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From Cheese to Pharma: A Designer Probiotic for IBD

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Probiotics are defined as live microorganisms which, when administered in adequate amounts, confer a health benefit on the host FAO/WHO report, 2001: (ftp.fao.org/es/esn/food/probio_report_en.pdf). Probiotic microbes are contained in various fermented food products that have been ingested for centuries. For example, Kefir is a fermented milk drink which contains a variable mixture of bacteria and yeast. It has been ingested in Russia and the Middle East for thousands of years. Probiotic microbes are now marketed widely for various health benefits but are considered food products and so are not subject to the strict regulations for testing or composition that are required for pharmaceuticals. Claims made for probiotic benefits are far ranging, not evidence-based, and in some instances, imaginative. Certain organisms such as lactobacilli, bifidobacteria, and some yeasts have demonstrated beneficial effects in clinical studies. In recent years, the interest of the scientific community in probiotics has been stimulated as laboratory studies have shown intriguing biologic effects of these microbes.

The interactions of the host with the microbial world are quite complex, but newer technologies are allowing these to be dissected, including the interactions of the host with its microbiota. The intestinal microbiota contains some 100 trillion microbes and only recently has this complex community begun to be defined using PCR-based genetic technologies ^{1, 2}. At the same time, the cellular and molecular mechanisms by which the host lives at peace with this huge number of colonizers are slowly being revealed ³. When these mechanisms go wrong, chronic inflammatory bowel disease can result ⁴. This has been shown clearly in experimental models and the same is likely true for humans with Crohn's disease and ulcerative colitis, i.e., these appear to be disorders of host-microbial homeostasis in the intestine. Probiotics enhance the normal homeostatic mechanisms in the intestine. Thus there is a scientific rationale for trials of probiotics in patients with IBD.

Probiotics transit through the gut once ingested, but most do not colonize or persist in the intestine for long because the existing microbial flora are fierce defenders of their space. Probiotics may allow a depleted flora to restore itself, i.e., in acute infectious diarrhea, but once the flora is restored the probiotic microbes leave the intestine. Nevertheless, while these organisms are in the intestine, they are able to interact with the epithelial layer and with the many types of immune cells lying below it ⁵. Many probiotics are derived from the commensal bacteria and some members of the commensal flora have similar effects although they are not considered traditional probiotics. These microbes can have numerous effects on host cells. For example, *Lactobacillus rhamnosus* GG prevents cytokine-induced apoptosis of intestinal epithelial cells *in vitro* by activation of anti-apoptotic molecules and inhibition of pro-apoptotic p38 MAP kinase ⁶. Other microbes target the epithelial cell NF- κ B pathway for inhibition, either by inhibiting ubiquitination of I κ B, thus preventing translocation of the NF- κ B p65/p50 heterodimer to the nucleus ⁷, or by upregulation of PPAR γ which enhances the nuclear removal of transcriptionally active NF- κ B p65/p50 heterodimer from the nucleus ⁸. The

probiotic VSL#3 has been shown to induce the production of the inhibitory cytokine, interleukin 10 (IL-10), in both human and mouse cells, and in the latter instance IL-10 induced regulatory T cells that were able to ameliorate experimental colitis^{9, 10}. These examples illustrate some of the complex effects that microbes can have on host cells and provide support for the idea that probiotic microbes may have beneficial effects even in inflamed intestine.

Most probiotics are naturally occurring microbes that have been selected empirically. Their safety is based on experience with them over the years, rather than on rigorous scientific testing, and thus they are categorized as “Generally Regarded as Safe” by the Food and Drug Administration. This empirical approach in selecting and validating potential probiotic organisms has been followed for centuries. However, why wait for nature to reveal probiotics with the desired properties? Why not engineer microbes with the desired traits, thus ensuring a more effective result? The first human testing of such a genetically-modified probiotic is reported in the June issue of *Clinical Gastroenterology and Hepatology*. Braat, et. al., have inserted the human IL-10 gene into the chromosome of *Lactococcus lactis*, in place of the thymidylate synthase gene (thyA), a gene required for replication and thus environmental persistence. *L. lactis* is a non-motile, non-sporulating gram positive bacterium used in the food industry for the generation of buttermilk, cheese, and other foods. The strain of *L. lactis* used in the present study, *L. lactis thy12*, has no plasmids or antibiotic resistance genes, and cannot replicate unless supplemented with thymine or thymidine, which would not occur outside the body. This organism can survive in the gut but does not colonize it. Overall these properties of this engineered strain seem ideal for the purpose of safe delivery of a biological agent to the intestinal mucosa.

The emphasis in the present paper is on the safety of this genetically-engineered microbe in patients with Crohn's disease. Ten patients with moderately active Crohn's disease that was moderately active despite conventional therapy were sequestered on a containment ward and given the *L. lactis thy12* producing IL-10 as a lyophilized preparation in capsules twice daily for 1 week. Recovery of the *L. lactis thy12* in stools indicated that >90% were dead and none of the viable *L. lactis thy12* in feces made synthesized IL-10. No *L. lactis thy12* DNA was detected in feces 2 days after ingestion ceased. There was no evidence of transfer of the IL-10 transgene to other bacteria in the gut. Thus the biologic containment of this organism that was predicted in theory was borne out experimentally. There were no untoward side effects in the patients from the ingestion of the microbe, and thus the expected safety of the microbe was verified, at least for short term exposure.

This phase I study cannot tell us much about the efficacy of this microbe in patients. IL-10 provided parenterally as a purified pharmaceutical was not effective in patients with Crohn's disease^{11,12}. This was disappointing and a bit surprising in that IL-10 plays a key role in intestinal homeostasis as demonstrated by the colitis that develops in mice deficient in IL-10. *L. lactis thy12* producing IL-10 have previously been shown to ameliorate colitis in two models of experimental colitis, providing proof of principle that topically delivered IL-10 can be therapeutically efficacious¹³. Will the same happen in humans? If it does, it would imply that the reason the parenteral IL-10 failed to work was that not enough of it got to the intestine. There were some beneficial effects in the present uncontrolled study, with a reduction of the mean CDAI from 300 to 230 during the week of ingestion. The patients enrolled were candidates for infliximab and would be a stiff challenging group in whom to test for any therapy. Moreover very few current therapeutics would have much effect in only 7 days. But more definitive data are needed before efficacy can be assessed.

One benefit of this approach is that one does not have to synthesize and purify the IL-10 or other biological delivered in this manner. This saves great expense and difficulty. However the dose delivered topically to the mucosa is undoubtedly an important variable in efficacy and

the dose delivered by microbes will be difficult to measure or control. This will provide a challenge for both clinicians and regulatory agencies, who pay close attention to dose and dose-response of traditional pharmaceuticals. If thymine is needed to keep the *L. lactis thy12* alive and producing IL-10, an obvious question is whether the amount of thymine in the intestine is variable among humans. And will rapid transit diminish or prevent the effects of this microbe? Many such questions remain to be resolved. However the present study seems to be a promising start into a new era of genetically modified, i.e., 'designer' probiotics for delivery of therapeutic agents into the intestine.

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