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Forum

Listeria as a vaccine vector

Kevin W. Bruhn [a,*], Noah Craft [a], Jeff F. Miller [b]

[a] *Department of Medicine, Division of Dermatology, Los Angeles Biomedical Research Institute at Harbor/UCLA Medical Center, 1124 W. Carson St., Torrance, CA 90502, USA*

[b] *Department of Microbiology, Immunology and Molecular Genetics, David Geffen School of Medicine, Molecular Biology Institute, University of California, Los Angeles, 10833 Le Conte Ave., Los Angeles, CA 90095, USA*

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Abstract

The immunostimulatory characteristics and intracellular niche of *Listeria monocytogenes* make it uniquely suitable for use as a live bacterial vaccine vector. Preclinical results supporting this idea, and current strategies to induce beneficial cell-mediated immunity to both infectious diseases and cancer with this vector, are discussed in this review.

Keywords: *Listeria monocytogenes*; Vaccine vector; Cancer immunotherapy

1. Introduction

The continued search for safer, more effective, therapeutic vaccines has led to multiple strategies to induce beneficial immune responses in the host. One strategy capitalizes on the immunostimulatory nature of live bacteria, utilizing them as vectors to deliver heterologous target antigens to the immune system. Basic research on the intracellular bacterium *Listeria monocytogenes* has revealed several unique biological and immunological characteristics that make it an ideal vaccine vector. *L. monocytogenes* triggers potent innate and adaptive immune responses in an infected host that are required for clearance of the organism. Its ability to inhabit both phagosomal and cytoplasmic compartments of host cells, combined with its inherent immunostimulatory capacities, results in enhanced antigen presentation and stimulation of effector and memory T cells. The efficiency of these cell-mediated immune responses has encouraged efforts to develop this bacterium as a recombinant antigen delivery vector to induce protective cellular immunity against infection or cancer.

Other viral and bacterial species have been utilized as live antigen delivery vectors, including vaccinia virus, *Salmonella*, *Shigella*, *Legionella*, *Lactococcus* and *Mycobacterium* (BCG), but several aspects of *L. monocytogenes* make it a uniquely attractive vaccine candidate. The bacterium can be grown under standard BSL2 laboratory conditions, and protocols to genetically manipulate the organism are well-established, allowing straightforward construction of recombinant vaccine strains. Large and/or multiple gene products can be expressed from a single recombinant strain. Much is known about the life cycle, genetics, and immunological characteristics of *L. monocytogenes*. This forms the foundation for the rational design of potent, specific, and safe vaccine platforms. Several laboratories have successfully induced protective immune responses by recombinant *L. monocytogenes* strains. At least two companies have initiated phase I/II clinical trials with specific *Listeria* vaccines. This review outlines relevant preclinical studies and their implications for the future development of recombinant *Listeria* as an effective clinical option to combat infectious diseases or tumor progression.

Abbreviations: LLO, Listeriolysin O; APC, Antigen presenting cell; CTL, Cytotoxic T lymphocyte; TLR, Toll-like receptor; NP, Nucleoprotein; TRP-2, Tyrosinase-related protein-2.

* Corresponding author. Los Angeles Biomedical Research Institute, Bldg. E-6, Rm. 145, 1124 W. Carson St., Torrance, CA 90502, USA. Tel.: +1 310 222 8011; fax: +1 310 222 5402.

E-mail address: kbruhn@ucla.edu (K.W. Bruhn).

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2. *Listeria* as an antigen delivery vehicle

2.1. Bacterial life cycle

Listeria is a facultative intracellular bacterium with a wide mammalian host range [1,2]. Its natural route of infection is oral, and translocation occurs across the gut epithelium prior to systemic dissemination. The bacterium can enter host cells via internalins, surface molecules that facilitate entry into non-phagocytic cells such as epithelial cells and hepatocytes. Disseminated bacteria are rapidly phagocytosed by macrophages and other phagocytic cells, predominantly in the liver (by Kupffer cells) and the spleen (by resident macrophages). Following internalization, fusion of phagosomal compartments with lysosomes normally leads to degradation of the invading bacterium. *Listeria*, however, is capable of escaping degradation by expressing the *hly* gene, which encodes a hemolysin called listeriolysin O (LLO). This enzyme degrades the phagosomal membrane and allows a small fraction of the bacteria to enter the cytoplasm. Once cytoplasmic, *Listeria* replicates, polymerizes host cell actin monomers to become motile, and ultimately spreads from cell to cell without leaving the intracellular compartment [3]. This ability of *Listeria* to inhabit the cytoplasmic niche facilitates its *in vivo* survival; bacteria that do not establish this niche (heat-killed bacteria, *hly* deletion mutants, LLO cytotoxicity mutants) are rapidly cleared.

2.2. Antigen presentation to $CD4^+$ and $CD8^+$ T cells

The ability of *L. monocytogenes* to inhabit the cytoplasm of host antigen presenting cells (APC) was a significant early rationalization for its use as an antigen delivery vector. Bacterial proteins secreted into the cytoplasm are directly accessible to the proteasomal machinery. This allows for protein degradation, transport of peptides into the endoplasmic reticulum, and loading onto MHC class I molecules (Fig. 1A) [4]. This endogenous pathway of sampling proteins for presentation to $CD8^+$ T cells (which normally displays self proteins) contrasts with the exogenous pathway, in which phagocytosed foreign material is directed into the MHC class II loading pathway and presented to $CD4^+$ T cells [5]. The presence of *Listeria* in both phagosomal and cytoplasmic compartments results in bacterial proteins having direct access to both MHC class II and class I molecules for antigen presentation to $CD4^+$ and $CD8^+$ T cells. Both of these T cell subsets are required for optimal clearance of *Listeria*. Effector $CD8^+$ cytotoxic T lymphocytes (CTL) expand following infection, mediate recognition and destruction of infected cells, then undergo a contraction phase which leaves a population of long-lived, protective memory T cells [6]. Activated $CD4^+$ helper T cells that differentiate into T_H1 effectors are critical for the production of cytokines, especially IFN-γ, that regulate development of persistent CTL. This effective T cell induction against *Listeria*-expressed proteins provides the rationale for the design

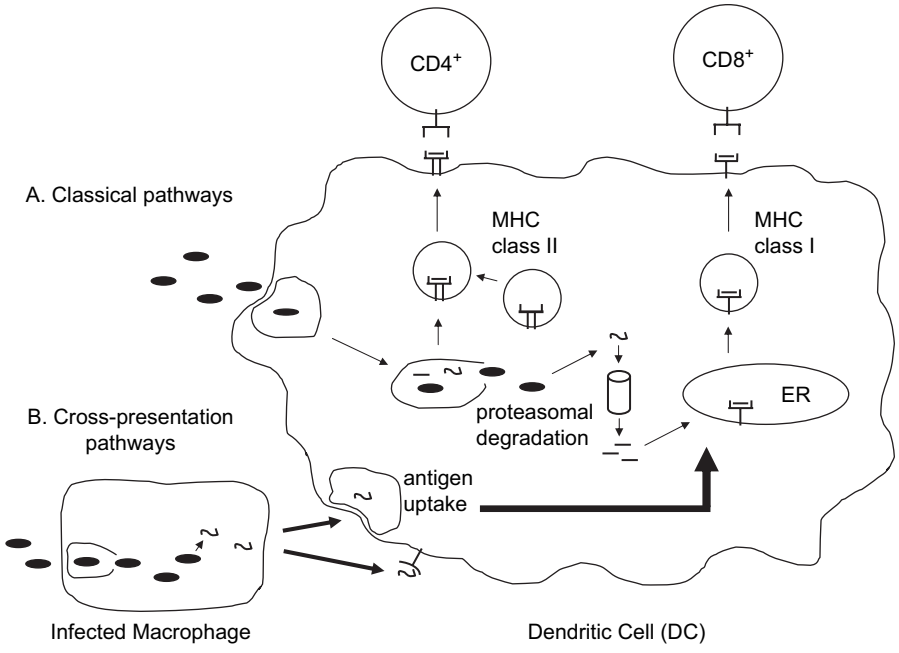

Fig. 1. Antigen presentation pathways for listerial antigens. (A) Phagocytosed bacteria are degraded in phagolysosomal compartments and protein fragments are directed into MHC class II-containing vesicles for loading and presentation to $CD4^+$ T cells (exogenous pathway) [5]. *Listeria* that escape into the cytoplasm secrete proteins that are processed by the cytosolic proteosomal machinery, and peptides are transported into the endoplasmic reticulum (ER) for loading onto MHC class I molecules and presentation to $CD8^+$ T cells (endogenous pathway) [4]. (B) Bacterial proteins originating from infected macrophages or other cell types can be acquired by dendritic cells (DCs) through a variety of mechanisms (phagocytosis, receptor-mediated endocytosis, etc.) and shuttled into both class II and class I pathways. These alternate means of acquiring and presenting antigen are termed "cross-presentation" [10].