In situ Delivery of Therapeutic Proteins by Recombinant *Lactococcus lactis*

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Chronic inflammatory bowel disease (IBD) such as Crohn's disease or ulcerative colitis, affects around 2 in every 1000 individuals in western countries and it's incidence, particularly amongst children, is increasing. The symptoms of IBD are extremely unpleasant and impact all aspects of quality of life. They include diarrhea, abdominal pain, rectal bleeding, fever, nausea, weight loss, lethargy and loss of appetite. If left untreated, IBD can even lead to death.

Conventional treatment of IBD involves powerful immunosuppressive chemotherapies and often surgical intervention during active, severe disease. Long-term anti-inflammatory medication is required where inflammation is less severe or following surgery as prevention against relapse of the disease. Administered orally or by injection, only a fraction of these drugs reaches the intended target site, the inflamed intestinal lining. This is not only an inefficient way to deliver drugs, but, more important, means that patients are often subject to a spectrum of unpleasant side effects.

Interleukin-10 (IL-10) is a cytokine that acts to suppress inflammation. Mice that cannot make IL-10 spontaneously develop IBD[1]. Therefore, IL-10 was considered a good candidate therapeutic in the treatment of IBD. Local delivery of recombinant IL-10 is strongly jeopardized by its extreme sensitivity. When however administered by injection, the high levels of IL-10 that are distributed throughout the body can lead to a number of important side effects.

Lactococcus lactis can be genetically engineered in such way that it secretes biologically active cytokines[2,3]. When applied to the mucosa, the engineered *L. lactis* strains can actively deliver such cytokines. Following intranasal inoculation of TTFC expressing *L. lactis* that either secrete IL-2 or IL-6, a marked increase of the immune response against TTFC was observed[4]. By use of this principle - active in situ delivery of a therapeutic agent via de novo synthesis by genetically engineered bacteria - we developed a new therapeutic approach for IBD[5]. Intragastric administration of *L. lactis* engineered to secrete murine IL-10 produced a 50% reduction in colitis induced in mice by periodic addition of dextran sulfate sodium, as well as prevented the onset of colitis in IL-$10^{-/-}$ mice. The use of the engineered *L. lactis* gets around the problem of delivering IL-10. This can now be achieved by active in situ synthesis in the intestine, which protects the therapeutic from breakdown along the way through the stomach and small bowel and also avoids its systemic distribution. By this strategy, doses of 10000-fold less IL-10 effectively cure IBD in mouse models as compared to doses adminstered by injection.

The inability to sense acute bacterial infection was shown to be a risk factor in the development of Crohn's disease[6,7]. Therefore, acute intestinal inflammation may offer a therapeutic window to

counteract IBD. Conventional anti-inflammatory therapy does not improve or can even aggravate the health condition of experimental acute inflammation in animals[8,9]. Trefoil factors (TFF) are involved in intestinal epithelial repair. Therefore they offer an intriguing tool to address acute colitis. There are few reports of successful use of TFF for the treatment of colitis and none report success following oral administration. We have constructed TFF-secreting *L. Lactis* and shown their effectiveness in preventing acute DSS colitis (Vandenbroucke, submitted).

This work has drawn a lot of attention in both the specialized and the popular press. Most commentators however expressed their–sincere-concern about the possible dangers of uncontrolled, deliberate release of genetically modified micro-organisms, as could occur when administered to humans. Therefore we engaged in the establishment of adequate means for biological growth control of engineered *L. lactis*.

The *thyA* gene product, thymidylate synthase, is a methyl transferase that provides the cell with thymine or thymidine by modifying uracil or uridine. Consequently the *thyA* gene is essential in *Escherichia coli*[10]. We therefore replaced the *L. lactis thyA* gene[11] with the human interleukin-10 (hIL-10) gene.Correct integration of hIL-10 and deletion of thyA was proven by PCR, southern blot and DNA sequencing. The resulting strains all showed hIL-10 production. ThyA deficiency led to rapid *L. lactis* death in the abscence of thymidine. This system for biological containment as well as its delivery capacity showed effective in pigs and will now be used in humans suffering from IBD (Steidler, submitted).

1. R. Kuhn, J. Lohler, D. Rennick, K. Rajewsky, W. Muller, *Cell* 75, 263-74 (1993).
2. L. Steidler et al., *Appl Environ Microbiol* 61, 1627-9 (1995).
3. L. Steidler, W. Fiers, E. Remaut, in *Metabolism and Application of Lactic Acid Bacteria.*, B. Ray and F. Bozoglu, Eds. (Springer-Verlag, Berlin Heidelberg New York, 1996), vol. 98.
4. L. Steidler et al., *Infect.Immun.* 66, 3183-9 (1998).
5. L. Steidler et al., *Science* 289, 1352-5 (2000).
6. Y. Ogura et al., *Nature* 411, 603-6 (2001).
7. J. P. Hugot et al., *Nature* 411, 599-603 (2001).
8. G. Kojouharoff et al., *Clin Exp Immunol* 107, 353-8 (1997).
9. W. Hans, J. Scholmerich, V. Gross, W. Falk, *Eur Cytokine Netw* 11, 67-74 (2000).
10. S. T. Chung and G. R. Greenberg, *J Bacteriol* 116, 1145-9 (1973).
11. P. Ross, F. O'Gara, S. Condon, *Appl.Environ.Microbiol.* 56, 2156-63 (1990).