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Salmonella enterica serovar Choleraesuis derivatives harbouring deletions in *rpoS* and *phoP* regulatory genes as vehicles for DNA vaccines

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ABSTRACT

We investigated the use of two previously described attenuated strains of *Salmonella enterica* subspecies *enterica* serovar Choleraesuis (*S.* Choleraesuis), Δ*phoP* and Δ*rpoS*, compared with the commercial attenuated SC-54 strain, as bactofection vehicles, to deliver an epitope model (3xFLAG) to the intestinal immune system. The gene encoding the epitope 3xFLAG was subcloned into the pCMVβm2A mammalian expression vector (creating pCMV3xFLAGm2A) and introduced into *S.* Choleraesuis strains. The 3xFLAG epitope was expressed efficiently in murine macrophage J774A.1 cell cultures infected with *Salmonella* Δ*phoP* and Δ*rpoS* vehicles but not with SC-54, as shown by gene-specific quantitative real-time reverse-transcriptase PCR. The stability of pCMV3xFLAGm2A in each strain was determined *in vitro* in the absence of antibiotic selection, and *in vivo* following oral immunisation of BALB/c mice. Administration of the DNA vaccine to mice led to the production of 3xFLAG-specific serum IgG and intestinal IgA antibody responses in Δ*rpoS* and SC-54, and spleen cell secretion of IFN-γ following specific 3xFLAG stimulation in Δ*phoP*. All together, these results indicate that Δ*phoP*, Δ*rpoS* and SC-54 that expressed 3xFLAG from pCMV3xFLAGm2A elicited a different biased immune response, in which the T-helper-1-like cellular immune response was predominant in Δ*phoP*, whilst IgA-related mucosal immunity predominated in Δ*rpoS* and SC-54. We conclude that Δ*phoP* and Δ*rpoS* of *S.* Choleraesuis are new promising candidates as vaccine bactofection vectors.

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1. Introduction

DNA vaccines offer several advantages over conventional methods of immunisation, in that they are easy to develop, are safe and well tolerated by laboratory and livestock animals, and are capable of stimulating cellular and humoral immune responses (Daudel et al., 2007). However, their relatively low efficacy, specifically in target species, is an important disadvantage, and several strategies have been employed to overcome this major stumbling block of DNA vaccines. The major challenge to improving DNA-based vaccines is to increase its transfection efficiency (Dietrich et al., 2003; van Drunen Littel-van den Hurk et al., 2004). An attractive alternative is the mucosal delivery of plasmid DNA by orally administered intracellular bacteria (Dietrich et al., 2003).

Attenuated bacteria can transfer plasmids that encode foreign antigens under the control of eukaryotic promoters to the host cells, and are considered as ideal candidates for the delivery of DNA vaccines (bactofection) (Daudel et al., 2007). Careful selection of an appropriate carrier strain and its attenuated mutations is an important means to fine-tune the quality of the immune response. Among other

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Table 1
Bacterial strains and plasmids used in this study.

Strain or plasmid	Description and relevant characteristics	Source (reference)
Salmonella enterica serovar Choleraesuis		
CECT[a] 915	Wild-type	Spanish Culture Type Collection
SCL[b] 00031	Δ*rpoS* from CECT 915	Dominguez-Bernal et al. (2008)
SCL 00032	Δ*phoP* from CECT 915	Dominguez-Bernal et al. (2008)
SC-54	Commercial Live Vaccine Strain	Boehringer Ingelheim (Roof and Doitchinoff, 1995)
SCL 00071	Wild-type/pCMVβm2A, Amp^R	This work
SCL 00072	Wild-type/pCMV3xFLAGm2A, Amp^R	This work
SCL 00068	Δ*rpoS*/pCMVβm2A, Amp^R	This work
SCL 00067	Δ*rpoS* pCMV3xFLAGm2A, Amp^R	This work
SCL 00066	Δ*phoP*/pCMVβm2A, Amp^R	This work
SCL 00064	Δ*phoP* pCMV3xFLAGm2A, Amp^R	This work
SCL 00073	SC-54/pCMVβm2A, Amp^R	This work
SCL 00072	SC-54/pCMV3xFLAGm2A, Amp^R	This work
Plasmids		
pCMVβm2A	Amp^R	Bauer et al. (2005), Datsenko and Wanner (2000)
pCMV3xFLAGm2A	Expressing 3xFLAG; Amp^R	This work

[a] CECT, Spanish collection of Type Cultures, Universidad de Valencia, Burjassot (Valencia), Spain.
[b] SCL, *Salmonella* Choleraesuis collection from the INBAVET group.

candidates, *Salmonella* has attracted considerable attention as an ideal orally administered antigen-delivery vector for plasmid DNA (Darji et al., 1997; Kwon et al., 2007). These *Salmonella*-based vaccines are fully capable of eliciting humoral, cell-mediated and mucosal immune responses, not only against their heterologous antigens, but also against the *Salmonella* carrier itself (Kwon et al., 2007).

We have recently reported the construction of two highly attenuated strains of *Salmonella* Choleraesuis, Δ*rpoS* and Δ*phoP*, with excellent properties as candidates for vaccine vectors (Dominguez-Bernal et al., 2008).

In the present study, we exploited these strains of *S.* Choleraesuis as bactofection vectors in a murine model. A previously described highly *Salmonella*-adapted eukaryotic expression low-copy-number vector, pCMVβm2A (Bauer et al., 2005), was chosen as a DNA delivery plasmid, which was administered to mice by the Δ*rpoS*, Δ*phoP* and the commercial attenuated SC-54 *S.* Choleraesuis strains. After DNA immunisation by bactofection, the mouse immune responses, including the systemic and mucosal antibody levels and T-cell response, were evaluated.

2. Materials and methods

2.1. Bacterial strains and growth conditions

The bacterial strains used in this study are listed in Table 1. *S.* Choleraesuis CECT 915 (ATCC 13312) was used as the wild-type (WT) strain. Bacteria were routinely cultured in Luria–Bertani (LB) broth and on LB agar plates. If required for the selection of recombinants or to maintain plasmids, 100 μg/ml ampicillin (Amp) was added. The reference vaccine was a commercially available live avirulent culture (Enterisol® SC-54) and was obtained from Boehringer Ingelheim.

2.2. Construction of recombinant plasmid encoding 3xFLAG epitope

pCMVβm2A has been described previously (Bauer et al., 2005). For the cloning of the *3xFLAG* fragment (encoding 3xFLAG) into pCMVβm2A, we first generated by PCR a 3xFLAG cassette by annealing the overlapping oligonucleotides: CFLA1D-X (Table 2) with a XhoI site and CFLA1D-N with a NotI site (Table 2). The amplicon was

Table 2
Primers used in this study.

Primer designation	Sequence (5′ → 3′)[a,b]	Orientation	Template plasmid/gene target
CFLA1D-X	CCGGTACTCGAG**ATGGACTACAAAGACCATGACGGTGATTA TAAAGATCATGATATCGATTACAAGGATGACGATGACAAG**	Forward	
CFLA1D-N	GGATCCCGCGGCCGC**CTTGTCATCGTCATCCTTGTAATCGA TATCATGATCTTTATAATCACCGTCATGGTCTTTGTAGTCCAT**	Reverse	
pcmv-1D	CCGCCTTGTCATCGTCATC	Forward	pCMVβm2A
pcmv-1R	GTTAACTTGTTTATTGCAGC	Reverse	pCMVβm2A
qFLAG-2D	GACTACAAAGACCATGACGG	Forward	3xFLAG
qFLAG-2R	GTCATCGTCATCCTTGTAATC	Reverse	3xFLAG
qmBactin-1D	GGCACCACACCTTCTACAATG	Forward	Mouse β-actin (Register et al., 2007; Varona et al., 2005)
qmBactin-1R	TGGATGGCTACGTACATGGCT	Reverse	Mouse β-actin (Varona et al., 2005)

[a] Enzyme restriction sites are underlined.
[b] Overlapping regions are depicted in bold.

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