiotics. During the first two weeks of treatment, all patients were also treated with antibiotics. The minimum quantity of milk to be consumed was set at 500 ml/day, a dose that the authors adjudged necessary to guarantee sufficient levels of probiotics in the gut (i.e. approx. $10^7$ cfu/g of intestinal content). Below this level, patients were excluded from the analysis. The frequency and consistency of the faeces were examined on a daily basis.

The children grew normally in both groups and no side effects were observed linked to the tested product. Of the 80 infants given the probiotic, 13 developed antibiotic-related diarrhoea, against 24 of the 77 control patients. The frequency of the diarrhoea was significantly superior in the control group (31.2 %) compared to the group taking the probiotics (16.3 %) (p=0.044).

This clinical study conducted on children shows that the simultaneous consumption of the probiotics *B. lactis* and *S. thermophila* during and after treatment with antibiotics enabled the frequency of antibiotic-related diarrhoea to be reduced. The tested product caused no side effects whatsoever.

The results confirm the advantages of probiotics as an auxiliary treatment for preventing antibiotic-associated diarrhoea in infants.

### Probiotics helping maintain the balance of the intestinal flora

A pilot study, conducted double blind and placebo controlled, analysed the impact of probiotics on the composition of the faecal flora in 30 patients infected with *H. pylori* but without symptoms. All the patients were subjected to the same antibiotic treatment (tritherapy) prescribed with the goal of eradicating the pathogen.

The 30 patients were split into 3 groups: one group received the placebo for 2 weeks, the second received the placebo for the first week then the probiotic for the second week, the third group received the probiotic for two weeks. Antibiotics were taken for the first 7 days of the test period. The probiotic mix, taken in the form of capsules in quantities of $2.5 \times 10^{10}$ cfu/day in total, was composed of *Lactobacillus acidophilus* CTL60, *Lactobacillus acidophilus* CUL21, *Bifidobacterium bifidum* CUL17 and *Bifidobacterium bifidum* Rhodia. The composition of the faecal bacterial flora was analysed on days 1, 7, 12, 17 and 27 after the start of the antibiotic treatment.

In the control group, the number of anaerobic bacteria in the faecal flora increased significantly between days 1 and 7 (period when the antibiotics were taken) and remained at a high level until day 27. In the group receiving the placebo until day 7, the development was identical for the first 7 days then the levels of anaerobic bacteria dropped significantly between days 7 and 27 in parallel with the consumption of the probiotic (days 8 to 15). In the group receiving the probiotic during the entire period, the levels of anaerobic bacteria remained stable.

This study had two main results. Firstly, consuming probiotics prevented a change in the flora while the antibiotics were being taken. Secondly, the effect was not only preventive - the probiotics taken after the antibiotics returned the flora to its initial state.

These results, although concerning a limited number of patients, provide a new argument in favour of using probiotics to preserve the balance of the intestinal flora during treatment with a broad-spectrum antibiotic.


Probiotics helping the balance of the intestinal flora

Preventing side effects caused by antibiotics

A study of the efficacy of an H. pylori eradication treatment and its gastro-intestinal tolerance also assessed the effect of consuming probiotics at the same time as antibiotics (3).

Forty-seven asymptomatic patients infected with H. pylori were divided - randomly and blindly - into a control group (n=24) consuming a fruity milk drink and a test group receiving the same product supplemented with a probiotic mixture (Lactobacillus rhamnosus GG, Lactobacillus rhamnosus LC705, Bifidobacterium breve Bb99 and Propionibacterium freudenreichii shermanii JS). The products were taken over 4 weeks, of which the first seven days coincided with the treatment period, the total quantity of probiotics consumed was between 6.5x10^10 cfu/day and 1.3x10^11 cfu/day.

During the antibiotic treatment period, the total quantity of probiotics was estimated using serology and the urea breath test. During the period that the tested drinks were consumed, the gas-tro-intestinal symptoms were noted on a daily basis by the patients. To count two of the administered probiotics (LGG and P. freudenreichii shermanii JS), the patients’ faeces were collected at the start of the study, after the antibiotic treatment, at the end of the period of taking the drinks and 6 weeks after the end of the study.

Although statistically insignificant (91 % vs. 79 %, p=0.42), the H. pylori eradication rate was greater in the group receiving probiotics than in the control group. However, when one considers the overall severity of the intestinal symptoms and the way these developed when the probiotics were taken, the probiotics improved the symptoms compared to the placebo (statistically significant difference between the two groups, p=0.038). The quantities of each of the studied probiotics in the faeces increased strongly during the administration period, and then returned to the base values at the end of the 6-week follow-up period. In the control group, the concentrations of these bacteria did not vary.

The results of this study highlight the efficacy of probiotics to reduce the side effects (gastro-intestinal symptoms) of taking antibiotics. However, no effects regarding H. pylori eradication were detected. It should also be noted that the probiotics survived in the digestive tract despite the presence of the broad spectrum antibiotics used in this study.

Improvement in resistance to pathogen infection through the consumption of lactobacilli has already been shown (4). A study by Gabriela Perdigon's team has highlighted the ability of a new probiotic mixture to limit infection from an entero-invasive strain of Escherichia coli in mice (5).

Milk fermented with the probiotics L. casei, L. delbrueckii bulgaricus and S. thermophilus was administered orally to mice over 2, 5 or 7 days. The administered product was diluted to 10^7 cfu/ml and was the only drink available to the animals. Control animals received diluted milk without containing probiotics. The non-specific immune response was assessed by measuring the phagocyte activity of the peritoneal macrophages and the number of IgA-producing cells in the intestine. After 5 days of probiotic or placebo administration, the mice were subjected to an oral dose of 10^9 cfu of an entero-invasive strain of E. coli. Besides the previous indicators, the authors also measured the bacterial translocation into the liver and spleen and the levels of pathogen specific IgA in the fluids of the small intestine.

After 5 days of fermented milk administration, the non-specific immune response had increased. In the E. coli infection test, the mice that had ingested probiotics showed less liver colonization than the control animals. Secretion into the intestinal fluids of E. coli specific IgA was greater in the mice given the probiotics.

Milk fermented with L. casei, L. delbrueckii bulgaricus and S. thermophilus limited E. coli infection in mice. These results suggest that the preventive effect could be based on the activation of innate, adaptive immunity in the intestinal mucosa.


This scientific letter “Yoghurts & fermented milks” is also available on the following website: www.maison-du-lait.com
Probiotics fighting inflammatory bowel diseases

The avenue researching the effectiveness of probiotics to fight inflammatory bowel diseases has received support from a study on a murine model

Ulcerative colitis is a chronic inflammation of the colon whose aetiology is not yet totally known but it is strongly suspected that the gut flora may start the inflammation. It is for this reason that, in recent times, consumption of probiotics has been put forward as possibly beneficial to remission and/or prevention of relapses. Studies on animal models of colitis suggest that certain bacteria of the gut microflora tend to cause inflammation whereas others might actually prevent it. An Italian team has attempted to clarify the immune mechanisms that come into play when controlling inflammation with probiotics (6).

The team used a mouse model in which colitis had been chemically induced. After an initial inflammatory attack, the mice were given either a mix of probiotics - VSL#3 (total 6x10^9 cfu/day) - or a placebo for 3 weeks. A second attack of colitis was then induced. Compared to the placebo, consumption of probiotics during the remission phase significantly reduced the severity of the second inflammatory attack and the associated mortality.

The protective effect caused by the probiotics would appear to involve lamina propria lymphocytes. This is shown by the following experiment: these lymphocytes, collected just before the second colitis attack from the mice treated with probiotics were injected into naïve mice (i.e. not treated with probiotics) in which it was then attempted to induce colitis. It was seen that the injection of lymphocytes from mice suffering from colitis and treated with probiotics had a protective effect on the naïve mice (less mortality than in the control animals).

Compared to the control, administering probiotics increased IL-10 secretion and the number of T LAP+** lymphocytes, i.e. lymphocytes bearing TGFβ in its inactive form on their surfaces. The T LAP+ lymphocytes would appear to be IL-10 dependent since an injection of anti-IL-10 inhibited their production.

It would also seem that T LAP+ lymphocytes are involved in the protective effect induced by probiotics. This is shown by two experiments. In the first, the injection of IL-10 blocking agents or TGFβ blocking agents and in the second, the exclusion of T LAP+ lymphocytes from the lamina propria lymphocyte pool put an end to the protection transferred to healthy mice via the injection of lymphocytes collected from the probiotic-consuming mice.

Besides showing the preventive effect of VSL#3 on recurrent colitis in mice, these experiments also propose a probable action mechanism for the probiotic. The role of lamina propria lymphocytes in this mechanism is clear. In precise terms, T lymphocytes carrying LAP and IL-10 appear to be at the heart of the mechanisms underlying the protective effect of probiotics against colitis.

Pouchitis is the inflammation of the pouch created by ileal-pouch anal anastomosis (IPAA) of the colon after the removal of a damaged part of the intestine in patients suffering from ulcerative colitis. In a previous study (7), Massimo Campieri et al. had examined the preventive effects of a VSL#3 probiotic mix. They have now put forward a preliminary explanation of the immune mechanism brought into play (8).

The initial study included 40 patients suffering from ulcerative colitis, who had undergone an operation to create an ileo-anal pouch. During the year that followed the operation, the patients were given either a placebo or a VSL#3 probiotic mix in a double blind, random study (1.8x10^9 cfu/day). A posteriori, the researchers analyzed the immune parameters of 16 patients that did not develop pouchitis during the follow-up period. The data came equally from the control group and the group taking the probiotic. The parameters studied were the presence of polymorphonuclear leukocytes in the intestinal mucosa and the expression of different cytokine genes (pro-inflammatory, Th1 and regulatory cytokines) also in the intestinal mucosa. A count of the polymorphonuclear leukocytes in patients suffering from pouchitis was used as a positive control.

In the patients who had been given probiotics, IL-1β, IL-8 and IFNy cytokine gene expression in the intestinal mucosa was significantly lower than that observed in the control patients. In those patients that had not suffered from pouchitis and had received the probiotic, the number of polymorphonuclear leukocytes in the mucosa was significantly lower than that seen in the patients who had been given the placebo and had not suffered from pouchitis and also in the patients who had suffered from pouchitis. TNFα, IL-6, IL-12 pro-inflammatory cytokine and IL-10 and TGFβ regulatory cytokine gene expression was similar in both the tested and control groups.

The lower expression levels of IL-8, the moderate number of polymorphonuclear leukocytes and the modification of IL-1β and IFNy gene expression all plead in favour of an anti-inflammatory effect.

This study provides explanatory elements on the probable action mechanism of VSL#3 in patients with an ileal-anal pouch. VSL#3 would appear to prevent the onset of pouchitis by regulating the influx of polymorphonuclear leukocytes into the mucous by inhibiting the production of IL-8, IL-1β and IFNy also appear to be involved. This study once again shows the expected correlation between IL-8 and polymorphonuclear leukocytes.

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Probiotics – in brief

Different hypotheses have been put forward to explain the way that ingested probiotics interact with the host’s cells. The modulation of the expression of certain genes could be involved. Gene expression in the mucosa of the small intestine was studied in a group of patients given antibiotics associated either with a placebo or with the probiotic, Lactobacillus GG.

Compared to the placebo, consumption of the probiotic resulted in the over- or under-expression of 334 and 92 genes respectively. The affected genes are involved in different functions - immunity, communication and cell growth.

Lactobacillus GG modulating intestinal mucosa gene expression

A study of 51 patients who had undergone ileal-pouch-anal-anastomosis due to ulcerative colitis has shown the beneficial effect of consuming a fermented milk containing the probiotics Lactobacillus La-5 and Bifidobacterium lactis Bb12 (daily total of 5x10^10 cfu).

The severity of the symptoms and the inflammation - determined by endoscopy - had reduced considerably after 4 weeks of taking the probiotics. However, as the study had no control group, a placebo effect cannot be excluded.

A fermented milk fighting inflammatory bowel disease

A study carried out on piglets has shown that some of the parameters regarding the way the digestive tract works have been improved in animals who received a probiotic (either Bifidobacterium breve + Bifidobacterium animalis or Lactobacillus acidophilus) via the enteral route compared to the control animals. An increase in the number of fibrocytes and fibroblasts in the mucosa and an increase in the number and activation rates of the endocrine cells in the stomach and small intestine were observed. In particular, the administration of Lactobacillus acidophilus resulted in an increase in the number of lymphocytes and lymphoid cells in the small intestine.

Effects of probiotics on the morphology of the digestive tract

Three probiotics were selected for their ability to inhibit in vitro production by the intestinal flora of β-glucuronidase, an enzyme that may be instrumental in liver cancer. These strains (Lactobacillus brevis HY7401, Lactobacillus acidophilus CSG and Bifidobacterium longum HY8001) were administered orally to mice along with the injection of an hepatotoxic product.

The administration of each probiotic reduced the concentration of hepatic damage markers (aspartate aminotransferase and alanine aminotransferase). The hepatoprotective effect expressed by these probiotics that inhibit β-glucuronidase was even more marked than that of an hepatoprotective agent sold in pharmacies.

Hepatoprotective effect of probiotics in mice

L. acidophilus has no effect on hypercholesterolemia in humans

The ability in vitro of certain bacteria to metabolize cholesterol has given hope of a cholesterol-lowering effect in vivo. However, a recent publication has confirmed the results of previous earlier studies that failed to show that probiotics could have a beneficial effect on cholesterol levels.

In this double-blind study, 80 volunteers with hypercholesterol consumed either a placebo or the probiotic Lactobacillus acidophilus (9x10^10 cfu/day) for 6 weeks. Despite the cholesterol-lowering properties observed in vitro, consumption of the probiotic did not result in any changes to the subjects’ plasma lipids.

L. acidophilus has no effect on hypercholesterolemia in humans


