Actobiotics for Healthcare: Precision Intervention in Human Diseases through Genetically Modified *Lactococcus lactis*

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We have developed ActoBiotics™, genetically modified (GM) bacteria that synthesize and deliver therapeutic proteins. ActoBiotics support a versatile topical and active delivery technology platform - TopAct™ - that provides an elegant solution for the oral delivery of therapeutic proteins and peptides.

By engineering *L. lactis* to express biologically active murine Interleukin-2 (mIL-2), we pioneered the use of recombinant *L. lactis* strains for the production of cytokines (*1*). In a subsequent study, we immunized mice intranasally with different strains of *L. lactis*, engineered to concomitantly accumulate tetanus toxin fragment C and secrete either mIL-2 or mIL-6 (*2*). The results showed a clear stimulatory effect for coinciding cytokine secretion, demonstrating that ActoBiotics can deliver cytokines to the mucosa. It now became possible to tailor immune responses through ActoBiotics that express the appropriate cytokines. We next designed IL-10-secreting *L. lactis* and found that oral administration substantially improved or prevented experimental colitis *in vivo* (*3*). The results provided unprecedented evidence for the therapeutic efficacy of *L. lactis* strains engineered to secrete cytokines, and established proof-of-concept for a new approach to the treatment of inflammatory bowel disease (IBD).

Trefoil factor (TFF) peptides enhance mucosal protection and healing, and could represent a new approach for mucosal healing (*4*). Vandenbroucke and colleagues showed that intragastric administration of TFF-secreting *L. lactis* bacteria resulted in efficient delivery of TFFs to the colonic mucosa, and prevented or healed experimental colitis *in vivo*. A TFF secreting *L. lactis* strain is currently being developed for treatment of oral mucositis as a consequence of induction chemotherapy.

Many clinical attempts to induce oral tolerance for therapeutic purposes have failed. In a study by Huibregtse and colleagues, *L. lactis* bacteria were engineered to secrete ovalbumin (OVA), and their ability to induce OVA-specific oral tolerance was evaluated in transgenic mice (*5*). The results indicated that engineered *L. lactis* bacteria could become an efficient tool for inducing antigen-specific tolerance, with possible application in the treatment of antigen-induced autoimmune diseases.

Clinical use of live GM bacteria requires stringent environmental containment. We developed a new, ingenious containment strategy with both bacteriostatic and bactericidal properties, centered on removal of thymidylate synthase gene (*thyA*) (*6*). In this particular case, the chromosomally-located *thyA* gene was replaced

with a synthetic human IL-10 (hIL-10) gene. Subsequently, the GM *L. lactis* strain (*L. lactis* Thy12) was shown to be environmentally contained and to secrete hIL-10 *in vivo*.

Following promising preclinical indications, *L. lactis* Thy12 was authorized for use in a pioneering Phase 1 clinical trial (*7*). The study was aimed at evaluating the safety, tolerability and containment of the hIL-10-secreting *L. lactis* strain in 10 patients with moderate to severe Crohn's disease. This first-ever human trial demonstrated that the use of GM bacteria for mucosal delivery of proteins could emerge as a viable strategy in IBD. In fact, a similar *L. lactis* strain is currently being tested in a Phase 2 clinical trial in ulcerative colitis patients, and if successful, could become the first oral protein drug available for IBD.

References

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