Development of a mucosal vaccine strategy against human dental caries based on the PstS protein of *Streptococcus mutans*.

Ferreira, E. L. [1], Batista, M. T. [1], Cavalcante, R. C. M. [1], Pegos, V. R. [3], Passos, H. M. [1], Silva, D. A. [1], Balan, A. [1,3], Ferreira, L. C. S. [1], Ferreira, R. C. C. [1]

[1]Department of Microbiology, Biomedical Science Institute, University of São Paulo, Brazil.

[2] Department of Pharmacy of Lagarto University of Sergipe, Brazil

[3]Biosciences National Laboratory (LNBio), Materials and Energy Research Center, Campinas, São Paulo, Brazil.

Bacterial ABC (ATP-Binding Cassete) transport systems play crucial role in physiology and can also influence pathogenicity. Therefore, components of ABC transporters have been tested as target antigens on vaccine approaches against different bacterial pathogens. In *Streptococcus mutans*, the main etiological agent of dental caries, it has been demonstrated that the absence of the phosphate binding protein (PstS), from the phosphate uptake system, leads to reduction on adherence to abiotic surface and biofilm production *in vitro*. Human dental caries is an infectious disease worldwide distributed and despite the knowledge of important *S. mutans* virulence factors, a vaccine formulation against this oral pathogen is still not available to human use. Thus, the main purpose of this work was the immunological characterization of a new anti-caries mucosal vaccine approach based on the PstS of *S. mutans* as target antigen. First, a recombinant soluble PstS form (rPstS), derived from *S. mutans* UA159 strain, was obtained in *Escherichia coli* after affinity chromatography purification. Circular Dichroism and fluorescence purified rPstS protein showed a stable secondary structure, similar to other binding proteins, and retained ability to interact with its ligand. Conserved antigenic epitopes, including heat-labile and heat-stable, were identified in rPstS by reactivity with serum raised against intact *S. mutans* cells in ELISA and Dot blot assays. Then, the immunogenicity of the rPstS protein was determined by administration via the sublingual route in combination with an adjuvant derived from the heat-labile toxin (LTK4R) of enterotoxigenic *Escherichia coli* (ETEC). Mucosal and systemic antibody responses were stimulated in Balb/c female mice immunized with both formulations determined by serum specific IgG and IgA secreting cells on spleen and cervical lymph nodes of animals. Finally, rPstS-specific IgG antibodies raised in mice interfered with the adhesion of bacteria to the oral cavity of naïve mice challenged with *S. mutans* NG8. Similarly, mice actively immunized with rPstS were partially protected to oral colonization after challenge with the same strain. Taken together, our results indicated that the recombinant PstS protein preserves structural and immunological characteristics of the native protein that can be exploited in vaccine strategies for the control of dental caries.

Key words: *Streptococcus mutans*, vaccine, PstS, sublingual immunization

Founding Agency: FAPESP