Probiotics and health: new facts and ideas
Philippe Marteau*, Philippe Seksik and Raymond Jian

Many trials on probiotics are now published that use established methods to demonstrate their clinical efficacy. Convincing progress has been made in the field of inflammatory bowel disease and allergy prevention in infants. Experimental studies show clear differences (and even sometimes opposite effects) between apparently closely related probiotics and suggest new mechanisms for the observed effects, such as immunostimulation by bacterial DNA and interaction with Toll-like receptors and dendritic cells in the gastrointestinal tract.

Introduction
Probiotics have been defined as non-pathogenic microorganisms that, when ingested, exert a positive influence on host health or physiology [1]. Many physicians have long been sceptical about their efficacy, as evidence for their efficacy was low and information on the stability of the strains in the products and their survival in the gastrointestinal tract was often lacking. However, a pharmacological approach has now been used to assess the effects and pharmacokinetics [2], and both scientists and health providers are showing renewed interest. Before 1990, 22 articles on probiotics were quoted in the Medline library, whereas more than 1000 have been published in the past ten years, including more than 200 in the first six months of this year. We now know that the pharmacokinetics of probiotics varies between strains and that many are indeed effective, as shown by positive double-blind randomized controlled trials (RCTs). Probably the most interesting and unexpected data were produced in the past few years in the field of immunomodulation with probiotics: treatment of allergic diseases or inflammatory bowel diseases. We summarize here recent data and ideas.

Probiotics and immunomodulation
The microorganisms present in the gastrointestinal tract, including the endogenous flora, interact with the mucosal cells including epithelial cells [3••] and immune cells. Progress has been made in our understanding of the mechanism of this interaction. Considerable interest has focused on dendritic cells. Dendritic cells belong to the group of antigen-presenting cells that initiate the local immune response in the intestinal mucosa and play a pivotal immunoregulatory role in the balance of T helper cells Th1, Th2 and Th3. Christensen et al. [4] showed that different species of lactobacilli exert very different activation patterns on the dendritic cells. Furthermore, Lactobacillus reuteri DSM12246 inhibited the activities of the other species. Clearly, not all probiotics share the same immunomodulating properties and can even have opposite effects on some parameters. Moreover, in this model the dose of probiotics also strongly influenced the nature of the immune response [4], which leaves us with new questions.

It has been recently discovered that bacterial DNA contains immunostimulatory sequences (ISS-DNA), especially non-methylated CpG motifs, which interact with Toll-like receptor-9 of epithelial cells; these sequences are potent activators of the innate immunity and exhibit anti-apoptotic properties in the mucosa [5••]. Rachmilewitz et al. [5••] showed that the administration of ISS-DNA was beneficial for the colonic mucosa in mice with chemically induced colitis. They then showed that the beneficial effect of the probiotic mixture VSL#3 (which contains three strains of bifidobacter, four strains of lactobacilli and a Streptococcus thermophilus) on this colitis model was derived from its DNA, as VSL genomic unmethylated DNA was effective whereas VSL methylated DNA and calf thymus DNA were ineffective [6]. These interesting and original results open avenues for the discovery of new treatments of inflammatory bowel disease and also demonstrate unsuspected mechanisms for some of the effects of probiotics. Madsen et al. [7] recently showed that bacterial VSL#3 DNA downregulated proinflammatory cytokine secretion by attenuation of the NF-kB pathway in intestinal epithelial cells.

Probiotics and intestinal inflammation
Bacteria in the gastrointestinal tract strongly influence intestinal inflammation and the majority of inflammatory bowel diseases, both in humans and animals, are more severe in the presence of the endogenous flora. Animal studies have demonstrated that some probiotics may significantly prevent or help cure inflammatory bowel diseases. Several RCTs have now confirmed a beneficial effect in humans with pouchitis, ulcerative colitis or Crohn’s disease. Gionchetti et al. [8••] performed a double-blind RCT comparing the effects of the probiotic VSL#3 and a placebo in preventing the recurrence of chronic relapsing pouchitis (an inflammatory bowel disease occurring after surgical resection of the colon). A total of 40 patients were treated for nine months; a relapse occurred in 15% of those in the VSL#3 group versus 100% in the placebo group (p the statistical probability <0.001) [8••]. This result was confirmed in a second multicentre double-blind RCT: the relapse at one year was 10% in the VSL#3-treated
patients versus 94% in the placebo-treated patients [9]. Interestingly, VSL#3 increased the tissue levels of interleukin 10 (IL-10) in these patients [10]. The same authors studied the effects of VSL#3 in the prevention of pouchitis [11]. Forty patients who had colectomy and ileo-pouch anal anastomosis for ulcerative colitis were randomized to receive either VSL#3 or placebo immediately after surgery and for one year. Pouchitis occurred in 10% of the patients in the VSL#3 group versus 40% in the placebo group (p <0.01). Three RCTs strongly suggested that *Escherichia coli* strain Nissle 1917 is as effective as mesalazine (i.e. the standard treatment) in preventing relapse of ulcerative colitis. The first two trials were criticized on the basis that the follow up was too short in the first study and that mesalazine was poorly effective in the control group [12,13]. However, the third trial was more conclusive: 222 patients with ulcerative colitis were treated for one year with mesalazine or *E. coli* Nissle 1917. The relapse rate at 1 year was 36.4% in the probiotic group versus 33% in the standard treatment group and the statistical analysis showed equivalence of the two treatments [14].

### Probiotics and allergy

Population-based studies suggest that increased exposure to bacteria in early life can be protective against allergy. Isolauri and coworkers [15,16] hypothesized that probiotics might be useful to treat or prevent allergy in infants and tested this hypothesis in two RCTs. In the first trial, 27 breast-fed infants suffering from atopic eczema were randomized to be weaned either with probiotic-supplemented extensively hydrolysed whey formula or with the same formula without probiotic [15]. The probiotics studied were *Bifidobacterium lactis* Bb12 and *Lactobacillus rhamnosus* GG. After two months of treatment, the SCORAD (SCORing Atopic Dermatitis) score, which measures eczema severity, was significantly lower in the two probiotic groups than in the placebo group (SCORAD 0, 1 and 13.4, respectively). In their second RCT, the authors assessed the possibility of preventing the occurrence of atopic eczema in infants at high risk [16**]. *L. rhamnosus* GG was given pellon tally to mothers who had at least one first degree relative with atopic eczema, allergic rhinitis or asthma. The probiotic was also administered to the infants for six months; 132 subjects completed the study. The frequency of atopic eczema was reduced by half in the probiotic group: 23% versus 46% (p = 0.008). The mechanism for this effect is still debated. Rautava et al. [17] reported that *L. rhamnosus* GG consumption increased the concentration of transforming growth factor β in the mothers’ milk. Studies on the composition of the intestinal flora of allergic infants or adults compared with non-allergic subjects failed until now to demonstrate conclusive results [18,19]. It is also unknown which probiotics would be effective and whether they would help cure allergy at other times of life. For example, *L. rhamnosus* GG administration did not prevent birch pollen allergy nor apple allergy in a series of allergic young adults or teenagers [20•]. Other trials are ongoing.

### Probiotics and intestinal microbes

Many researchers and clinicians are interested in trying to prevent or cure intestinal infections with probiotics, especially those caused by *Clostridium difficile* or *Escherichia coli* O157:H7 (see [2] and references therein). Colonization of the gastric mucosa by *Helicobacter pylori* is the main cause of gastritis and ulcers, and is strongly associated with gastric lymphoma and cancer. Some probiotics exert antigenic properties against *H. pylori* in *vivo* [21]. Recent RCTs have assessed the efficacy of some probiotic strains and/or probiotic culture supernatants in decreasing gastric colonization by *H. pylori* and/or the urease activity of this microorganism in *vivo* [22–24]. Results are encouraging, but still conflicting, and more studies are needed. One mechanism of action could be the inhibition of the binding of *H. pylori* to its receptors by some probiotics, such as some (but not all) *L. reuteri* strains [25].

**Probiotics to deliver active ingredients to specific targets in the gastrointestinal tract**

Probiotics can be considered as an original way to deliver active constituents to targets in the gastrointestinal tract. Natural or genetically modified probiotics are a source of these constituents and also act as a vector that can protect the activities from acid in the stomach and deliver them at the site in the intestine where they should be active. It was established that yoghurt bacteria, which are easily destroyed by bile in the duodenum, deliver their intracellular lactase activity in the human gastrointestinal tract and help lactase-deficient subjects to digest lactose [26]. Drouault et al. [27**] showed that lactococci that had been genetically modified to contain high quantities of intracellular lipase (by insertion of the *Staphylococcus hyicus* lipase gene) helped lipid digestion in an animal model of pancreatic insufficiency. This vector was chosen because it is easily lysed by bile; this study demonstrates that bile-sensitive probiotics can be used to deliver active ingredients in the small intestine (probably in the duodenum). *Oxalobacter formigenes* is a bacterium that colonizes the gastrointestinal tract and degrades oxalate. Sidhu et al. [28] showed that gavage of rats with *O. formigenes* reduced urinary oxalate excretion. Such an approach seems feasible for the prevention of oxalate kidney stone disease.

Genetically modified lactic acid bacteria have been proposed as a vehicle to deliver vaccines in the gastro-intestinal tract. Their potential benefits could be protection against antigen digestion, programmed release in the gut lumen (targeting), increased immunogenicity in comparison with isolated antigens, and the potential for including (by recombination) several antigens allowing combined vaccinations. Several secretion-expression probiotic vectors have been constructed and are currently being tested in animal models [29]. The cytokine IL-10 has shown promise in clinical trials for the treatment of inflammatory bowel disease, especially Crohn’s disease. It influences the inflammation cascade, especially the balance between Th1 and Th2 responses in the inflammatory lesions. Steidler et al. [30]
modified the genome of a *Lactococcus lactis* strain to make it produce high levels of IL-10. Intragastric administration of this IL-10-secreting probiotic caused a 50% reduction in mice with colitis induced with dextran sodium sulfate and prevented the onset of colitis in IL-10 knockout mice (two models of inflammatory bowel disease). Thus, the probiotic vector could provide a way to deliver the active ingredient (IL-10) at the site of inflammation. Other probiotics carrying different immunomodulating molecules are currently being tested (e.g., superoxide dismutase, trefoil peptides). As Crohn’s disease mainly affects the distal ileum and colon in humans, a probiotic vector with good pharmacokinetics to this target should be selected for future trials.

**Conclusions**

There is now real evidence that probiotics significantly influence health; however, they are not a panacea. Negative trials have also been published and should be encouraged, as one might learn from them and because no information should be hidden. New molecular tools, especially DNA microarray techniques, will allow us to further our understanding of how commensal or exogenous microorganisms modulate the expression of genes involved in several important intestinal functions including immunity.

**References and recommended reading**

Papers of particular interest, published within the annual period of review, have been highlighted as:** of special interest** and •• of outstanding interest


This paper shows that commensal bacteria modulate the expression of some of the host genes involved in several important intestinal functions, including mucosal barrier fortification. These findings provide perspectives about the interactions between resident or ingested microorganisms and their hosts.


A well-designed double-blind placebo-controlled study showing that a probiotic preparation is very effective in the treatment of chronic relapsing pouchitis (an inflammatory bowel disease that occurs in humans after ileal anastomosis and in which the endogenous flora is thought to have a role).


This double-blind randomized placebo-controlled study shows that probiotic administration in mothers before birth, during lactation and in infants significantly decreases the risk of developing atopic eczema in the first two years of life. This result is in keeping with the theory that an excessive hygiene (or a too low bacterial priming of the immune system) in infancy is associated with an increased risk of allergy.


This paper shows that *Lactobacillus rhamnosus* GG is not effective in every case of allergy, as no effect was observed in adults or teenagers with birch pollen and apple allergy.


This paper shows that bacteria that are lysed by bile in the upper small intestine may be used to transport enzymes and cure disease. L. lactis that was genetically modified to contain high levels of lipase helped lipid digestion in a pig model with pancreatic insufficiency. Clinical applications can be expected, especially in patients with cystic fibrosis and insufficient response to classical pancreatic enzyme supplementation. Genetically modified probiotics could constitute new treatment strategies.

