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Listeria monocytogenes as novel carrier system for the development of live vaccines

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Abstract

Listeria monocytogenes is a facultative intracellular bacterium that enters a variety of non-professional mammalian cells by triggered phagocytosis (''zipper mechanism'') and replicates in the cytosol of the infected host cells. Therefore, it is a promising vaccine vector for the presentation of passenger antigens to the MHC class II and especially class I pathways. Here, we review recent progress made in our laboratory on the development of novel attenuated *L. monocytogenes* carrier strains for the delivery of heterologous antigens or antigen-encoding DNA and RNA to eukaryotic host cells. Based on the deletion of the chromosomal copy of the tryptophanyl-tRNA synthetase gene (*trpS*) and plasmid-based in trans complementation of the same, we were able to establish a balanced-lethal plasmid system in *L. monocytogenes*. Safety concerns in the antigen delivery in vivo were addressed by chromosomal deletion of genes in the basic branch of the aromatic amino acid pathway, resulting in safe, attenuated *L. monocytogenes* carrier strains. Furthermore, plasmid-based expression of a cytosolically expressed phage lysin resulted in a self-destructing carrier strain that has been successfully used for the delivery of antigens as well as antigen-encoding plasmid DNA and particularly mRNA, therefore overcoming bottlenecks that have been shown to exist for bacteria-mediated DNA delivery.

Keywords: Vaccination; *Listeria monocytogenes*; DNA delivery; RNA delivery; Antigen delivery

Introduction

Since the successful eradication of smallpox in 1979 it has become evident that vaccines are among the most important achievements in modern medicine (WHO, 1980). Through nationwide vaccination strategies, the incidence rates of most of the common child-related diseases like mumps, measles, German measles, and polio could be lowered to up to 100% (Robinson and Amara, 2005). Induction of an immune response against certain pathogens is achieved by administration of either a dead or an attenuated live vaccine leading to protection of the host against a subsequent challenge with the respective wild-type strain. Depending on the

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type of vaccination, different immune reactions may be induced. In general, dead vaccines lead to the establishment of humoral immunity whereas live vaccines can also trigger mucosal and cellular immune responses, depending on the location inside the host (Zinkernagel, 2003).

Besides immunization against a wild-type variant of the vaccine strain itself, the use of live bacteria for the delivery of antigens and antigen-encoding DNA vaccines for the induction of protective immunity also against heterologous pathogens has been well established over the last decades (for recent reviews see Detmer and Glenting (2006), Daudel et al. (2007) and the references given therein). To this end, a quite diverse set of different bacterial carrier strains have already been investigated including non-pathogenic species such as *Staphylococcus carnosus*, *Lactococcus lactis* or *Streptococcus gordinii*, as well as virulence-attenuated strains of pathogenic species like e.g. *Staphylococcus aureus*, *Salmonella enterica* serovar Typhimurium or *Shigella flexneri* (Detmer and Glenting, 2006; Daudel et al., 2007). *Listeria monocytogenes* – as a Gram-positive facultative intracellular pathogen of humans – combines a number of advantageous features that makes its use as a live carrier system for heterologous antigens very promising.

Its life cycle in cell culture as well as the infection biology and immune response in animal model systems have been characterized in great detail over the past 20 years and therefore, *L. monocytogenes* has become a paradigm for an intracellularly replicating bacterial pathogen (for recent reviews see Vazquez-Boland et al. (2001), Pamer (2004)).

In addition, the complete genome sequences of two strains have been determined (Glaser et al., 2001; Nelson et al., 2004) which makes it possible to rationally design and generate defined virulence-attenuated strains (Darji et al., 2003; Schmid et al., 2005).

L. monocytogenes is normally taken up by the oral route mainly by contaminated food, and enters the underlying cells by penetration of the mucosal linings. In the murine model of systemic listeriosis, oral application of *L. monocytogenes* resulted in the generation of a strong mucosal CD4 as well as CD8T cell response (Huleatt et al., 2001; Pope et al., 2001; Kursar et al., 2002, 2004).

Besides its ability to infect a wide variety of different cell types inside the host (Vazquez-Boland et al., 2001), *L. monocytogenes* displays a natural tropism for dendritic cells (Pron et al., 2001) resulting in a potent priming of specific T cells (Feng et al., 2005). By virtue of its intracellular replication cycle, *L. monocytogenes*-derived antigens are subsequently presented mainly via the MHC-I pathway triggering a strong cellular immune response (Pamer, 2004).

Furthermore, as a Gram-positive bacterium, *L. monocytogenes* does not express lipopolysaccharide (LPS) and therefore is less likely to induce an LPS-mediated septic shock in contrast to Gram-negative bacteria like *S. flexneri* (Karima et al., 1999).

Of major importance for its practical application as vaccine carrier was the observation that virulence attenuation or pre-existing anti-listerial immunity in a murine model do not affect its ability to elicit effective immune responses against heterologous passenger antigens (Bouwer et al., 1999; Starks et al., 2004; Stevens et al., 2005).

The suitability as a potential vaccine carrier in humans of an attenuated Δ*actA*/Δ*plcB* mutant strain of *L. monocytogenes* was evaluated in a phase I safety trial in adult volunteers demonstrating that *L. monocytogenes* can be applied in high enough doses to elicit humoral, mucosal, and cellular immune responses without serious long-term health sequelae (Angelakopoulos et al., 2002).

Finally, the fact that *L. monocytogenes* can be easily cultivated to large numbers in simple growth media renders the production of larger quantities of vaccine strains in an industrial setting economically feasible. In the same vein, *L. monocytogenes*-derived carrier strains do not require an intact cold chain for storage which is often a serious logistical problem for many other live vaccines particularly in developing countries.

Therefore, *L. monocytogenes* has already been used successfully for the delivery of antigens and antigen-encoding plasmid vaccines in diverse model systems (for recent reviews see Dietrich et al. (2002), Paterson and Johnson (2004), Singh and Paterson (2006)).

This report summarizes recent advances made in our group evaluating attenuated *L. monocytogenes* as suitable carrier strains for the delivery of antigens and antigen-encoding DNA or RNA for vaccination strategies. A model summarizing our current understanding on how infection with *L. monocytogenes* carrier strains might lead to the presentation of the delivered heterologous antigens is presented in Fig. 1.

Delivery of antigen-encoding expression plasmids to eukaryotic host cells with *L. monocytogenes*

Besides the more common approach of immunization with purified or recombinant peptides and proteins, plasmid DNA encoding a vaccine antigen for expression in eukaryotic cells has also been successfully applied for the development of prophylactic or therapeutic vaccines (Gurunathan et al., 2000). Vaccination plasmids are usually composed of an origin of replication in bacteria, a selection marker and a eukaryotic expression cassette(s) which encode protein-antigens and allow their expression in mammalian cells. Usually highly purified, naked DNA is used for these approaches. However,