Recombinant *Vibrio cholerae* ghost as a delivery vehicle for vaccinating against *Staphylococcus aureus*

Adrià Auladell Martin
Degree in microbiology, 2014. Universitat Autònoma de Barcelona

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**Initial hypothesis and objectives**

The main objective of this project is to create a vaccine able to confer protection against heterologous strains of *Staphylococcus aureus*. It will induce a cellular immune response based on the polarization of CD4+ helper cells to Th1/Th1. The initial hypothesis is that the Bacterial Ghost methodology used in the construction of the vaccine is successful, and that the selected antigens (IdSa, IdsD and SdrD) generate a specific protective immunity in the individuals who was administered.

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**Background**

*Staphylococcus aureus* is a Gram-positive and ubiquitous bacterium from the phylum Firmicutes. It is known to be present as a commensal in the skin and nasal microbiome in 25–30% of humans, and it is also a common pathogen, causing skin and soft tissue infections, such as cellulitis, impetigo, and folliculitis. Although these infections are usually originated in the skin, invasive and life-threatening infections such as bacteremia, pneumonia, meningitis, endocarditis, toxic shock syndrome (TSS) and sepsis may ensue. During the last decade, the treatment of these infections has been difficulted by the irruption of resistant strains, such as methicillin resistant strain (MRSA-S.aureus) or vancomycin resistant strain (VRSa.S.aureus)1. Due to this fact, in United States, S.aureus represents one of the major causes of death. Indeed, it is involved in over 18,000 deceases per year; about 290,000 hospitalizations and almost 12 million of medical treatments and 1.

Different studies established a link between Th1 cells and neutrophils. Interleukin 17A (IL-17A) is known to be important during the neutrophils' recruitment facilitating chemotaxis, eliminating the bacterium easily. Th1 polarization is also required for the neutrophils' activation at the infection side because, for instance, an intracellular stage is known to appear sometimes in S.aureus infections2,3. Finally, it has demonstrated by Stranger Jones et al of that IdSa, IdsD and SdrD confer protection against S.aureus4.

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**Materials and methods**

**Obtaining of vaccine subunits**

Chemical synthesis of the genes using *V.cholerae* codon usage

- Digestion of pMAL-A and IdSa with *Hind* III
- Digestion of pKSEL-2, IdsD and SdrD with the respective restriction enzymes
- Ligation \((T4)\) and transformation in E.coli JM109

**Bacterial ghost formation**

- Selection of clones (Lb agar + Ampicillin + Clobaromecin). Confirmation by colony PCR, sequencing plasmid.

**Project dissemination**

After the concession of the project and its realization, a patent application will be submitted. Once the patent is granted, the project will be disseminated to the scientific community through a paper publication in a high impact journal such as Clinical Vaccine Immunology (CVI) or Vaccine.

**Expected results**

The vaccine formed by the rVCG expressing IdSa, IdsD and SdrD should provide an effective immune response against S.aureus and a memory response. The protective response must be mediated by Th1 and Th2 cells, increasing the production of IL-17 and IFN-γ in the vaccinated individuals compared to the control ones. This protection will be effective against S.aureus due to the use of three different antigens all of them present in most of the pathogenic strains. During the lethal challenge, a survival increase in immunized mice is expected.

**Bacterial ghost**

Bacterial ghosts are empty cell envelopes produced from Gram-negative bacteria controlling E protein expression. This control is due to the cloned PNA374 lysis E gene. E protein forms a tunnel structure spanning the whole cell wall complex, through which the cytoplasmic contents are expelled4,5. Recombinant DNA technology facilitates the development of multivalent protein or DNA vaccines. The expression of recombinant proteins can be localized in the outer membrane (OM), the inner membrane (IM) and the periplasmic space (PS)6.

**Protection studies**

- **Group 1**: Intramuscular dose of 3 mg rVCG in 50 μl of PBS
- **Group 2**: Im dose of 3 mg VCG in 50 μl of PBS
- **Group 3**: Im dose of 1 mg VCG in 50 μl of PBS
- **Group 5**: Im dose of 50 μl of PBS

ELISA to determine antibody titer, IFN-γ and Flow citometry analysis to determine Th1/Th2 cells levels

**Statistical analysis** (1 student test)

This project provides a direct solution to one of the major problems in public health of the developed world: S.aureus infections are increasing the morbidity and mortality every year having a special importance the MRSA strains infections. The creation of a feasible vaccine has failed so far because there was a lack of information about the immune response able to establish an effective clearance of the infection.

To sum up, this vaccine will be cheaper than other approaches because of the simple production, it does not depend on complex purification processes, and the stock produced can be logistically made cold storage unnecessary.

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1. Lyczard A, Skou ST. *Staphylococcus aureus* virulence factors are shown to act in concert to escape the immune response. *Virulence* 2010;1(4):288-300.