Virologica Sinica 38 (2023) 627–630

Contents lists available at ScienceDirect

Virologica Sinica

journal homepage: www.keaipublishing.com/en/journals/virologica-sinica
www.virosin.org

Letter

Delta (B.1.617.2) inactivated vaccine candidate elicited neutralizing antibodies to SARS-CoV-2 and circulating variants in rhesus macaques

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Dear Editor,

The coronavirus disease 2019 (COVID-19) pandemic has resulted in 763 million cases and over 6 million deaths as of April 2023. Given the high prevalence and widespread distribution of coronaviruses, the genetic diversity and frequent recombination of their genomes, as well as the increased activity at the human-animal interface, are critical for global public health and socioeconomic impact (Zhu et al., 2020). Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variant Delta was confirmed in India in October 2020 (Edara et al., 2021). Studies have shown that the viral load of those infected with the Delta variant is up to 1260 times higher than that of people infected with the prototype, and the incubation period is shortened from 6 days to 4 days compared with that of the prototype, meaning that it has the characteristics of high viral load and short incubation time (Li et al., 2022). This made the Delta variant the most threatening and dangerous variant in the world before the advent of Omicron.

Currently, a variety of vaccines against SARS-CoV-2 (inactivated vaccines, recombinant protein vaccines, adenovirus vector vaccines, DNA vaccines, and mRNA vaccines) have been developed. However, SARS-CoV-2 mutations might weaken vaccine-induced protective immune responses. CoronaVac or BBIBP-CorV showed only 59% protection against the SARS-CoV-2 variant Delta (B.1.617) (Edara et al., 2021). Two doses of AZD1222 and BNT162b2 vaccines are estimated to be 60% and 88% effective for the Delta variant, respectively (Lopez Bernal et al., 2021b). This emphasizes the need for accelerated development of a vaccine against Delta.

Previous research has shown that Delta is more likely to infect people who receive only a single dose of the vaccine, while a second dose provides better protection (Lopez Bernal et al., 2021b). Therefore, we developed an inactivated vaccine based on the Delta variant and used a two-vaccination immunization schedule in the rhesus macaque model for preclinical virological, immunological, and pathological anti-infection characterization according to the need for protective immunity to rechallenge and demonstrate the preclinical efficacy and safety of the vaccine.

Zhejiang Provincial Center for Disease Control and Prevention carried out the screening of Delta variant strains from samples of imported cases in Zhoushan in 2021 and obtained the candidate strain (accession number: SARS-CoV-2/Vero/LXG/2021/ZJ28 (Delta/B.1.617.2/EPI_ISL_1,911,196) (Supplementary Fig. S1, Fig. 1A). To obtain a domesticated virus stock with high fecundity, we purified the Delta variant and evaluated the genetic stability of the strain, and virus titer determination demonstrated relatively stable results (Supplementary Fig. S2A). The MOI range for virus inoculation was set at 0.001–0.01, and the virus was harvested at 48–96 h (Supplementary Fig. S2B). The virus was inactivated using a ratio of 1:4000 β-propiolactone at 2°C–8°C for 24–28 h. The harvested samples from three batches all exhibited a virus titer of 0, and no cytopathic changes were found in the three blind passages, confirming the stability and repeatability of the inactivation process (Supplementary Fig. S2C). Negative-stained electron micrographs revealed oval-shaped virus particles with diameter from 60 nm to 220 nm (Fig. 1B). Furthermore, Western blot analysis demonstrated the presence of structural proteins of the virus in the vaccine stock solution (Fig. 1C and D).

To evaluate the vaccine immunogenicity in rodent, BALB/c mice and SD rats were intramuscularly received two immunization schedules (vaccinated on D0/D14 or D0/D28), respectively. Each immunization regimen included six groups: low-dose (5 μg/dose), medium-dose (10 μg/dose), high-dose (20 μg/dose) of vaccine with adjuvant, medium-dose

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https://doi.org/10.1016/j.virs.2023.05.009
Received 28 February 2023; Accepted 23 May 2023
Available online 25 May 2023

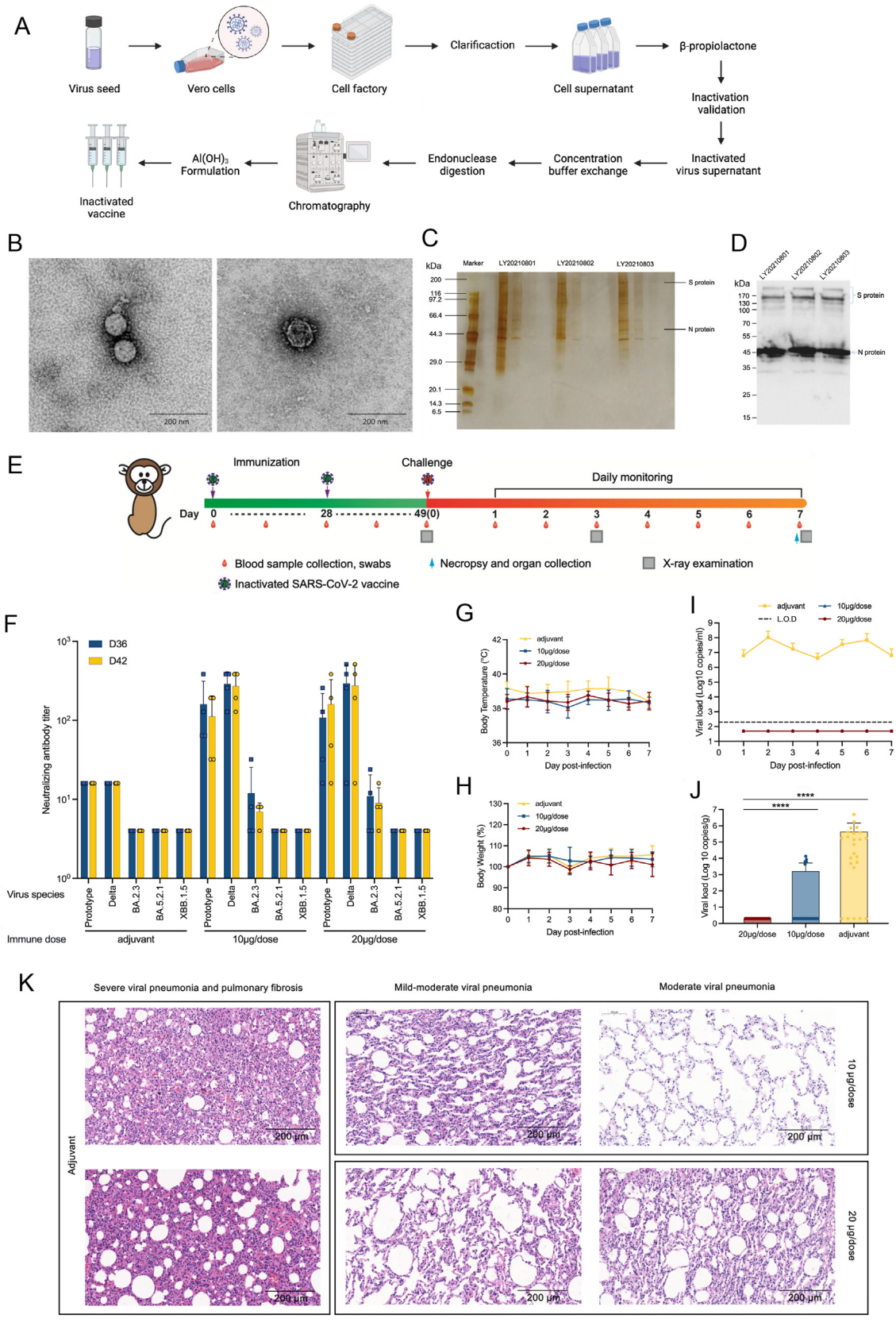

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Fig. 1. Characterization of the inactivated vaccine (Vero cells, Delta variant) candidate for SARS-CoV-2. **A** Flowchart of the inactivated vaccine preparation. **B** Electron microscopy results of the vaccine master and working seed lot. **C** Protein composition and purity evaluation of the inactivated vaccine using SDS-PAGE. Protein bands were stained with silver staining. The figure shows the results of three batches of virus harvested liquids diluted by different times (from left to right: stock solution, 2-fold dilution and 4-fold dilution). **D** Confirmation of proteins in vaccine stock by Western blotting. **E–K** Immunogenicity and protective efficacy of the inactivated vaccine (Vero cells, Delta variant) in rhesus macaques (n = 12). **E** Schematic of evaluating the immunogenicity and protective efficacy of SARS-CoV-2 inactivated vaccine (Vero cells, Delta strain) in rhesus macaques. **F** Rhesus monkey sera were collected on day 8 (D36) and day 14 (D42) after the second immunization and tested for NAb titers against prototype, Delta, Omicron BA.2.3, Omicron BA.5.2.1, and Omicron XBB.1.5. Data are expressed as geometric mean and standard deviation. **G–K** Protective efficacy of inactivated vaccine against SARS-CoV-2 Delta (B.1.617.2) challenge was assessed in rhesus monkeys 21 days after the second immunization. Changes in clinical signs, including body temperature (**G**) and body weight (**H**), were recorded following vaccination. **I** The amount of virus in daily throat swabs obtained from rhesus monkeys for one week after inoculation. **J** Viral load in all seven lung lobes collected from all rhesus monkeys on day 7 post-infection as determined by real-time PCR. **K** Histopathological changes in the lungs of macaques at day 7 post-infection. Kruskal-Wallis test with Dunn's multiple-testing correction (**G–J**) was applied, $^{***}P < 0.0001$. Limit of detection (L.O.D): 200 copies/mL.

(10 μg/dose) of vaccine without adjuvant, adjuvant control and 0.9% NaCl control. SARS-CoV-2 *S*-specific immunoglobulin G (IgG) developed quickly in the serum of the vaccinated mice and increased significantly after the second dose, with the low-dose and mid-dose groups reaching the highest antibody level at week 8, while the high-dose group reached the highest antibody level at week 7 and maintained high antibody titers up to D57. Following a one-way ANOVA with Tukey's multiple comparisons test, it was observed that both the D0/D14 and D0/D28 immunization schedules resulted in similarly high levels of antibodies, with no significant differences between the two schedules (Supplementary Fig. S3A, S3B). Similar results were obtained in the Sprague-Dawley (SD) rat group (Supplementary Fig. S3C, S3D).

The level of neutralizing antibody (NAb) against Delta (B.1.617.2) in each dose group increased gradually with the extension of the post-immunization time. At 56 days after the first immunization, the peak NAb titers of the low (geometric mean titer, GMT 2125), medium (GMT 2803), and high doses (GMT 3613) under the D0/D28 immunization schedule were much higher than those of the D0/D14 schedule (GMT 1292, 1192, and 1610, respectively), which indicated that the D0/D28 immunization schedule is more effective than the D0/D14 immunization schedule (Supplementary Fig. S3E, S3F). The negative control group (non-vaccinated) and the adjuvant group did not produce NAbs during the whole process. However, the results between the D0/D14 and D0/D28 immunization schedules were similar in SD rats (Supplementary Fig. S3G, S3H).

The results of the animal serum cross-NAb assay demonstrated that the vaccine could induce SARS-CoV-2-specific neutralization activity in mice and had good cross-neutralization activity against the prototype, Alpha variant, and Omicron variant (Supplementary Fig. S3I, S3J). It is worth noting that the cross-NAb levels of different doses in the D0/D28 immunization program were higher than those of the D0/D14 program. However, there were no significant differences in NAb levels against the SARS-CoV-2 variants between the dose groups. The results in SD rats were similar to those in BALB/c mice. And the levels of cross-NAbs were closer between the two immunization procedures at different doses for Omicron BA.1 and Omicron BA.2 variant (Supplementary Fig. S3K, S3L). By comparing the NAb levels against Delta and other variants, we found that the neutralizing potency of the anti-serum against other SARS-CoV-2 variants was lower than Delta, but it still remained at a relatively high level which also indicates that the vaccine has a good broad spectrum.

We next assessed the immunogenicity and protective efficacy of the inactivated vaccines in rhesus monkeys challenged with Delta variant (Fig. 1E). Twelve rhesus monkeys were divided into three groups (2/gender/group): adjuvant group; low-dose vaccine group (10 μg); high-dose vaccine group (20 μg). All macaques were immunized on days 0 and 28. At 21 days after the second immunization (D49), SARS-CoV-2 Delta was administered by the intratracheal route with a viral load of 1×10^5 $TCID_{50}$. The results showed that the serum NAb induced by the vaccine has a broad-spectrum protective effect against different SARS-CoV-2 variants (Fig. 1F). Blood cell count remained unchanged after rhesus inoculated with live virus (Supplementary Fig. S4A–S4D). No significant changes in body temperature or body weight of rhesus monkey was observed after being infected with the virus (Fig. 1G and H). The control group exhibited consistently high viral loads in their throat swabs throughout the evaluation period following the virus infection. While all macaques in the vaccine-immunized group tested negative for the virus in their throat swabs (Fig. 1I). Upon autopsy, the viral RNA was not detected in the lungs of the animals in the high-dose immunization group, but there were occasional positive results in the lung tissues of the animals in the low-dose immunization group (Fig. 1J). The pathological analysis showed that the virus caused significantly more severe lung damage in the control group than in the vaccine-immunized group (Fig. 1K). Overall, the results suggest that both low-dose and high-dose inactivated vaccines were effective in protecting rhesus macaques from severe infection with SARS-CoV-2 mutant virus and reduced pathological damage to the lungs.

Single-dose toxicity was assessed in SD rats by administering four doses (10 μg/dose) of the inactivated vaccine (Vero cell, Delta strain) by a single intramuscular injection. No significant toxicity was observed, and the maximum tolerated dose (MTD) was $\geq$ 4 doses per rat (10 μg/dose). The dose administered in this study was 1200 times of the proposed clinical dose, based on human adult weight (60 kg) and rat weight (0.2 kg).

Repeated-dose toxicity was evaluated in cynomolgus monkeys. The animals were divided into three groups and intramuscularly administrated by 0.5 mL 0.9% NaCl (negative control), 1 dose/animal in an injection volume of 0.5 mL, containing 10 μg of inactivated vaccine (Vero cell, Delta strain), or 5 doses/animal in an injection volume of 2.5 mL (containing 50 μg). Each injection was administered at D1, D15, D29 and D43, respectively, for a total of four injections. After the first and/or last injection, there were increases in body temperature, account of eosinophils ($\times 10^9$/L), and levels of fibronectin, and the cytokine IL-6, which were associated with acute reactions, local injection site reactions, and immune responses. No significant systemic toxic reactions were observed, and the no-observed-adverse-effect-level (NOAEL) was five doses per monkey. Local irritation reactions were observed after injection in both groups, but these reactions recovered four weeks after treatment discontinuation.

A systemic active allergic reaction test was conducted in guinea pigs. Sensitization was induced by intramuscular injection of the inactivated vaccine (Vero cell, Delta strain) at dose of 0.1 dose, and the stimulation was 0.2 dose in the low-dose group. In the high-dose group, the sensitization dose was 1 dose, and the stimulation was 2 doses. The concentration/content of each dose was 10 μg/0.5 mL. Sensitization was administered by intramuscular injection once on D1, D3, and D5, and a total of three injections were administered. Three animals in each group were stimulated 14 days after the last sensitization (D19) and other six animals in each group were stimulated 21 days after the last sensitization (D26). The guinea pigs in both low- and high-dose groups showed negative allergic reactions.

The inactivated vaccine elicited a high-intensity immune response, producing high titers of NAbs and *anti*-S1 protein-specific IgG antibodies similar to BBIBP-CorV (Wang et al., 2020). By comparing the immunogenicity of two doses of inactivated COVID-19 vaccine given 14 and 28 days apart, we found that both doses resulted in good antibody positivity rates, which is consistent with previous findings (Xia et al., 2020, 2021;

Zhang et al., 2021). Appropriately extended interval vaccination resulted in a more robust humoral immune response, which did not affect overall immunogenicity and even improved NAb responses to prototype, Alpha, Delta, and Omicron variants. Many countries have adopted immunization strategies with delayed dosing intervals, which have shown commendable clinical efficacy (Lopez Bernal et al., 2021a).

The Omicron BA.4 and BA.5 variants have caused a new wave of global infections (Xiang et al., 2022), with their S sequences highly related to BA.2. Several recent studies have generated and tested Omicron-specific vaccine candidates with different vaccine antigen designs and components. However, the sera NAbs triggered by these Omicron-specific vaccines *in vivo* exerted very limited cross-protection ability against different variants of SARS-CoV-2 and did not have broad-spectrum activity (Lee et al., 2022; Zang et al., 2022; Zhang et al., 2022). The inactivated vaccine we designed has excellent infection-neutralization ability against SARS-CoV-2 variants of concern (VOCs), including VOCs with immune escape properties. Our Delta inactivated vaccine induced relatively high neutralizing antibody levels against Omicron BA.2 (GMT 1024), prototype (GMT 786), and Alpha (GMT 563) in animal models, indicating that it might be proved to be an ideal candidate vaccine. A recent study reported that Delta infection also induced cross-neutralization against Omicron (Suryawanshi et al., 2022). Therefore, it is reasonable to design next-generation vaccines based on Delta RBD sequences. Although the Delta is no longer the main circulating variant, its inactivated vaccine can still be used as a reserve vaccine to deal with future outbreaks. In addition, we will further study the sequential immunization of this Delta-originated vaccine combined with those originated from other variants (prototype or Omicron), and other types of vaccine (mRNA or protein vaccines), to explore the best immunization scheme for current or possible future outbreaks.

For SARS-CoV-2, the goal of current vaccines is to prevent symptomatic COVID-19. By decreasing the viral load in the upper and lower respiratory tracts, the risk of transmission is reduced, and this worldwide epidemics would be curbed. Overall, in the absence of effective antiviral drugs against the Delta variant, the present vaccine has good efficacy and safety, providing a potential solution for the COVID-19 pandemic.

Footnotes

This study was supported by the Science and Technology Program of Zhejiang Province (#2022C03017), Key Research and Development Program of Zhejiang Province (#2021C03044), Major Health Science and Technology Projects of Zhejiang Province (#WKJ-ZJ-2105), and National Key R&D Program of China (#2021YFC2301200). This study was approved by the Ethical Review Committee of Zhejiang Provincial Center for Disease Control and Prevention, China (2021-002-01). Participant signed a written consent form. All experimental procedures with mice, rats, guinea pigs, and cynomolgus monkeys were conducted according to Chinese animal use guidelines and were approved by the Institutional Animal Care and Use Committee (IACUC). The approval number for the toxicity test of the SARS-CoV-2 inactivated vaccine (Vero cells, Delta strain) given to SD rats by single intramuscular injection is ACU21-2368. The approval number for the toxicity test of the SARS-CoV-2 inactivated vaccine (Vero cells, Delta strain) given to cynomolgus monkeys by repeated intramuscular injection for 6 weeks and a recovery period of 4 weeks is ACU21-2324. The approval number for the systemic active allergic reaction test of the SARS-CoV-2 inactivated vaccine (Vero cells, Delta strain) administered to guinea pigs is ACU21-2578. Rhesus macaque studies were performed in an animal biosafety level 4 (ABSL-4) laboratory and approved by the State Key Laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences with approval number WIVA42202002-01. We thank all the researchers, doctors, nurses, medical technicians, front-line workers, and public health officials for their hard work during this pandemic. The authors declare that they have no conflict of interest. The study protocol was approved by the Ethics Committee of the Zhejiang Provincial Center of Disease Control and Prevention.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.virs.2023.05.009.

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