Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Vaccine 39 (2021) 6980–6983

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Short communication

Ameliorated immunity elicited by intradermal inoculation in individuals vaccinated with inactivated SARS-CoV-2 vaccine

Shengtao Fan, Dandan Li, Heng Zhao, Li Yu, Pingfang Cui, Lichun Wang, Ying Zhang, Yun Liao, Xingli Xu, Guorun Jiang, Qihan Li *

Institute of Medical Biology, Chinese Academy of Medical Sciences & Peking Union Medical College, Yunnan Key Laboratory of Vaccine Research and Development on Severe Infectious Diseases, Kunming 650118, China

ARTICLE INFO

Article history:
Received 19 May 2021
Received in revised form 13 September 2021
Accepted 19 October 2021
Available online 22 October 2021

Keywords:
SARS-CoV-2
Antibody
Antigen inoculation
Intradermal immunization

ABSTRACT

In clinical trials, antibodies against SARS-CoV-2 were almost eliminated in participants six months after immunization with an inactivated SARS-CoV-2 vaccine. The short duration of antibody persistence is an urgent problem. In this study, the problem was solved by intradermal inoculation with trace antigen. Within 72 h after intradermal inoculation, slight inflammatory reactions, such as redness and swelling, were observed at the inoculation site of the participants. On the 7th, 60th and 180th days after inoculation, the antibodies of the participants were detected, and it was found that the neutralizing antibody and ELISA (IgGs) anti-S antibody levels rapidly increased and were maintained for 6 months. These results indicate that there was a SARS-CoV-2-specific immune response in the participants immunized with an inactivated SARS-CoV-2 vaccine, which could be quickly and massively activated by intradermal trace antigen inoculation to produce an effective clinically protective effect.

1. Introduction

The number of individuals with coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection has exceeded 221 million worldwide (as of September 08, 2021, COVID-19.who.int). The world's economic and social development is facing unprecedented challenges. To date, coronavirus disease is currently developing, and the disease prevention and control situation is still urgent [1]. Vaccines are the most effective way to prevent acute infectious diseases [2]. Since the COVID-19 outbreak, many countries have made great efforts to develop an effective vaccine against SARS-CoV-2 [3]. To date, there have been four types of SARS-CoV-2 vaccines: inactivated vaccines [4], adenovirus vector vaccines [5], recombinant protein vaccines [6] and mRNA vaccines [7], which cause different immune responses and antibody persistence effects [8]. There are no methods to evaluate SARS-CoV-2 vaccine effectiveness. Based on previous research, it is generally believed that neutralizing antibodies is still the gold standard to evaluate vaccines, but for SARS-CoV-2 in particular, ELISA (IgGs) anti-S/N antibodies are also important in vaccine evaluation [9]. As SARS-CoV-2 is novel, little is known about the induced immune response. Compared with the production induced by conventional vaccines [10,11], the production of SARS-CoV-2 antibodies induced by intramuscular inoculation decreases too fast [9]. In this study, we will provide insight into how to elicit antibodies from humans immunized with an inactivated SARS-CoV-2 vaccine.

2. Methods

The study protocol was approved by the Ethics Committee of the West China Second University Hospital, Sichuan University (approval number: Y2020008). Fifty participants were randomly selected and immunized with inactivated SARS-CoV-2 vaccine via the intramuscular route. The SARS-CoV-2 inactivated vaccine was developed by the Institute of Medical Biology (IMB), Chinese Academy of Medical Sciences (CAMS). Briefly, the KMS-1 strain (MT226610.1) was inoculated into Vero cells. Dual inactivation was performed with formaldehyde (1:4000) to partially disrupt the viral membrane, followed by beta-propiolactone (1:2000) to disrupt the structure of the viral genome. The viral antigen content was measured via enzyme-linked immunosorbent assay [12]. In all, 150 U of SARS-CoV-2 antigens and 0.125 mg of aluminium adjuvant were contained in a 0.5 ml/dose. A booster immunization was performed 14 days after the first dose. On the 14th, 28th and 180th days after immunization, serum samples were collected to detect neutralizing antibodies and ELISA (IgGs) anti-S/N antibod-

* Corresponding author.
E-mail address: liqihan@imbcams.com.cn (Q. Li).

https://doi.org/10.1016/j.vaccine.2021.10.043
0264-410X/

ies. In brief, inactivated serum was serially diluted 2-fold and incubated with the KMS-1 strain (100 $lgCCID_{50}$/well) for 2 h at 37 °C, followed by inoculation into Vero cells for cytopathic effect (CPE) observation. The neutralizing antibody titers of the serum were defined by CPE assay. ELISAs were conducted with antibodies against the S protein and the N protein that were developed by this institute. S and N proteins were used to coat 96-well ELISA plates at a concentration of 5 μg/well and then incubated with serum samples. The OD values were measured using an ELISA plate reader [12]. On the 186th day after the two intramuscular injections, 20 participants were randomly assigned to a group inoculated with 10 U of SARS-CoV-2 antigen (0.1 ml/dose) via the intradermal route with a MicronJet 600 Microneedle (NanoPass Technologies, Ltd.) according to the manufacturer's instructions. The occurrence time and diameter of redness were recorded. On the 7th, 60th and 180th days after intradermal immunization, serum samples were collected to detect neutralizing antibodies (NAb) and ELISA (IgGs) anti-S/N antibodies (Fig. 1).

3. Results

In the phase I clinical trial of an inactivated vaccine, the antibody level of healthy adults aged 18–59 years reached a relatively high level on days 14 and 28 after immunization according to the 0- and 14-day two-dose intramuscular immunization procedure, including that of neutralizing (34.1 and 29.3) (Fig. 2A), ELISA (IgGs) anti-S (2700 and 2314) (Fig. 2B), and ELISA (IgGs) anti-N (457 and 400) (Fig. 2C) antibodies, but there was no significant difference between the two time points. In a follow-up investigation, we found that the antibody level was not durable and was in a state of rapid decline. On the 180th day after immunization, both neutralizing and ELISA (IgGs) anti-S antibody levels decreased to a relatively low level, especially those of neutralizing antibodies, and ELISA (IgGs) anti-S antibody levels were significantly different from those on the 14th and 28th day after immunization (Fig. 2).

To find an effective way to stimulate the immune response, 20 randomly selected participants were intradermally immunized. Twenty-four hours after inoculation, the inoculation site of all participants turned red (diameter: 0.93 ± 0.03 cm). Over time, the redness area gradually increased. At 36 h (diameter: 1.38 ± 0.13 cm), 48 h (diameter: 1.83 ± 0.08 cm) and 72 h (diameter: 0.83 ± 0.03 c m) (Fig. 3), the temperature was normal for all the participants, the inoculation site did not hurt or itch, and there was no adverse reaction.

On the 7th, 60th and 180th days after intradermal immunization, the neutralizing antibody and ELISA (IgGs) anti-S antibody levels of all participants were determined again. On the 7th day after intradermal inoculation, the neutralizing antibody level was 12.5, which was 8 times that before inoculation ($P < 0.01$); on the 60th day after antigen stimulation, the neutralizing antibody level reached 53.3, which was 33 times that before antigen stimulation ($P < 0.0001$). Furthermore, on the 180th day after antigen stimulation, the neutralizing antibody level reached 13.3, which was 8.5 times that before antigen stimulation ($P < 0.01$) (Fig. 4A). On the 7th day after antigen stimulation, the ELISA (IgGs) anti-S antibody level was 2200, 10 times higher than that before antigen stimulation ($P < 0.05$); on the 60th day after antigen stimulation, the ELISA (IgGs) anti-S antibody level was 21333, 97 times higher than that before antigen stimulation ($P < 0.0001$). On the 180th day after antigen stimulation, the ELISA (IgGs) anti-S antibody level was 2667, 12 times higher than that before antigen stimulation ($P < 0.05$) (Fig. 4B). On the 7th day after antigen stimulation, the ELISA (IgGs) anti-N antibody level was 2400, 8 times higher than that before antigen stimulation ($P < 0.05$); on the 60th day after antigen stimulation, the ELISA (IgGs) anti-S antibody level was 17600, 59 times higher than that before antigen stimulation ($P < 0.0001$). On the 180th day after antigen stimulation, the ELISA (IgGs) anti-N antibody level was 2667, 9 times higher than that before antigen stimulation ($P < 0.05$) (Fig. 4C). These results indicate that the memory immune response was activated rapidly and maintained for 6 months after a single intradermal injection.

4. Discussion

To effectively solve the problem that antibody levels cannot be maintained after vaccination with a SARS-CoV-2 vaccine, inspired by the classical tuberculin test [13], we adopted the skin test method to detect the cellular immune response to the SARS-CoV-2-specific antigen. If the skin test results are positive, there are immune cells specific to the tested antigen, such as sensitized Th1 cells [14] and antigen-specific B cells [15]. In our experiment, all the participants had mild inflammatory reactions, such as local redness at the inoculation site. According to the classical immunology theory [16–18], the specific immune reaction in all the participants was activated, and there were SARS-CoV-2-specific antibodies in their bodies. In particular, neutralizing antibodies and ELISA (IgGs) anti-S antibodies are produced in large quantities in a short period of time and can be maintained for a long time, which may be due to the rapid activation of antigen-specific B cells [19]. These results further verify our conjecture. Of course, this method of inducing rapid activation of the immune response has been tested for only inactivated vaccines, and whether it functions similarly with other types of vaccines still needs further verification.

In this work, we conducted a preliminary study on how to induce or strengthen the immune response of the body and provided an idea and solution to the problem that antibody levels cannot be maintained after vaccination with an inactivated SARS-CoV-2 vaccine, which has important guiding significance for the establishment and maintenance of herd immunity after mass vaccination with SARS-CoV-2 vaccines in the future.

Funding

This study was supported by the Yunnan Health Training Project for High Level Talents (H-2017035). This study was supported by the Yunnan Fundamental Research Projects (grant No. 202001AS070047 and 2019FB101) and Yunnan Province technology innovation talent training object project (202005AD160006). This study was funded by the Major Science and Technology Spe-

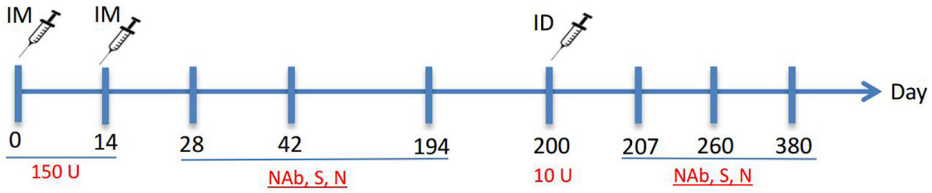

Fig. 1. Schematic depicting the immunization schedule.

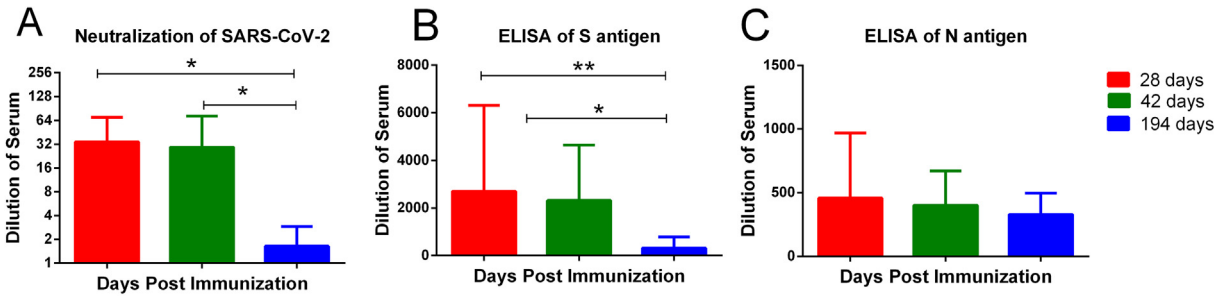

Fig. 2. Immune response induced by intramuscular immunization with an inactivated SARS-CoV-2 vaccine in adults. Neutralizing antibodies (A), ELISA (IgGs) anti-S antibodies (B) and ELISA (IgGs) anti-N antibodies (C) whose production was induced by an inactivated vaccine in a clinical trial in participants assigned to the 0- and 14-day schedule at 28, 42 and 192 days after intramuscular immunization. Statistical significance was assessed by unpaired t tests (*p < 0.05, **p < 0.01).

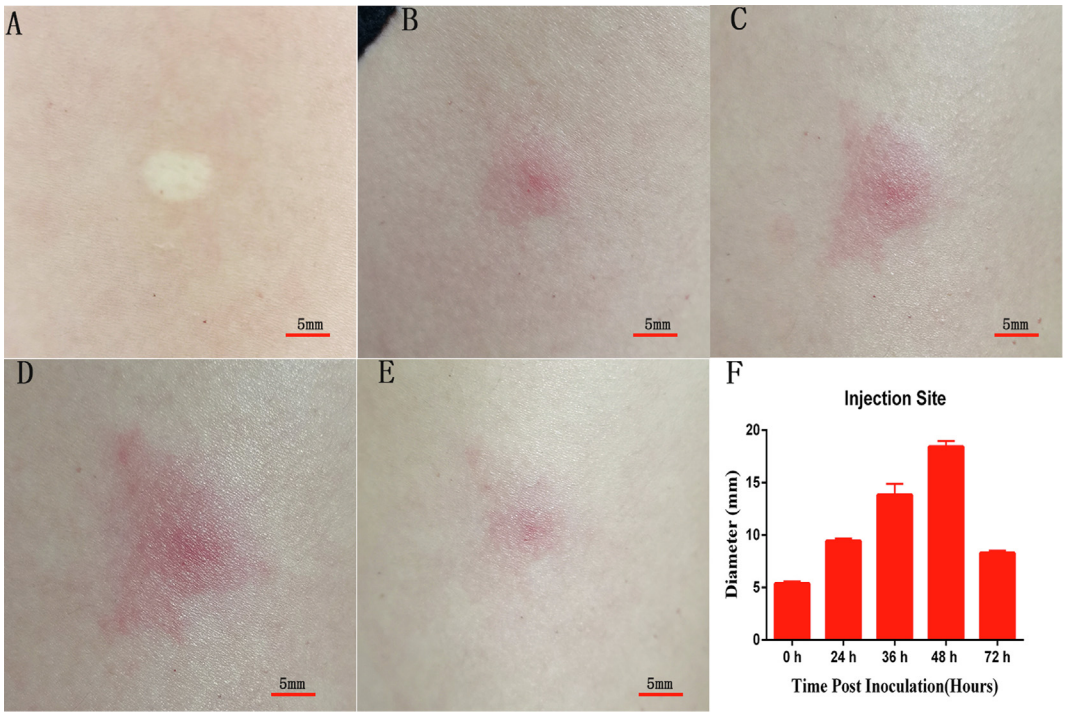

Fig. 3. Clinical observations at the inoculation site in participants Clinical observation of redness in the appearance of the skin at 0 h (A), 24 h (B), 36 h (C), 48 h (D) and 72 h (E) post inoculation. The red scale is 0.5 cm. Statistical analysis of skin redness in participants at 0 h, 24 h, 36 h, 48 h and 72 h post inoculation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

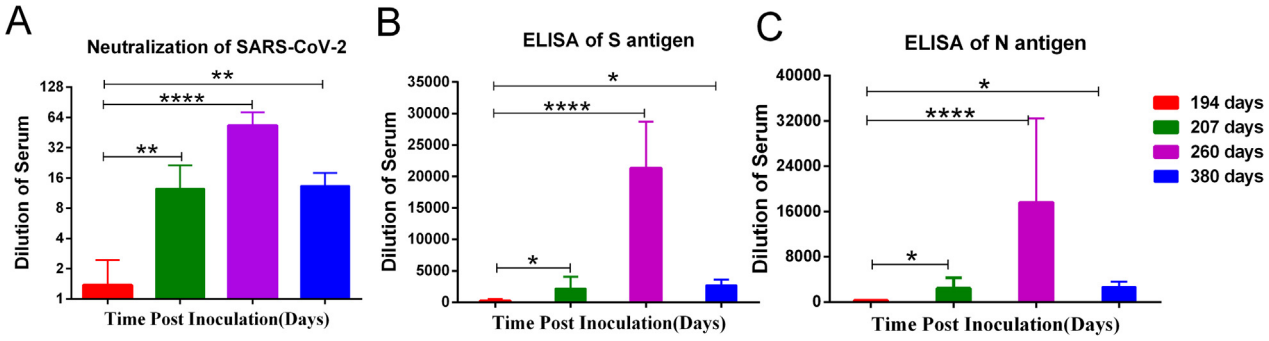

Fig. 4. Immune response induced by trace antigen in individuals vaccinated with an inactivated SARS-CoV-2 vaccine Neutralizing antibodies (A), ELISA (IgGs) anti-S antibodies (B) and ELISA (IgGs) anti-N antibodies (C) whose production was induced by an inactivated vaccine in individuals 7, 60 and 180 days after intradermal injection. Statistical significance was assessed by unpaired t tests (*p < 0.05, **p < 0.01, ****p < 0.0001).

cial Projects of Yunnan Province (202002AA100009) and funds from the Yunnan Key Laboratory of Vaccine Research & Development in Severe Infectious Diseases.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] Chung JY, Thone MN, Kwon YJ. Covid-19 vaccines: The status and perspectives in delivery points of view. Adv Drug Deliv Rev 2021;170:1–25.
[2] Awadasseid A, Wu Y, Tanaka Y, Zhang W. Current advances in the development of sars-cov-2 vaccines. International journal of biological sciences 2021;17:8–19.
[3] Ura T, Yamashita A, Mizuki N, Okuda K, Shimada M. New vaccine production platforms used in developing sars-cov-2 vaccine candidates. Vaccine 2021;39:197–201.
[4] Zhang Y, Zeng G, Pan H, Li C, Hu Y, Chu K, et al. Safety, tolerability, and immunogenicity of an inactivated sars-cov-2 vaccine in healthy adults aged 18–59 years: A randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. Lancet Infect Dis 2021;21:181–92.
[5] Folegatti PM, Ewer KJ, Aley PK, Angus B, Becker S, Belij-Rammerstorfer S, et al. Safety and immunogenicity of the chadox1 ncov-19 vaccine against sars-cov-2: A preliminary report of a phase 1/2, single-blind, randomised controlled trial. Lancet 2020;396:467–78.
[6] Keech C, Albert G, Cho I, Robertson A, Reed P, Neal S, et al. Phase 1–2 trial of a sars-cov-2 recombinant spike protein nanoparticle vaccine. New Engl J Med 2020;383:2320–32.
[7] Baden LR, El Sahly HM, Essink B, Kotloff K, Frey S, Novak R, et al. Efficacy and safety of the mrna-1273 sars-cov-2 vaccine. New Engl J Med 2021;384:403–16.
[8] Krammer F. Sars-cov-2 vaccines in development. Nature 2020;586:516–27.
[9] Liao Y, Zhang Y, Zhao H, Pu J, Zhao Z, Li D, et al. Intensified antibody response elicited by boost suggests immune memory in individuals administered two doses of sars-cov-2 inactivated vaccine. Emerging Microbes Infect 2021;10:1112–5.
[10] Zhu F, Xu W, Xia J, Liang Z, Liu Y, Zhang X, et al. Efficacy, safety, and immunogenicity of an enterovirus 71 vaccine in china. New Engl J Med 2014;370:818–28.
[11] Liao G, Li R, Li C, Sun M, Jiang S, Li Y, et al. Phase 3 trial of a sabin strain-based inactivated poliovirus vaccine. J Infect Dis 2016;214:1728–34.
[12] Pu J, Yu Q, Yin Z, Zhang Y, Li X, Yin Q, et al. The safety and immunogenicity of an inactivated sars-cov-2 vaccine in chinese adults aged 18–59 years: A phase i randomized, double-blinded, controlled trial. Vaccine 2021;39:2746–54.
[13] Askenase PW, Atwood JE. Basophils in tuberculin and "jones-mote" delayed reactions of humans. J Clin Investig 1976;58:1145–54.
[14] Commandeur S, Coppola M, Dijkman K, Friggen AH, van Meijgaarden KE, van den Eeden SJ, et al. Clonal analysis of the t-cell response to in vivo expressed mycobacterium tuberculosis protein rv2034, using a cd154 expression based t-cell cloning method. PLoS ONE 2014;9:e99203.
[15] Thom ML, Hope JC, McAulay M, Villarreal-Ramos B, Coffey TJ, Stephens S, et al. The effect of tuberculin testing on the development of cell-mediated immune responses during mycobacterium bovis infection. Vet Immunol Immunopathol 2006;114:25–36.
[16] Castro-Rodriguez JA, Mallol J, Andrade R, Munoz M, Azzini I. Comparison of tuberculin skin test response after three modalities of neonatal bcg vaccination. Trans R Soc Trop Med Hyg 2007;101:493–6.
[17] Soysal A, Millington KA, Bakir M, Dosanjh D, Aslan Y, Deeks JJ, et al. Effect of bcg vaccination on risk of mycobacterium tuberculosis infection in children with household tuberculosis contact: A prospective community-based study. Lancet 2005;366:1443–51.
[18] McNeil MM, DeStefano F. Vaccine-associated hypersensitivity. J Allergy Clin Immunol 2018;141:463–72.
[19] Kim E, Weisel FJ, Balmert SC, Khan MS, Huang S, Erdos G, et al. A single subcutaneous or intranasal immunization with adenovirus-based sars-cov-2 vaccine induces robust humoral and cellular immune responses in mice. Eur J Immunol 2021.