

## BRUCELLOSIS VACCINE BASED ON MICROPARTICLES

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### Introduction

In general, vaccines currently used on livestock (i.e. *B. abortus* S19 and *B. melitensis* Rev 1) offer adequate protective effects. However, they display a number of drawbacks, mainly related to their incomplete avirulence in animals and humans, and the interference in the diagnostic with the current serological tests (1).

Another possibility to design and prepare new, sure and effective vaccines may be the use of subcellular compounds loaded in microparticles, rather than the whole alive microorganism. This approach would permit to overcome the main drawbacks related with the use of live vaccines and to potentiate the immune response to subcellular antigens, avoiding booster doses.

The aim of this work was to determine if the HS antigenic extract from *Brucella ovis* in microparticles induces an adequate immune response and hence confers protection against experimental brucellosis in mice.

### Materials and Methods

#### Materials

Polyvinyl alcohol (PVA) and organic solvents were from BDH-Supplies. Pluronic F-68 was from Sigma.  $\beta$ -cyclodextrin and poly- $\epsilon$ -caprolactone (PEC) from Aldrich. Hot Saline (HS) extract was obtained from whole *B. ovis* cells (2).

#### Preparation and characterization of HS-loaded microparticles (HS-MP)

HS was mixed with  $\beta$ -cyclodextrin and dispersed in an aqueous solution containing Pluronic F-68 6%. PEC was dissolved in methylenechloride 4% and emulsified with the antigen aqueous phase.

This emulsion was dispersed in an aqueous phase containing 0.5% PVA. The organic solvent was eliminated and the resulting carriers purified by centrifugation and, finally, freeze-dried (3).

Microparticles were sized in a Mastersizer-S<sup>®</sup>. The antigen content was determined by the BCA protein assay after digestion of microparticles with NaOH 0.1 M.

#### Cytokine assay

Experimental groups with 3 BALB/c mice per group were immunized orally or subcutaneously with HS-MP containing 20  $\mu$ g HS. On day 15<sup>th</sup>, mice were sacrificed by cervical dislocation and their spleen removed and treated to recover spleen cells. Using 96-well round bottom microtiter plates, cells were plated, at a concentration of 800,000 cells/well, and incubated with the HS extract (100  $\mu$ g/well) for cytokine induction. Cells were cultured for 24 h for IL-2 accumulation, and for 48 h for IFN- $\gamma$  and IL-4 release. Samples were tested using commercial sandwich ELISA kits (Biosource Int.).

#### Experimental protection against infection challenge

Groups of 20 female BALB/c mice, 8-10 weeks old, were vaccinated orally (10 mice) or s.c. (10 mice) with: i) HS-PEC particles containing 20  $\mu$ g HS/animal; ii) free HS (20  $\mu$ g/animal); iii) empty PEC particles; iv) 5x10<sup>4</sup> CFU/mouse of the Rev 1 reference vaccine strain; or v) buffer solution alone (unvaccinated control group). Eight weeks after vaccination, mice were challenged i.p. with 5x10<sup>4</sup> CFU/mouse of the smooth virulent *B. abortus* 2308 (10 mice/group) or of the rough

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virulent *B. ovis* PA (10 mice/group) reference strains. Mice were sacrificed at 2 (for *B. abortus* 2308) or 3 (for *B. ovis* PA) weeks after challenge, spleens were removed and plated on culture medium for viable counts. Protection was expressed by the log of mean (SD) CFU /spleen of the virulent challenge strain.

#### Results and Discussion

HS-MP displayed a mean size of  $1.44 \pm 0.53 \mu\text{m}$  with unimodal distribution. The yield of the process was 71%, obtained by the final dry weight of microparticles respect to the initial amount of the polymer. Finally, the encapsulation efficiency was around 30% and the HS loading  $5.18 \pm 1.52 \mu\text{g}/\text{mg}$  microparticles.

The subcutaneous administration of HS-MP gave high amounts of IFN- $\gamma$  and IL-2 but low quantities of IL-4 (data not shown). The antibody isotype immunization suggested the induction of a Th1 response.

The vaccine administered either subcutaneously or orally protected mice against *B. ovis* infection. Such protection was similar to that provided by the reference living attenuated *B. melitensis* Rev 1 vaccine that was equally effective by both vaccination routes (see Table 1).

**Table 1.** Protection conferred against *B. abortus* infection in BALB/c mice. Logs CFU/spleen (SD).

Vaccination	Oral	Subcutaneous
HS-MP	6.16 (0.28)	3.90 (0.67) **
Empty MP	5.91 (0.43)	4.79 (1.38)
Free HS	5.74 (0.39)	5.42 (0.66)
Rev 1	3.29 (1.59)*	2.86 (1.13) **
PBS	5.95 (0.44)	5.95 (0.44)

\*P<0.05 and \*\*P<0.01 vs. unvaccinated control group by Dunnett's test.

In contrast, only the subcutaneous vaccination with microparticles containing HS was as effective as Rev 1 in conferring protection against *B. abortus* infection (see Table 2). The use of free HS or empty microparticles did not produce any protective effect.

Consequently, the data suggest that subcutaneous administration of subcellular vaccine based on poly- $\epsilon$ -caprolactone microparticles are effective and safe against

brucellosis. Additional research must be performed to establish the protective value of this innocuous rough subcellular vaccine against challenge with other *Brucella* strains and in natural hosts.

**Table 2.** Protection conferred against *B. ovis* infection in BALB/c mice. Logs CFU/spleen (SD).

Vaccination	Oral	Subcutaneous
HS-MP	4.77 (2.61) *	3.12 (1.83) **
Empty MP	6.92 (0.16)	5.24 (1.74)
Free HS	6.51 (0.51)	7.20 (0.30)
Rev 1	4.88 (2.03)*	3.72 (1.61) **
PBS	7.24 (0.30)	7.24 (0.35)

\*P<0.05 and \*\*P<0.01 vs unvaccinated control group by Dunnett's test.

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