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.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Short Communication

Immunological memory and neutralizing activity to a single dose of COVID-19 vaccine in previously infected individuals

Mitnala Sasikala\textsuperscript{a,}\textsuperscript{*}, Jaggaiahgari Shashidhar\textsuperscript{a}, Gujarlapudi Deepika\textsuperscript{b}, Vishnubhotla Ravikanth\textsuperscript{c}, Vemula Venkata Krishna\textsuperscript{a}, Yelamanchili Sadhana\textsuperscript{c}, Kottapalli Pragathi\textsuperscript{d}, Duvvur Nageshwar Reddy\textsuperscript{a}

\textsuperscript{a} Institute of Translational Research, Department of Molecular Biology, Asian Healthcare Foundation, AIG Hospitals Complex, Mindspace Road, Gachibowli, Hyderabad, 500032, Telangana, India

\textsuperscript{b} Department of Biochemistry, AIG Hospitals, Mindspace Road, Gachibowli, Hyderabad, 500032, Telangana, India

\textsuperscript{c} Department of Microbiology and Virology, AIG Hospitals, Mindspace Road, Gachibowli, Hyderabad, 500032, Telangana, India

\textsuperscript{d} Department of Microbiology and Infection Control, AIG Hospitals, Mindspace Road, Gachibowli, Hyderabad, 500032, Telangana, India

\textbf{A R T I C L E  I N F O}

Article history:
Received 16 April 2021
Received in revised form 12 May 2021
Accepted 16 May 2021

Keywords:
SARS-CoV-2
COVID-19
Memory T-cells
Memory B-cells

\textbf{A B S T R A C T}

\textbf{Background:} The efficacy of COVID-19 vaccines to generate immunological memory post-vaccination has not previously been studied.

\textbf{Objective:} To assess immunological memory in previously SARS-CoV-2 infected individuals after a single dose of mRNA vaccine.

\textbf{Patients and methods:} Healthcare workers (n = 280) were enrolled after obtaining written informed consent and grouped under previously infected and no prior exposure (reverse transcription-polymerase chain reaction positive and negative, respectively). Blood was drawn at baseline and post-vaccination (single dose of COVISHIELD) for enumerating neutralizing antibodies by chemiluminescence and memory T- and B-cells by flow cytometry.

\textbf{Results:} Post vaccination, compared with the no prior exposure group, the previously infected group had higher levels of: antibody response (1124.73 ± 869.13 vs 94.23 ± 140.06 AU/ml, p = 0.0001); CD4 memory T-cells: central memory CCR7+CD45RA− (p = 0.0001), effector memory CCR7−/CD45RA− (p = 0.01); total CD8+ T-cells (p = 0.004); CD8+ naïve T-cells CCR7+/CD45RA+ (p = 0.01); and memory B-cells CD20+/CD27+ (p = 0.0001).

\textbf{Discussion:} Single-dose vaccination elicited higher neutralizing antibody response and protective immunity in individuals who had recovered from SARS-CoV-2 infection compared with those with no prior exposure.

\textcopyright 2021 The Authors. Published by Elsevier Ltd on behalf of International Society for Infectious Diseases. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

\textbf{Introduction}

The ongoing COVID-19 pandemic caused by SARS-CoV-2 has resulted in unprecedented mortality and morbidity globally. In the absence of specific therapeutic drugs to treat COVID-19, vaccines are currently the only option to control SARS-CoV-2 infection (Krammer, 2020). COVID-19 vaccination programs initiated worldwide have reduced disease severity (Rinott et al., 2021). Vaccinating huge populations require evidence-based strategies for successful implementation to control the pandemic. Although individuals who have had COVID-19 have been shown to have a higher antibody response to a single dose of mRNA vaccine than non-exposed individuals (Krammer et al., 2021; Saadat et al., 2021), their efficacy to generate immunological memory and protection against reinfection of SARS-CoV-2 is not yet reported. If a single dose could induce adequate immunological memory in previously infected individuals in addition to a higher neutralizing activity, the second dose could be diverted to vaccinate and protect a larger population. Therefore, our aim was to assess immunological memory in previously infected individuals after a single dose of vector-based vaccine.

\textbf{Participants and methods}

We enrolled healthcare workers (n = 280) who were vaccinated between 16 January and 5 February 2021, at AIG Hospitals,
Hyderabad, India (a hospital recognized by the Indian Council of Medical Research to test, treat and vaccinate for COVID-19) for assessing immunological memory response. Participants who were reverse transcription-polymerase chain reaction (RT-PCR) positive for SARS-CoV-2 and recovered formed the previously infected group, and participants who were RT-PCR negative formed the no prior exposure group. All participants were given COVISHIELD (AstraZeneca vaccine ChAdOx1/AZD1222 manufactured by Serum Institute of India) in two doses, 28 days apart, as per the (then) guidelines. Side effects after the single dose of vaccine were noted for all the participants. Blood was drawn from all the participants at day 0 (baseline) and 28 (post single-dose). Serum samples were tested for neutralizing antibodies (immunoglobulin G) by chemiluminescence (Shang et al., 2020), and day 28 samples were analysed for neutralizing antibodies and memory cells by flow cytometry (Yang et al., 2020). The Institutional Ethics Committee of AIIMS Hospitals approved the study, and all participants provided written informed consent. Students t test and Z proportion test were used to test differences between the groups. A P-value of < 0.05 was considered significant.

Results

Participant demographics, baseline serostatus and frequency of side effects after the single dose of vaccination are provided in Table 1. Of the 280 individuals enrolled in the study, 131 were RT-PCR positive with mild to moderate disease, and 50 required hospital admission. All 131 (46.78%) were seropositive, and 149 (53.22%) were seronegative prior to the first vaccination dose.

Higher antibody response in previously infected individuals with single dose

Baseline neutralizing antibodies were significantly higher in previously infected individuals compared with the no prior exposure group (61.58 ± 46.88 vs 5.03 ± 2.54 AU/ml, p = 0.0001). All the seropositive (100%, n = 131) and 94.6% (n = 141) of seronegative participants developed neutralizing antibodies by day 28 after the first dose of COVISHIELD. The previously infected group mounted greater antibody response (1124.73 ± 869.13 vs 94.23 ± 140.06 AU/ml, p = 0.0001) to a single dose of COVISHIELD vaccine compared with the no prior exposure group (Figure 1).

Single-dose vaccine elicited higher memory T- and B-cell responses in previously infected individuals

Memory CD4+ T-cell responses elicited by a single dose of COVISHIELD were significantly higher in the previously infected group compared with the no prior exposure group: CD4+ T-cells (p = 0.001); CCR7+/CD45RA- central memory T-cells (p = 0.0001); CCR7+/CD45RA+ effector memory CD4+ T-cells (p = 0.01). Effector memory CD4+ T-cells CCR7-/CD45RA+ were not significantly different between the groups (p = 0.10) (Figure 1). Total CD8+ T-cells (p = 0.004) and CD8+ naïve T-cells CCR7+/CD45RA+ (p = 0.01) were significantly higher in previously infected individuals, while CD8+ central memory T-cells CCR7+/CD45RA- (p = 0.07) and CD8+ effector memory T-cells CCR7-CD45RA- (p = 0.58) showed an increasing trend at day 28. Effector memory CD8+ T-cells CCR7-/CD45RA+ (p = 0.64) were similar in both groups. Significantly higher B-cell responses were seen in previously infected individuals: CD45+CD20+ B-cells (p = 0.0005); CD20+CD27+ memory B-cells (p = 0.0001); CD20+HLADR + activated B-cells (p = 0.0001) (Figure 1).

Discussion

Our results demonstrate that previously infected individuals mounted higher immune and memory responses to a single dose of vector-based vaccine compared with those with no prior exposure. Earlier studies reported immune memory to SARS-CoV-2 infection (Rodda et al., 2021; Dan et al., 2021) and higher antibody response to a single vaccine dose in previously infected individuals (Rinott et al., 2021). Our study reports higher memory T and B-cell responses in addition to higher antibody response with a single dose of COVISHIELD given at 3–6 months after recovery from COVID-19. These results suggest protective immune memory in previously exposed individuals after a single dose of vaccine. Such individuals could mount memory recall response on a subsequent

### Table 1

Demographics, serostatus and post vaccination symptoms of participants.

|                          | Previously infected | No prior exposure | Significance* |
|--------------------------|---------------------|-------------------|--------------|
| Participants (n)         | 131                 | 149               |              |
| Male                     | 79 (60.30%)         | 98 (65.77%)       | 0.34         |
| Female                   | 52 (39.69%)         | 51 (34.22%)       | 0.34         |
| Age (years)              |                     |                   |              |
| Male                     | 20–58               | 18–58             |              |
| Female                   | 19–53               | 18–60             |              |
| Baseline seropositivity (%)|                   |                   |              |
| Overall                  | 46.78               |                   |              |
| Male                     | 44.6                |                   |              |
| Female                   | 50.4                |                   |              |
| Duration between infection and vaccination (months) | 5.0 ± 2.0 | 5.0 ± 2.0 | 0.04 |
| Time to vaccination after recovery (months) | 4.0 ± 2.0 | 4.0 ± 2.0 | 0.004 |
| Type of vaccine | COVISHIELD          | COVISHIELD        |              |
| Symptoms (n and % of participants) |              |                   |              |
| Fever                    | 38 (29.6%)          | 28 (18.79%)       | 0.04         |
| Headache                 | 13 (9.2%)           | 15 (10.06%)       | 0.96         |
| Body pain                | 38 (29%)            | 25 (16.77%)       | 0.01         |
| Chills                    | 1 (0.76%)           |                   |              |
| Back pain                | 5 (3.81%)           | 2 (1.34%)         | 0.18         |
| Cold, cough              |                     | 2 (1.34%)         |              |
| Fatigue                  | 90 (68.70%)         | 60 (40.26%)       | 0.0001       |
| Local side effects       | 13 (9.92%)          | 15 (10.6%)        | 0.96         |

n – number; % – percentage.

* Z-test.
encounter with antigen as they have developed adaptive immune memory. Thus, individuals who have had COVID-19 and recovered would have adequate protection with a single dose of vaccine. Our results demonstrate evidence to support a single-dose vaccination strategy for previously infected individuals to increase coverage and protect a larger number of populations. All the individuals with no prior exposure to COVID-19 would be required to take the second vaccination dose. In addition, longitudinal follow-up studies are necessary to assess the longevity of protective memory in order to determine the timing of the second dose.

Conflict of interest

None to declare.
Funding source

None.

Ethical approval

The study was approved by the Institutional Ethics Committee of AIG Hospitals, Hyderabad, India.

Acknowledgement

We acknowledge internal funding from Asian healthcare foundation.

References

Dan JM, Mateus J, Kato Y, Hastie KM, Yu ED, Faliti CE, et al. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. Science 2021;371(6529):eabf4063.

Krammer F, Srivastava K, Alshammary H, Amoako AA, Awaeda MH, Beach KF, et al. Antibody responses in seropositive persons after a single dose of SARS-CoV-2 mRNA vaccine. N Engl J Med 2021;384(14):1372–4.

Rinnert E, Youngster I, Lewis YE. Reduction in COVID-19 patients requiring mechanical ventilation following implementation of a national COVID-19 vaccination program — Israel, December 2020–February 2021. MMWR Morb Mortal Wkly Rep 2021;70:326–8.

Rodd LB, Netland J, Shehata L, Pruner KB, Morawski PA, Thouvenel CD, et al. Functional SARS-CoV-2-specific immune memory persists after mild COVID-19. Cell 2021;184(1):169–183.e17.

Saadat S, Tehrani ZR, Logue J, Newman M, Frieman MB, Harris AD, et al. Binding and neutralization antibody titers after a single vaccine dose in health care workers previously infected with SARS-CoV-2. JAMA 2021;325(14):1467–9.

Shang W, Yang Y, Rao Y, Rao X. The outbreak of SARS-CoV-2 pneumonia calls for viral vaccines. NPJ Vaccines 2020;5:18.

Yang Q, Zhang M, Chen Q, Chen W, Wei C, Qiao K, et al. Characterization of human tissue-resident memory T cells at different infection sites in patients with tuberculosis. J Immunol 2020;204(9):2331–6.