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Immunogenicity and safety of a RBD vaccine against SARS-CoV-2 in a murine model

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ABSTRACT

Introduction: Although more than half of the world's population is already vaccinated, the appearance of new variants of concern puts public health at risk due to the generation of new immunogens against the virus as a crucial and relevant strategy in the control of these new variants.

Methods: A preclinical study used a potential vaccine candidate (RBD, SARS-CoV-2). Four groups of BALB/c mice were used, a control group, an adjuvant group, a group inoculated with one dose of RBD subunit protein, and the fourth group inoculated with two doses of RBD subunit protein.

Results: No inflammatory or cellular changes were shown in the mice's anatomopathological evaluation. Higher kinetics and 75% seroconversion were obtained in the mice inoculated with two doses of RBD ($P < 0.0001$).

Conclusions: The application of two doses of the RBD vaccine candidate in BALB/c mice proved safe and immunogenic against SARS-CoV-2.

1. Introduction

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is a beta coronavirus that has caused more than 383 million infections and the death of almost six million people worldwide since 2019 [1]. To date, 61.1% of the world population has received at least one dose of one of the vaccines available against SARS-CoV-2 [2]. However, new variants of concern have recently appeared, such as Omicron (B.1.1.529, BA.1, BA.2) [3], with the ability to partially evade neutralization mediated by IgG1-IgG3 type antibodies against the Spike (S) protein of SARS-CoV-2 [4].

The appearance of new concern variants pushes public health at risk in the large urban centers where a vital part of the world's economic activity occurs. Generating new immunogens with rapid synthesis, low toxicity, and high thermal resistance will play a relevant role in controlling the pandemic and the resurgence of new variants. In addition, many developing countries have not reached vaccination coverage

levels to reduce community transmission of SARS-Cov-2. In this sense, the elaboration of new vaccines designed from the Receptor Binding Domain (RBD), a highly immunogenic protein present in the S, is the key for the virus to enter the host cells [5]. The study of RBD could be a valuable strategy to strengthen the humoral immune response against SARS-CoV-2 and reduce the rate of SARS-CoV-2 infection.

This work aimed to develop the preliminary phase of a preclinical in vivo safety and immunogenicity study of a vaccine candidate RBD segment against SARS-CoV-2.

2. Materials and methods

An in vivo preclinical study was carried out in a murine model to determine the safety and immunogenicity of a protein segment of RBD as a vaccine candidate against SARS-CoV-2. BALB/c mice were used, and the sample size was made for convenience.

Experimental design. The study was carried out for seven weeks,

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and the RBD subunit of the *Spike* protein was used. The vials used for this study as vaccines were donated from Stoecker Labor GmbH (Luebeck - Germany) and were synthesized by Icosagen™. The produced peptide was expressed in a CHO-based cell line and had a total length of 225 amino acids (319–541 plus alanine and serine (AS) at the N-terminus) and a His-6 tag at the C-terminus and a molecular weight of 26.37 kDa, which was affinity purified to >95%. The peptide was obtained from the surface glycoprotein sequence of SARS-CoV-2 Wuhan-Hu-1 (Accession ID: YP_009724390.1). Sixty-four mice were included, of which 33 were inoculated with the RBD protein segment and a non-immunized control group of 22 mice (Fig. 1). The experimental group immunized with RBD

(n = 33) was inoculated intraperitoneally with 15 µg/mL of protein +0.02% adjuvant (aluminum hydroxide). The control group (n = 22) was inoculated with 0.02% adjuvant intraperitoneally. Three animals were euthanized on the seventh day in the experimental group inoculated with the RBD and the non-inoculated control group. Additionally, on day 21 of the trial, 12 mice were inoculated with the second dose of RBD peptide [15 µg/mL]. In addition, the third group of 9 mice was evaluated without inoculating them with the RBD recombinant peptide or adjuvant. In this group, one animal was euthanized on day zero, three on day seven, and then one weekly from day 21 (Fig. 1A).

Ethical aspects. The work was approved by the Animal Ethics

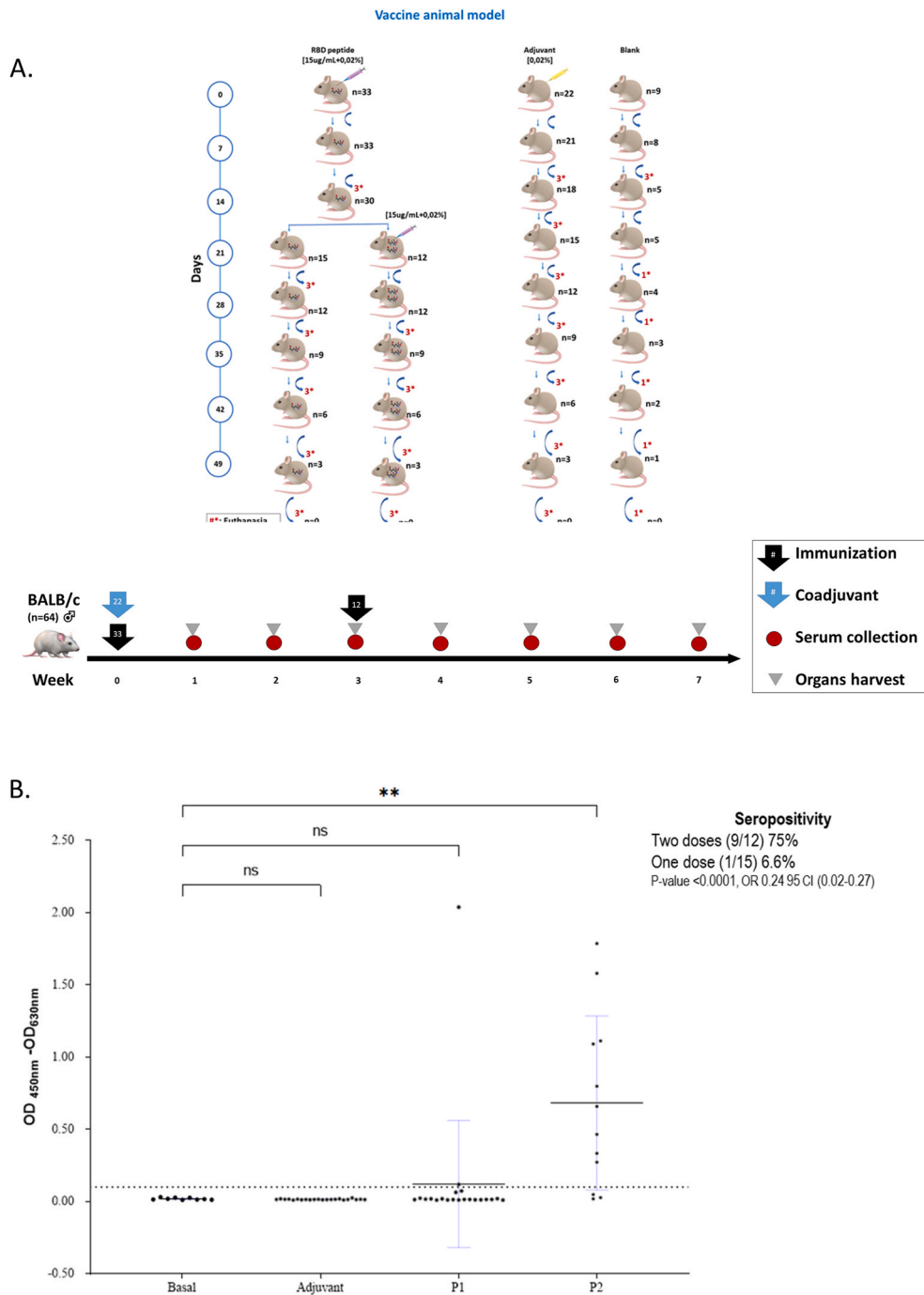


Fig. 1. A. BALB/c animal model experimental design. B. Kinetics and seropositivity of IgG-anti RBD in the assessed groups. A statistically significant difference in kinetics and seroconversion can be noted in BALB/C inoculated with two doses of the immunogen. *P < 0.05, **P < 0.001, ***P < 0.0001, P1: one dose, P2: two doses.

Committee of the University of Córdoba (Memorandum of ethical study 005, May 26, 2021) and the Ethics Committee for Experimentation on Animals (CEEA) of the University of Antioquia (Memorandum of ethical study 141, May 3, August 2021). Euthanasia was carried out following the protocols of the World Organization for Animal Health (OIE) and the American Veterinary Medical Association (AVMA) [6,7].

Histopathological safety analysis. Safety evaluation of the RBD peptide was performed through pathology analysis using hematoxylin-eosin (H&E) staining of the lung, liver, kidney, spleen, and brain. A medical pathologist performed the procedure unaware of the group to which the evaluated mouse tissues belonged. Each histological section was evaluated following the different international societies' recommendations regarding evaluating toxicological pathology for clinical safety [8–10].

Immunogenicity analysis. The peptide's immunogenicity was evaluated through a commercial ELISA, mouse Anti-SARS-CoV-2

Antibody IgG titer serologic assay kit (Acrobiosystems, Cat RAS-T018), which was carried out following the instructions of the manufacturer [11].

Analysis of data. The data were analyzed using the Statistical Package for the Social Sciences version 27 (SPSS). Univariate analysis for qualitative variables was performed by calculating absolute and relative frequencies. In quantitative variables, measures of central tendency were calculated. In addition, the normality of these variables was determined with the Kolmogorov-Smirnov test. The bivariate analysis for qualitative variables was carried out through Pearson's Chi-square test. For variables of a qualitative-quantitative nature, the Kruskal Wallis test was used, and a post hoc test was carried out (Dunn's test). The significance of the P-value was set to <0.05 for all analyzes performed. In addition, the risk was approximated by calculating the odds ratio (OR) with its confidence interval (95% CI).

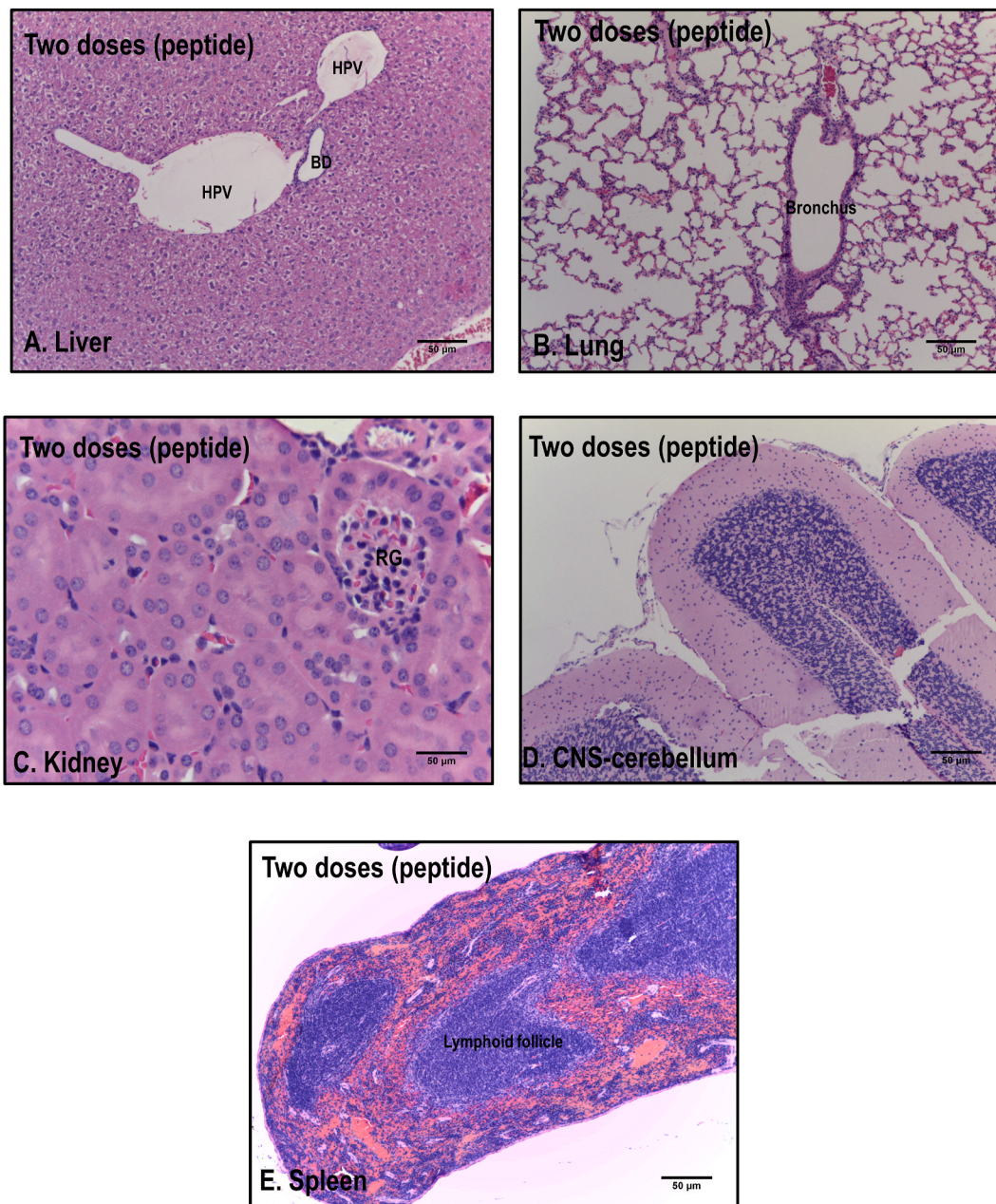


Fig. 2. A-E. Anatomopathological evaluation in BALB/c mice vaccinated with two doses of RBD. No inflammatory or tissue alterations were generated in the liver, lung, kidney, cerebellum, and spleen after applying two RBD doses. BD: Bile Duct, HPV: Hepatic Portal Vein, RG: Renal Glomerulus.

3. Results

Immunogenicity assessment, detection of anti-RBD antibodies against SARS-CoV-2. The immunogenicity evaluation in this study was performed by detecting IgG antibodies against the RBD protein of SARS-CoV-2. In the BALB/c mice of the control group (not inoculated) and those that received only the adjuvant, no anti-RBD IgG antibodies against SARS-CoV-2 were observed, and none of these mice seroconverted against this protein. On the other hand, in the BALB/c groups inoculated with one or two doses of the RBD peptide, higher anti-RBD IgG kinetics was observed in the mice inoculated with two doses of the peptide, and this difference was statistically significant when compared with the other groups evaluated ($P < 0.001$). In addition, it was shown that 75% (9/12) of the BALB/c that received two doses of RBD seroconverted, while in the mice that received a single dose, the seroconversion rate was 6.6% (1/15) ($P < 0.0001$). Applying two doses of the peptide could have a protective factor against this infection (Fig. 1B).

Peptide safety and histopathological description. The anatomopathological findings of the different organs analyzed did not show alterations (Fig. 2). The different doses applied to RBD have not induced morphological alterations in the organs of animals of the inoculated group. Morphological analysis did not show differences between the inoculated and the control groups (supplementary material). These organs seem efficient in multisystemic asset damage, showing the morphological tools' sensitivity and applicability to evaluate vaccines' safety. All the samples were evaluated using the H&E staining, and as no alterations were evidenced, complementary analysis with immunohistochemistry or electron microscopy was unnecessary [12].

4. Discussion

The safety findings of this study are compatible with other studies carried out in mice inoculated with RBD, in which no pathological changes induced by the application of this protein have been seen compared to the control group [13]. These findings confirm that RBD is a safe peptide and that its application does not promote the appearance of inflammatory changes or tissue alterations in the host. Our findings are compatible with Yang et al. [14], who reported in phase 1 and 2 studies for the ZF2001 vaccine. They also used the RBD peptide as an immunogen and found a seroconversion of 79%, evidenced in the individuals who received two doses of the peptide. However, they used three doses, two at 25 µg/ml and the third at 50 µg/ml.

In addition, our data on the IgG kinetics of the vaccine candidate are similar to those observed in other animal models that have evaluated the immunogenicity of RBD [13,15]. These studies have shown higher kinetics of IgG antibodies after applying a second dose of the protein. However, there are differences in the number of residues with the other RBDs studied, whose residues were between 319–541 and 319–537 (16). The RBD used in the present work has 223 residues (319–541), plus two extra amino acids (AS) in N-terminus and His-6 tag at C-terminus. The data show that the RBD protein subunit contains promising epitopes that can be considered vaccine candidates with high immunogenic activity after applying two doses of the peptide. The seroconversion of anti-RBD antibodies against SARS-CoV-2 is achieved earlier than 49 days after inoculation.

On the other hand, it could be inferred that the expression of specific IgG isotypes against the RBD protein of SARS-CoV-2 in mice inoculated with two doses of this vaccine candidate would have the ability to bind to the RBD protein segment, neutralizing the possible virus interaction. With ACE-2 receptors and thus blocking the infection of cells in these hosts [5,16]. In addition, these antibodies could also mediate the destruction of the virus through the balanced activation of other immunological mechanisms such as antibody-mediated cell cytotoxicity and the activation of the membrane attack complex and opsonization processes mediated by the classical pathway of complement [17]. The

present study has limitations, such as the non-evaluation of a cellular immune response towards the Th1 pattern. The other limitation is that neutralizing anti-RBD IgG antibodies against SARS-CoV-2 was not carried out. However, the presence of anti-RBD IgG antibodies allows us to indirectly infer the expression and activation of this cellular immune pattern. Since the Th1 response allows the expression of IFN- γ , the cytokine that induces in plasma cells the change of isotype of IgM antibodies to IgG1-IgG3 [18], it is essential to mention that this study is part of the preliminary phase of a preclinical trial in which the initial evaluation of the safety and efficacy of a protein segment of RBD as a vaccine candidate against SARS-CoV-2 was carried out.

On the other hand, in October 2020, the World Bank provided \$12 billion for developing countries to finance the purchase and distribution of COVID-19 vaccines, tests, and treatments [19]. However, the technological gap increases in developing countries since their vaccination coverage is low and the constant dynamics of SARS-CoV-2 mutations [20]. In other words, it is not just about buying vaccines from large pharmaceutical multinationals. Efforts are required for biotechnological autonomy based on research and development, leading to their vaccines. Biotechnological autonomy in developing countries will make it possible to increase coverage, overcome inequity in access to vaccines, and deal with the appearance of new variants with modified vaccines.

In conclusion, applying two doses of the RBD vaccine candidate in BALB/c mice proved safe and immunogenic against SARS-CoV-2. Besides the laboratory results, the present work demonstrates the importance of the development and production of vaccines in developing countries such as Colombia.

Ethical considerations

This research was carried out following the international ethical standards given by the World Health Organization and the Pan American Health Organization, supported by the Declaration of Helsinki and national legislation, resolution 008430 of 1993 of the Ministry of Health. Health of Colombia regulates health studies. In addition, this work was endorsed by the Animal Ethics Committee of the University of Córdoba and the Ethics Committee for Animal Experimentation (CEEA) of the University of Antioquia.

Financial support

The current study did not obtain any financial support from the authors.

Availability of data and materials

The databases generated and analyzed during this study will be publicly available after making a reasonable request to the authors.

Consent for publication

Not applicable.

CRediT authorship contribution statement

Andrés Díaz: designed the study, All authors read and approved the manuscript. **Héctor Serrano-Coll:** Data curation, Formal analysis, performed the data analysis, Writing – original draft, wrote the manuscript, All authors read and approved the manuscript. **Yesica Botero:** carried out the murine model, All authors read and approved the manuscript. **Alfonso Calderón:** designed the study, All authors read and approved the manuscript. **Ariel Arteta-Cueto:** performed the histopathological evaluation, All authors read and approved the manuscript. **Bertha Gastelbondo:** Data curation, Formal analysis, performed the data analysis, All authors read and approved the manuscript. **Camilo Guzmán:** Writing – review & editing, made the critical review, All

authors read and approved the manuscript. **Ricardo Rivero**: performed the serological evaluation, All authors read and approved the manuscript. **Caty Martínez**: performed the serological evaluation, All authors read and approved the manuscript. **Tania Marquez**: carried out the murine model, All authors read and approved the manuscript. **Germán Arrieta**: Writing – review & editing, made the critical review, All authors read and approved the manuscript. **Salim Mattar**: designed the study, Writing – original draft, wrote the manuscript, This research was directed by SM, All authors read and approved the manuscript.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.tmaid.2022.102427>.

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