Available online at www.sciencedirect.com
ScienceDirect
Vaccine 25 (2007) 2279–2287
Vaccine
www.elsevier.com/locate/vaccine

# Immunization with non-replicating *E. coli* minicells delivering both protein antigen and DNA protects mice from lethal challenge with lymphocytic choriomeningitis virus

Matthew J. Giacalone [a,b], Juan C. Zapata [c], Neil L. Berkley [a], Roger A. Sabbadini [a,b], Yen-Lin Chu [b], Maria S. Salvato [c], Kathleen L. McGuire [a,b,*]

[a] *Vaxiion Therapeutics, Inc., San Diego, CA, USA*
[b] *Department of Biology, Center for Microbial Sciences, San Diego State University, 5500 Campanile Drive, San Diego, CA 92182-4614, USA*
[c] *Institute of Human Virology, University of Maryland Biotechnology Institute, Baltimore, MD, USA*

Received 21 September 2006; received in revised form 20 November 2006; accepted 30 November 2006
Available online 26 December 2006

**Abstract**

In the midst of new investigations into the mechanisms of both delivery and protection of new vaccines and vaccine carriers, it has become clear that immunization with delivery mechanisms that do not involve living, replicating organisms are vastly preferred. In this report, non-replicating bacterial minicells simultaneously co-delivering the nucleoprotein (NP) of lymphocytic choriomeningitis virus (LCMV) and the corresponding DNA vaccine were tested for the ability to generate protective cellular immune responses in mice. It was found that good protection (89%) was achieved after intramuscular administration, moderate protection (31%) was achieved after intranasal administration, and less protection (7%) was achieved following gastric immunization. These results provide a solid foundation on which to pursue the use of bacterial minicells as a non-replicating vaccine delivery platform.


*Keywords:* Minicells; Vaccine; Immune response; Mucosal delivery; LCMV

## 1. Introduction

A major goal in vaccine development is the production of safe and efficient delivery mechanisms capable of eliciting protective immune responses. In the past decade, the use of attenuated bacterial pathogens such as *Listeria monocytogenes*, *Shigella flexneri* and a variety of attenuated *Salmonella* serovars have proven useful in the generation of both antibody and cell-mediated protective immunity in animal infection or tumor models [1–3]. In most cases, constitutive expression of heterologous protein antigens by these bacterial carriers *in vivo* elicits protective immune responses [4–6]. In addition to this strategy, *Salmonella typhimurium* and *Shigella flexneri* have been used to deliver plasmid DNA vaccines to elicit protective immune responses in mice [2,7]. In light of these successes, it should be noted that major safety concerns such as pathogenic reversion, horizontal gene transfer and adverse inflammatory responses with respect to using these approaches in human beings still exist [8–10].

The use of bacterial minicells presents a unique, alternative approach in furthering the development of safe and efficacious vaccine delivery. Minicells are small (100–400 nm), quazi-spherical, achromosomal bacterial particles that result from the disruption of the normal bacterial cell cycle [11,12]. Minicells contain many components of their parental cells including lipopolysaccharides (LPS), an

* Corresponding author at: Department of Biology, Center for Microbial Sciences, San Diego State University, 5500 Campanile Drive, San Diego, CA 92182-4614, USA. Tel.: +1 619 594 7191; fax: +1 619 594 5676.
*E-mail address:* kmcguire@sunstroke.sdsu.edu (K.L. McGuire).

0264-410X/$ – see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.vaccine.2006.11.069

intact peptidoglycan layer (cell wall), as well as any recombinantly expressed proteins and plasmid DNA molecules [11,13–16]. This is the same combination of components and properties that makes attenuated pathogenic strains effective as vaccine carriers. Minicells have already been used to express plasmid encoded antigens from a wide variety of pathogens [17–19] and have previously been used as vaccine carriers [20]. In those studies, heterologous protein antigens delivered by minicells resulted in antigen specific antibody responses. Studies using attenuated pathogenic bacteria to deliver DNA vaccines have been shown to elicit antigen specific cellular immune responses [2,21,22] suggesting that DNA vaccine delivery using minicells could potentially elicit similar responses.

There is mounting evidence to suggest that the administration of both DNA and the corresponding protein antigen vaccines, either sequentially or in combination, is more effective at generating robust immune responses [23–25]. Preliminary studies suggest that minicells co-delivering a eukaryotic expression plasmid and the corresponding protein antigen could generate much higher antigen-specific serum IgG antibody responses when administered intramuscularly (i.m.) [26]. More importantly, administration of minicells via the intranasal (i.n.) or oral (p.o.) routes of administration resulted in the production of both antigen-specific serum IgG and mucosal IgA.

In this report, bacterial minicells derived from a non-pathogenic *E. coli* K-12 strain capable of the simultaneous delivery of both recombinant protein antigen and the corresponding DNA vaccine are evaluated for their ability to elicit protective cell-mediated immunity against a lethal challenge with lymphocytic choriomeningitis virus (LCMV) in mice following i.m., i.n., or p.o. administrations. Eighty-nine percent of mice survived a lethal intracranial challenge when the vaccine was administered i.m., in comparison to 31 or 7% when administered i.n. or p.o., respectively. The investigation into the underlying cellular responses mediating immunity and survival is also described. Together, the results of this study demonstrate for the first time that non-replicating bacterial minicells can simultaneously deliver heterologous protein antigens and the corresponding plasmid DNA vaccine to a mucosal surface to elicit protective, systemic immunity.

## 2. Materials and methods

### 2.1. Bacterial strains

MPX1B9 [$F^-$, $\lambda^-$, *ilvG*, *rfb-50*, *rph-1*, *zac*::*aph*, *lacI*$^q$, $P_{tac}$ *ftsZ20*, Δ*phoA*] [26,27] is a genetically stable, minicell producing *E. coli* strain that was obtained from Vaxiion Therapeutics, Inc. Growth of MPX1B9 in Luria-Bertani (LB) broth requires 15 μM IPTG to support normal cell division and minicell induction requires additional IPTG inducer as described below.

### 2.2. Plasmid construction

The rhamnose inducible prokaryotic expression plasmid pRHA-67 was obtained from Vaxiion Therapeutics, Inc. This pUC18 derivative contains a multiple cloning site placed downstream of the *E. coli rhaB* promoter sequence as well as the two tandemly encoded *rhaB* regulatory genes *rhaR* and *rhaS* [28].

The coding sequence for the LCMV NP protein was PCR amplified using pCMV-NP as template DNA [29]. The forward primer was designed to introduce a *Sal*I restriction endonuclease site and the reverse primer designed to replace the natural stop codon with a FLAG® tag sequence positioned in front of a bacterial stop codon followed by an *Xba*I restriction endonuclease site. The appropriately sized 1712 bp product was subcloned into pRHA-67 that was previously digested with *Sal*I and *Xba*I to complete pMJG28. In a separate PCR reaction, the entire sequence for the eukaryotic expression cassette driving LCMV NP protein was amplified using pCMV-NP as a template. Both the forward and reverse primers of this reaction were designed to introduce flanking *Kpn*I sites. This 2774 bp product was subcloned into the unique *Kpn*I site of pMJG28 to complete pMJG30.

Prokaryotic expression of FLAG-tagged LCMV NP from pMJG30 was tested in *E. coli* Top 10 cells [F- *mcrA* Δ(*mrr-hsdRMS-mcrBC*) Φ*80lacZΔM15 ΔlacX74 recA1 araD139* Δ(*ara-leu*)*7697 galU galK rpsL* ($Str^R$) *endA1 nupG*] (Invitrogen, Carlsbad, CA) or in minicells derived from MPX1B9 harboring this plasmid as described below. Eukaryotic expression of LCMV NP from pMJG30 was confirmed in Cos-7 cells.

### 2.3. Minicell isolations

Plasmid DNA was introduced into MPX1B9 cells by electroporation using a Bio-Rad Gene Pulser™ apparatus and transformants selected for on LB agar plates containing 100 μg/mL ampicillin and 15 μM IPTG. Cultures of MPX1B9 cells harboring pMJG30 were started from a single distinct colony and grown overnight in 3 mL LB broth containing 100 μg/mL ampicillin and 15 μM IPTG at 37 °C (LB-Amp/IPTG). After overnight incubation, cultures were diluted 1:125 into 400 mL of LB-Amp/IPTG in a 1 L baffled shake flask. Cultures were grown to an optical density of 600 nm ($A_{600}$) = 0.1 at which time minicell production was induced by the addition of IPTG for a final concentration of 45 μM. MPX1B9 cells harboring pMJG30 were co-induced to produce minicells with C-terminus FLAG-tagged LCMV NP protein by the simultaneous addition of 1 mM rhamnose. Cultures were allowed to grow overnight for 15 h from time of induction.

Minicells were enriched by differential centrifugation and further purified by linear sucrose gradients as described previously [11] with LB broth containing 1 μg/mL ciprofloxacin substituted as the buffer. Minicell quantification was performed measuring $A_{600}$ and applying the equation: No.