Expert Opinion

Transgenic probiotica as drug delivery systems: the golden bullet?

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Functional human proteins are constitutively produced in genetically modified bacteria that survive on human mucosal surfaces, to the benefit of the host. The successful Phase I clinical trial with IL-10-producing *Lactococcus lactis* for Crohn's disease has opened new avenues for the use of transgenic bacteria as delivery vehicles. The major advantage of this novel strategy is the avoidance of systemic side effects associated with conventional therapies. This methodology opens up an alternative method for local delivery of therapeutic proteins to various mucosal tissues.

Keywords: cytokines, lactic acid bacteria, transgenic probiotica

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1. Introduction

Due to technological advances, the insight into the molecular basis of disease is rapidly expanding. As a consequence, a multitude of possible therapeutic proteins, including cytokines and signalling molecules, have been identified. However, the therapeutic use of these possibly clinically relevant proteins is hampered by functional and economical considerations. The most important of these considerations are delivery and production of these proteins. It has recently emerged that by using genetically engineered bacteria, important barriers in this respect can be circumvented. Conventionally, therapeutic proteins are purified to become endotoxin free (which is very expensive), and are subsequently systemically administered with a suitable carrier into the bloodstream. The obvious drawbacks of this conventional strategy are the unwanted side effects arising as a consequence of the systemic delivery, and often prohibitively high production costs. Genetically modified (GM) bacteria that are well suited to co-existing with humans, and produce human therapeutic proteins are opening new ways for those who are suffering from disease conditions: the delivery of proteins in the tractus (e.g., intestinal tract) circumvents the need for endotoxin-free preparations. By their very nature bacteria are cheap producers of recombinant proteins and for digestive tract related diseases, delivery can be topical rather than systemic.

2. Studies of transgenic bacteria as delivery vehicles

Microbes have long been consumed in a variety of fermented foods and drinks, and reside in the intestinal milieu, to the benefit of the host – these probiotic microbes are classified as GRAS (generally regarded as safe) organisms. Furthermore, these food-grade organisms have been genetically engineered to produce various foreign proteins for immune modulation. For example, antigens from pathogens for use as a prophylactic immunization, and proteins such as cytokines, interleukins and interferons, have been expressed. Local delivery of recombinant therapeutical proteins to the affected intestine in many disease conditions has many advantages; targeting to the mucosa can be easily

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achieved by administering GM bacteria that produce therapeutic proteins to prevent and combat infections. In the oral cavity, GM *Streptococcus mutans* were used to prevent dental caries by a novel approach postulated as replacement therapy [1]. A different approach was reported by Paton *et al.* who used engineered *E. coli* that express a range of toxin-binding receptors to capture bacterial toxins to prevent enteric infections [2-4]. Similarly, GM *Streptococcus gordonii* have been used to produce single chain antibodies (ScFv) specific for *Candida albicans* [5], as well as recombinant IL-1Ra [6]. The ScFv *S. gordonii* could eliminate experimental *C. albicans* in rats after successful colonization in the vagina along with the anti-inflammatory effects of the IL-1Ra expressing *Streptococcus gordonii*. In addition, oral immunization of *Listeria monocytogenes* expressing the Gag protein of the HIV results in increase of Gag specific $CD8^+$ cells in mice [7]. Non-pathogenic strains of *Salmonella typhimurium* are used to express virulence factors of *L. monocytogenes*. A second use of GM bacteria is to restore metabolic enzyme deficiencies or other human proteins. For example, *L. lactis* expressing eukaryotic lipase were effective in treating pancreatic insufficiency in pigs [8]. Third, GM bacteria can be used to modulate the immune system. *Lactobacillus casei* is used to deliver bovine β-lacto-globulin during neonatal colonization, in order to to prevent milk allergic reactions in mice [9]. Likewise, *Lactobacillus* is extensively used in active vaccination and other immune interventions, which are discussed below. Thus, both by niche occupation, and by the production of recombinant proteins, GM bacteria may be clinically interesting.

It is especially interesting in this context that, next to pancreatic insufficiency, *L. lactis* has been used to deliver different therapeutic proteins orally. Trefolin factors involved in the protection and repair of the intestinal epithelium were expressed in *L. lactis*, and when delivered orally, had a striking protection against DSS-colitis in mice [10]. *S. gordonii*, that were genetically modified to express IL-1Ra (pro-inflammatory), improved the condition of $IL\text{-}2^{-/-}$ mice that spontaneously developed ulcerative-colitis-like pathology. The delivery of IL-10 locally by *L. lactis* has proven to have many advantages over systemic administration in mice. This improved status in mice with inflammatory bowel disease (IBD) was followed by the first clinical trial of human IL-10 producing *L. lactis* in IBD patients (see below) [11,12].

Likewise, *Lactobacillus jensenii* that were modified to secrete cyanovirin-N successfully inhibited HIV when delivered vaginally [13]. *Lactobacillus zeae*, engineered to produce a ScFv against *S. mutans* antigen I/II adhesion molecule, markedly reduced *S. mutans* counts and caries scores in a rat model [14]. Intranasal application of live bacteria that express antigens has the beneficial effects of increasing the antigen-specific antibodies during infection [15], and at the same time lowering the levels of antibodies for the allergic epitope [16].

Braat *et al.* conducted the first successful human trial with a GM *L. lactis* strain, in which the thymidylate synthase gene was replaced with a synthetic sequence encoding mature human IL-10 [11]. This novel treatment was safe, with minor adverse events and a decrease in disease activity. Fecally recovered *L. lactis*-thy12 were dependent on thymidine for growth, and IL-10 production indicated that the containment strategy was efficient. Bacterial-based topical delivery of biologically active proteins is a novel and highly promising avenue for combating mucosal disease.

One possible disadvantage of the usage of GM probiotica is that the delivery of powerful recombinant proteins is not targeted further within the intestinal tract. Potentially, this could lead to unwanted side effects. Therefore, a new generation of targeted GM Lactococci are now being designed.

3. Expert opinion

The execution of the first clinical trial is obviously of importance for further development of this field, but future studies are likely to include more specifically targeted recombinant proteins, for example by membrane expression instead of continuous secretion of the recombinant protein by GM organisms. In support of this view, membrane expression of ScFv, instead of secretion, turned out to be more efficient in the removal of *S. mutans* from the oral cavity [14]. Nevertheless, the present generation of clinically interesting GM organisms (GMOs) has already achieved substantially better local delivery of heterologously expressed proteins, compared with conventional systemic strategies. In this context, it is important to note that whereas GM-organism-delivered IL-10 seemed effective in the first placebo-uncontrolled trial, systemic IL-10 delivery did not seem clinically interesting, dramatically highlighting the power of local delivery. Further refinement of targeting strategies by this novel class of therapeutics will only further add to the promise of GMOs in clinical medicine. They are particularly attractive as an alternative to conventional therapy, given the increasing problem of side effects and the high production cost of conventional therapy. The most convincing evidence for the clinical effectiveness of recombinant probiotica certainly comes from the first clinical trial using recombinant *L. lactis* secretion of IL-10, and other animal studies. One important point to remember is that the various transgenic probiotica are different and each may have a different clinical effect in a specific disease. As well as modulating the immune response, transgenic probiotica are also used to deliver growth factors, silencer RNA, and peptides that inhibit appetite. Significant safety issues will need to be addressed. There is a seemingly limitless area for future research on the use of transgenic probiotica, and this novel technology clearly has great potential. Broad usage of such golden bullets, both as therapeutics and as functional food, may revolutionize the usage of probiotica in the near future.

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