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The antibiotic resistance-free vaccine based on the non-replicative pPAL vector is fully protective against SARS-CoV-2 in the murine model

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Background. The main objective of this work is the development of a DNA vaccine against the SARS-CoV-2 virus based on the non-replicative antibiotic resistance marker gene-free the plasmid vector pPAL.

Methods. We designed pPAL-Sfs and pPAL-structural protein constructs. A PCR cloning procedure was carried out to obtain the pPAL-based recombinant vaccine and laboratory-scale batches of pPAL-based SARS-CoV-2 vaccine constructs were produced. Transfection was performed on the human HEK293 cell line with the pPAL-based recombinant vaccine. Expression was evaluated by Western blot. Evaluation of protection experiments against a lethal dose of 105 pfu of SARS-CoV-2 (Wuhan-Hu-1 and Delta strains) in K18-hACE2 female mice vaccinated intramuscularly with a prime/boost regimen was carried out by assessing both humoral and cellular immune responses. ELISA was used to evaluate humoral immunity, namely total IgG, as well as IgG1 and IgG2c subclasses. The cellular immune response was evaluated by quantifying the rate of IFN-γ producing splenocyte clones used ELISpot. In addition, characterization of the cellular response was carried out by intracellular staining (ICS) to identify of the rate of IFN-γ and TNF-α producing TCD4+ lymphocytes, as well as the proportion of TCD8+ lymphocytes. Determination of viral load in the main target organs was done by RT-PCR (lungs, heart, and brain). Virus replication capacity was also evaluated in target organs tissues. In vitro assays were performed out to determine the levels of neutralizing antibodies against SARS-CoV-2 virus.

Results. The results show 100% protection of vaccinated animals in terms of symptomatology, animal weight, level of neutralizing antibodies against the virus and the rate of IFN-γ and TNF-α producing splenocyte clones. The analysis of IgG subclasses shows a predominance of IgG2c over IgG1, indicating the activation of a specific and cytotoxic Th1 protective cellular immune response and immunological memory. Finally, a reduction of viral load has been observed in vaccinated animals, with a clear reduction of virus replication in the main target organs. Furthermore, there is a synergistic effect increasing protection using the two plasmids p-PALSfs + pPAL-structural protein (under patent).

Conclusions. The DNA vaccine pPAL-Sfs + pPAL-structural protein is fully protective in the mouse model in terms of maintenance of body weight, absence of significant clinical signs, viral load clearance in target organs and immune response. The immune response included neutralizing antibodies, predominance of IgG2c over IgG1 ratio, a Th1 response, and a multifunctional cytotoxic cellular response.

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