DEVELOPMENT OF A MALARIA RNA VACCINE WITH SEQUENCES FROM TWO DIFFERENT PLASMODIUM VIVAX ANTIGENS

Denis A Molina[1]; Gabriela de Assis B. Caldas[1]; Natália S. Hojo de Souza[2]; Renata S. Fernandes[1]; Rodolfo Ferreira[3]; Carla E. Cunha[2]; Hiromi Muramatsu[4]; Ricardo Gazzinelli[1,2]; Norbert Pardi[4]; Irene da Silva Soares[3]; Santuza Teixeira[1]

[1]Centro de Tecnologia Vacinas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brasil.
[2]Instituto René-Rachou, Fiocruz Minas, Belo Horizonte, MG, Brasil.
[3]Facultade de Ciências farmacêuticas, Universidade de São Paulo (USP), São Paulo, SP, Brasil.
[4]Department of Microbiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, 19104, USA

E-mail: moldenis@gmail.com

Abstract: mRNA-based vaccines have emerged as a promising alternative to conventional approaches due to their rapid development, design flexibility, cost-effectiveness, and ability to induce robust humoral and cellular immune responses. Additionally, RNA vaccines allow the combination of sequences encoding different antigens in a single formulation. Objective: To evaluate mRNA formulations encoding *Plasmodium vivax* antigens expressed at different stages of the parasite life cycle, aiming at a multistage vaccination strategy for malaria control. Methods: Codon-optimized coding sequences of modified versions of the *P. vivax* Circumsporozoite protein (PvCSP) and Reticulocyte Binding Protein 1a (PvRBP1a) were synthesized and cloned into vectors containing a T7 RNA polymerase promoter and appropriate 5′ and 3′ regulatory sequences. In vitro–transcribed mRNAs include a poly(A) tail, m1Ψ-5′-triphosphate uridine substitution and a 5′ cap co-transcriptionally added using a trinucleotide cap1 analog (CleanCap) were encapsulated into LNPs. Antigen expression was confirmed by Western blot and immunofluorescence after transfection of HEK293T cells. C57BL/6 mice were immunized intramuscularly with three doses of mRNA-PvCSP, of mRNA-PvRBP1a, and with a mixture of both encapsulated mRNAs. Humoral immune responses were evaluated by ELISA and cellular responses were assessed by measuring interferon-γ (IFN-γ) production in PvCSP-stimulated splenocytes. Results: HEK293T cells efficiently expressed of both antigens. Immunization with mRNA-PvCSP induced high titers of PvCSP-specific antibodies and significantly increased IFN-γ production compared to control groups and recombinant protein-immunized mice, indicating a strong Th1-type response. Analysis of the immune response of animals immunized with mRNA-PvRBP1a is underway. Conclusion: mRNA-PvCSP vaccination induces robust immune responses, supporting the mRNA platform as a promising multistage strategy for a P. vivax malaria vaccine.

Sources of financial support; CNPq, INCTVacinas