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COMMENTARY

RECOMBINANT PROBIOTICS AND THEIR POTENTIAL IN HUMAN HEALTH

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ABSTRACT: ***The use of probiotics in the form of whole bacteria to achieve health benefits in humans is evolving by the development of recombinant probiotic bacteria as carriers of specific genes achieving a probiotic effect. Studies have demonstrated that bacteria carrying either the gene for IL-10 or for trefoil factors when given in an animal model or as part of a human trial can ameliorate inflammatory conditions of the colon. Such an approach has the advantages of a long-term delivery, the potential for fewer side effects and utility in many other conditions including other autoimmune diseases, dental caries, candidiasis and allergies. Other health benefits are also under investigation in relation to altering the fatty acid composition of adipose tissue by colonizing the intestine with a bacterium carrying the gene involved in fatty acid metabolism. Not only is the lean body mass advantageously affected but the enzyme is active in inducing apoptosis in cancer cells. Benefits may also be obtained not only by adding a gene but deletion of a gene from a bacterium which modulates the production of pro-inflammatory cytokines or may make the probiotic bacterium act as a more effective delivery system by enhancing colonization.***

KEY WORDS: Probiotic Bacteria, Recombinant Probiotics, Recombination

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The effects of probiotic bacteria on human health have been investigated mainly during the last two decades (Seegers, 2002; Wells and Mercenier, 2003; Hanniffy et al., 2004), but the potential for future developments in this field remains impressive. As a corollary of the investigations on probiotics, two very promising areas of study have emerged in recent years: i) bacterial derived molecules having probiotic properties (Caselli et al., 2011) and ii) the generation of recombinant probiotic strains able to express molecules with favourable pharmacological properties (Blaise Corthesy et al., 2007). In the present article we will focus our attention on this last issue. We will take into consideration findings related to bacteria stably transfected with genes coding for molecules able to prevent or cure different pathological conditions. Colonizing (e.g. *Streptococcus gondii*, *Lactobacillus plantarum*, *Lactobacillus paracasei*, *Lactobacillus casei*, *Lactobacillus acidophilus*) as well as non-colonizing (e.g. *Lactobacillus lactis*) bacterial species have been investigated both as live vaccine vehicles (acting as carriers for protective antigens) and as active producers of molecules with known pharmacological properties.

In respect to the use of these microorganisms as carriers for antigens, the most complete studies have been carried out with the 50kDa carboxyl-terminal fragment of tetanus toxin (TTFC) (Grangette et al., 2002); this approach has now been extended to additional antigens e.g. the B subunit of cholera toxin (Seegers, 2002; Wells and Mercenier, 2003; Hanniffy et al., 2004).

On the other hand, transfected bacteria have been used to deliver cytokines, but this technique was recently used to investigate other biological properties. Steidler chose to construct recombinant *Lactobacillus lactis* strains secreting murine IL-10 (Steidler et al., 2000). These authors demonstrated that these recombinant strains were able to prevent and treat inflammation in two murine models of colitis. Significantly, these same effects were obtained with much lower doses of IL-10 than those

required when IL-10 itself was used as a drug. The same authors further constructed a safe (no antibiotic resistance markers and a chromosomally integrated transgenic) strain of *Lactobacillus lactis* secreting IL-10 of human origin (Steidler et al., 2003). Authorization by a local ethical committee to carry out a phase 1 clinical study on voluntary patients has been obtained in the Netherlands (Braat et al., 2006). In this study, Crohn's disease patients were treated with recombinant *Lactobacillus lactis* (LL-THY 12) in which the thymidylate synthase gene was replaced with a synthetic sequence encoding mature human IL-10. The oral administration of this strain was safe and a decrease in disease activity was observed. The authors concluded that the use of genetically modified bacteria for mucosal delivery of therapeutic proteins is a feasible strategy in human beings. This strategy avoids systemic side effects and appears suitable as maintenance treatment for chronic intestinal diseases. Novel therapeutic strategies for acute and chronic colitis based on recombinant probiotics were also assessed by the generation and in vivo evaluation of *Lactobacillus lactis* strains secreting bioactive murine trefoil factors (TFF) (Van den Broucke et al., 2004). The authors demonstrated that intra gastric administration of this bacterial strain, but not of purified TFF, led to prevention and healing in the acute dextran sodium sulfate (DSS)-induced murine model of colitis, and were similarly effective in reducing established chronic DSS colitis. It has also to be remembered that production and mucosal delivery of different bioactive molecules such as allergens, digestive enzymes and single-chain Fw antibodies have been achieved using lactic acid bacteria (3). Targeted diseases included vaginal candidiosis (Beninati et al., 2000), dental caries (Ktuger et al., 2002), allergies (Kruisselbrink et al., 2001; Chatel et al., 2001; Repa et al., 2003; Daniel et al., 2006), autoimmune diseases (Maassen et al., 1999; Madsen et al., 1999), HPV-induced tumors (Bermudez-Humaran et al., 2005) and pancreatic insufficiency (Drouault et al., 2002). More recently, Rosberg-Cody (Rosberg-Cody et al., 2010) investigated whether a recombinant strain of *Lactobacillus paracasei*, previously isolated from the human gastrointestinal tract, expressing conjugated linoleic acid (CLA) isomerase from *Propionibacterium acnes,* could influence the fatty-acid composition of different tissues in the mouse. Ingestion of the *Lactobacillus paracasei* strain expressing CLA isomerase was associated with a 4-fold increase ($p > 0.001$) in t10c12 CLA in adipose tissues of the mice when compared with animals that received the non CLA producing isogonics strain. These data show that a single gene encoding CLA isomerase expressed by an intestinal bacterium can influence the fatty-acid composition of the host adipose tissue. This t10c12 CLA isomer is also associated with decreased body fat and increased lean body mass in various animal species (Park et al., 1997; Cherian et al., 2005; Yamasaki et al., 2003; Navarro et al., 2006; Ostrowska et al., 1999) and, to some extents, human beings (Blankson et al., 2000; Mougios et al., 2001; Riserus et al., 2001; Smedman and Vessby, 2001; Thom et al., 2001). It is also well know that t10c12 CLA isomer is the most potent isomer in terms of potential to prevent cell proliferation and induce apoptosis in cancer cells (Cho et al., 2005; Cho et al., 2006; Kim et al., 2002a; Lee et al., 2006a; Ochoa et al., 2004) notably, when the microbial derived t10c12 CLA was incubated with SW480 colon cancer cells for 5 days, cell viability was decreased by 92% (Rosberg-Cody et al., 2007), and it is possible that a CLA-producing probiotic will may be able to keep colon cancer cells in check. Although commensal *Bifidobacterium* and Lactobacillus species from the gastro-intestinal tract have been shown to produce CLA in vitro (Barrett et al., 2007; Coakley et al., 2003; Rosberg-Cody et al., 2004), the majority of these studies have demonstrated the production of c9t11 CLA from linoleic acid, while only a few bacteria such as *Propionibacterium acnes* (Verhulst et al., 1987), the rumen bacterium *Megasphera elsodenii* (Kim et al., 2002b), and the human derived *Lactobacillus rhamnosus* PL60 and *Lactobacillus plantarum* PL 62 (Lee et al., 2006b; Lee et al., 2007) have been reported to produce t10c12 CLA. Modulation of fatty acid production by bacteria may represent a very important probiotic activity and recombinant probiotics may become useful for this in the near future.

Recombinant probiotics may be linked not only to the addition of one or more genes but also to the deletion of one or more genes. In fact, to study the molecular mechanisms involved in the induction and repression of intestinal inflammation, Mohamadzadeh (Mohamadzadeh et al., 2011) has recently deleted the phosphoglycerol transferase gene that plays a key role in lipoteichoic acid (LTA) biosynthesis in *Lactobacillus acidophilus* NCK 56.

The results of these authors show that the *Lactobacillus acidophilus* LTA^- not only down regulated IL 12 and TNF alfa, which are known pro-inflammatory cytokines, but also significantly enhanced IL -10 production by dendritic cells (DC) and controlled the regulation of co-stimulatory DC functions, resulting in their inability to induce CD 4+ T cell activation. The treatment of mice with *L. acidophilus* LTA^-, compared with *L. acidophilus* LTA^+, significantly counteracted DSS-induced colitis. These authors concluded that directed alteration of cell-surface components of *L. acidophilus* represents a potential new strategy for the treatment of inflammatory intestinal disorders.

Moreover, efforts have been devoted to improve the efficacy of probiotic bacteria as delivery systems; in this context cell wall mutants of *Lactobacillus plantarum* and *Lactobacillus lactis* defective in alanine racemase (*alr* gene) were constructed (Palumbo et al., 2004; Steen et al., 2005): each of these mutants behaved as a substantially improved antigen delivery system compared with its wild-type counterpart (Grangette et al., 2004). The potency of the *Lactobacillus plantarum* Alr^- mutant was further confirmed using a weak immunogenic, such as *Helicobacter pylori* urease B, as a protective antigen; a significant reduction of the *Helicobacter pylori* load in the mouse stomach was achieved after immunization with the recombinant mutant *Lactobacillus plantarum* strain in contrast to results obtained with its wild-type counterpart (Blaise-Corthesy et al., 2005).

Any gene coding for an active molecule, potentially useful for human health, may be used to generate recombinant probiotic bacteria; in this context, an impressive number of

options are available to be investigated in *in vitro* and *in vivo* studies. It is worthy of note, however, that several gene products need glycosylation, phosphorylation or other more complex chemical changes; these may require the enzymatic machinery of eukaryotic cells. Thus, although current available genomic information should greatly facilitate the generation of useful recombinant probiotics, several technical issues and biologically limiting factors have to be taken attentively in consideration. In any case, the use of rapidly evolving genomic technology will surely help to evolve this intriguing and fascinating field and we can expect that from the present pioneering status we will soon progress to the generation of innovative therapeutic tools.

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