New insight into the direct role of live lactic acid bacteria in lactose digestion

Nadine Cerf-Bensussan

Lactase deficiency is a widespread problem occurring in approximately 70% of the world’s population. Consumption of milk by lactase-deficient individuals results in digestive troubles.

One of the beneficial effects of yoghurts is the improvement of lactose tolerance and thus milk digestion. Numerous studies suggested that this beneficial effect is specific of yoghurts, which contain live bacteria. In fact, consumption of heated yoghurt does not lead to this improvement (1). However, as for the most beneficial effects of lactic acid bacteria, the underlined mechanisms are not well known.

A neat work conducted in INRA by Gérard Corthier’s team (and supported by the Scientific Mission of Syndifrais) shows for the first time, in a mice model, that yoghurt lactose digestion is performed by enzymes of the lactic acid bacteria during the intestinal transit (2).

In order to demonstrate that lactic acid bacteria play a direct role, these researchers genetically modified Streptococcus thermophilus (one of the two yoghurt starters). The first modification was to fuse the luciferase genes (a light producing protein) to the lactose operon (which encodes the enzymes responsible for lactose hydrolysis). When lactose activates the lactose operon, luciferase is also activated and thus produce a quantifiable light. Secondly, genes involved in lactose hydrolysis are also modified i.e. lactose permease and β-galactosidase, respectively involved in transport and hydrolysis of lactose. Thus three types of bacteria are available: one bears a functional lactose operon and the two others have an inactive lactose operon.

When the functional-lactose operon bacteria and lactose are given by oral gavage to mice, a substantial luciferase activity is measured in faeces. This first experiment demonstrates that lactose is able to activate lactose operon and that bacteria are able to remain alive during their transit in the digestive tract of mice.

The second experiment demonstrates that live bacteria directly hydrolyse lactose. The remaining amount of lactose is measured in faeces of three groups of mice: some of them received water or lactose as a drinking solution and the third group received lactose and one of the three modified live bacteria. Oral administration of bacteria bearing a functional lactose operon to mice those mice fed with lactose resulted in a significant decrease of lactose in faeces compared to those mice who received lactose alone. No decrease was observed in mice inoculated with the bacteria modified for permease or β-galactosidase.

This careful study demonstrated that live lactic acid bacteria play a direct role in lactose digestion. It sensibly reinforces the observation that yoghurt (containing live flora) intake improves lactose digestion. We hope that in the future, models of this quality may be proposed in order to elucidate the mechanisms underlined by the beneficial effects of lactic acid bacteria, particularly in the field of gut immunity.

1• Syndifrais (2002), Yoghurts & Fermented Milks, Letter n°6, p.1
Preventing infections by probiotics

Protection against lung infection: studies in a rodent model

Assuming that oral immune stimulation can induce immunity in mucosal sites remote from the gut, the aim of the works presented below was to study the effect of oral or intranasal administration of probiotics on prevention of lung infection in rodents.

Two publications were from the CERELA (Reference Centre for Lactobacillus) in Argentina and the third one was from Yakult Central Institute for Microbiological Research.

In the first work (3), young (3 weeks) or old (8 weeks) mice were challenged with Pseudomonas aeruginosa (from human origin) which is an important pathogen in neonates and in individuals with compromised respiratory functions. Adult animals were able to clear the pathogen from their lungs 24 h after infection, whereas young mice still demonstrated a high colonization after this time.

Young mice were fed with yoghurt or with a suspension of Lactobacillus casei CRL 431 during 2, 5 or 7 consecutive days prior to infection with Pseudomonas aeruginosa.

In young mice, administration of L. casei for 2 days and yoghurt for 7 days increased P. aeruginosa clearance from the lungs after challenge. Sixteen hours postchallenge, the treated animals had completely cleared pathogen from their lungs. At this time, yoghurt administration for 5 days had a significant effect but it was not enough to achieve total bacterial clearance.

At the same time, there occurred an increase in phagocytic activity of alveolar macrophages, with values two times higher than in control mice (without probiotic intake) and the white blood cells differential counts did not show any detectable modification of the percentage of polymorphonuclear cells and lymphocytes between control and treated groups. Furthermore it appeared that previous administration of L. casei or yoghurt induced an increase in IgA and IgG levels in bronchoalveolar lavages after P. aeruginosa infection, although there was no relationship with the serum values.

In summary, yoghurt and L. casei administration had a beneficial effect on pulmonary infection with P. aeruginosa in weaned mice. The treatments studied in this experiment were able to induce an activation of alveolar macrophages and an increase in IgA levels in the respiratory tract, which improve the immunological reaction against an acute infection with P. aeruginosa.

In the second work (4), the preventive effect of intranasally inoculated Lactobacillus fermentum against Streptococcus pneumoniae was studied using a mouse experimental model. Adult mice were intranasally inoculated with L. fermentum (isolated mice origin) before the intranasal inoculation of Streptococcus pneumoniae (human origin). The L. fermentum treatment decreased the number of S. pneumoniae throughout the respiratory tract and increased the number of activated macrophages in lung and a higher lymphocyte population in the tracheal lamina propria. Anti-S. pneumoniae antibodies increased in lactobacilli-treated animals compared to the non-treated lactobacilli mice. The increase in antibody levels suggests that the mucosal immune system could be involved in the protective effect.

From the results obtained in these works, it can be suggested that the intervention of the immune system could be involved as one of the main mechanisms responsible for the protective effect exerted on lung infection. Furthermore, the immunisation against pulmonary pathogens induced by lactobacilli may be mediated via gastrointestinal or respiratory route.

The purpose of another study (5), conducted by a team from Yakult Central Institute for Microbiological Research, was to investigate whether oral administration of Lactobacillus casei Shirota activates not only the systemic immune system but also the local immune system and whether it ameliorates influenza virus infection in the upper respiratory tract of aged mice.

The mice received the probiotic in the diet for four months prior to the infection with the virus. The results show that compared to the control group: the virus titre is reduced in the probiotic group (P<0.05); natural killer (NK) activity of splenocytes and lung cells of the probiotic group is increased (P<0.01 and P<0.05); potent induction of gamma interferon (γ IFN) and tumour necrosis factor α (TNFα) which play an important role in excluding influenza virus, was evident in nasal lymphocytes. In fact, these findings suggest that oral administration of L. casei Shirota activates not only systemic cellular immunity but also local cellular immunity and that ameliorates influenza infection.

References:
Protection against atopic disease

A previous work published in the Lancet by E Isolauri’s team from Finland showed that perinatal administration of Lactobacillus rhamnosus GG halved the subsequent occurrence of atopic eczema in at-risk infants (6). Recently, in a double-blinded, placebo-controlled study of 62 mother-infant pairs (7), these researchers show that administering Lactobacillus rhamnosus GG to the pregnant and lactating mother increased the immunoprotective potential of breast milk as assessed by the amount of anti-inflammatory transforming growth factor β2 (TGF-β2) in the milk of mothers receiving probiotics compared to those receiving placebo (2885 pg/ml vs. 1340 pg/ml, P=0.18). The risk of developing atopic eczema during the first two years of life in infants whose mothers received probiotics was reduced in comparison with that infants whose mothers received placebo (15% and 47% respectively, P=0.0098).

Thus, administering Lactobacillus rhamnosus GG during pregnancy and breast-feeding offers an effective mode of promoting the immunoprotective potential of breast-feeding and provides protection against atopic eczema during the first two years of life. If indeed microbes have a role in the aetiology of allergy these data raise the question as to whether the composition of gut microflora in allergic infants could be associated with the development of allergic symptoms and could consequently constitute a new treatment target.

With the view to answer to this question, the same team assessed the faecal microflora of ten healthy infants and 27 infants with atopic eczema during breast-feeding and after weaning (8). The most prominent result is that atopic infants had less Gram-positive species among the most predominant aerobes and smaller total cell count. Thus, these data revealed that the composition of the intestinal microflora is of some importance in the apparition of atopic eczema and raised the question that whether Gram-positive bacteria may have distinctive importance in protection against atopic sensitization. Further studies are aimed of answering this question.

Enhancement of immunity in the elderly

The immune defence system is known to be adversely affected by the ageing process and there is substantial evidence that a poorly functioning immune system can contribute to decreased disease resistance (9,10). An attractive means of restoring immune function is dietary intervention. The present publication (11) describes the outcome of a 9 week dietary intervention trial designed to determine the effects of Bifidobacterium lactis HN019 on cellular immunity in the elderly. Thirty healthy volunteers (median 69 years) participated in the trial. During the first 3 and the last 3 weeks they consumed milk and during the median period they consumed milk supplemented with the probiotic (5x10^8 or 5x10^9 cells per day).

After consumption of the probiotic, a slight increase in the proportion of total, , helper (CD4+) and activated (CD25+) T lymphocytes is measured. The ex vivo phagocytic capacity of mononuclear and polymorphonuclear phagocytes and the tumoricidal activity of natural killer cells were also elevated after probiotic consumption. The authors conclude that B. Lactis HNO19 could be an effective dietary supplement for enhancing some aspects of cellular immunity in the elderly. However it remains questionable to determine if the modification of the immune response indicated by these results is of physiological significance for the host.
It is widely accepted that the intestinal microflora plays an important role for the health of the host and possesses immunomodulating capacity. Elucidation of the mechanisms by which intestinal microorganisms, including potential probiotics, modulate the immune system remains an open question.

In clarifying the mechanisms behind the immunoregulatory effect of the gut flora (including probiotics) little attention has been focused on their effect on dentritic cells which play a pivotal immuno-regulatory role in the Th1, Th2 and Th3 balance and are present throughout the gastrointestinal tract.

In a work published in Journal of Immunology (12), researchers tested the hypothesis that some species of *Lactobacillus* which are major components of the commensal gut flora differentially activate dentritic cells. Bone marrow derived murine dentritic cells were exposed to various lethally irradiated *Lactobacillus* species and resultant culture supernatants were analyzed for IL6, IL10, IL12 and TNFα. The most significant differences among the tested lactobacilli species were observed for IL12 and TNFα, with the following ranking of the species:

- L. casei >> L. plantarum Lb1 > L. fermentum Lb20 = L. johnsonii La1 = L. plantarum 299v >> L. reuteri.

Cytokine responses ranged from no induction observed for *L. reuteri* to responses induced by *L. casei* far exceeding that achieved by LPS stimulation. Similar but less pronounced differences were observed among lactobacilli in the induction of IL6 and IL10.

Although all strains up-regulated surface MHC class II and CD86, which is indicative of dentritic cell maturation, those lactobacilli with greatest capacity to induce IL12 were most effective. Remarkably, *L. reuteri*, a poor IL12 inducer, inhibited IL12, IL6 and TNFα induction by the otherwise strong cytokine inducer *L. casei*, while IL10 production remained unaltered. In analogous fashion, *L. reuteri* reduced *L. casei*-induced up-regulation of CD86.

These results suggest that different species of *Lactobacillus* exert very different dentritic cell activation patterns and, furthermore, at least one species may be capable of inhibiting activities of other species of the genus. Thus, the potential exists for Th1/Th2/Th3-driving capacities of the dentritic cells to be modulated according to composition of gut flora, including ingested probiotics.

In a large scale clinical study (13), children from day-care centres received either yoghurt, milk fermented by yoghurt symbiosis and *Lactobacillus casei* (DN 114 001), or jellified milk as diet supplement during 30 day supplementation two periods separated by one 30 day period without supplementation. Faeces were collected before, during and after the second supplementation period in order to measure the levels of IgA.

All faecal samples contained significant IgA levels. IgA levels decreased transiently in children receiving yoghurt, and increased in children receiving jellified milk, but did not vary in the group of children who were given the fermented milk. This data suggests that lactic acid bacteria can trigger immune responses. The authors hypothesised that supplementation with *L. casei* suppresses these responses, which can be interpreted as the generation of mucosal tolerance to environmental flora.
Probing new abilities for probiotics

Efficiency of Lactobacillus rhamnosus GG against dental caries

Carious process has been associated to many variables within microbiological parameters such as streptococci and lactobacilli, the most abundant of the acidogenic species resident in the oral cavity, are associated with the presence and onset of dental caries (14). On the other hand, Lactobacillus rhamnosus GG (LGG) has been shown to temporarily colonize the mouth and to inhibit in vitro a caries pathogen Streptococcus sobrinus (15). This probiotic belongs to the homofermentative lactobacilli, which cannot ferment sucrose or lactose. It does not enhance caries and could therefore be safe for the teeth.

The hypothesis of a Finnish study (16) was that LGG would affect oral microbiota in a positive way and that children receiving the probiotic in milk would develop fewer caries than children receiving normal milk. This randomized, double-blind, placebo controlled intervention was conducted for 7 months with 594 children aged from 1-6 years and was the first clinical long-term trial on the effects of a probiotic on dental caries and caries risk. The caries risk was calculated on a basis of clinical and microbiological data, comprising mutans streptococcus levels from dental plaque and saliva. The results showed fewer dental caries in the LGG group and lower mutans streptococcus count at the end of the study. LGG was found to reduce the risk of caries significantly (odds ratio = 0.56, P=0.01). The effect was particularly clear in the 3 to 4 year-olds (compared to 1-2 years or 5-6 years).

Thus, milk containing the probiotic LGG may have beneficial effects on children’s dental health and may be a promising preventive method for combating caries and reducing caries risk.

Probiotics against Helicobacter pylori infections:

The gastric pathogen Helicobacter pylori is the principal cause of peptic ulcers and the major risk factor for gastric cancers in humans. Several studies investigated the effects of probiotics on H. pylori. It seems that probiotic supplementation has a beneficial effect on H. pylori infections in humans (17). However the mode of action of the probiotics on H. pylori is not yet elucidated.

A team from Kitasato, University in Japan examined in vitro the competition of binding of Lactobacillus reuteri and Helicobacter pylori to gangliotetracyceramide (asialo-GM1) and sulfatide which are putative glycolipid receptor molecules of H. pylori (18).

It was shown that strains of L. reuteri bind in vitro to asialo-GM1 and sulfatide, and inhibit binding of H. pylori to both glycolipids. This is the first study to have shown that L. reuteri possesses the cell surface protein that inhibits in vitro the binding of H. pylori to glycolipid receptors. This result suggests that in vivo L. reuteri strains might be an effective competitor to H. pylori at the receptor site, and thus at the early stage of infection.


LAB-DOC bibliographic selection

The data base LAB-DOC organised by SYNDIFRAIS, brought together the bibliographic references of the international scientific publications accompanied by the authors’ summaries.


22- Elkins CA, Moser SA, Savage DC. (2001) Genes encoding bile salt hydrolases and conjugated bile salt transporters in Lactobacillus johnsonii 100-100 and other Lactobacillus species. Microbiology;147(Pt 12):3403-12


