Remarkable immunogenicity and protective efficacy of BBV152, an inactivated SARS-CoV-2 vaccine in rhesus macaques

Pragya Yadav
ICMR-NATIONAL INSTITUTE OF VIROLOGY

Raches Ella
Bharat Biotech International Limited

Sanjay Kumar
Dept. of Neurosurgery, Command Hospital (Southern Command), Armed Forces Medical College (AFMC)

Dilip Patil
Indian Council of Medical Research-National Institute of Virology

Sreeleekshmy Mohandas
Indian Council of Medical Research-National Institute of Virology

Anita Shete
Indian Council of Medical Research-National Institute of Virology

Gaurav Bhati
Army Institute of Cardio -Thoracic Sciences

Gajanan Sapkal
National Institute of Virology

Himanshu Kaushal
Indian Council of Medical Research-National Institute of Virology

Savita Patil
Indian Council of Medical Research-National Institute of Virology

Rajlaxmi Jain
Indian Council of Medical Research-National Institute of Virology

Gururaj Rao Deshpande
Indian Council of Medical Research-National Institute of Virology

Nivedita Gupta
Indian Council of Medical Research

Kshitij Agarwal
Indian Council of Medical Research

Mangesh Gokhale
Indian Council of Medical Research-National Institute of Virology

Basavaraj Mathapati
Sharda Sharma  
Indian Council of Medical Research-National Institute of Virology

Rashmi Gunjikar  
Indian Council of Medical Research-National Institute of Virology

Abhinendra Kumar  
Indian Council of Medical Research-National Institute of Virology

Kaumudi Kalele  
Indian Council of Medical Research-National Institute of Virology

V K Srinivas  
Bharat Biotech International Limited

Krishna Mohan  
Bharat Biotech International Limited

Raman Gangakhedkar  
Indian Council of Medical Research

Krishna Ella  
Bharat Biotech International Limited

Priya Abraham  
Indian Council of Medical Research-National Institute of Virology

Samiran Panda  
Indian Council of Medical Research

Balram Bhargava (✉ balrambhargava@yahoo.com)  
Indian Council of Medical Research

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Article

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Abstract

The COVID-19 pandemic is a global health crisis that has severely affected mankind and posed a great challenge to the public health system of affected countries. The availability of a safe and effective vaccine is the need of the hour to overcome this crisis. Here, we have developed and assessed the protective efficacy and immunogenicity of an inactivated SARS-CoV-2 vaccine (BBV152) in rhesus macaques (Macaca mulata). Twenty macaques were divided into four groups of five animals each. One group was administered a placebo while three groups were immunized with three different vaccine candidates at 0 and 14 days. All the macaques were challenged with SARS-CoV-2 fourteen days after the second dose. The protective response was observed with increasing SARS-CoV-2 specific IgG and neutralizing antibody titers from 3rd-week post-immunization. Viral clearance was observed from bronchoalveolar lavage fluid, nasal swab, throat swab, and lung tissues at 7 days post-infection in the vaccinated groups. No evidence of pneumonia was observed by histopathological examination in vaccinated groups, unlike the placebo group which showed features of interstitial pneumonia and localization of viral antigen in the alveolar epithelium and macrophages by immunohistochemistry. Data from this study substantiate the immunogenicity of the vaccine candidates and BBV152 is being evaluated in Phase I clinical trials in India (NCT04471519).

Introduction

The pandemic of coronavirus disease 2019 (COVID-19) has caused an unprecedented public health burden in several countries across the globe\(^1\). The spread of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is has infected more than 23 million people until August 2020\(^2\). With all the public health measures in place, including behavioural modifications such as the use of masks, hand sanitization; pharmaceutical interventions such as antiviral drugs or safe and effective vaccines seem to be the only means of stopping this raging pandemic. With the release of the first genome sequence of SARS-CoV-2 from China on 11 January 2020, the race against the virus and time had begun for the development of an effective COVID-19 vaccine. Multiple vaccine development platforms from traditional to next-generation approaches are being used by different research groups worldwide. Purified inactivated viruses have been traditionally used in vaccine development. These inactivated vaccines are safe and effective in the prevention of diseases i.e., rabies, polio, hepatitis A, and influenza\(^3\). Here, we report the assessment of immunogenicity and protective efficacy of three formulations of a purified whole-virion inactivated SARS-CoV-2 (BBV152) vaccine candidate in the rhesus macaques.

Results

Anti-SARS-CoV-2 IgG and neutralizing antibody response

Adverse events were not seen in animals immunized with a two-dose vaccination regimen. We evaluated anti-SARS-CoV-2 Immunoglobulin-G (IgG) antibody and neutralizing antibody (NAb) titers from the serum samples during the immunization phase (0, 12, 19, 26 and 28 days) and after SARS-CoV-2 infection (0, 1,
3, and 7) (Figure 1A). IgG levels were detectable from 3rd-week post-immunization and were found increasing till 35th day [7 days post-infection (DPI)] (Figure 1B, 1C). Group III showed the highest IgG titer (1:25600) compared to group II and IV (1:1600-1:6400) (Figure 1D). The highest NAb titers of 1:209 to 1:5,217 were detected in group III after the SARS-CoV-2 challenge. The NAb titers for groups II and IV were (1:87.4 - 1: 3974) and (1:29.5 -1: 3403) respectively (Figure 1E, 1F). These NAb titers correlated with the IgG antibody titers (Figure 1B-F). NAb and IgG response was not detectable in the placebo group (Figure 1B-F).

**Viral load in the nasal swab, throat swab and bronchoalveolar lavage fluid**

Genomic RNA (gRNA) was detected from nasal swab (NS) specimens of all animals in the placebo group from 1 to 7 DPI. Viral clearance was observed in NS specimens of all the animals from the vaccinated group on 7 DPI. (Figure 2A). Subgenomic RNA (sgRNA) was detected in two of five animals at 3 DPI and one of five animals at 7 DPI of the placebo group. sgRNA was detected in the NS sample of only one animal of the vaccinated group IV on 5 DPI (Figure 2A).

Throat swab (TS) specimens of the placebo group were tested positive for gRNA at 1, 3, 5, and 7 DPI. Vaccinated groups had a detectable level of gRNA from 1 to 5 DPI with viral clearance on 7 DPI (Figure 2B). sgRNA was not detected in TS specimens of animals from either group. Bronchoalveolar lavage (BAL) fluid specimens of the animals from the placebo group were positive for gRNA from 1 to 7 DPI. In the vaccinated groups, gRNA was detected in BAL specimens until 3 DPI (Figure 2C). sgRNA was detected in BAL specimens of four out of five animals of the placebo group, while it was not detected in BAL specimens of vaccinated groups. Except for the placebo group, none of the vaccinated groups showed the presence of gRNA in lung lobes (Figure 2D). The comparisons of viral copy numbers of the NS, TS, and the BAL fluid samples of the vaccinated as compared to the placebo group were found to be statistically significant using the two-tailed Mann-Whitney test.

**Viral load in the respiratory tract, lungs, and extra-pulmonary organs**

On 7 DPI, animals from all the groups were sacrificed and swab samples, BAL, and various organs were collected. The animals of the placebo group at 7 DPI showed bronchopneumonic patches and consolidation in the lungs at necropsy (Figure 3A and 3B). In the placebo group, gRNA was detected in the trachea (3/5), nasopharyngeal mucosa (2/5), oropharyngeal mucosa (3/5) and nasal mucosa (1/5) specimens (Figure 3C). Four out of five animals had detectable gRNA and sgRNA in multiple lobes of the lungs (Figure 3D). Lung specimens of all animals from the vaccinated groups were found negative for gRNA and sgRNA. In the placebo group, gRNA was detected in skin, ileum, colon, gall bladder, stomach, urinary bladder, and pancreas. Only one animal from group IV showed the presence of gRNA in ileum and caecum. Heart, liver, kidney, spleen, and brain were tested negative for gRNA in all animals.

**Clinico-radiological analysis**
Weight loss, pyrexia, and worsening of SpO2 at room air, lethargy, reduced food and water intake, reduced self-grooming was observed in the placebo group and persisted till 7 DPI whereas these features resolved in the other group II and IV (Supplementary Table 1). The chest radiograph of the three animals in the placebo group showed infiltrates, bronchopneumonia, or lobar pneumonia which persisted till 7 DPI. Similar chest radiographic abnormalities were detected in two out of five animals in group II and IV, but resolved by 5 DPI (Extended Data Figure 1A-D). No clinical or radiographic abnormalities were detected in group III animals.

**Histopathological examination and immunohistochemistry**

Lung tissue showed mild to moderate interstitial pneumonia in the animals of the placebo group (Table 2) characterized by thickening of alveolar septa, hyaline membrane formation, accumulation of edematous fluid, and fibrin. Occasionally, certain foci of bronchioles showed necrosis and loss of epithelium with neutrophils and macrophage infiltration. Moderate to severe disease was present in 3 and mild disease in 2 animals of the placebo group with the involvement of four to six lung lobes. In group II, two animals had a single lobe of the lung affected (Table 3). Viral antigens were detected in the alveolar epithelium by immunohistochemistry (IHC) in the placebo group suggestive of SARS-CoV-2 infection. These findings indicate significant protection of the lungs from the vaccinated group as compared to the placebo group. Group II, III, and IV had significantly lower disease burden compared to the placebo group (Table 3).

**Virus isolation**

NS, TS, BAL fluid, urine, stool, and lung tissue specimens (1 to 7 DPI) from placebo and vaccinated groups were processed for virus isolation. Cytopathic effect (CPE) was observed in TS and NS specimens of the placebo group on 1 and 3 DPI. BAL, urine, stool, and lungs specimens did not show CPE in any group. Two TS and one NS specimen of group II and IV respectively yielded virus isolation on 1 DPI.

**Lymphocyte subset and cytokine/chemokine profile**

Lymphocytes were found to be significantly low in the placebo (mean ± SD; 14.51±9.31, p < 0.05) compared to the vaccinated macaques (group II, mean ± SD; 31.88±15.85) on 1 DPI. The assessment based on the surface marker of gated lymphocytes from lysed whole blood showed no statistically significant difference between placebo and vaccinated groups in terms of the percentage of cells expressing CD3+, CD8+ and CD20+ till 7 DPI (Extended Data Figure 2). IL-6 was found to be of significantly higher in the placebo group (mean ± SD; 22.78±23.21, p < 0.001) compared to the group's II (mean ± SD; 2.97±4.85, p < 0.001) and III (mean ± SD; 2.65±1.98, P < 0.001). Additionally, the anti-inflammatory cytokine IL-5 was significantly higher in group II (mean ± SD; 3.14±2.41, p < 0.001) compared to the placebo group on 1 DPI. IL-8 was found significantly higher in group III on 3 DPI and 5 DPI (mean ± SD; 4653.65±3088.34, p < 0.05, mean ± SD; 4715.5±2864.15, p < 0.01) compared to the placebo group (mean ± SD; 1891.16±878.11, 3 DPI; mean ± SD; 1216.11±757.64, 5 DPI). IL-2, TNF-α, and IFN-γ were not detectable in all the animals.
Discussion

A safe and effective vaccine is the need of the hour to overcome the COVID-19 pandemic. In the global race for the development of vaccines, few research groups have reported the preclinical studies of viral vector vaccines (ChAdOx1 nCoV-19 and Ad-26.COV.2.S)\(^4,5\), mRNA vaccine (mRNA-1273)\(^6\), DNA vaccine (INO-4800)\(^7\) and inactivated vaccines (PiCoVacc and BBIBP-CorV)\(^8,9\). Phase I/II clinical trial are either completed or on-going for these vaccines. Here, we report the two-dose vaccination regimen of inactivated SARS-CoV-2 vaccine candidates found to induce a strong immune response and protection of animals from the infection of SARS-CoV-2.

Neutralizing antibodies and IgG responses were observed from 3\(^{rd}\)-week post-immunization in vaccinated groups. IgG titer rose in an increasing pattern with the highest response in group III. The presence of gRNA in NS was observed in the placebo group until 7 DPI. Vaccinated groups had no detectable gRNA in NS on 7 DPI indicating the ability of vaccine candidates to limit upper respiratory tract viral replication, which is a key factor determining the virus transmission. gRNA and sgRNA were not detected in the BAL fluid from 5 DPI suggesting that vaccination hindered virus replication and enabled faster clearance from lower airway protecting the animals. gRNA was detected in multiple organs at necropsy in the placebo group, whereas it was found to be cleared in the vaccinated groups.

Wang et al. and colleagues reported immunogenicity of the two-dose vaccination regimen with effective NAb response in low and middle vaccine doses of inactivated SARS-CoV-2 vaccine and noted virus clearance\(^9\). Similarly, Gao et al. and colleagues also reported rising titers of NAb with three dose vaccinations regimen and protection of animals by virus clearance from pharynx or lungs at 7 DPI with high vaccine dose\(^8\).

An elevated level of IL-6 is highly consistent in COVID-19 disease and lymphopenia may be associated with a high level of IL-6\(^10,11\). Our experimental data showed an elevated level of IL-6 in the placebo group compared to vaccinate groups (II and III) on 1 DPI, suggestive of protection against the SARS-CoV-2 infection. The transient lymphopenia in the placebo group could be suggestive of suboptimal cellular immunity in the placebo group or may be due to the recruitment of lymphocytes to the inflamed respiratory tract\(^12,13\). Furthermore, lymphopenia is specifically CD8+ T cells biased\(^12,14\), this possibly explains the elevated proportion of CD4+ T cells in the placebo group\(^12\). IL-5, a Th\(_2\) cytokine, is associated with eosinophilia, and evidence of eosinophils for antiviral activity is documented\(^15\). Our study demonstrated an elevated level of IL-5 in vaccinated group II compared to the placebo group. IL-8, a key chemokine, responsible for the recruitment of neutrophils and other immune cells was substantially high in vaccinated group III indicative of infection.
Organ viral load, interstitial pneumonia, and detection of viral antigen by IHC in lung tissue strengthen the evidence of SARS CoV-2 induced pulmonary disease in placebo animals. Group III (3 µg, Adjuvant-A) animals had no clinical/radiological evidence of disease activity demonstrating significant protection from SARS-CoV-2. These findings correlate well with protection from disease demonstrated by serological, gross pathological, and HPE findings.

Altogether this study demonstrates that a two-dose vaccination regimen using 3µg dose of the vaccine candidate with adjuvant B induce a significant immune response and provide effective protection in animals challenged with SARS-CoV-2. Data from this study substantiate the immunogenicity of BBV152 which is being evaluated in Phase I clinical trials in India (NCT04471519).

**Declarations**

**Acknowledgment**

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**Author’s Contribution**

PDY, KME, KM, PA, NG, and BB conceived and designed the study. KME, KM, RE, VKS, and SPBS performed vaccine design and production. PDY, SK, SLM, and DRP performed the planning of the animal experiment. SK, DRP, SLM, GB, KA, SMM, BM, and MDG performed animal experimentation. PDY, ASA, GS, HK, DN, RJ, GD and DYP performed the laboratory work planning and data analysis. ASA, PDY, and RJ developed the in-house ELISA. GD, CM, HK, SP, RJ, ANK, YJ, TM, PS, SB, DM, KK, SS, RG, PG, and HD performed sample processing in the laboratory. SF, AS, MK, AK, SD, and SG assisted in animal experimentation. PDY, SK, ASA, SM, and DYP have drafted the manuscript. PDY, SP, ASA, DYP, SK, PA, NG, and BB substantively revised it. All authors reviewed the manuscript and agree to its contents.
References


**Methods**

**Ethics statement**

The study was approved by the Institutional Project Review Committee, and Institutional Biosafety Committee, ICMR-National Institute of Virology (NIV), Pune, The study was recommended by the Institutional Animal Ethics Committee (Registration 43/GO/ReBi/SL/99/CPCSEA) and further approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi letter No. V11011 (13)/7/2020-CPCSEA-DADF dated 08,06,2020. The permission from the Office of Principal Chief Conservator of Forests (PCCF), Maharashtra state, and Central India was obtained for capturing of *Rhesus Macaques* from the wild. The research was conducted in compliance with the guidelines laid down by CPCSEA, Government of India."
Generation of vaccine

Bharat Biotech has established biosafety level 3 (BSL3) manufacturing facilities with a highly characterized and safe vero cell manufacturing platform that were readily deployed towards process development and large-scale manufacturing. Vero CCL-81 cells were initially grown in tissue culture flasks and cell stacks using Dulbecco's Modified Eagle Medium (DMEM) (Sigma-Aldrich, India) containing 5-10% newborn calf serum (NBCS). Virus propagation was done in bioreactors at the temperature of 36 ± 1°C and was harvested at 36-72 h post-infection and supernatants were collected, clarified, and aliquoted. The virus was inactivated with ß-propiolactone at a ratio of 1:2000 – 1:4000 at 2-8°C for 24-32 h. It was further purified by column chromatography and concentrated using a tangential flow filtration system. A total of three vaccine formulations were prepared, namely BBV152A (3µg+alum+ imidazoquinoline), BBV152B (6µg+alum+ imidazoquinoline), and BBV152C (6µg+alum). Imidazoquinoline are TLR7 / TLR8 agonists, which are highly immunostimulatory in nature.

Study design and experiments on rhesus macaques

Rhesus macaques (Macaca mulatta) were housed in individual cages at the animal facility of ICMR-NIV, Pune. The animals were maintained on commercial pelleted feed, fruits, vegetables, and ad-libitum potable drinking water with a 12h/12h dark/light cycle. All the animals were clinically evaluated for skin/systemic disorders, hemoglobin, total leukocyte count, differential leukocyte count, platelet count, packed cell volume, biochemical parameters (AST, ALT, bilirubin, serum proteins, alkaline phosphatase, LDH, BUN, creatinine, cholesterol, triglycerides, sodium, potassium, glucose), abdominal ultrasonography, chest X-ray, tuberculin test and were found fit for the study. Animals were screened for Kyasanur forest disease virus and SARS-CoV-2 and IgG antibodies and found to be negative\textsuperscript{17,18}. Biomedic data systems temperature transponder was implanted in the interscapular region subcutaneously for monitoring of body temperature during the study.

Twenty adult animals aged 3 - 12 years were divided into 4 groups of five animals (3 M, 2 F) each viz. the placebo (group I), group II, III, and IV. The placebo group was administered Phosphate buffer saline (PBS), group II, III, and IV were immunized with formulations of purified inactivated SARS-CoV-2 vaccine candidate 6µg+Adjuvant-A (BBV152C), 3µg+Adjuvant-B (BBV152A), and 6µg+Adjuvant-B (BBV152B) respectively. Animals were administered with two doses of vaccine/placebo on days 0 and 14 respectively intramuscularly in the deltoid region. Blood samples were collected on 0, 12, 19, 26, and 28 days for assessing the anti-SARS IgG antibody and NAb titers (Figure 1A).

After completion of twenty-eight days of immunization, animals were shifted to animal biosafety level-4 facility. Animals were challenged with 1 ml of SARS-CoV-2 (P-3, NIV-2020770, TCID50 10\textsuperscript{6.5}/ml)\textsuperscript{19} intratracheally and 0.25 ml in each nostril. Animals were monitored twice daily and clinical scoring was performed based on parameters as listed in Supplementary Table 3. Clinical examination was done on 0, 1, 3, 5, and 7 DPI along with body temperature, body weight, pulse rate, and oxygen saturation at room air (Supplementary Table 3). NS, TS, rectal swab, chest X-ray, blood specimens, and BAL fluid were collected
on 0, 1, 3, 5, and 7 DPI. BAL fluid collection was performed using a flexible pediatric bronchoscope (Pentax Medical India Private Limited) under general anesthesia. The bronchoscope was inserted into the trachea and was guided through bronchus past the 3rd bifurcation; 5 ml of normal saline was instilled and aspirated from the lower/middle lobes of the lungs bilaterally. On 7 DPI, a detailed bronchoscopy and BAL fluid collection from lobes of the lungs bilaterally were performed.

During necropsy, the following organs; brain, nasal mucosa, tonsil, nasopharynx, oropharynx, cervical lymph node, trachea, lungs, mediastinal lymph node, heart, spleen, liver, kidneys, urinary bladder, gastrointestinal tract and skin along with underlying deltoid muscle from the immunization site and cerebrospinal fluid (CSF) were collected.

**Enzyme-linked immunosorbent assay for detection of anti-SARS-CoV-2 IgG antibody**

Immunoplates (Maxisorp, Nunc) were coated with 100 μL/well of SARS CoV-2 antigen overnight at 4°C in the carbonate buffer. Subsequently, wells were blocked with liquid plate sealer (CANDOR Bioscience GmbH, Germany) for two hours at room temperature (25-30°C). One hundred μL of diluted rhesus macaque serum samples (1:100 in 1% bovine serum albumin in 1×PBS containing 0.1% Tween (PBST) were added to duplicate wells and incubated at 37°C for one hour. To each well, 100 μL of a 1:15000 dilution of goat anti-monkey IgG peroxidase-conjugated antibodies (Jackson ImmunoResearch, USA) was added wells and incubated at 37°C for one hour. The plates were washed 5 times with a wash buffer (1×PBST) post all incubations. One hundred microliters of (3,3',5,5'-Tetramethylbenzidine (TMB) substrate was added and incubated for 10 min. The reaction was stopped by 1N H2SO4 and absorbance was measured at 450 nm18. The sample was considered positive when the P/N ratio was more than 1.5. and optical density values with the SARS-CoV-2 antigen was above 0.2.

**Plaque reduction neutralization test**

The plaque reduction neutralization test (PRNT) was performed as described earlier20. A four-fold serial dilution of rhesus macaque's serum samples was mixed with an equal amount of virus suspension containing 50-60 plaque-forming units (PFU) in 0.1 ml. After incubating the mixtures at 37°C for 1 h, each virus-diluted serum sample (0.1 ml) was inoculated onto one well of a 24-well tissue culture plate containing a confluent monolayer of Vero CCL-81 cells. After incubating the plate at 37°C for 60 min, an overlay medium consisting of 2% carboxymethyl cellulose (CMC) with 2% fetal calf serum (FCS) in 2× MEM was added to the cell monolayer, and the plate was further incubated at 37°C in 5% CO2 for 5 days. Plates were stained with 1% amido black for an hour. Antibody titers were defined as the highest serum dilution that resulted in >50 (PRNT50) reduction in the number of plaques.

**Detection of SARS-CoV-2 genomic and subgenomic viral RNA**

The organs harvested during necropsy were uniformly weighed and homogenized (Tissue homogenizer, Qiagen, Germany) using 1 ml of sterile MEM (GIBCO, Thermo Fisher Scientific, USA). Two hundred μl of each specimen (NS, TS, rectal swab, whole blood, BAL fluid, urine, CSF, and tissue homogenates) was
used for RNA extraction using MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit (Thermo Fisher
Scientific, USA). SARS-CoV-2 real-time RT-PCR was performed and a standard curve was plotted using in
vitro transcribed RNA for E gene (gRNA) as described earlier\textsuperscript{21,22}. Subgenomic RNA for the E gene was
amplified using real-time RT-PCR from the above specimens using earlier published literature\textsuperscript{23}.

**Lymphocyte subset and cytokine/chemokine profile**

To analyze phenotype and proportion of T helper, T cytotoxic and B cells anti-coagulated (0.1ml) whole
blood was surface-stained with appropriate fluorochrome-conjugated antibodies along with their
corresponding isotype controls. Two sets of sample tubes were prepared, one for T cell (CD3-FITC, CD8-
PE, CD4-APC) and another for B cell (CD45-PerCP, CD3-FITC, CD20-PE). After incubation for 30 min at 4\textdegree C
in dark, 2 ml of RBC lysing buffer were added to each tube, vortexed, and incubated at room temperature
for 12 min. Two milliliters of washing solution was added to each tube. The sample tubes were
centrifuged at 200 x g for 5 min. and the supernatant was carefully aspirated out. The cell pellets were
suspended in 500μl wash buffer and vortexed. Y, α Y, α

**Virus isolation from clinical/necropsy specimens**

One hundred microliters of each specimen were inoculated onto 24-well Vero CCL-81 cell monolayers
maintained in MEM (Gibco, UK), and incubated for 1 h at 37°C with rocking every 10 min. Subsequently,
the media was removed and cells were washed with 1× PBS. Media with 2% FBS was added to each well
and was incubated in a CO\textsubscript{2} incubator at 37°C for 5 days. The culture plate was examined daily for CPE
using an inverted microscope (Nikon, Eclipse Ti, Japan)\textsuperscript{19}. The cell culture supernatant from the wells
showing CPE was further confirmed by real-time RT-PCR\textsuperscript{21}.

**Histopathological examination and Immunohistochemistry**

Tissue sections from lungs were immersion-fixed in 10% neutral buffered formalin. Tissue processing
and embedding were performed by techniques described earlier\textsuperscript{25}. Four micrometers thick tissue sections
were used for hematoxylin and eosin staining. Anti-SARS-CoV-2 immunoreactivity in the tissues was
assessed using mouse polyclonal serum. For IHC, mouse polyclonal serum was used as the primary
antibody (1:500) and anti-mouse HRP antibody was used as a secondary antibody\textsuperscript{25,17}.

**Statistical analysis**

Clinical, virological, hematological, biochemical, and immunological data were analyzed using GraphPad
Prism software version 8.4.3 (GraphPad, San Diego, California) and Stata 14 software. (StataCorp LLC,
USA). The clinical, virological, and serological data for the different groups were initially compared using
the non-parametric Kruskal-Wallis test. The groups that were significant using the Kruskal-Wallis test were
further assessed using the Mann-Whitney test. A group-wise comparison was performed to assess
significance between the placebo and the other vaccinated groups using a two-tailed Mann-Whitney test.
The p-values less than 0.05 were considered significant and are marked on the figures. Non-significant
values are not depicted in the figures. The log10 plots below the detection limits are depicted as log10 (1) for the illustration purpose. The detection limits are marked with the dotted lines on the respective figures.

**Reporting summary**

The nature research reporting summary provided with this paper contains the information on research design.

**Data availability**

All the data other than those presented in the article are provided in the form of supplementary files.

**Figures**

![Figure 1](image-url)
Experimental summary and humoral response in vaccinated animals (A) Experiment summary of the workflow. Two doses of three different inactivated SARS-CoV-2 vaccine formulations were administered to the three groups of animals. Two doses of placebo were administered to the fourth group of the animals, which were the controls in the study. All the animals were challenged 14 days after the second dose. Samples were collected at different time point pre-challenge and post-infection (B) Anti-SARS-CoV-2 IgG response during a two-dose vaccine regime for four groups of animals observed from 1st to 4th week of immunization. (C) Anti-SARS-CoV-2 IgG response at post-infection (0, 1, 3, 5, and 7 DPI) for four groups of animals. (D) Anti-SARS-CoV-2 IgG titers in animals at 7 DPI. (E) NAb titers in animals from 1st to 4th week of immunization. (F) NAb titers in animals at 0, 1, 3, 5, and 7 DPI. The statistical significance was assessed using the Kruskal-Wallis test followed by the two-tailed Mann-Whitney test between two groups; p-values of less than 0.05 were considered to be statistically significant. The dotted line in figure D indicates the limit of detection of the assay.

Figure 2

Genomic viral RNA detection in respiratory tract specimens. Genomic viral RNA load in (A) nasal swab, (B) throat swab, and (C) BAL at 1, 3, 5, and 7 DPI. (D) Genomic viral RNA load in different lobes of lungs at 7 DPI. The statistical significance was assessed using the Kruskal-Wallis test followed by the two-tailed Mann-Whitney test between the two groups; p-values less than 0.05 were considered to be statistically significant. The dotted lines indicate the limit of detection of the assay.
Figure 3

Gross pathology of lungs and viral RNA load in different tissues. (A) Lungs showing extensive involvement of the RUL, RLL, LUL and LLL (group I), and (B) normal lung (group III) (C) Genomic viral RNA of respiratory tract tissues, (D) lungs tissue and (E) extrapulmonary organs at 7 DPI. The statistical significance was assessed using the Kruskal-Wallis test followed by the two-tailed Mann-Whitney test between the two groups; p-values less than 0.05 were considered to be statistically significant. The dotted lines indicate the limit of detection of the assay.
Figure 4

Histopathological and immunohistochemical findings. (A) Lungs showing moderate acute inflammatory changes with hemorrhages and infiltration of inflammatory cells. Alveolar septa showed hyaline membrane formation (asterisks) with inflammatory cells and RBCs. The adjacent alveolar interstitium is thickened by edema (black arrow) and moderate infiltration of lymphocytes, neutrophils, and macrophages (white broad arrow). H&E, ×400. (B) Lungs showing normal histomorphological features of alveolar septa with type I pneumocyte (white arrow) and occasional alveolar macrophages (black arrow) along the alveolar lumen in group II H&E, ×400. (C) Lungs section depicting normal histomorphological features of alveolar septa with type I pneumocyte (white arrow) and occasional alveolar macrophages (black arrow) along the alveolar lumen in group III H&E, ×400. (D) Lung section depicting normal histomorphological features of alveolar septa with type I pneumocyte (white arrow) and occasional alveolar macrophages (black arrow) along the alveolar lumen in group IV H&E, ×400. (E) Lungs section of placebo group showing the presence of viral antigen by IHC in type-I pneumocytes (arrow) of alveolar septa and alveolar macrophages (arrow) (×400). Lung section of group II (F), group III (G), and group IV (H) showing the absence of viral antigen by IHC.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarytable123.docx
- ExtendedDataFigure1and2.pdf