

ORIGINAL ARTICLE

Heterologous booster vaccination with CoronaVac following prime vaccination with mRNA vaccine

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Abstract

Objective. Despite the high vaccine efficacy of mRNA COVID-19 vaccines, there are individuals who developed excessive reactogenic and/or allergic responses after the first mRNA dose and were considered ineligible for further mRNA doses. CoronaVac, an inactivated SARS-CoV-2 vaccine, is recommended in Singapore as an alternative. **Methods.** Individuals, ineligible for further mRNA vaccines (BNT162b2 or mRNA-1273) because of excessive reactive responses to prime mRNA vaccination, were recruited and offered two doses of CoronaVac as booster vaccination 38–224 days post their mRNA vaccine dose. Individuals who did not develop any excessive reactive responses after the prime mRNA vaccination were also recruited and given another

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mRNA vaccine as booster vaccination. Blood samples were collected at days 0, 21 and 90 post first CoronaVac dose and mRNA dose, respectively, for analysis. **Results.** We showed that two CoronaVac booster doses induced specific immunity in these mRNA vaccine-primed individuals. Although the spike-specific antibody response was lower, their memory B cell response against the receptor-binding domain (RBD) of the spike protein was similar, compared with individuals who received two BNT162b2 injections. The spike-specific memory T cell response also increased following CoronaVac booster doses. However, specific immunity against the Omicron variant was low, similar to individuals with two BNT162b2 doses. **Conclusion.** Our findings showed that while mRNA vaccine-primed individuals can opt for two subsequent doses of CoronaVac, an additional dose may be necessary to achieve protection, especially against newly emerging immune escape variants such as Omicron.

Keywords: Allergic, Antibodies, B cells, CoronaVac, COVID-19, Delta, Omicron, S protein, SARS-CoV-2, T cells

INTRODUCTION

Since December 2019, Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has rapidly evolved into a global pandemic. The pandemic has devastated healthcare systems across the world and caused major socioeconomical disruptions. As of 7 April 2022, there have been 493 million confirmed cases and 6.2 million fatalities globally.¹

The rapid development of effective vaccines has been an essential step in reducing morbidity and mortality from the COVID-19 pandemic. Within 9 months from the first reports of the disease, inactivated vaccines from Sinovac (CoronaVac) and Sinopharm (BBIBP-CorV) had received emergency use authorization in China. By December 2020, mRNA vaccines from Pfizer-BioNTech (BNT162b2) and Moderna (mRNA-1273) were authorised in many regions and COVID-19 vaccination programmes began in earnest. Both mRNA vaccines demonstrated 95% vaccine efficacy in pivotal clinical trials.^{2,3} An estimated 116 000 (73.1%) SARS-CoV-2 infections, 19 467 (79.1%) COVID-19-related hospitalisations and 4 351 (79%) deaths have been averted among fully vaccinated persons in Israel.⁴

Despite the huge success of COVID-19 mRNA vaccines, mild or severe adverse effects, including pain, local redness or swelling, fever, fatigue, headache, muscle pain, nausea, vomiting, itching and chills, are common.^{5–7} More severe

hypersensitivity reactions are uncommon, with anaphylaxis reported in 2.5–11.1 cases per million doses.⁸ Overall, hypersensitivity-related adverse events have been reported in 0.63% of individuals who received BNT162b2 and 1.5% of individuals who received mRNA-1273, compared with 0.51% and 1.1%, respectively, in the placebo groups.^{9,10} Individuals with a history of a severe allergic reaction to any component of the vaccine are generally contraindicated from receiving further doses of the same vaccine.

As part of Singapore's COVID-19 vaccination programme, CoronaVac, a two-dose inactivated SARS-CoV-2 vaccine with 90% and 86% efficacy against intensive care admission and death, respectively,¹¹ is the only authorised alternative that is recommended for individuals who develop allergic or excessive reactogenicity responses after the first mRNA dose. Here, we examined the immune responses following CoronaVac vaccination. We also investigated the binding and neutralisation efficacy of the induced immune response against recent emerging variants such as the Delta and Omicron variants.

RESULTS

CoronaVac-induced specific antibody responses against both the N and S protein

We recruited a cohort of 103 individuals ([Supplementary table 1](#)), who developed allergic or

excessive reactogenic responses following mRNA vaccination, and were ineligible for further mRNA doses (Figure 1). They were offered, instead, two additional doses of inactivated SARS-CoV-2 vaccine, CoronaVac, 38–224 days post their mRNA vaccine dose. At baseline (day 0), the mRNA-primed individuals did not have anti-N antibodies (Figure 2a) but had anti-RBD (Figure 2b) and anti-S antibodies (Figure 2c), demonstrating the spike specificity and the effectiveness of the mRNA vaccine in antibody induction. After CoronaVac administration, there was a lag in anti-N response, with an increase only following the second CoronaVac dose (day 90). In contrast, there was an increase in anti-RBD and anti-S antibodies at day 21 but not at day 90. We observed a negative correlation between anti-S antibody response at day 0 and time interval from the mRNA dose to the first CoronaVac dose (Supplementary figure 1a). Interestingly, the anti-S response at day 90 positively correlated with the time interval, showing that individuals with a longer time interval had a better vaccine-induced anti-S response at day 90 (Supplementary figure 1b).

At day 90, despite having one more dose (mRNA vaccine, BNT162b2 or mRNA-1273), individuals primed with one mRNA dose and boosted with two doses of CoronaVac (mRNA/CV2) had an anti-S response against the wildtype (WT) S protein that was lower than that of individuals who received two mRNA (BNT162b2) doses (mRNA/mRNA) (Figure 3a, Supplementary figure 2). Similarly, mRNA/CV2 individuals had an anti-S response against Delta variant that was lower than that of mRNA/mRNA individuals (Figure 3b). The binding efficacy against Omicron S protein for both mRNA/mRNA individuals and mRNA/CV2 individuals was low, with no statistical difference between the two groups (Figure 3c). The anti-S antibody data were supported by functional neutralisation data. The mRNA/CV2 individuals had neutralisation titers against WT (Figure 3d) and Delta (Figure 3e) pseudoviruses that were lower than that of the mRNA/mRNA individuals. Both groups had similarly low neutralisation titers against the Omicron pseudovirus (Figure 3f).

CoronaVac-induced specific memory B and T cell responses against S protein.

We next examined the RBD-specific memory B cell (MBC) following vaccination. At day 21, 11/28

(39.3%) of the mRNA-primed individuals who received their first CoronaVac dose, had an RBD-specific MBC above their baseline. There was a significant increase in RBD-specific MBC levels between days 21 and 90 (Figure 4a). At day 90, 17/19 (89.5%) have an RBD-specific MBC above their baseline at day 0 (Figure 4b). We did not observe any significant difference in RBD-specific MBCs between mRNA/CV2 and mRNA/mRNA individuals (Figure 4c). Following the first CoronaVac dose, there was an induction of S peptide-specific activated Tfh ($CD4^+ CXCR5^+ CD154^+$) response (Supplementary figure 3a). At day 21, 2/24 (8.3%) had an activated Tfh response above their baseline. At day 90, 8/24 (33.3%) had an activated Tfh response above their baseline (Supplementary figure 3b).

We then analysed the SARS-CoV-2 S peptide-specific T cell response by ELISpot. There were no significant increases in peptide-specific CD3, CD8 and CD4 Th1 responses at days 21 and 90 following the first CoronaVac dose, compared with their baseline (Figure 4d). However, the CD4 Th2 cell response was significantly different after the first CoronaVac dose and boosted after the second CoronaVac dose (Figure 4d). At day 21, 24% (6/25) had values above their baseline and over 80% (20/25) mounted a CD4 Th2 cell response at day 90. While we found that mRNA/CV2 individuals had lower S peptide-specific CD3 and CD8 responses, they have higher CD4 Th2 responses than mRNA/mRNA individuals (Figure 4e).

DISCUSSION

Vaccination is one of the best ways to prevent and manage infectious diseases. This is evident in recent data, where an estimated 116 000 (73.1%) SARS-CoV-2 infections, 19 467 (79.1%) COVID-19-related hospitalisations and 4 351 (79%) deaths were averted among fully vaccinated persons in Israel.⁴ Hence, for individuals who are ineligible for further mRNA doses because of excessive reactogenic and/or allergic reactions to the first mRNA vaccine, it is important to understand the alternative vaccine options and how they elicit an immune response in these individuals.

In this study, we showed that, following two CoronaVac vaccine doses, mRNA vaccine-primed individuals were able to mount a specific immune response. All individuals developed a robust anti-N and anti-RBD antibody response. We also

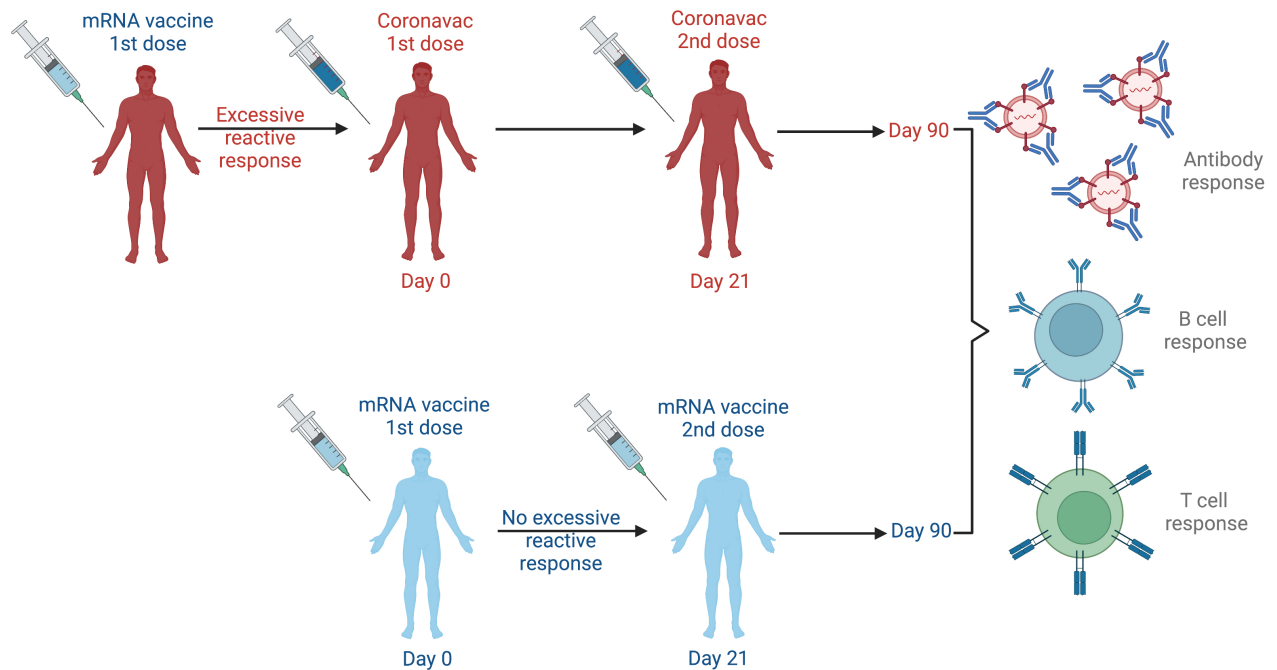


Figure 1. Study design. Individuals, ineligible for further mRNA vaccines because of excessive reactive responses to prime mRNA vaccination, were recruited and offered two doses of CoronaVac as booster vaccination. Individuals who did not develop any excessive reactive responses after the prime mRNA vaccination, received another mRNA vaccine as booster vaccination. Blood samples were collected at days 0, 21 and 90 post first CoronaVac dose and mRNA dose, respectively, and the antibody profiles, B and T cell responses, were analysed. The figure was created with Biorender.com.

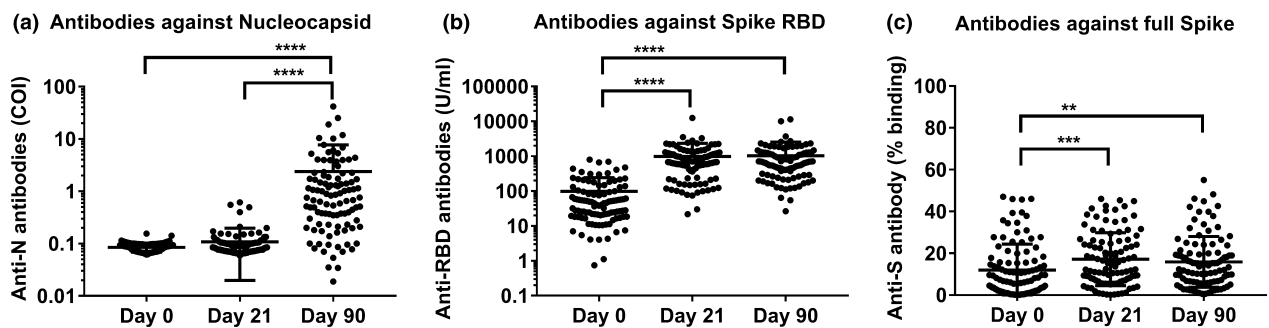


Figure 2. Antibody responses following CoronaVac vaccination. Individuals, who developed excessive reactogenic and/or allergic responses following mRNA vaccination (BNT162b2 or mRNA-1273) doses, were recruited and offered two doses of CoronaVac 38–224 days post their mRNA vaccine dose. Plasma samples ($n = 103$) were collected at three timepoints, day 0 (before CoronaVac vaccination, on the day of first CoronaVac dose), day 21 (before the second CoronaVac dose) and day 90 (70 days after the second CoronaVac dose). The plasma samples were screened for **(a)** anti-N antibodies using the commercial Elecsys® Anti-SARS-CoV-2 N (Roche N) immunoassay for the detection of antibodies against the SARS-CoV-2 nucleocapsid (N) antigen, **(b)** anti-RBD antibodies using the commercial Elecsys® Anti-SARS-CoV-2 S (Roche S) immunoassay for detection of antibodies against the SARS-CoV-2 spike (S) antigen and **(c)** anti-S antibodies using the SFB assay that detect antibodies against the full length S protein. Data are represented as mean + standard deviation. To compare between the different timepoints, Kruskal–Wallis tests and *post hoc* tests using Dunn's multiple comparison tests were used. ** P -value < 0.01, *** P -value < 0.001, **** P -value < 0.0001.

examined the antibody response against whole Spike, as we have previously shown that mutations beyond the RBD can exert long-range allosteric structural changes that can influence

antibody binding and neutralisation.¹² Although the induced anti-Spike antibody response was lower than that in mRNA/CoV2 individuals, the memory B cell responses were comparable to

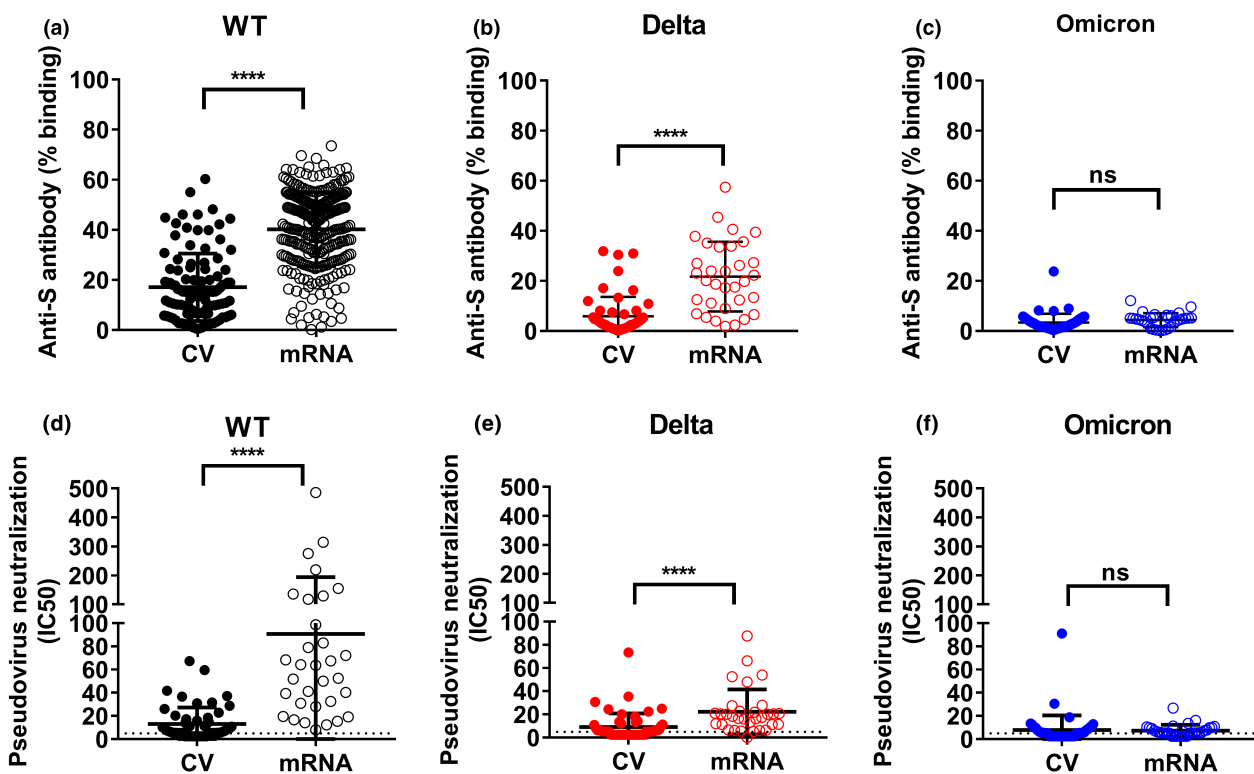


Figure 3. Comparison of antibody responses. Using the SFB assay that detect antibodies against the full-length S protein, the plasma samples were also screened for anti-S IgG. **(a)** Comparison of IgG responses against wildtype (WT) S protein at 70 days following last vaccine dose in individuals who received one mRNA dose and two CoronaVac doses (CV) or two mRNA doses (mRNA). Samples are age-matched, with median age for both CV and mRNA cohorts at 47 years. CV, $n = 103$; mRNA, $n = 286$. Comparison of IgG responses against **(b)** Delta and **(c)** Omicron variant S protein at 70 days following last vaccine dose. CV, $n = 54$ (median age: 47); mRNA, $n = 32$ (median age: 51). Neutralisation capacity against **(d)** WT, **(e)** Delta and **(f)** Omicron pseudoviruses, IC50 titre, were compared. CV, $n = 54$ (median age: 47); mRNA, $n = 32$ (median age: 51). Dotted line indicates limit of detection (LoD). For samples that do not exhibit 50% inhibition at the lowest dilution tested, we report the IC50 as a value of $\frac{1}{2}$ LOD (the limit of detection). Data are represented as mean + standard deviation. To compare between CV and mRNA groups, the Mann–Whitney U -test was used. *** P -value < 0.001 , **** P -value < 0.0001 .

levels in individuals who received two mRNA doses. There was also a significant induction of Th2 cellular response. This is in contrast with mRNA vaccination, where Th1 cellular response predominants.^{13–15} Because of limited sample availability, we were not able to analyse samples from the same individuals across all assays examined in this study. However, regardless of the subset of samples used (either for B cell comparison in Figure 4c or T cell comparison in Figure 4e), we confirmed that the induced anti-Spike antibody response in mRNA/CV2 individuals was also lower than that in mRNA/mRNA individuals (Supplementary figure 2). This is similar to the comparison done in Figure 3a. While it is noteworthy that the mRNA/CV2 individuals had an additional dose (the mRNA dose before the two CoronaVac doses), our data

suggest that mRNA vaccine-primed individuals who mount excessive reactogenic and/or allergic reactions to mRNA vaccine could opt for CoronaVac vaccination. Interestingly, similar to studies examining extended dosing interval of BNT162b2 vaccination,^{16–18} we also showed that mRNA/CV2 individuals with a longer time interval from the mRNA dose to the first CoronaVac dose have enhanced vaccine-induced anti-S antibody response at day 90, despite a lower anti-S antibody response at day 0.

In today's COVID-19 landscape with fast evolving and newly emerging variants such as Omicron, we have observed low antibody response and correspondingly reduced pseudovirus neutralisation titre against the Omicron variant in our cohort of Asian individuals receiving two doses of mRNA vaccines, which is consistent with

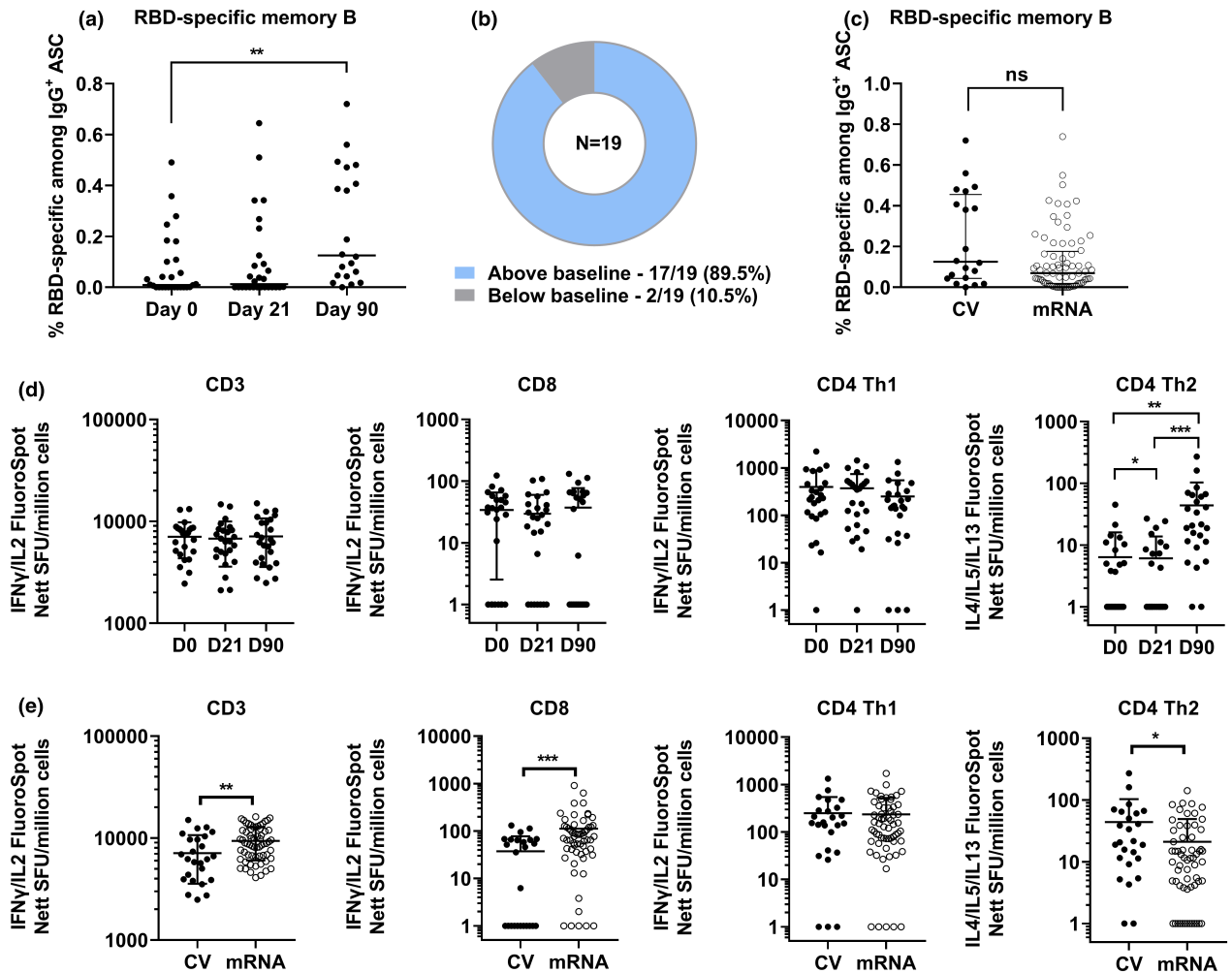


Figure 4. B and T cell responses following vaccination. **(a)** RBD-specific MBC among IgG⁺ antibody-secreting cells (ASC) in mRNA vaccine-primed individuals at day 0 (before CoronaVac vaccination, $n = 28$), day 21 (following the first CoronaVac dose, $n = 28$) and day 90 (following the second CoronaVac dose, $n = 20$). **(b)** Proportion of mRNA vaccine-primed individuals with a higher RBD-specific MBC response at day 90, compared with their corresponding baseline at day 0 ($n = 19$). **(c)** RBD-specific MBC among IgG⁺ ASC in mRNA vaccine-primed individuals following two CoronaVac doses (CV, $n = 20$) and in individuals who received two mRNA doses (mRNA, $n = 76$). Median age for CV and mRNA cohorts at 45 and 47 years, respectively. **(d)** PBMCs from mRNA vaccine-primed individuals ($n = 25$) at day 0 (before CoronaVac vaccination), day 21 (following the first CoronaVac dose) and day 90 (following the second CoronaVac dose) were stimulated with SARS-CoV-2 S peptide pools, and spike protein-specific CD3, CD8, CD4 Th1 or CD4 Th2 cells were assayed by IL2/IFN- γ ELISPOT using 9-mer or 15-mer pool peptides, respectively. **(e)** Comparison of Spike protein-specific CD3, CD8, CD4 Th1 or CD4 Th2 responses in mRNA vaccine-primed individuals following two CoronaVac doses (CV, $n = 25$) at day 90 following the first CoronaVac dose and in individuals who received two mRNA doses (mRNA, $n = 63$) at day 90 following the first mRNA dose. Median age for both CV and mRNA cohorts at 47 years. Data presented are spot forming units (SFU) per million PBMC from paired samples at three timepoints. Each data point represents the normalised mean spot count from duplicate wells, after subtraction of medium-only control. FluoroSpot results were analysed with the Welch's t -test for parametric unpaired comparisons. Data are represented as mean + standard deviation. To compare between CV and mRNA groups, the Mann-Whitney U -test was used. * P -value < 0.05, ** P -value < 0.01, *** P -value < 0.001.

published data.^{19–21} More importantly, our study showed that mRNA/CV2 individuals, who have received one mRNA and two CoronaVac doses, also had similarly low antibody and corresponding pseudovirus neutralization titers against Omicron. Hence, similar to individuals who received two

mRNA-dose vaccination, it is pertinent for mRNA boost-ineligible individuals, who have been advised against mRNA vaccination and opted for CoronaVac vaccination, to receive another vaccine dose to achieve protection, especially against newly emerging variants such as Omicron. While a

single additional mRNA dose has been shown to significantly increase the titers of anti-Omicron antibodies,^{22–24} it remains unknown how many additional CoronaVac booster doses are needed to achieve the same effect. Vaccines of other non-mRNA platforms can also be considered. Novavax's protein-based NVX-CoV2373 vaccine has recently been authorised in Singapore (February 2022) and has a reported vaccine efficacy of 92.6%.²⁵ Similar to the mRNA vaccines, NVX-CoV2373 also induced a Th1 response.²⁶ The levels of induced live virus neutralising antibody and cellular response were reported to be lower in individuals who received NVX-CoV2373 as a booster following a two-dose BNT162b2 primary vaccination series, as compared with individuals who received another BNT162b2 dose as booster.²⁷ Despite a lower immune induction by NVX-CoV2373 (compared with BNT162b2), it is unknown how that compares with CoronaVac. Whether NVX-CoV2373 is a better alternative vaccine than CoronaVac, especially for mRNA boost-ineligible individuals, remains to be determined.

METHODS

Ethics statement and study population

A cohort of 103 individuals (Supplementary table 1), who developed allergic or excessive reactogenic responses following the first mRNA vaccination (BNT162b2 or mRNA-1273) and were thus considered ineligible for further mRNA doses, was recruited and offered two doses of inactivated SARS-CoV-2 vaccine (CoronaVac) 38–224 days post their mRNA vaccine dose (Figure 1). Blood was collected at day 0 (before the first CoronaVac dose), 21 (before the second CoronaVac dose) and 90 (post first dose). A cohort of 286 individuals (Supplementary table 1), who received two mRNA vaccines (BNT162b2), were also recruited with blood sampling at days 0, 21 and 90 post first dose (Figure 1). None of the participants had known or reported SARS-CoV-2 infection.

Commercial serological assays for the detection of anti-SARS-CoV-2 antibodies

Samples were analysed for antibodies against the spike (S) protein RBD and the nucleocapsid (N) antigen by Elecsys® Anti-SARS-CoV-2 S (Roche S) and Elecsys® Anti-SARS-CoV-2 (Roche N; Roche, Basel, Switzerland) immunoassays, respectively, using Roche Cobas e411 Analyser (Roche, Basel, Switzerland), following the manufacturer's instructions. For Roche S assay (Roche, Basel, Switzerland), the electrochemiluminescent signal representing the level of antibodies in titrated samples was measured and assigned a value. Samples with antibody levels ≥ 0.8 U mL⁻¹ were considered

positive. For Roche N assay, the cut-off index (COI) was derived from the measured signal, where samples with COI ≥ 1.0 were considered reactive.

Spike protein flow cytometry-based assay (SFB assay) for antibody detection

The SFB assay was performed as previously described.^{28,29} Cells, expressing the spike protein of either WT, Delta or Omicron variants, were seeded at 1.5×10^5 cells/well in 96-well V-bottom plates (ThermoFisher Scientific, Waltham, USA). Cells were incubated with human serum (diluted 1:100 in 10% FBS; HyClone, Chicago, USA) followed by a secondary incubation with a double stain, comprising Alexa Fluor 647-conjugated anti-human IgG (1:500 dilution; ThermoFisher Scientific) and propidium iodide (PI; 1:2500 dilution; Sigma Aldrich, Burlington, USA). Cells were acquired using a BD Biosciences (New Jersey, USA) LSR4 laser and analysed using FlowJo (Tree Star, BD Biosciences). Cells were gated on: (1) FSC-A/SSC-A to exclude cell debris, (2) FSC-A/FSC-H for single cells, (3) FSC-A/PI for live cells (PI-negative population), (4) FITC/Alexa Fluor 647 (Supplementary figure 4). Binding is determined by the percentage of GFP-positive S protein-expressing cells bound by antibody, indicated by Alexa Fluor 647- and FITC-positive events. The assay was performed as two independent experiments, each with technical duplicates. The amount of spike protein expressed on the cell surface was verified by ACE-2-Human Fc binding (Supplementary figure 5). For assays examining the variants, a subset of age-matched samples was randomly selected and examined ($n = 54$ for CV day 90; $n = 32$ for mRNA group day 90) because of limited sample availability.

SARS-CoV-2 pseudotyped lentivirus production

The pTT5LnX-CoV-SP (expressing SARS-CoV-2 Spike protein, Genbank: YP_009724390.1) was used as a template plasmid to generate Spike gene of B.1.617.2 Delta and B.1.1.529 Omicron variant using QuickChange Lightning Multi Site-Directed Mutagenesis Kit (Agilent, Santa Clara, USA). Pseudoviruses were generated and titered as previously described³⁰ using a third-generation lentivirus system.

Pseudovirus neutralisation assay

The pseudotyped lentivirus neutralisation assay was performed as previously described,^{30,31} with modifications. A stable cell line expressing human ACE2, CHO-ACE2,³² was used. Four-fold serially diluted heat-inactivated plasma samples (1:5–1:5120) were incubated with pseudovirus expressing S proteins of either WT, Delta or Omicron variant (5 ng p24 per well), before being added to preseeded CHO-ACE2 cells in duplicate. After 48 h, cells were lysed and luciferase activity was quantified on a GloMax Luminometer (Promega, Madison, USA). For samples that do not exhibit 50% inhibition at the lowest dilution tested,⁵ we report the IC₅₀ as a value of $\frac{1}{2}$ LOD (the limit of detection). A subset of age-matched samples

was randomly selected and examined ($n = 54$ for CV day 90; $n = 32$ for mRNA group day 90) because of limited sample availability.

Memory B cell ELISpot

SARS-CoV-2 RBD-specific memory B cell numbers were counted using ELISpot Path: Human IgG (SARS-CoV-2, RBD) ALP kit (Mabtech, Cincinnati, USA), following the manufacturer's instructions. PBMCs were resuspended in RPMI + 10% FBS + $1 \mu\text{g mL}^{-1}$ R848 + 10 ng mL^{-1} IL-2 and incubated at 37°C for 5 days for differentiation into antibody-secreting cells. To determine RBD-specific memory B cell numbers, 100 000 or 400 000 live cells were taken for ELISpot plating. Total IgG-secreting cells were detected by plating 1500 or 3000 live cells to normalise the results. Plates were then read on an IRIS ELISpot reader (Mabtech). Spots were calculated based on the average of two wells. Because of limited cell availability, a subset of age-matched samples were randomly selected and examined ($n = 28$ for CV day 0 and 21; $n = 20$ for CV day 90; $n = 76$ for mRNA group day 90).

Extracellular and intracellular profiling of T cells with flow cytometry

Profiling of SARS-CoV-2-specific activated Tfh subsets was performed as previously described with modifications.³³ PBMCs were rested overnight at 37°C in RPMI-1640 + 5% human serum, followed by stimulation with PMA (100 ng/mL) (Sigma Aldrich) and ionomycin ($1 \mu\text{g mL}^{-1}$) (Sigma Aldrich) or pooled SARS-CoV-2 PepTivator® S and S1 peptides (0.6 nmol mL^{-1} each) (Miltenyi Biotec) for 6 h. Brefeldin A and Monesin (ThermoFisher Scientific) were added at 2 h poststimulation. Cells were stained with surface markers for 30 min (Supplementary table 2, #1–21), followed by 30 min fixation and permeabilization with Fopx3/Transcription Factor Staining Buffer Set (ThermoFisher Scientific). Permeabilized cells were then stained for intracellular cytokines for 30 min (Supplementary table 2, #22–29). Cells were acquired with the Cytex™ Aurora (SpectroFlo®) and analysed using FlowJo. Gating strategy is described in Supplementary figure 6. Because of limited cell availability, a subset of age-matched paired samples was randomly selected and examined ($n = 24$ for the CV group).

IFN- γ /IL-2/IL-4/IL-5/IL-13 FluoroSpot assays

PBMCs were incubated overnight in RPMI-1640 + 10% Human AB Serum + 1% Penicillin Streptomycin + 1% 200 g mL^{-1} D-glucose (ThermoFisher Scientific). FluoroSpot assays were used to measure CD8, CD4 Th1 and Th2 responses. PBMCs were stimulated in duplicates with SARS-CoV-2 spike peptide pool¹⁴ (JPT Peptide Technologies, Berlin, Germany) with $0.1 \mu\text{g mL}^{-1}$ co-stimulator anti-CD28 (mAb CD28A). CD8 and CD4 Th1 and CD4 Th2 responses were measured using Human IFN- γ /IL-2 FluoroSpot PLUS kits and custom Human IL-4/IL-5/IL-13 FluoroSpot FLEX kits, respectively, following the manufacturer's instructions (MabTech). Plates were analysed with Mabtech IRIS

FluoroSpot and ELISpot reader using FITC filter for IFN- γ and IL-13, Cy3 filter for IL-2 and IL-4, and Cy5 filter for IL-5. Because of limited cell availability, a subset of age-matched paired samples was randomly selected and examined ($n = 25$ for CV; $n = 63$ for mRNA groups).

Statistical analysis

Statistical analysis was performed using GraphPad Prism 7. To compare between different timepoints, Kruskal–Wallis tests and *post hoc* tests using Dunn's multiple comparison tests were used. Unpaired comparisons (between CV and mRNA groups) were performed using the Mann–Whitney *U*-test. FluoroSpot results were analysed with the Welch's *t*-test for parametric unpaired comparisons. A Spearman correlation was used to examine the correlation between the anti-S antibody and time interval between the mRNA and the first CoronaVac dose. All tests were two-tailed and a *P*-value < 0.05 was considered statistically significant.

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CONFLICT OF INTEREST

A patent application for the SFB assay has been filed (Singapore patent #10202009679P: A Method Of Detecting Antibodies And Related Products). The authors declare no other competing interests.

AUTHOR CONTRIBUTIONS

Yun Shan Goh: Conceptualization; data curation; formal analysis; investigation; methodology; supervision; validation; visualization; writing – original draft; writing – review and editing. **Siew-Wai Fong:** Conceptualization; data curation; formal analysis; methodology; validation; writing – original draft; writing – review and editing. **Angeline Rouers:** Conceptualization; data curation; formal analysis; methodology; validation; writing – original draft; writing – review and editing. **Zi Wei Chang:** Conceptualization; data curation; formal analysis; methodology; validation; writing

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Supporting Information

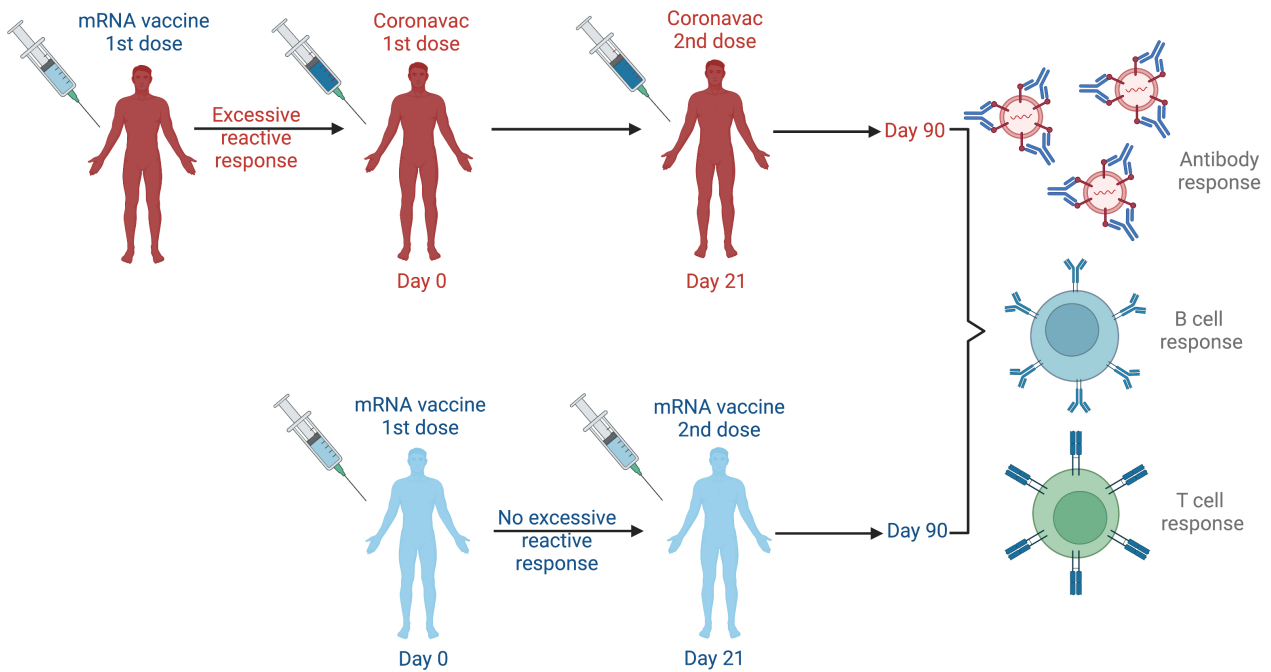
Additional supporting information may be found online in the Supporting Information section at the end of the article.



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Graphical Abstract

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Individuals, ineligible for further mRNA vaccines because of excessive reactive responses to prime mRNA vaccination, were recruited and offered two doses of CoronaVac as booster vaccination. Individuals who did not develop any excessive reactive responses after the prime mRNA vaccination received another mRNA vaccine as booster vaccination. Blood samples were collected at days 0, 21 and 90 post first CoronaVac dose and mRNA dose, respectively, and the antibody profiles, B and T cell responses, were analysed.