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Chapter 7

Genetically Engineered Bacteria in Gene Therapy — Hopes and Challenges

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http://dx.doi.org/10.5772/61042

Abstract

The main concern of gene therapy is to target the gene of interest to intended cell tissues for optimizing treatment efficiency. Genetically engineered bacteria have been developed as shuttle vectors for localized delivery of therapeutics. Their success depends upon their tropism to target cells and the efficiency of the engaged delivery system. Bodies of evidence clearly indicate the great potential of recombinant bacteria in gene therapy, although most of the studies were just looking for proof-of-concept rather than a ready-to-use final product. This part will provide an overview of our current understanding of bacteria-based delivery of therapeutic genes and heterologous antigens for prophylactic strategies.

Keywords: Recombinant bacteria, Gene delivery, Gene therapy, Immunoprophylaxy

1. Introduction

1.1. Concept of genetically engineered microorganisms as delivery vectors

Although significant progress has been made in physical and chemical methods for gene delivery, these nonmicrobial strategies still present some drawbacks related to specificity and efficiency of gene transfer [1–6]. For example, new formulations of lipid nanoparticles have led to great improvement in gene stability and transfer, yet there remains a lack of a targeting system that would favor the gene transfer to particular cell tissues [7]. Live avirulent microbial vectors such as viruses and bacteria are a promising approach for gene delivery that may serve

to fill in those blanks [8–14]. As such, microbial vectors are able to not only serve as cell factories for the production of the transgene but also as vehicles that deliver the transgene to specific cells for which they have a naturally high tropism. Gene transduction with recombinant viruses is generally based on the use of an expression cassette encompassing a transgene [8–11], while in bacteria, the classic approach of gene transfer is based on plasmid-encoded genes [12–14]. The gene of interest must be delivered to the cell's nucleus to allow an efficient manufacturing of the corresponding protein. DNA escape from intracellular bacteria to host cell cytosol may occur following their phagocytosis and lysosomal degradation within the cell. This is, however, not the case for intracellular bacteria that resist or subvert the phagolysosomal processing such as *Salmonella* or *Listeria* [15,16] and for extracellular bacteria that behave as commensals within a specific cellular niche. Commensal bacteria might be, however, of particular interest if the treatment strategy aims at delivering a gene product to targeted cell tissue through a potent delivery machinery. The delivery system used by avirulent vectors is therefore a critical point for optimizing the success of any therapy.

2. Bacteria as delivery vectors in gene therapy

Recombinant bacteria are being considered as an in vivo cell factory that could be used for the delivery of therapeutic genes to target cells. In this process known as "bactofection," a number of bacterial species have been developed as delivery vectors for their application in different therapeutic approaches.

2.1. Attenuated mutant bacteria

The most known bacteria for such purposes are *Salmonella typhimurium* strains that have proven to be useful in DNA vaccination approach. The strategy is based on the transformation of an attenuated strain with a plasmid DNA bearing the gene of interest. It has been shown that oral administration of such transformants into mice induced a robust immune response against gene-encoding antigen [17]. This study is the first to describe the possible transfer of a plasmid DNA from bacteria to host cells resulting in antigen processing and induction of specific immunity. This DNA vaccination approach has proven to be useful in prophylactic settings against tumor antigens. In the murine melanoma model, it has been shown that oral administration of attenuated *S. typhimurium* harboring gene sequences encoding tumoral peptide epitopes fused to murine ubiquitin gene could confer protection against tumor growth through the induction of a type I protective immunity [18]. This strategy of DNA delivery allowed an optimized antigen processing for vaccine development.

2.2. Naturally occurring nonpathogenic bacteria

The genus *Clostridium* comprises a group of nonpathogenic species that are strictly anaerobic and largely distributed in the environment. They are able to produce endospores that can selectively germinate under hypoxia. Given these characteristics, wild-type *Clostridium* has been used to target tumors that are known as poorly oxygenated tissues [19,20]. Various

http://dx.doi.org/10.5772/61042

experimental studies have reported the usefulness of clostridia in cancer therapy [21–25]. The injection of either whole *Clostridium* or spores into tumor tissues resulted in tumor destruction as a consequence of the multiplication of bacteria within colonized tumors. Subsequently, more elaborate strategies were developed for the potential use of *Clostridium* as a carrier to deliver prodrug converting enzymes into tumor tissues. Following systemic administration of the prodrug, the latter can be locally activated by the enzyme within tumor tissues, hence promoting a targeted effect against cancer cells. Therefore, selective exposure of tumor tissues to the effect of the prodrug is a promising strategy that may have broad applications in clinical studies. Likewise, recombinant spores of *Clostridium* or *Bacillus subtilis* have been used as a model for surface expression of vaccine antigens. This is based on insertion into chromosomal DNA of bacteria of the gene of interest which is fused to a gene encoding a spore surface protein. This stable genetic construction has allowed an efficient assembly and expression of a variety of fused proteins on the surface of the forming spores. The strategy of recombinant spores has been mainly tested for the development of mucosal vaccines [26].

Gene therapy in cancer has been also investigated using a food-grade microorganism *Bifidobacterium infantis*, which is a nonpathogenic and anaerobic bacterium that can proliferate in the hypoxic environment of tumor tissues as well. *B. infantis* has been applied as a gene delivery system in various cancer models such as bladder cancer including melanoma [27] thanks to its specific targeting property to the anaerobic environment of tumor cells. This bacterium has been successfully used for antitumor suicide gene therapy in a murine model of renal cell carcinoma [27,28]. This strategy is based on the use of the herpes simplex virus thymidine kinase/ganciclovir system to selectively kill tumor cells. Recombinant bacteria bearing virus thymidine kinase gene can replicate within tumor tissue and locally express the enzyme which, in turn, catalyzes the nontoxic precursor ganciclovir to a toxic form resulting in tumor cell killing through termination of DNA replication.

Lactococcus lactis is another food-grade bacterium that has been engineered for gene therapy in inflammatory bowel diseases (IBD). As this bacteria tends to naturally colonize the intestinal epithelium, they were used as vectors for localized delivery of anti-inflammatory mediators. In murine model of induced colitis, oral administration of recombinant *L. lactis* expressing IL-10 [29], IL-27 [30] or anti-TNF nanobody [31] could reduce intestinal inflammation, thereby offering a safe and reliable strategy for the treatment of IBD.

3. Type III delivery system: A promising strategy for targeting intended cell tissues

A broad spectrum of pathogenic bacteria (*Salmonella*, *Shigella*, *Yersinia*, *Pseudomonas*,...) use the type III secretion system (TTSS) to deliver their effector molecules to the membrane or into the host cell's cytosol to subvert the signaling pathways [32,33]. Most of the effector proteins are produced and stored inside bacteria before their secretion by the TTSS upon contact with host cells [34]. This elaborate process allows the bacteria to optimize the function of delivered molecules and, therefore, to resist the host defense mechanisms and proliferate within their

niche. The potential of TTSS in gene therapy has been investigated in various experimental models for localized delivery of vaccine antigens or therapeutic molecules.

3.1. Application in immunoprophylaxy

The first attempt in using the TTSS for the delivery of heterologous antigens for vaccine purposes was performed with attenuated *Salmonella*. It has been shown that recombinant *Salmonella* harboring a heterologous gene from pathogenic microorganisms fused to a *Salmonella* effector protein-encoding gene or to a small DNA sequence coding for bacterial signal peptide was able to deliver the hybrid protein into the host cytosol [35]. When injected into mice, these recombinant bacteria induced a protective cytotoxic T lymphocyte (CTLs) response against infection. Thus, the engagement of the hybrid protein by the TTSS allows their subsequent engagement by the major histocompatibility class-I pathway and generation of CTLs that are required for effective immunity against intracellular pathogens [36–38]. The use of the TTSS vaccination approach has been proven to work in different infectious models. In parasitic models of *Plasmodium berghei* infection, TTSS-dependent delivery of a dominant CD8 epitope by *Salmonella* conferred protection from infection in mice [39]. In a similar way, *Yersinia* has been used in vaccination studies in murine models to deliver antigens from a pathogenic protozoan parasite *Entamoeba histolytica* [40]. In this model, it has been shown that TTSS can mediate the delivery of high-molecular-weight antigen that induced significant protection against infection through promoting specific type 1 immune response.

The experimental approach of the bacterial TTSS in vaccination studies has been investigated in cancer models as well. Studies in mice indicated that oral administration of recombinant *Salmonella* expressing tumor antigens induced $CD8^+$ T cell-mediated control of tumor progression [41,42]. *Pseudomonas aeruginosa* was also evaluated as a live attenuated vector for TTSS delivery of antigen in antitumor vaccine experiments. Inoculation of recombinant *Pseudomonas* delivering ovalbumin to mice was shown to induce specific CD8+ T cell response that was associated with a significant resistance against ovalbumin-expressing tumor [43]. These experimental investigations underline the efficacy of this delivery system in antitumor immunoprophylaxy.

Besides their role in the delivery of heterologous antigens, bacterial vectors present major advantages over nonmicrobial adjuvant vaccines in that they are endowed with the ability to induce innate immunity through pathogen-associated molecular patterns (PAMPs). These specific microbial motifs include lipoproteins, lipopolysaccharides, single-strand RNA, and nonmethylated DNA sequences that can trigger the maturation process of antigen-presenting cells through binding to their specific Toll-like receptors and consequently induce the production of inflammatory cytokines [44]. This is particularly interesting for vaccination strategies aiming to optimize the protection efficacy [45].

3.2. Application in therapeutic development

Optimal efficiency of any microbial vector in gene therapy relies particularly on its ability to deliver a sufficient amount of the drug to targeted cell tissues while preserving healthy tissue.

http://dx.doi.org/10.5772/61042

The fact that *Shigella* specifically colonizes the colon and activates the TTSS upon contact with epithelial cells prompted their use as a candidate for localized delivery of anti-inflammatory mediators in inflammatory bowel diseases. Ulcerative colitis and Crohn's disease are characterized by the massive production of inflammatory cytokines such as TNF-α and IL-1β that mediate colon tissue destruction. Anti-inflammatory recombinant IL-10 was used successfully for treatment of IBD, although high doses and repeated administrations were necessary for minimal therapeutic efficacy [46–50]. *Bacteria* TTSS-mediated delivery of IL-10 may offer a good alternative of treatment targeting the colon. The proof-of-principle of this strategy was shown in inflammatory models of *Shigella* infection. When IL-10 was fused to a bacterial signal peptide, the hybrid protein was shown not only to be delivered by the TTSS of *Shigella* but also to be biologically active. Injection of IL-10 recombinant *Shigella* to mice induced a marked reduction of inflammatory symptoms as compared to wild-type *Shigella* and this was associated with a significant local reduction of TNF-α, a major inflammatory cytokine [51]. IL-1 receptor antagonist is a natural inhibitor that antagonizes the inflammatory potential of IL-1β. Imbalance between IL-1β and IL-1 receptor antagonist is associated with acute intestinal inflammation [52,53]. In keeping with this, it has been shown that delivery of recombinant IL-1 receptor antagonist in the intestine blocks IL-1β-mediated colitis in rabbits [54]. Localized delivery of IL-1 receptor antagonist by the TTSS of *Shigella* was shown to be as efficient as IL-10 in reducing the inflammatory symptoms within invaded tissues [51]. As outlined elsewhere, the treatment of experimental colitis could be partially achieved using IL-10 recombinant *Lactobacillus* that colonizes all the intestine. Nevertheless, *Shigella* may provide a useful alternative as a live vector thanks to its specific targeting to the site of IBD, the colon. Yet, due to safety concerns, this is possible only with the use of highly attenuated *Shigella* that can be biologically contained [55]. Furthermore, the efficiency of such an approach awaits additional insight into experimental intestinal models of *Shigella* [56,57]. Taken together, the use of bacterial TTSS for localized delivery of immunogenic antigens or therapeutic molecules may offer alternative options in improving the effectiveness of gene therapy.

4. Issues to overcome for better translating the generated proofs-of-concept to effective treatments in human

Bodies of evidence clearly indicate that bacterial vectors are a promising strategy for gene delivery. Many experimental investigations have shown proof-of-concept examples of the feasibility of such an approach, yet steps forward are still needed not only to translate these concepts into effective treatments for humans but also to find the perfect delivery system for each disease situation.

For safety reasons, nonpathogenic food-grade bacteria remain more attractive as live vectors for vaccine and therapeutic strategies. Some concerns exist, however, about targeting issues which is crucial for optimal efficiency. The best example is the potential use of *Lactobacillus lactis* for the delivery of IL-10 in the treatment of IBD. As this anti-inflammatory cytokine has a pleiotropic immunosuppressive effect, it is particularly crucial to target the inflammatory site while preserving healthy tissues. On the other hand, studies related to the potential

application of some microbial vectors in gene therapy are on hold for safety issues. Although research on attenuated bacteria has led to significant progresses in gene therapy, there remain some limitations that preclude their use in immunocompromised populations as well as in infants. The challenge is how to emphasize the benefits while controlling the disadvantages of these microbial vectors. With this regard, recent studies highlighted new lines of development of TTSS-based delivery in avirulent vectors. Interestingly, the gene locus coding for TTSS of *Vibrio parahaemolyticus* has been cloned into a nonpathogenic *E. coli* K-12 strain and shown to be efficient in the delivery of heterologous peptides. The generation of a nonpathogenic *E. coli* displaying an active TTSS is an important step that opens the way for applicability of TTSS-dependent delivery of foreign molecules [58]. In the same way, it has been shown that bacterial minicells derived from aberrant cell division of a mutant strain of *S. typhimurium* may assemble functional TTSS. These nonreplicating nanoparticles were shown to deliver antigen by the TTSS and to promote Th1 immune response, thereby offering an alternative strategy of antigen delivery platform for vaccine and immunotherapeutic developments [59].

5. Perspectives

Recombinant bacteria have shown great potential in the preclinical trials. Their clinical potential relies on their safety and biological containment. Most of the studies were just looking for proof-of-concept rather than a final product that could be put directly to use. Given the global needs, future research challenges should focus on the balance between the optimization of gene therapy through effectiveness of gene delivery to target cells and the biological control of recombinant bacteria to ensure not only an appropriate shutoff mechanism but also to minimize the risks of insertional mutagenesis and aberrant genomic location of delivered genes.

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