VAC_01 - Assessment of protective immunity of a bivalent vaccine candidate based on a recombinant influenza virus against *Streptococcus pneumoniae* and influenza

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Introduction: *Streptococcus pneumoniae* is a major cause of pneumonia and meningitis, resulting in great mortality worldwide. In addition, secondary pneumococcal infections are the main complication in influenza infected patients, resulting in poor prognosis. The licensed pneumococcal vaccines, despite reducing the death rates, are serotype-specific, becoming non-protective with the circulation of new strains. Thus, to overcome this problem, we generated a recombinant influenza virus carrying a highly immunogenic and conserved pneumococcus surface protein (nicknamed SP protein), aiming the development of a bivalent vaccine against *S. pneumoniae* and influenza infections.

Objective: To evaluate the potential of a vaccination protocol using a recombinant influenza virus encoding the SP protein (Flu-SP) to induce protective immune response against pneumococcus and influenza, in mice.

Methodology: The recombinant influenza viruses were constructed by reverse genetics and characterized by PCR, sequencing and titration. Posteriorly, C57BL/6 mice were intranasally immunized with: Flu-SP followed by boost with adjuvanted SP protein (alum); Flu-Control (Flu-CT) and boost with alum; or PBS (two inoculations). Blood samples were collected and serum anti-SP and anti-influenza antibodies were assessed by ELISA. Furthermore, the ability of anti-SP antibodies to bind to different pneumococcal strains was analyzed by flow cytometry. Finally, to evaluate the protective capacity against pneumococcus, the immunized mice were intranasally challenged with a letal dose (5xMLD50) of a highly virulent pneumococcal strain (ATCC 6303). Moreover, to assess the protection against influenza, C57BL/6 mice was inoculated with Flu-SP, Flu-CT or PBS (one dose) and challenged with a letal dose (100xMLD50) of influenza virus (H1N1). The survival was monitored for 10 days. Differences ($\rho < 0.05$) between groups and survival curves were assessed by ANOVA and Log-rank test, respectively.

Results: The results showed that our vaccination protocol (primed with Flu-SP and boosted with adjuvanted SP protein) has induced high levels of anti-SP and anti-influenza IgG antibodies. In addition, an efficient binding of anti-SP antibodies to the surface of different pneumococcal strains were observed. After the pneumococcal lethal challenge, our immunization protocol protected almost 65% of vaccinated mice, whereas the animals of the control groups did not present relevant protection rates. Furthermore, immunization with recombinant viruses (Flu-CT or Flu-SP) resulted in 100% protection against a challenge with influenza, whereas all animals inoculated with PBS died. It's known that specific antibodies play a pivotal role in defense against pneumococcal and influenza infections. Therefore, it is possible that the higher anti-pneumococcal and anti-influenza IgG titers induced by immunization might have contributed to the protection from lethal challenges, resulting in a more effective bacterial opsonophagocytosis and virus neutralizing, respectively.

Conclusion: In short, these results indicate that our immunization protocol was able to induce specific and protective immune response against *S. pneumoniae* and influenza in mice and represents a promising bivalent vaccine strategy against these respiratory pathogens.

Keywords: Recombinant influenza virus; Protective immunity; Bivalente vaccine