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Preclinical evaluation of the RBD-Trimeric vaccine: A novel approach to strengthening biotechnological sovereignty in developing countries against SARS-CoV-2 variants

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ABSTRACT

New immunogens against emerging new virus variants are essential for controlling new variants.
Methods: A preclinical study in which a receptor-binding domain (RBD) trimer was designed in silico with information from the Beta (B.1.351), Omicron (BA.5), and Wuhan 1 variant. A three-dimensional model of the RBD-trimer was made, and the synthesis of the trimer was based on the RBD domain of the S protein of Beta and Omicron. For the experimental trials, 63 BALB/c mice were immunized and divided into three groups: control (n = 15), adjuvant (n = 15), and RBD-trimer (n = 33).
Results: 81 % (13/16), 90 % (9/10), and 85 % (6/7) of BALB/c mice that received one dose, two doses, and three doses, respectively, seroconverted. Significant statistical differences ($p < 0.001$) were found between the experimental group vaccinated with the RBD-trimer, the group with adjuvant, and the control group. The booster did not show significant differences ($p > 0.05$. No inflammatory or cellular changes were observed, highlighting the safety of the RBD vaccine candidate. Kinetics and seroconversion of 75 % were obtained in the mice with two doses of tri-RBD. ($P < 0.0001$).
Conclusions: Applying two doses of the RBD vaccine candidate in BALB/c mice was safe and immunogenic against SARS-CoV-2. This study provides support for the country's biotechnological sovereignty and its potential contribution to public health in Colombia.

1. Introduction

Coronaviruses are a family of positive-sense single-stranded RNA viruses. The coronavirus subfamily is divided into four genera: alpha, beta, gamma, and delta coronaviruses. SARS-CoV-2 belongs to the betacoronavirus genus, which includes SARS-CoV and MERS-CoV [1]. Like other coronaviruses, the SARS-CoV-2 RNA genome encodes four major structural proteins: the spike (S), envelope (E), membrane (M), and nucleocapsid proteins (N).

The Spike (S) protein is the primary entry mechanism of SARS-CoV-2 into host target cells. The spike is a glycoprotein that mediates the fusion of the virus with the cell membrane, forming homotrimers attached to the virus's surface [2]. The spike monomers have a molecular weight of approximately 180 KDa with two subunits known as S1 and S2. The S1 subunit folds independently as a domain, whose portions at the level of the N and C terminal regions mediate the binding of these RBD domains

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to receptors such as angiotensin-converting enzyme 2 (ACE2). The S1 subunit is mainly responsible for binding to the receptor. It can be subdivided into the N-terminal domain, the C-terminal domain (CTD), and two additional domains (subdomains 1–2). SARS-CoV-2 uses the CTD to bind to the cellular receptor ACE2. Besides, the configurations of the RBD can be additionally regulated by glycans, fatty acids, and physical temperature, or individual mutations within the S1 subunits, such as amino acid substitution at the D614G or altered hydrophobicity at S375F. Nevertheless, the S1 subunit senses the entry signal derived from target cell receptor binding and translates it into the activating signal for the S2 subunit [3].

An essential aspect of understanding the mechanism of SARS-CoV-2 infection is related to the interaction between RBD and ACE2. The RBD of SARS-CoV-2 has a stronger binding affinity to the cellular receptor ACE2 than binding to the RBD of SARS-CoV. Therefore, comparative studies reveal distinct structural features that determine different ACE2 binding affinity of the two RBDs [4].

The innate immune response against SARS-CoV-2 is triggered by pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs) or retinoic acid-inducible gene I (RIG-I) component receptors (RLRs) that allow the virus to be detected. This triggers a downstream signaling cascade, leading to the secretion of cytokines such as type I/III interferons (IFN), tumor necrosis factor-alpha (TNF-a), interleukin-1 (IL-1), and IL-6, among others. These cytokines induce antiviral activity in host cells and adaptive immune responses. Additionally, IFN-I can effectively control SARS-CoV infection. Data from in vitro studies suggest that SARS-CoV-2 is susceptible to pretreatment with IFN-I/III. Other molecules of the innate immune system, such as the lymphocyte antigen six complex loci E (LY6E) and the proteins of the IFN-induced transmembrane family (IFITM), can inhibit SARS-CoV-2 [5].

The humoral immune response to SARS-CoV-2 infection, neutralizing antibodies, has been identified as a fundamental immune component against viral infections. The primary function of neutralizing antibodies is antigen binding and interaction with cells carrying Fc receptors [5]. This response is mediated by antibodies that target the glycoproteins of the viral surface, mainly the Spike (S) glycoprotein and the nucleocapsid protein. These antibodies are detectable approximately six days after confirmation of infection by RT-qPCR, and those directed RBD demonstrate neutralizing capacity and, therefore, eliminate the virus and prevent infection. After acute SARS-COV-2 infection, the time for the appearance of early IgM and late IgG antibodies ranges between 6 and 28 days, longer in milder cases. IgG, IgM, and IgA antibody responses to the cysteine-like protease of SARS-CoV-2 are also reported in patients with COVID-19, and these responses correlate with antibody titers against the nucleocapsid protein. Viral clearance and recovery likely require coordinated B and T cell function. However, it is unclear whether it provides long-lasting immunity and protection against reinfection, which is crucial when considering variable immune responses and the prospects for protective vaccination.

SARS-CoV-2 has shown a high capacity for mutations, leading to variants such as Delta and Omicron, which are known for their increased transmissibility, and in some cases, partial resistance to current vaccines [6]. Although approved vaccines have effectively prevented severe disease and reduced mortality, their efficacy against these variants has diminished. The continued development of new vaccines capable of neutralizing the current and possible future variants is essential. The creation of effective long-range vaccines is a priority for disease control, especially given the possibility of more resistant variants or those with more severe characteristics.

The objective of the present study was to promote and contribute to the country's biotechnological independence by generating functional peptides as a vaccine candidate for the prevention of SARS-CoV-2 infection. With the execution of the project, we sought to analyze a recombinant RBD-trimer of SARS-CoV-2, evaluate its immunogenicity and safety in murine models, which would have the utility as a vaccine candidate against the Omicron (B2.5), Beta (B.1.351) and Wuhan 1 of SARS-CoV-2.

2. Materials and methods

2.1. The type of study, geographical location, and sequence designs of a trimer are based on the RBD domains of the SARS-CoV-2 S protein

A meticulous in silico design process was undertaken, incorporating information from the Beta (B.1.351), Ómicron (BA.5), and Wuhan 1 variant of SARS-CoV-2 to create an RBD-trimer (318–541). Histidine sequences were strategically linked to amino acid sequences, forming connecting loops. This RBD-trimer was then inoculated into BALB/c mice for potential vaccine candidate analysis, and an immunoassay was also employed. The amino acid sequences were as follows:

2.1.1. Beta RBD amino acid (B.1.351)

RVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWRKRISNCVA-
DYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVADSFVIRGDEVR-
QIAPGQTGNIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNY-
LYRLFRKSNLKPFERDISTEIY-
QASTPCNGVKGFNCYFPLQSYGFQPTYGVGYQPYRVVVLSFELLHA-
PATVCGPKKSTNLVKNKCVNF.

2.1.2. Amino acids of the RBD of Omicron (BA.5)

RVQPTESIVRFPNITNLCPFDEVFNATRFASVYAWNRKRISNCVA-
DYSVLYNFAPFFAKCYGVSPTKLNDLCFTNVADSFVIRGNEVS-
QIAPGQTGNIADYNYKLPDDFTGCVIAWNSNKLDSKVGGNYNYR-
YRLFRKSNLKPFERDISTEIY-
QAGNKPCNGVAGVNCYFPLQSYGFRPTYGVGHQPYRVVVLSFELLHA-
PATVCGPKKSTNLVKNKCVNFNFNGL.

2.1.3. Wuhan RBD 1 amino acid

RVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVA-
DYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVR-
QIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNY-
LYRLFRKSNLKPFERDISTEIY-
QAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHA-
PATVCGPKKSTNLVKNKCVNF.

2.2. Obtaining and evaluating the three-dimensional model of the RBD trimer

With the amino acid sequences that comprise each of the proposed antigens, the 3D models were obtained using homology-based modeling provided by the Swiss model server and Alphafold [7,8]. Furthermore, the latter were compared with those obtained by modelers [9]. The models were evaluated using Ramachandrán Plot and Z score [10,11]. According to the results of the number of allowed residues, specific modifications of residues in the amino acid sequence were made to achieve potentially stable models. Then, the root median square deviation (RMSD) values between the overlap of the alpha carbon skeletons of the antigens and the S1 protein were obtained to determine if the original folding of the protein was conserved using the Chimera UCSF software [12]. Parameters such as isoelectric point and molecular size were analyzed using the Protparam tool of the Expasy.org server [13]. The B cell epitopes of the models were analyzed using the Ellipro 2.0 server [14].

2.3. Trimer synthesis based on the RBD domain of the Beta and Omicron S protein

Based on the in silico trimeric model, trimeric glycoprotein was synthesized in Chinese hamster ovary cells (CHOEBNALT85) using Icosagen CRDMO. The glycoprotein spanned amino acids 14–1211 of the SARS-CoV-2 Spike and included the mutations T19I, L24S, del25/27, del69-70, G142D, V213G, G339D, S371F, S373P, S375F, T376A,

D405N, R408S, K417N, N440K, L452R, S477N, T478K, E484A, F486V, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, and N969K. Two additional amino acids (AS) were added at the N-terminus, a trimerization domain, and a His-6 tag at the C-terminus to improve stability and facilitate purification. A GS bond linked the protein and the trimerization domain, and a GSG bond was incorporated between the trimerization domain and His tag. Additionally, the furin cleavage site (RRAR) at the interface between the S1 and S2 subunits was mutated to GSAS to avoid unwanted cleavage. The tagged glycoprotein was purified by affinity chromatography, gel filtration, and HPLC, which allowed exclusion of inclusion bodies and unwanted proteins. Finally, the His tag was removed by passing the protein through an imidazole-loaded column to obtain a high-purity glycoprotein suitable for analysis and experimental assays [15].

2.4. Preparation of the experimental groups and inoculation of the immunogen immunogenicity of the SARS-CoV-2 RBD trimer

Sixty-three male and female BALB/c mice, three months old and free of specific pathogens, were chosen as the experimental model. The BALB/c strain is commonly used in immunology research due to its high susceptibility to pathogens and ability to produce a robust immune response. They were immunized and divided into three groups: control (n = 15), adjuvant (n = 15), and RBD-trimer (n = 33) for experimental trials. The experimental group was inoculated with 25 μg of SARS-CoV-2 RBD-trimer plus 0.02 % of the aluminum hydroxide adjuvant intraperitoneally [16,17]. The vaccine candidate was administered in three different regimens. The first group received a single dose (n = 16), the second group received two doses (n = 10), and the third group received three doses (n = 7). The doses were given on days 0, 21, and 28, respectively. The vaccine control group was inoculated with adjuvant intraperitoneally at the exact times as the experimental group. Finally, a non-vaccine control group was included, which did not receive a dose of adjuvant or RBD-trimeric.

2.5. Ethical aspects

The Committee on Ethics for Animal Experimentation (CEEA) approved the work of the University of Antioquia (Session No. 152, June 1, 2023). In all cases, the Bioethical protocols of the World Organization for Animal Health [18] and the American Veterinary Medical Association [19] were strictly adhered to.

2.6. Sample collection and pathological analysis

For a comprehensive evaluation of safety and immunogenicity, euthanasia and organ dissection were meticulously performed. Lung, liver, kidney, spleen, brain, and serum samples were collected at specific intervals following the protocols of the World Organization for Animal Health [16] and the American Veterinary Medical Association [17]. For histopathological analysis, tissues were fixed and stained with hematoxylin-eosin (H&E). The tissues were analyzed by a pathologist external to the study.

2.7. Evaluation of the humoral response in a murine model

The detection of anti-RBD-trimeric antibodies in mice was carried out using an in-house ELISA for the detection of antibodies against RBD of SARS-CoV-2 developed by the Tropic Biological Research Institute (IIBT) of the Universidad Córdoba**.** The indirect ELISA methodology was executed with the ASSIST PLUS-Pipetting robot from INTEGRA Biosciences. For this, coating solutions composed of 1X PBS pH 7.2 and the RBD trimer vaccine candidate were prepared, with concentrations between 1 and 5 μg/ml, added to a high binding 96-well flat-bottom plate using a volume of 50 μL/well followed by incubation at 4 °C overnight. Subsequently, the plate was washed once with 300 μL of washing solution (PBS+0.05 % tween 20) in the Bio-Rad immunowash automated washer. For blocking the plate, 200 μL of 1 % BSA and 2 % sucrose in 1X PBS were added to each well and incubated at room temperature for 2 h. The samples, positive controls, and negative controls were diluted in PBS in a concentration range of 1:10 to 1:100. A volume of 100 μL was added to each well and incubated at room temperature for 1 h.

Like the samples, the secondary anti-IgG antibody (Anti-Mouse, HRP Conjugate, Promega®) labeled with peroxidase was diluted in PBS to a final concentration of 1:10,000, and 100 μL was added to each well. The plate was incubated for 30 min at room temperature and washed as described in the previous step. Then, 100 μL of TMB was added to each well. The plate was incubated for 15 min at room temperature in the dark, and 100 μL of 1M HCl to stop the reaction was added. The reading was made at a wavelength of 450 nm with a correction of 620 nm in the CLARIOstar equipment from BMG LABTECH with an interval of less than 30 min after the reaction. The cut-off point was calculated based on the results obtained from the control group.

2.8. Validation of the in-house ELISA test with a commercial counterpart and evaluation of cross-reactivity

From post-pandemic sera (n = 50) and six groups of pre-pandemic sera (n = 36) collected between 2018 and 2019 (Group 1: patients positive for Zika virus (n = 4) in acute and convalescent stages; group 2: patients positive for Chikungunya virus (n = 7); group 3: patients positive for Rickettsia infection (n = 10); group 4: Patients with active Dengue virus infection diagnosed with NS1 (n = 10); Hantavirus-positive patients (n = 5). Anti-RBD IgG was detected with the commercial Anti-SARS-CoV-2 ELISA (IgG) (Euroimmun, Germany) according to the manufacturer's instructions. The sera were analyzed using the in-house ELISA described above. In all cases, the sera were tested in duplicate. According to the results, the cut-off point, sensitivity, and specificity were adjusted under a Receiver Operator model—curve (ROC).

2.9. Statistical analysis

The data collected in this study were analyzed using the R Project for Statistical Computing software (version 4.4.0) [20]. The univariate analysis of the qualitative variables was carried out by calculating absolute and relative frequencies. The analysis of the qualitative variables was carried out through the Pearson Chi-square test. Multivariate analysis of variance was performed using the Kruskal-Wallis test. For all tests, p-values less than 0.05 were considered significant.

3. Results

3.1. Modeling the RBD-trimer of SARS-CoV-2 as a vaccine candidate

The three-dimensional structure of the RBD-trimer was modeled from the protein sequences of the three variants by homology using SWISS-MODEL (Fig. 1A–C).

Structural similarity analyses showed a high structural concordance between the models, indicating that 84.97 % of the residues had an average score in the 3D-1D profile greater than or equal to 0.1. This suggests that most of the residues in the model were in structural conformations compatible with the sequence, which is a good indicator that the model is reliable for further analysis. This finding suggests high predictive confidence, which reinforces the reliability of our research. In addition, conservation values were calculated across the three variant proteins, showing that most of the variation was found in the receptor-biding motif (RBM) of the protein. These findings suggest that the designed peptide has the potential to elicit a broad neutralizing response against viral variants via immune escape.

The physicochemical parameters derived from the designed

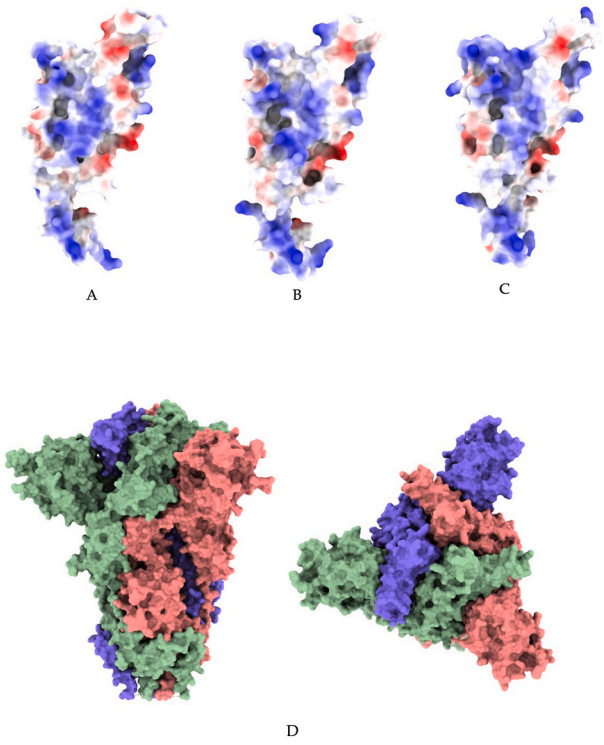

Fig. 1. 3D models of the monomer RBD of SARS-CoV-2 obtained by homology. (A) Beta-variant RBD monomer. (B) RBD monomer of the Omicron variant (BA.5). (C) monomer-RBD of Wuhan 1 variant. D. AlphaFold Structural Prediction of the RBD Trimer Based on the Designed Monomers.

monomers indicated that the trimeric vaccine model has an approximate molecular weight of 75 kDa and an isoelectric point of 8.8259, suggesting a net positive charge at neutral pH. Compositional analysis revealed a diverse distribution of amino acids, with a high content of polar amino acids (43.5 %) and non-polar amino acids (56.5 %). Additionally, charge distribution analysis indicated no significant clusters of positive, negative, or mixed charges along the protein sequence, and no high-scoring segments, either charged or uncharged, were identified. These characteristics reinforce the stability and rational design of the model based on the obtained monomers. The trimer was observed to generate nine predicted B epitopes with high immunogenic capacity, further reassuring the reliability of the findings (Table 1).

In Fig. 1D, a prediction generated by AlphaFold based on the designed monomers is presented. This prediction allowed us to observe the relative positions of the RBD subunits and their structural organization in the context of the spike protein, despite potential differences in sequences and conformations. The results demonstrated that the RBD assembled coherently and functionally with the full-length S protein, supporting its structural integrity in the context of the trimeric protein.

The results of the structural prediction using AlphaFold show an ipTM (intra- and inter-chain TM-score) = 0.84 and a pTM (predicted TM-score) = 0.85, indicating high quality in the predicted structure. The ipTM of 0.84 suggests correct alignment and interaction between the subunits of the model, while the pTM of 0.85 reflects high similarity with reference experimental structures, validating the accuracy of the prediction. Additionally, the molecular weight of the predicted structure is approximately 397.83 kDa.

The synthesized protein had a molecular weight of 410.252 kDa, concentration of 1 mg/ml, and purity of >80 %. However, the HPLC analysis revealed the presence of two additional peaks related to impurities (Fig. 2).

3.2. Immunogenicity of the SARS-CoV-2 RBD-trimer

According to the biological parameters, the mice maintained average growth and gained weight after immunization compared to initial values ($p > 0.001$). The anatomopathological analyses of the different organs studied did not reveal any abnormalities. The various administered doses of the RBD-trimeric did not cause morphological changes in the organs of the animals in the inoculated group (Fig. 3).

3.3. Evaluation of the humoral response of the experimental groups

According to the control group's results, a cut-off points of 0.217 was defined to discriminate between positive and negative sera. BALB/c mice seroconversion was obtained, one dose 81 % (13/16), two doses 90 % (9/10), and three doses 90 % (9/10). Significant statistical differences ($p < 0.001$) were found between the experimental group vaccinated with the RBD-trimer compared to the group with adjuvant and the control group (Fig. 4A). The adjuvant did not generate an immunological reaction. Regarding the booster doses, there were no significant differences ($p > 0.05$) between the groups that received different doses of the vaccine candidate (Fig. 4B).

3.4. Evaluation of the humoral response of the experimental groups

According to the results obtained by Euroimmun's commercial Anti-SARS-CoV-2 ELISA (IgG), 31 post-pandemic sera were positive, five were doubtful, and 14 were negative. No cross-reactivity was evident with pre-pandemic sera. Based on the results, the true negatives (pre-pandemic sera) were validated with the commercial ELISA, and a cut-off point of 1.22 (OD) was determined for the in-house ELISA. Forty-seven sera showed positive results and three negative values. Like the Euroimmun ELISA, there was no cross-reactivity with any pre-pandemic sera evaluated.

The comparison between the commercial ELISA and the in-house RBD-Trimeric ELISA revealed **62** % and 78 % concordance for positive and negative sera results, respectively. The in-house ELISA showed a sensitivity of 93.9 % and a specificity of 79.3 %. Meanwhile, a Positive Predictive Value (PPV) of 0.70 and a Negative Predictive Value (NPV) of 0.96 were obtained. The ROC-AUC curve indicated that in 81 % of the cases, the test had an ideal discrimination criterion for binary classification (positive-negative) of the tested sera (Fig. 5).

Table 1
Predicted linear epitope and Ellipro for the SARS-CoV-2 RBD trimer vaccine candidate.

Epítope #	Beginning	End	Sequence	Residues	Score
1	155	179	DISTEIYQAGSTPCNGVEGFNCYFP	25	0.818
2	243	257	KFASVYAWNRKHHHP	15	0.803
3	216	228	KKSTNLVKNKCVN	13	0.694
4	183	194	YGFQPTNGVGYQ	12	0.69
5	125	139	NSNNLDSKVGGNYNY	15	0.663
6	232	238	HCPFGEV	7	0.645
7	143	151	LFRKSNLKP	9	0.588
8	53	62	YSVLYNSASF	10	0.575
9	31	35	NATRF	5	0.511

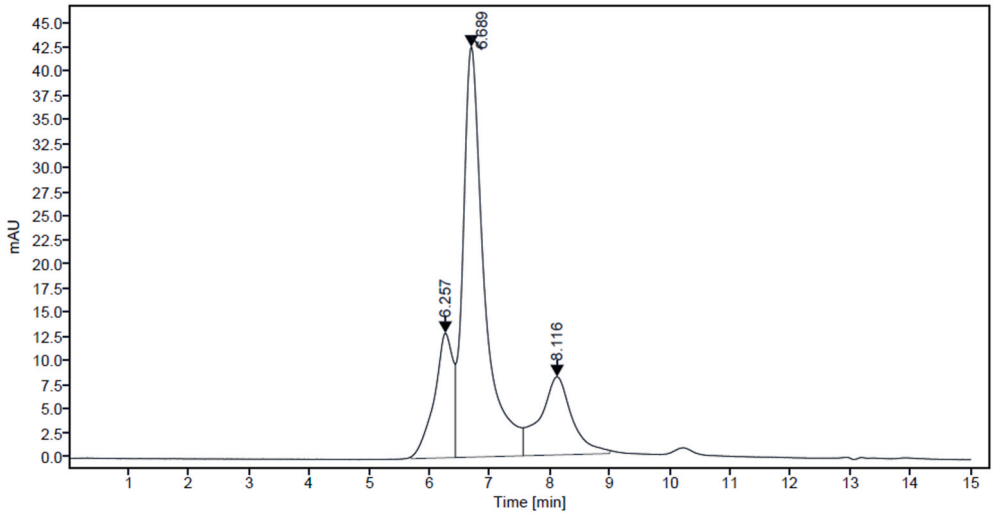

Fig. 2. Chromatogram of the final product of trimeric glycoprotein purification.

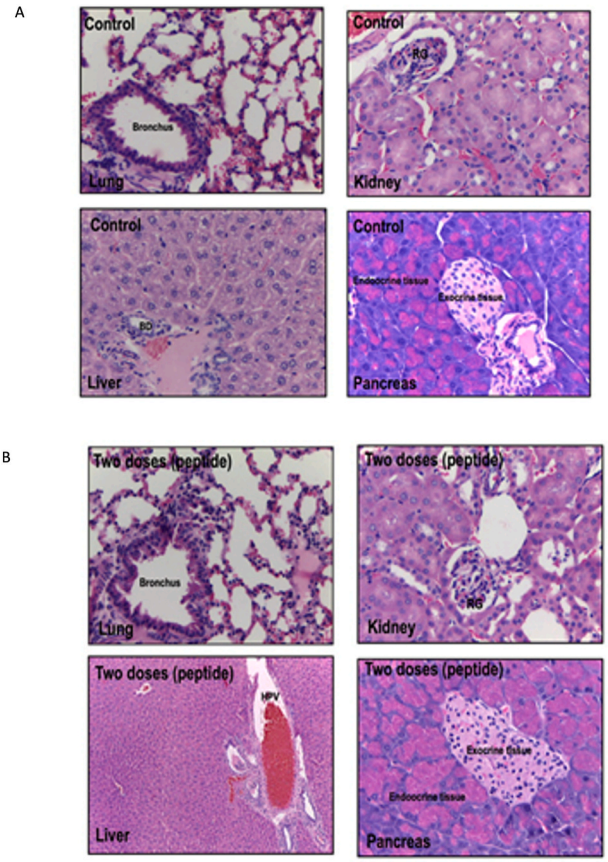

Fig. 3. Pathological evaluation in BALB/c mice in the control group (A) and vaccinated with two doses of the trimeric RBD vaccine candidate (B). No inflammatory or tissue alterations were observed in the liver, lung, kidney, and pancreas after applying two doses of RBD. BD: Biliary Duct, HPV: Hepatic Portal Vein, RG: Renal Glomerulus.

4. Discussion

In this preliminary study, we have successfully designed and evaluated the immunogenic potential of a vaccine candidate based on the RBD of SARS-CoV-2. Our findings provide crucial insights into the safety and effectiveness of the vaccine. The initial tests in the BALB/c animal model have shown that the RBD-trimeric does not generate tissue damage or morphological alterations in the lung, liver, kidney, spleen, and brain. Although histopathological evaluation of the intraperitoneal inoculation site in the mice was not performed, macroscopic follow-up revealed no significant signs of inflammation or induration in the abdomen. These findings are consistent with those reported by Leal et al. [21], who identified pain and inflammation at the inoculation site as the main complications when evaluating RBD heterodimers as vaccine candidates. These significant findings confirm the vaccine candidate's safety [22–24] and pave the way for further research. The safety results are consistent with those obtained by our research group in a previous study [25].

In the study perfomed by Díaz et al. [25], the researchers used a trimer based on the RBD of SARS-CoV-2 and obtained a seroconversion of 75 % of the animals with two doses. The present study obtained seroconversion in 81 % of the mice from the first dose. The results indicate that a vaccine model based on a trimer not only confirms safety but also stimulates the immune system to a greater extent, producing antibodies and achieving seroconversion in 90 % of individuals with two doses of 25 μg of the RBD-trimer. This promising result suggests the potential of our vaccine model. It is possible that the first dose of RBD-trimer elicited a detectable immune response, and additional doses did not significantly increase antibody production. Nevertheless, booster doses increased the seroconversion rate.

These results are comparable with those published by Rina-Su et al. [26]. who evaluated a vaccine candidate based on an RBD trimer and only managed to induce a robust humoral response after two doses. However, the RBD trimer used in this study generated an immune response with a single dose, representing an advantage in safety and in reducing adverse events associated with vaccination [26]. However, implementing the RBD trimer has been shown to induce a more robust humoral immune response than RBD monomers, making this antigenic scaffold a promising candidate for future human or veterinary vaccines. In addition, it offers a new perspective for the design of other vaccines [27].

The robust humoral immune response described in this work, driven by IgG antibodies, could indirectly demonstrate the differentiation of $CD4^+$ T cells into Th1 and Th17 effector phenotypes. These phenotypes are characterized by the release of TNF-α, IFN-γ, and IL-17, and these cytokines play a relevant role in plasma cells during the isotype switching to IgG (neutralizing antibody) [28]. Moreover, it is important to mention that since a large part of the global population has been exposed to the virus naturally or through vaccines, the study of the cellular immune response becomes less relevant in this context.

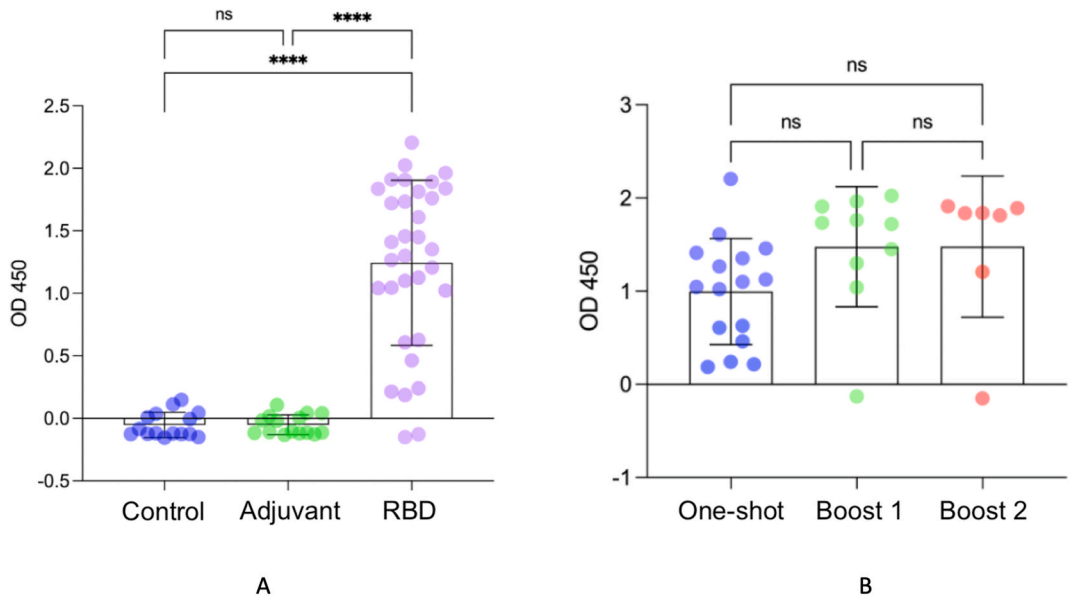

Fig. 4. (A) Absorbance results of the experimental groups. (B) Comparison between the different vaccine doses of trimer-RBD administered.

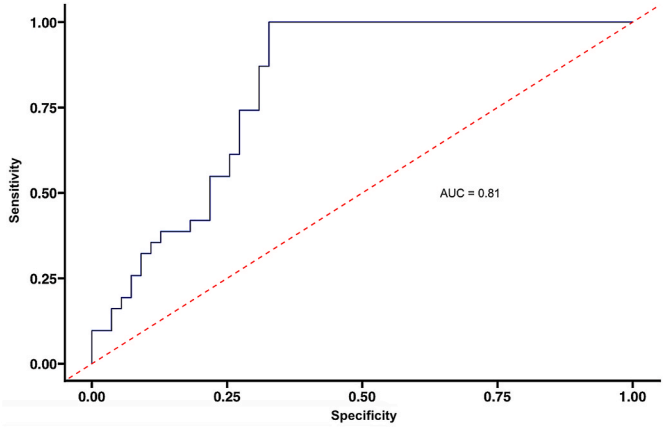

Fig. 5. Area under the Receptor Operating Characteristic Curve (AUC-ROC) of the *in house* RBD-Trimeric ELISA.

The results of the in-house ELISA indicate good performance for detecting IgG antibodies against the RBD of SARS-CoV-2 generated in a murine model and humans. The test can confirm the absence of specific antibodies against RBD with 96 % confidence. Furthermore, it did not show cross-reactivity with sera positive for Hantavirus, Dengue, Zika, Chikungunya, and Rickettsia as presented by some tests marketed and used as diagnostic techniques during the first peaks of the pandemic in the Colombian Caribbean [18]. This confirms that the test discriminates IgG antibodies against SARS-CoV-2 concerning antibodies generated by microorganisms that cause tropical diseases. However, the in-house ELISA prototype showed moderate specificity (79.3 %). This value indicates that the test could lead to false positives, requiring methodological adjustments such as incubation time and temperature [19,22]. Another factor to consider is the number of serums processed. More data obtained from sera with confirmed clinical diagnosis could normalize the values and increase reliability regarding the test's specificity [29]. However, the ELISA design effectively measured the humoral response stimulated by the vaccine candidate in a murine model. Using the same antigen for both processes proved ideal for detecting antibodies specific to the vaccine candidate [22,29]. This suggests that the methodology can be applied to other microorganisms.

This could signify a momentous leap towards biotechnological sovereignty for developing countries, empowering them to face future public health challenges with confidence. The COVID-19 pandemic has starkly exposed the structural weaknesses and vulnerability of health systems, particularly the biotechnology shortage. However, the World Bank's disbursement of funds to facilitate vaccine acquisition and distribution in developing countries was a step towards addressing this issue. The disparity in technological resources led to low vaccination coverage and the virus's continued evolution [30].

By developing and validating vaccines internally, developing countries can reduce their dependence on imports, which is often costly and subject to supply constraints. Moreover, the present study underscores the potential long-term benefits of investing in the development of technical skills and research capabilities within the country, both in terms of economic and scientific development. The progress shown in this work could encourage short-term research for local development that contributes to the biotechnological sovereignty of Colombia. By developing and producing vaccines and diagnostic tests locally, Colombia can ensure a faster and more effective response to health threats and even monitor some endemic diseases.

This research is a scientific endeavor and a strategic step in supporting Colombia's current efforts to strengthen its biotechnological sovereignty [31]. The recent construction of a vaccine plant is a tangible example of the country's investment in infrastructure and technology to develop local capabilities in vaccine production. This project, driven by the Ministry of Science, Technology and Innovation of Colombia, underscores the importance of collaboration between the government, academia, business people, and civil society. The National Science, Technology, and Innovation (CTeI) policy has paved the way for significant achievements, such as the ones highlighted in this study, to strengthen biotechnology and recover the country's pharmaceutical independence. This underscores the importance of designing, testing, and evaluating prototypes in the context of Colombia's biotechnological sovereignty and the potential of this research to contribute to these national objectives.

Finally, these results show high seroconversion and a robust immune response. However, to gain a more comprehensive understanding of the efficacy of this vaccine candidate, it is necessary to evaluate the stability, durability, affinity, and neutralizing capacity of the generated antibodies in the future. Additionally, these advancements are crucial for addressing local health challenges, and fostering collaboration between the government, academia, and the private sector will strengthen

biotechnology in Colombia, contributing to a more effective response to health emergencies.

5. Conclusions

The study confirms that the RBD-trimeric vaccine candidate is safe and effective in a murine model. It significantly increases antibody levels and demonstrates strong potential for preventing SARS-CoV-2 infection.

The ELISA developed in this study showed good performance in detecting IgG antibodies against the RBD of SARS-CoV-2 in murine and human models. Despite moderate specificity (79.3 %), the test proved useful in discriminating between specific antibodies against SARS-CoV-2. Although this study provides valuable information on the safety and initial efficacy of vaccine candidates, further research is needed to assess the stability, durability, and neutralizing capacity of the generated antibodies. In addition, neutralization assays are essential to determine the protection of vaccine candidates against viral infections, which will be a crucial aspect in future studies.

CRediT authorship contribution statement

Luis Flórez: Writing – review & editing, Writing – original draft, Methodology, Data curation. **Daniel Echeverri-De la Hoz:** Writing – original draft, Formal analysis, Data curation. **Alfonso Calderón:** Methodology, Conceptualization. **Hector Serrano-Coll:** Investigation, Conceptualization. **Caty Martinez:** Methodology, Investigation, Conceptualization. **Camilo Guzmán:** Investigation, Conceptualization. **Bertha Gastelbondo:** Methodology, Investigation, Conceptualization. **German Arrieta:** Methodology, Investigation, Conceptualization. **Ariel Arteta:** Investigation. **Tania Márquez:** Methodology, Investigation. **Ricardo Rivero:** Methodology, Investigation. **Salim Máttar:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Informed consent statement

The human sera and the informed acknowledgment were obtained to use in the ELISA tests.

Institutional review board statement

The Ethics Board of the Faculty of Veterinary Medicine, University of Cordoba, Acta 003, of June 7, 2022, approved the animal experimental model.

Data availability statement

Informed consent was obtained from all subjects involved in the study.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Salim Mattar reports financial support was provided by University of Cordoba. Salim Mattar reports a relationship with University of Cordoba that includes: employment. No apply If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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