# Recombinant influenza virus carrying surface protein A (PspA) of *Streptococcus pneumoniae* as a bivalent vaccine platform against influenza and pneumococcal pneumonia

**Kimberly Freitas Cardoso[1*], Lara Regina Alves de Souza[2], Beatriz Senra Álvares da Silva Santos[3], Ketyllen Reis Andrade de Carvalho[1]; Eliane Namie Miyaji[3]; Márcio Sobreira Silva Araujo[1]**

*1 Instituto René Rachou/Fiocruz Minas, Belo Horizonte, MG, Brasil;*
*2 UFMG - Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brasil;*
*3 Instituto Butantan, São Paulo, SP, Brasil.*
** Corresponding author. E-mail: kimberlycardoso@hotmail.com*

*S. pneumoniae* and influenza virus are respiratory tract pathogens that cause high mortality worldwide. The influenza virus causes flu, while pneumococcus causes pneumonia and meningitis, also secondary infections in flu patients, worsening their clinical condition. The licensed pneumococcal vaccines are serotype-specific, resulting in serotype replacement by non-vaccine serotypes. Therefore, aiming to develop a bivalent vaccine against both these pathogens, we constructed, by reverse genetics, a recombinant influenza virus carrying the pneumococcal PspA protein (Flu-PspA virus). Thus, this work aimed to evaluate the efficacy of a heterologous *prime-boost* protocol with the Flu-PspA virus and the rPspA protein, in the murine model via intramuscular route. For this, C57BL/6 mice were primed with Flu-PspA and boosted with rPspA+Alum (vaccine group). In the control groups, animals were primed with control virus (Flu-CT) and boosted with rPspA+Alum or Alum; or two doses of PBS. Later, fourteen days after the last immunization, anti-PspA and anti-influenza serum IgG antibodies were quantified by ELISA. Subsequently, the ability of anti-PspA antibodies to bind and deposit complement (C3 component) on the surface of different strains of *S. pneumoniae* was evaluated by flow cytometry. Then, twenty-one days after the last dose, animals were lethally challenged with 7xLD50 of the pneumococcus invasive strain ATCC6303 or with 100xLD50 of influenza A/PR8/34 virus, and survival was monitored for ten days. In addition, three days after the lethal challenge, the bacterial load present in bronchoalveolar lavage (BALF) was quantified by titration, and inflammatory cytokines were quantified by CBA. Thus, we observed that the animals of the vaccine group showed high levels of anti-PspA and anti-influenza IgG antibodies in serum, and the anti-PspA antibodies were able to effectively deposit complement in different strains of pneumococcus, demonstrating the response of broad spectrum of the vaccine. Furthermore, we observed 100% protection after lethal pneumococcal and influenza challenges, a two to three $logs_{10}$ reduction of bacterial load in the BALF, and lower levels of inflammatory cytokines in BALF. Therefore, this heterologous *prime-boost* protocol showed high protection and immunogenicity, being a promising bivalent vaccine strategy against influenza and pneumococcal pneumonia.

**Key words**: Recombinant influenza virus; Bivalent Vaccine; *Streptococcus pneumoniae*.

**Vírus influenza recombinante carreando a proteína de superfície A (PspA) de *Streptococcus pneumoniae* como plataforma vacinal bivalente contra gripe e pneumonia por pneumococo**

*Streptococcus pneumoniae* e o vírus influenza são patógenos respiratórios que causam alta mortalidade globalmente. Para contornar isso, geramos um vírus da influenza recombinante carreando a proteína PspA do pneumococo (Flu-PspA) e avaliamos uma estratégia vacinal usando o Flu-PspA e a proteína PspA recombinante, em um modelo murino via intramuscular. O grupo vacinal apresentou altos níveis de anticorpos anti-PspA e anti-influenza, com deposição eficaz de complemento em cepas do pneumococo. Além disso, observamos 100% de proteção contra desafios letais com pneumococo e influenza, e redução da carga bacteriana e citocinas inflamatórias após desafio pneumocócico, demonstrando o potencial dessa abordagem vacinal bivalente.

**Palavras-chave**: Vírus influenza recombinante; Vacina bivalente; *Streptococcus pneumoniae*.

Acknowledge*: This work was developed having support from CNPq, CAPES, FAPEMIG, FIOCRUZ and INCTV*.