

Invited Commentary

Immunology of pre- and probiotic supplementation

(First published online 25 June 2008)

The notion that ingestion of so-called ‘good’ bacteria (‘probiotics’) or food components favouring growth of probiotic bacteria (‘prebiotics’) can have advantageous effects on human health arouses strong opinions, both in agreement and against. Remarkably few data have been fuelling this debate. Nevertheless, two important studies have now appeared, one published in *The Lancet* showing that probiotic supplementation in patients with acute pancreatitis is deleterious⁽¹⁾ and one in this issue of the *British Journal of Nutrition* by Arribas *et al.*⁽²⁾, showing the beneficial effects of *Lactobacillus fermentum* in an experimental model of septic shock. Together with existing studies on the immunological effects of pre- and probiotic food supplementation, the main effects of such treatment seems localised to inducing anergy in the T cell compartment. Health claims of pre- and probiotic treatment should be interpreted within this framework.

The mucosal surface of the intestine represents the major contact of the body with microbiological stimuli and in this compartment is most of the immunological activity of the body. Despite the bewildering number of bacteria in the intestine, especially the colon, in most cases the human immune system acts to tolerate harmless bacteria while eliminating pathogenic bacteria. A variety of immunological mechanisms, which include the existence of an efficient colonic columnar epithelial barrier, the production of antibacterial peptides (for example, the production of α -defensins by the Paneth cells), constant sentinel-like surveillance by CD16^{low} monocytes and efficient recruitment of granulocytes to areas in which barrier integrity has been compromised mediates protection of the body against the microbiological onslaught on the mucosal surface⁽³⁾. Counterintuitively, reduced activity of these innate mechanisms, as a consequence of genetic defects, seems responsible for chronic intestinal inflammation in Crohn’s disease, which is associated with an abnormally exaggerated mucosal immune response to an otherwise normal intestinal flora⁽⁴⁾. The sheer size of the mucosal immune system as compared with other parts of the immune system makes the notion that influencing the mucosal immune system by dietary means plausible.

It usually assumed that the flora along the intestinal tract is established early in life and in mammalian organisms resembles the flora of the mother and is stable. Nevertheless, pre- or probiotic treatment seems capable of at least temporally altering this composition. Probiotics are a group of bacteria of which the *Lactobacilli* and *Bifidobacteria* are the most prominent members and it is claimed that they have anti-inflammatory properties. Accordingly, such probiotic bacterial strains protect against experimental colitis in rodents^(5–7) as well as exacerbations of

inflammatory bowel disease⁽⁸⁾ and topical allergy^(9,10) in human patients. In agreement, food supplements specifically enhancing the growth of probiotic bacteria are recognised to be beneficial in a variety of inflammatory conditions including inflammatory bowel disease^(11,12) and genetically engineering plants for the production of prebiotics has become an industry in its own right^(13,14) despite limited insight into the immunological mechanisms induced by such food supplementation. In contrast to most micro-organisms, some probiotic bacteria are capable of impairing the immunological reaction to their mucosal presence in particular and have a dampening effect on the adaptive immune system in general. To a certain extent probiotics exert their action by niche occupation and thus preventing colonisation of the bowel by pathogenic bacterial species^(15–17). Moreover, it has become clear that probiotic bacteria directly influence host physiology, especially barrier function^(18,19), but their anti-inflammatory effects in inflammatory bowel disease⁽²⁰⁾ and topical allergic disease^(20,21) suggest that a direct effect on the immune system is involved as well. As inflammatory bowel disease displays mainly Th1 characteristics and topical allergies are characterised by a Th2 phenotype and both benefit from probiotic supplementation, the immunological effects of probiotic bacteria probably do not involve altered Th1/Th2 polarisation. There is evidence from a double-blind placebo-controlled study that *Lactobacillus reuteri protectis* reduces short-term sick-leave from work caused by respiratory or gastrointestinal infections^(22–24). This effect is also not easily explained via an influence on altered Th1/Th2 polarisation but suggests effects of probiotic treatment at a more fundamental level of human immunobiology.

The study of Arribas *et al.*⁽²⁾, which documents a preventive effect of *Lactobacillus fermentum* in a murine model of septic shock, provides important clues as to what these mechanisms might be. The authors document markedly reduced splenic T cell responses, suggesting that the T cell compartment might be the relevant target for many of the effects observed by probiotics. Also, the earlier observation reported by the same laboratory that different probiotic bacteria, as well as prebiotics, are beneficial in a trinitrobenzenesulfonic acid model of rat colitis (which represents a classical T cell-mediated inflammation) points in this direction. Interestingly, both *in vitro* as well as in human volunteers or patients with Crohn’s disease, *Lactobacillus rhamnosus* induced an anergy-like T cell hyporesponsiveness which was apparently mediated via reduced co-stimulatory activity in dendritic cells⁽²⁵⁾, which fits well with a scheme that pre- and probiotics act through diminished T cell activation⁽²⁵⁾. This hypothesis also proves exceedingly useful in explaining the clinical

efficacy in Crohn's disease of *Lactococcus lactis* expressing IL-10 where intravenous application of this IL is clinically not useful^(26,27); the bacterial context of the IL-10 might result in combined T cell anergy and IL-10-dependent induction of tolerance and thus be synergistic. In this context it should prove highly interesting to compare the efficacy of *Lactococcus lactis* expressing IL-10 to the mother organism not expressing IL-10 in Crohn's disease, but such a study has not been performed.

The molecular basis of the immunological modulation by pre- and probiotics on T cell reactivity remains unaddressed, but may lie in the secretion of soluble factors interfering with dendritic cell specific intercellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN)⁽²⁸⁾. This may provide the bacterium with an evasive strategy with respect to the immune system and hence a competitive advantage at the mucosal surface and thus provide the bacterium with a niche for cell growth in the bowel. How bacteria prevent other micro-organisms from profiting from this niche is unclear, but evidence exists, for instance, that *Lactobacillus rhamnosus* is able to suppress the growth of a diverse range of other bacteria via a variety of strategies^(29–31).

Strikingly, in the recent trial of probiotics in human acute pancreatitis⁽¹⁾ the supermortality in the probiotic-treated group seemed mainly due to enhanced bowel ischaemia in these patients. Since there is good evidence linking bowel ischaemia severity to reduced T cell reactivity⁽²⁷⁾, effects of probiotic bacteria on T cell reactivity may be linked to negative effects of probiotics in this disease.

In conclusion, also based on the study by Arribas *et al.*⁽²⁾, a picture emerges in which pre- and probiotics exert a class effect on reactivity in the T cell compartment, possibly mediated via reduced co-stimulatory activity of dendritic cells. In view of this class effect, the current emphasis in the field that each different pre- and probiotic should be considered individually and that results obtained with different strains should not be extrapolated to related strains may be somewhat overemphasised. In other words, the bioanalytical profiling of both host microbiota and probiotic will be crucial for defining how best to apply prebiotics and probiotics: optimal dosage, frequency, duration, and consequently providing a rational basis for supporting the belief that their consumption results in health benefits.

The authors have no conflict of interest to declare.

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