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Vaccine development

USE OF A STAPHYLOCOCCUS AUREUS SMALL COLONY VARIANT AS AN ATTENUATED LIVE VACCINE FOR THE PREVENTION OF INTRAMAMMARY INFECTIONS

J. CÙtÈ-Gravel[1], E. Brouillette[1], N. Obradovic[1], M. Allard[1], B. Talbot[1], C. Ster[1], F. Malouin[1]

[1]Biologie, Université de Sherbrooke, Sherbrooke, Canada

Objectives. *Staphylococcus aureus* is a leading cause of bovine mastitis. *S. aureus* intramammary infections (IMIs) are particularly difficult to treat by antibiotherapy. Also, no vaccine formulation has shown high protective efficacy to date, possibly because of some of the intracellular aspects of *S. aureus* infections. *S. aureus* small colony variants (SCVs) are easily internalized in host cells and persist without generating invasive infections. Our approach aims at taking advantage of SCVs by customizing a live vaccine vector for intracellular delivery of antigens thus permitting stimulation of a cell-mediated immune response. The objectives of this study were therefore to engineer a *S. aureus* SCV live vaccine vector by deletion of the *hemB* gene and to obtain further attenuation of virulence through inactivation of gene SACOL720. Gene 720 was previously shown by our team to be important for virulence in experimental bovine IMI. Subsequent objectives were then to evaluate persistence of the live vaccine vector in a cell culture assay and to attest attenuation and safety in a murine IMI model.

Methods. The Δ720, Δ*hemB* and double Δ720Δ*hemB* mutants were obtained in *S. aureus* ATCC 29213. Δ720 was generated using the TargeTron gene knockout system by insertion of a group II intron in SACOL0720. The deletion of gene *hemB* was achieved by gene replacement with an *emrA* cassette in parental and Δ720 strains. Mutations were confirmed by phenotypic characterization and PCR. All four strains were assayed for persistence in bovine mammary gland epithelial cells (MAC-T). After a 3-h incubation of cells with bacteria at a MOI of 10, cells were incubated in presence of lysostaphin to kill extracellular bacteria for an additional 12h before cells were lysed for intracellular bacterial counts. The virulence of the Δ720Δ*hemB* mutant was assayed in a murine IMI model. Mice were inoculated through the teat canal of the large R4 and L4 mammary glands using 100 CFU of the double mutant or parental strain. CFUs per gram of harvested glands were evaluated after 1, 2, 4 and 7 days of infection.

Results. Δ720Δ*hemB* and Δ720 mutants showed significantly lower CFUs in MAC-T cells compared to the parental strain (P≤0.01). Following experimental IMI, the Δ720Δ*hemB* mutant was strongly attenuated compared to its parental strain. The bacterial counts for the double mutant were reduced by 5 log10 to near bacterial clearance at day 7. In comparison, the parental strain provoked severe invasive infections, killing 5 of 12 mice and maintained high viable counts (9 log10 CFUs/g) up to day 7.
Conclusions. We have successfully obtained an attenuated *S. aureus* strain. This Δ720Δ*hemB* mutant is a stable SCV that could be used for intracellular expression of key antigens. Such a live vaccine vector could enhance cell-mediated immunity against *S. aureus*.