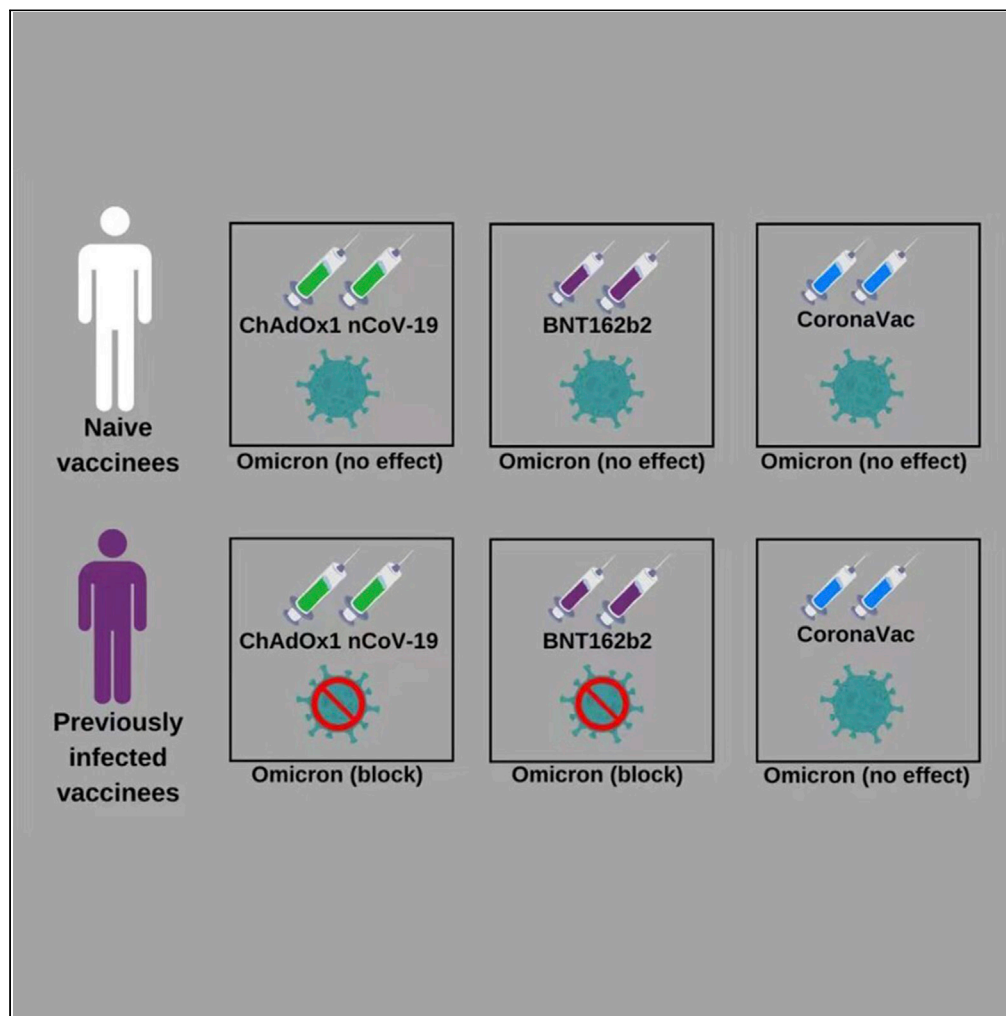


Article

Susceptibility to SARS-CoV-2 omicron following ChAdOx1 nCoV-19 and BNT162b2 versus CoronaVac vaccination



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Highlights

ChAdOx1 nCoV-19, BNT162b2, and CoronaVac did not protect against Omicron variant

Combination of prior infection and ChAdOx1 nCoV-19 or BNT162b2 cross-protected against Omicron variant

The CoronaVac vaccine had no protective effect against Omicron regardless of infection status

Prolonged target antigen exposure and target diversification are key for next SARS-CoV-2 vaccines

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Article

Susceptibility to SARS-CoV-2 omicron following ChAdOx1 nCoV-19 and BNT162b2 versus CoronaVac vaccination

Jia Xin Chua,^{1,5,*} Lindy Gillian Durrant,^{1,2} Yin Ling Chok,¹ and Oi Ming Lai^{3,4}

SUMMARY

The emergence of SARS-CoV-2 variants raises concerns of reduced COVID-19 vaccine efficacy. We investigated the humoral immunity in uninfected and previously infected ChAdOx1 nCoV-19, BNT162b2 and CoronaVac vaccinees, who have received complete regimes of vaccines by means of a SARS-CoV-2 surrogate virus blocking test. The ChAdOx1 nCoV-19 ($p = 0.0013$) and BNT162b2 ($p = 0.0005$) vaccines induced significant higher blocking activity with longer durability against the Spike (S) protein receptor binding domain (RBD) of wild type SARS-CoV-2 than the CoronaVac vaccine in uninfected vaccinees. Prior infection improved protection in the CoronaVac vaccinees. Subsequent investigation on the breadth of SARS-CoV-2 vaccine-induced antibody blocking responses, revealed that all vaccine platforms cross-protected uninfected vaccinees against all variant of concerns, except Omicron. Prior infection protected the ChAdOx1 nCoV-19 and BNT162b2 vaccinees against Omicron but not CoronaVac vaccinees. Our study suggests that vaccines that induce broader sterilizing immunity are essential to fight against fast-emerging variants.

INTRODUCTION

To fight the COVID-19 pandemic, many vaccine candidates have been developed, using various strategies, encompassing both traditional method (inactivated whole virus, live-attenuated virus, and protein subunit of the virus) and next-generation techniques (mRNA, DNA, and viral-based) (Caddy, 2020; Callaway, 2020; van Riel and deWit, 2020). To date, six COVID-19 vaccines have been approved by at least one World Health Organization (WHO) recognized authority for emergency or full use, including ChAdOx1 nCoV-19 (Oxford-AstraZeneca), BNT162b2 (Pfizer/BioNtech), mRNA-1273 (Moderna), Ad26-COV2.S (Janssen), CoronaVac (Sinovac), and Sputnik V (Gamaleya) (Baden et al., 2021; Folegatti et al., 2020; Logunov et al., 2021; Polack et al., 2020; Sadoff et al., 2021; Zhang et al., 2021b). Although these approved vaccines have been shown to elicit robust humoral and/or cell-mediated response toward WT SARS-CoV-2 (Wuhan Hu-1) (Baden et al., 2021; Folegatti et al., 2020; Jones et al., 2021), the quick emergence of SARS-CoV-2 variants raised concerns about the effectiveness of current approved vaccines.

The WHO has designated 5 SARS-CoV-2 variants as variants of concerns (VOCs), including Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), and Omicron (B.1.1.529). The S glycoprotein of the SARS-CoV-2 virus facilitates viral entry via interaction with the host cellular ACE-2 receptor and is therefore the primary target for most COVID-19 vaccines (Cevik et al., 2021). Thus, mutations within S glycoprotein, especially at the RBD, may affect the effectiveness of vaccines and increase disease severity. Some of the most critical mutations include the D614G at the C-terminal region of S1 domain, and N501Y, L452R, K417N/K417T, E484K/E484A at the RBD. These mutations contribute to increase viral infectivity and transmissibility by enhancing binding affinity of S glycoprotein to ACE-2 (N501Y, L452R, and D614G), conformational change of S glycoprotein (N501Y and K417N/K417T), immune escape and decrease in sera neutralization (E484K/E484A and L452R) (Garcia-Beltran et al., 2021; Greaney et al., 2021; Starr et al., 2021; Zhang et al., 2020, 2021a). In November 2020, Omicron variant was reported. In just a few months' time, it has overtaken the previously dominant Delta variant, driving the current waves of the COVID-19 pandemic worldwide, owing to its shorter doubling time (Karim and Karim, 2021) and highly transmissibility (Callaway and Ledford, 2021).

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Table 1. Characteristics of the study cohort

| | Naïve n= 2 | ChAdOx1 nCoV-19 n= 33 | BNT162b2 n= 17 | CoronaVac n= 34 |
|--|-------------|-----------------------|---------------------|----------------------|
| Female; uninfected [median age (min-max)] | n = 1 43 | n = 15 40 (23-54) | n = 7 48 (18-72) | n = 13 43 (29-68) |
| Female; prior infection [median age (min-max)] | N/A | n = 2 42 (30-53) | n = 2 31 (19-43) | n = 2 35 (29-40) |
| Male; uninfected [median (min-max)] | n = 1 28 | n = 14 42 (22-64) | n = 7 39 (20-63) | n = 14 42 (22-73) |
| Male; prior infection [median age (min-max)] | N/A | n = 1 41 | n = 1 37 | n = 5 40 (28-52) |

This study aims to investigate and compare the anti-S protein RBD blocking antibody activity of Malaysian vaccinees (with or without previous infection) against SARS-CoV-2 WT virus and variants.

RESULTS

The ChAdOx1 nCoV-19 and BNT162b2 vaccines induced better vaccine efficacy against WT and delta variants compared to the CoronaVac vaccine

A total of 84 sera from three groups of vaccinees with or without prior infection (Table 1) were examined for blocking antibody activity, against the WT and Delta variant of SARS-CoV-2 S protein RBDs (Figures 1A and 1B). Serum dilution used was 1:10 dilution, and we considered >30% of inhibition of SARS-CoV-2 S protein RBD binding to hACE-2 receptors as sufficient, based on manufacturer's protocol and on a previously established cut-off value (Tan et al., 2020). Although the uninfected ChAdOx1 nCoV-19 (WT: 78%, Delta: 74%) and BNT162b2 (WT: 86%; Delta: 82%) vaccinee sera demonstrated effective blockade of SARS-CoV-2 WT- and Delta-RBD binding to hACE-2 receptors, the uninfected CoronaVac (WT: 48%, Delta: 40%) vaccinee sera showed significant lower level of blocking activity (WT: ChAdOx1 nCoV-19 VS CoronaVac, $p = 0.0013$; BNT162b2 VS CoronaVac, $p = 0.0005$ and Delta: ChAdOx1 nCoV-19 VS CoronaVac, $p = 0.0007$; BNT162b2 VS CoronaVac, $p = 0.0004$). Previous infection improved blocking activity in all groups, especially in CoronaVac vaccinees, of which 1.5- and 1.8-fold increase in blocking activity toward WT- and Delta-RBD, respectively was seen as compared to naive CoronaVac vaccinees. No antibody blocking activity was detected in unvaccinated individuals.

The previously infected vaccinee sera displayed better WT RBD-hACE-2 blocking in a dose dependent manner, compared to uninfected vaccinee sera

Subsequently, 3 uninfected or previously infected vaccinees whose sera demonstrated high RBD-hACE-2 blocking activity (80–100%) at 1:10 dilution were selected from each vaccine group and performed a dose dependent WT RBD-hACE-2 blocking assay. Serum dilutions used were 1:10, 1:30, 1:100, 1:300 and 1:1000 dilutions. The uninfected vaccinees were selected from those who had received second dose vaccines approximately within a month (ChAdOx1 nCoV-19: Day 7–34; BNT162b2: Day 18–31 and CoronaVac: Day 24–25). Because of limited number of samples for previously infected vaccinees, we had to include samples collected more than a month after second dose of vaccination (ChAdOx1 nCoV-19: Day 12–46; BNT162b2: Day 14–47 and CoronaVac: Day 34–55). Results were presented as the mean values of the three vaccinees within each group (Figure 2).

All uninfected vaccinees showed dose dependent increase in antibody blocking activity, with BNT162b2 vaccinees showing the highest blocking antibody titer (endpoint titer of 1:1000), followed by ChAdOx1 nCoV-19 and CoronaVac vaccinees (endpoint titer of 1:100). At 1:10 and 1:30 dilutions, the BNT162b2 vaccinees demonstrated highest level of antibody blocking activity of 96 and 93%, followed by ChAdOx1 nCoV-19 (93 and 80%) and CoronaVac (87 and 71%). At 1:100, 1:300 and 1:1000 dilutions, the BNT162b2 vaccinees exhibited significant higher antibody blocking activity of 1.8-fold (87 VS 49%; $p = 0.0295$), 2.8-fold (76 VS 27%; $p = 0.0025$) and 5.8-fold (52 VS 9%; $p = 0.0071$), respectively, as compared to that of ChAdOx1 nCoV-19; and 2-fold (87 VS 44%; $p = 0.0089$), 2.6-fold (76 VS 29%; $p = 0.0041$) and 7.4-fold (52 VS 7%; $p = 0.0052$), respectively, as compared to that of CoronaVac vaccinees.

Previous infection improved antibody blocking activity, giving high blocking antibody titer across all three vaccine platforms (endpoint titer of 1:1000). Previously infected vaccinees who received BNT162b2 vaccines

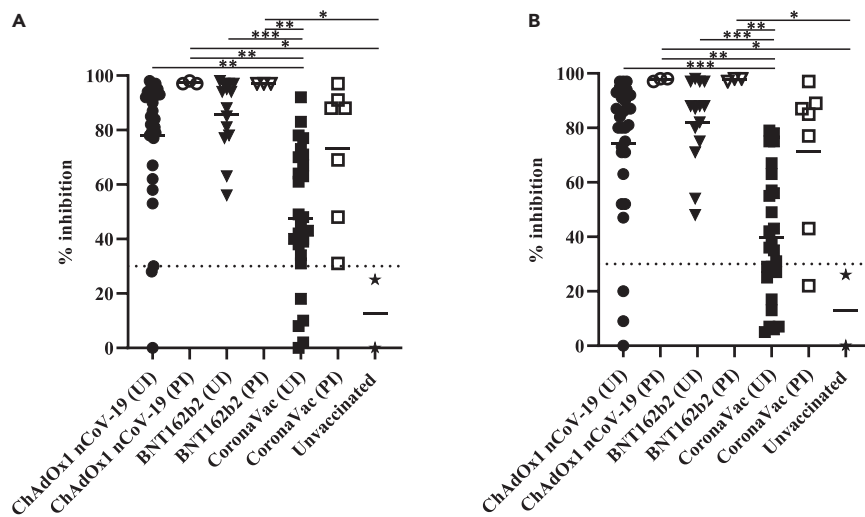


Figure 1. ELISA analysis of the vaccinee sera-mediated inhibition of WT and Delta SARS-CoV-2 RBD binding to hACE-2 receptors

(A) The sera (1:10 dilution) were pre-incubated with HRPO-conjugated WT-RBD, before adding into wells pre-coated with hACE-2 receptors. Sera from two unvaccinated individuals were included as controls. Each data point represents a different serum sample from a vaccinee. The dotted line indicates the inhibition of RBD-hACE-2 interaction by sera cut-off at 30% and the mean inhibition shown as line. Comparative analysis of the ELISA results was established by ANOVA followed by unpaired Kruskal-Wallis test with values of *P* calculated accordingly, using the GraphPad Prism 8 software. (*, $p < 0.05$; **, $p < 0.005$; ***, $p < 0.005$ and ****, $p < 0.0001$). Annotations: UI, uninfected; PI, previously infected.

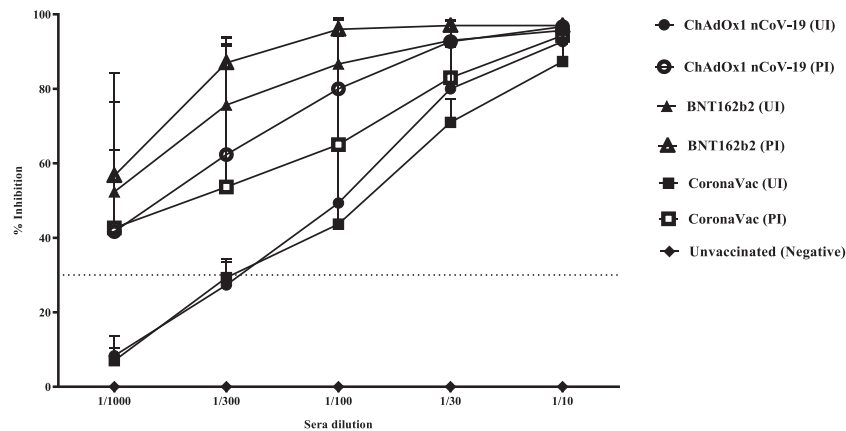
(B) The sera (1:10 dilution) were pre-incubated with HRPO-conjugated Delta-RBD, before adding into wells pre-coated with hACE-2 receptors. Sera from two unvaccinated individuals were included as controls. Each data point represents a different serum sample from a vaccinee. The dotted line indicates the inhibition of RBD-hACE-2 interaction by sera cut-off at 30% and the mean inhibition shown as line. Comparative analysis of the ELISA results was established by ANOVA followed by unpaired Kruskal-Wallis test with values of *P* calculated accordingly, using the GraphPad Prism 8 software. (*, $p < 0.05$; **, $p < 0.005$; ***, $p < 0.005$ and ****, $p < 0.0001$). Annotations: UI, uninfected; PI, previously infected.

showed 2-fold (96 VS 49%; $p = 0.0038$), 3.2-fold (87 VS 27%; $p = 0.0001$) and 5.7-fold (51 VS 9%; $p = 0.0025$) higher antibody blocking activity compared to ChAdOx1 nCoV-19 uninfected vaccinees; 2.2-fold (96 VS 44%; $p = 0.001$), 3-fold (87 VS 29%; $p = 0.0002$) and 8.1-fold (57 VS 7%; $p = 0.0019$) higher antibody blocking activity compared to CoronaVac uninfected vaccinees, at 1:100, 1:300 and 1:1000 dilutions, respectively. At 1:300, previously infected ChAdOx1 nCoV-19 vaccinees induced 2.3-fold (62 VS 27%; $p = 0.0465$) higher antibody blocking activity, compared to ChAdOx1 nCoV-19 uninfected vaccinees; at 1:1000 dilution, previously infected CoronaVac vaccinees demonstrated 5-fold (42 VS 7%; $p = 0.0409$) higher antibody blocking activity, compared to CoronaVac uninfected vaccinees.

The ChAdOx1 nCoV-19 and BNT162b2 vaccines induced blocking antibody activity with longer durability compared to the CoronaVac vaccine in uninfected vaccinees

Next, we investigated whether timing after second dose vaccination had an impact on the level of antibody blocking activity against WT- (Figures 3A–3C) and Delta-RBD (Figures 3D–3F). Vaccinees were divided into two groups, sera collected within 30 days (Group 1) and, more than 30 days (Group 2), after the second dose vaccinations (Table 2).

For uninfected ChAdOx1 nCoV-19 vaccinees ($n = 30$), the mean sera collection day for Group 1 ($n = 8$) and 2 ($n = 22$) was day 21 and 53, respectively. All vaccinees from Group 1 showed effective blocking antibody responses, giving an average of 88 and 85% of inhibition against WT- and Delta-RBD, respectively. A minimal reduction in blocking antibody response was seen in Group 2 against WT- (average of 74%) and Delta-RBD (average of 70%), but it was not statistically significant. Notably, among the uninfected ChAdOx1 nCoV-19 vaccinees, 3/22 of them showed lower (55–70%) and another 3/22 showed no (<30%) blocking antibody response. These six vaccinee sera were collected between one and three months after the second dose vaccination, indicating that a minority of the vaccinees who have received



| Statistics | | |
|---|---------|---------|
| Sample | P value | Summary |
| 1:100 dilution | | |
| BNT162b2 (N) VS ChAdOx1 nCoV-19 (N) | 0.0295 | * |
| BNT162b2 (PI) VS ChAdOx1 nCoV-19 (N) | 0.0038 | ** |
| BNT162b2 (N) VS CoronaVac (N) | 0.0089 | ** |
| BNT162b2 (PI) VS CoronaVac (N) | 0.001 | *** |
| ChAdOx1 nCoV-19 (PI) VS CoronaVac (N) | 0.0360 | * |
| 1:300 dilution | | |
| BNT162b2 (N) VS ChAdOx1 nCoV-19 (N) | 0.0025 | ** |
| BNT162b2 (PI) VS ChAdOx1 nCoV-19 (N) | 0.0001 | *** |
| BNT162b2 (N) VS CoronaVac (N) | 0.0041 | *** |
| BNT162b2 (PI) VS CoronaVac (N) | 0.0002 | *** |
| ChAdOx1 nCoV-19 (PI) VS ChAdOx1 nCoV-19 (N) | 0.0465 | * |
| 1:1000 dilution | | |
| BNT162b2 (N) VS ChAdOx1 nCoV-19 (N) | 0.0071 | ** |
| BNT162b2 (PI) VS ChAdOx1 nCoV-19 (N) | 0.0025 | ** |
| BNT162b2 (N) VS CoronaVac (N) | 0.0052 | ** |
| BNT162b2 (PI) VS CoronaVac (N) | 0.0019 | ** |
| ChAdOx1 nCoV-19 (PI) VS CoronaVac (N) | 0.0495 | * |
| CoronaVac (PI) VS CoronaVac (N) | 0.0409 | * |

Figure 2. Assessment of dose dependent antibody blocking activity against WT-RBD-hACE-2 interaction

The sera (1:10, 1:30, 1:100, 1:300 and 1:1000 dilutions) were pre-incubated with HRPO-conjugated WT-RBD, before adding into wells pre-coated with hACE-2 receptors. Unvaccinated individual sera (negative) were included as negative controls. The dotted line indicates the inhibition of RBD-hACE-2 interaction by sera cut-off at 30% and the mean inhibition shown as line. The results were expressed as mean \pm SEM ($n = 3$ for vaccinees and $n = 2$ for unvaccinated individuals). Comparative analysis of the ELISA results was established by two-way ANOVA using the GraphPad Prism 8 software. (*, $p < 0.05$; **, $p < 0.005$; ***, $p < 0.005$ and ****, $p < 0.0001$). Annotations: UI, uninfected; PI, previously infected.

the ChAdOx1 nCoV-19 vaccine, may require a booster dose as early as one month after second dose vaccination.

For uninfected BNT162b2 vaccinees ($n = 14$), the mean sera collection day for Group 1 ($n = 3$) and 2 ($n = 11$) was day 19 and 84, respectively. The Group 1 showed effective blocking antibody responses against WT- and Delta-RBD, giving an average of 96 and 94%, respectively; minimal reduction seen in Group 2 against WT- (average of 83%) and Delta-RBD (average of 79%), which was statistically insignificant. Among Group 2 vaccinees, 4/11 of them whose sera were collected between three to six months after second dose vaccination, still showed high blocking antibody responses (>70%) against WT- and Delta-RBD, indicating that BNT162b2 vaccine offers longer protection of possibly more than six months.

For uninfected CoronaVac vaccinees ($n = 27$), the mean sera collection day for Group 1 ($n = 9$) and 2 ($n = 18$) was day 23 and 49, respectively. The blocking antibody activity observed in this group was lower compared to the other two vaccine platforms, with Group 1 showed an average of 68 and 54% inhibition against WT- and Delta-RBD, respectively. Further, Group 2 showed 1.8- ($p = 0.0181$) and 1.6-fold reduction in antibody

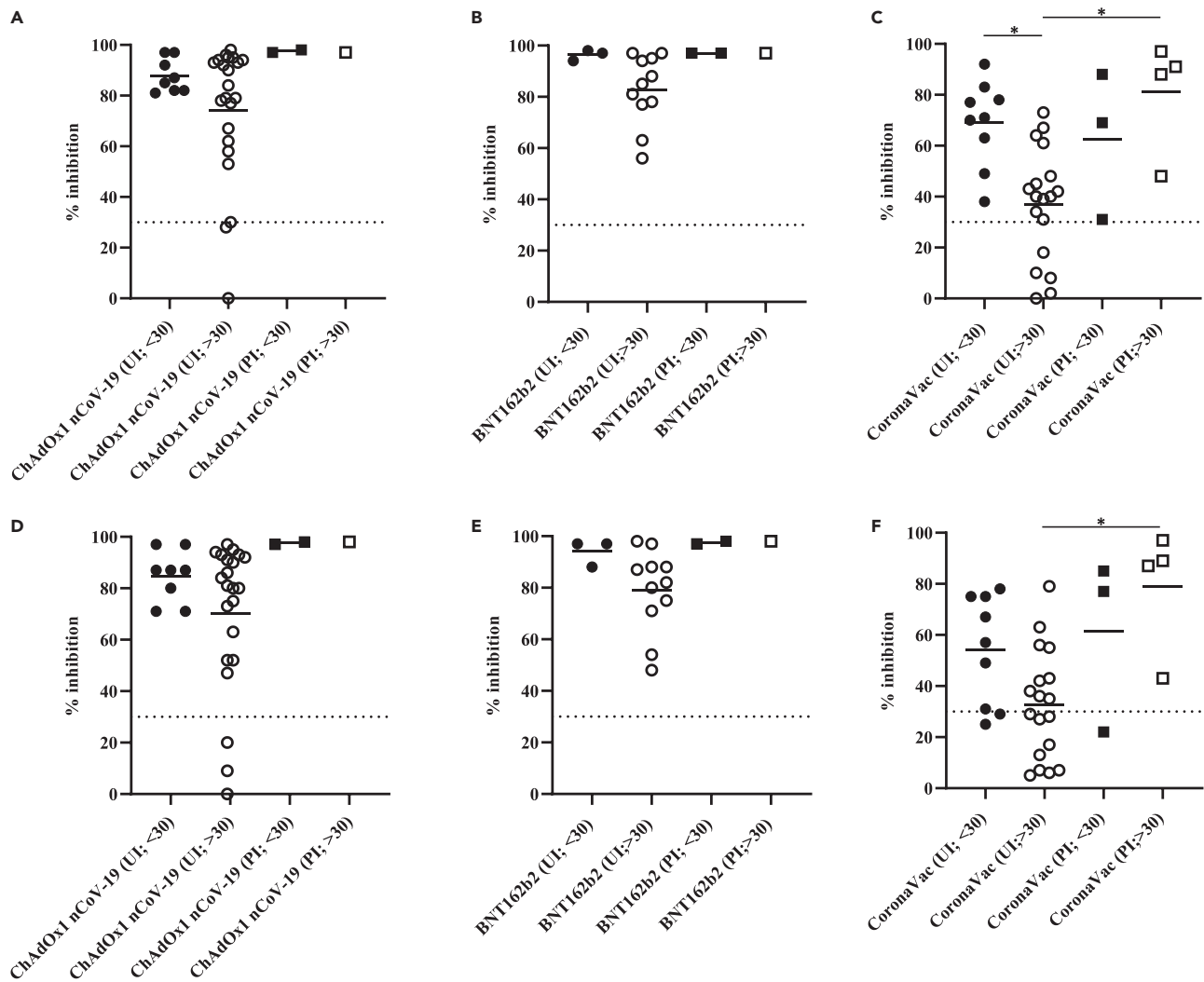


Figure 3. ELISA analysis of the potential impact of durability and timing after second dose vaccination on the potency of antibody neutralizing activity

(A–C) The sera (1:10 dilution) were pre-incubated with HRPO-conjugated WT-RBD, before adding into wells pre-coated with hACE-2 receptors. Each data point represents a different serum sample from a vaccinee. The dotted line indicates the inhibition of RBD-hACE-2 interaction by sera cut-off at 30% and the mean inhibition shown as line. Comparative analysis of the ELISA results was performed by ANOVA followed by unpaired Kruskal-Wallis test with values of *P* calculated accordingly, using the GraphPad Prism 8 software. (*, $p < 0.05$; **, $p < 0.005$; *** and $p < 0.005$). Annotations: UI, uninfected; PI, previously infected.

(D–F) The sera (1:10 dilution) were pre-incubated with HRPO-conjugated Delta-RBD, before adding into wells pre-coated with hACE-2 receptors. Each data point represents a different serum sample from a vaccinee. The dotted line indicates the inhibition of RBD-hACE-2 interaction by sera cut-off at 30% and the mean inhibition shown as line. Comparative analysis of the ELISA results was performed by ANOVA followed by unpaired Kruskal-Wallis test with values of *P* calculated accordingly, using the GraphPad Prism 8 software. (*, $p < 0.05$; **, $p < 0.005$; *** and $p < 0.005$). Annotations: UI, uninfected; PI, previously infected.

blocking activity against WT- and Delta-RBD, respectively compared to Group 1, indicating that blocking antibody against S protein RBD, wanes within a month after second dose vaccination.

Natural infection prolonged blocking antibody durability in vaccinees regardless of vaccine platforms

Next, we sought to assess whether natural infection in combination of vaccination would improve blocking antibody durability. Vaccinees were divided into two groups, sera collected within 30 days (Group 1) and, more than 30 days (Group 2), after second dose vaccinations (Figure 3, Table 2).

Table 2. Sera collection timeline after second dose vaccination

| | ≤ 30 days | >30 days |
|--|--------------------|----------------------|
| ChAdOx1 nCoV-19 (uninfected; n = 30) median day (min-max) | n= 9 21 (7-30) | n= 21 55 (33-82) |
| ChAdOx1 nCoV-19 (prior infection; n = 3) median day (min-max) | n= 2 18 (12-24) | n= 1 46 (46) |
| BNT162b2 (uninfected; n = 14) median day (min-max) | n= 3 19 (18-21) | n= 11 84 (31-188) |
| BNT162b2 (prior infection; n = 3) median day (min-max) | n= 2 22 (14-29) | n= 1 47 (47) |
| CoronaVac (uninfected; n = 27) median day (min-max) | n= 9 23 (21-25) | n= 18 49 (32-69) |
| CoronaVac (prior infection; n = 7) median day (min-max) | n= 3 27 (26-28) | n= 4 38 (33-43) |

For ChAdOx1 nCoV-19 (n = 3) and BNT162b2 vaccinees (n = 3) with prior infection, the mean sera collection day for Group 1 and 2 was day 18 and 46, respectively for ChAdOx1 nCoV-19 vaccinees and day 22 and 47, respectively for BNT162b2 vaccinees. Two groups of both vaccine platforms showed effective neutralizing antibody responses, with near to 100% of inhibition against WT- and Delta-RBD.

For CoronaVac vaccinee with previous infection (n = 7), the mean sera collection day for Group 1 (n = 3) and 2 (n = 4) was day 27 and 38, respectively. There was no significant difference in neutralizing antibody activity in Group 1 vaccinees for naive (WT:68%, Delta:54%) or previously infected (WT:63%, Delta:61%) CoronaVac vaccinees. In contrast, Group 2 CoronaVac vaccinees with prior infection showed significant 2.2- (p= 0.015) and 2.4-fold (p= 0.0149) of increase in neutralizing antibody activity against WT- and Delta-RBD, respectively, as compared to the uninfected CoronaVac vaccinees.

Our results showed that ChAdOx1 nCoV-19 and BNT162b2 vaccines induced superior blocking antibody reactivity against WT and Delta variant, with better durability, compared to that of CoronaVac vaccinees; previous infection greatly improves antibody potency and durability in the CoronaVac vaccinees.

All vaccine platforms failed to induce cross-protection against omicron variant in uninfected vaccinees

At the time of this study, the Delta variant was found predominantly in Malaysia. To examine the neutralizing antibody activity against other SARS-CoV-2 variants, uninfected vaccinees whose sera showed excellent neutralizing activity against the WT- and Delta- RBD, were randomly selected from each vaccine group and assessed for their cross-protection against other VOCs (Alpha, Beta, Gamma, and Omicron) and a variant of interest (VOI) Epsilon (Figure 4). The uninfected ChAdOx1 nCoV-19 vaccinee sera demonstrated effective neutralizing antibody reactivity against WT (92%) and all variants (Delta: 91%, Epsilon 78%, Alpha: 72%, Gamma: 60%, and Beta: 59%) although lower activity was seen against Gamma and Beta variants. The BNT162b2 uninfected vaccinee sera showed overall similar neutralizing antibody response as the ChAdOx1 nCoV-19 uninfected vaccinee sera, except that BNT162b2 sera gave better protection against Epsilon, Beta and Gamma variants (WT: 90%, Delta: 88%, Alpha: 72%, Epsilon: 85%, Beta: 75%, and Gamma: 67%). In contrast, the CoronaVac uninfected vaccinee sera showed effective response toward WT (81%) and Delta (75%) only; with significant loss of activity seen against the Alpha (39%), Epsilon (63%), Beta (48%), and Gamma (38%). All of the uninfected vaccinee sera failed to neutralise (<30%) the newly emerged Omicron variant.

Previously infected vaccinees who received ChAdOx1 nCoV-19 or BNT162b2 vaccines were cross-protected against omicron variant but not for those who have received the CoronaVac vaccines

Subsequently, we investigated whether natural infection would improve antibody blocking activity against all variants and induce protection against Omicron variant in vaccinees across three vaccine platforms (Figure 4).

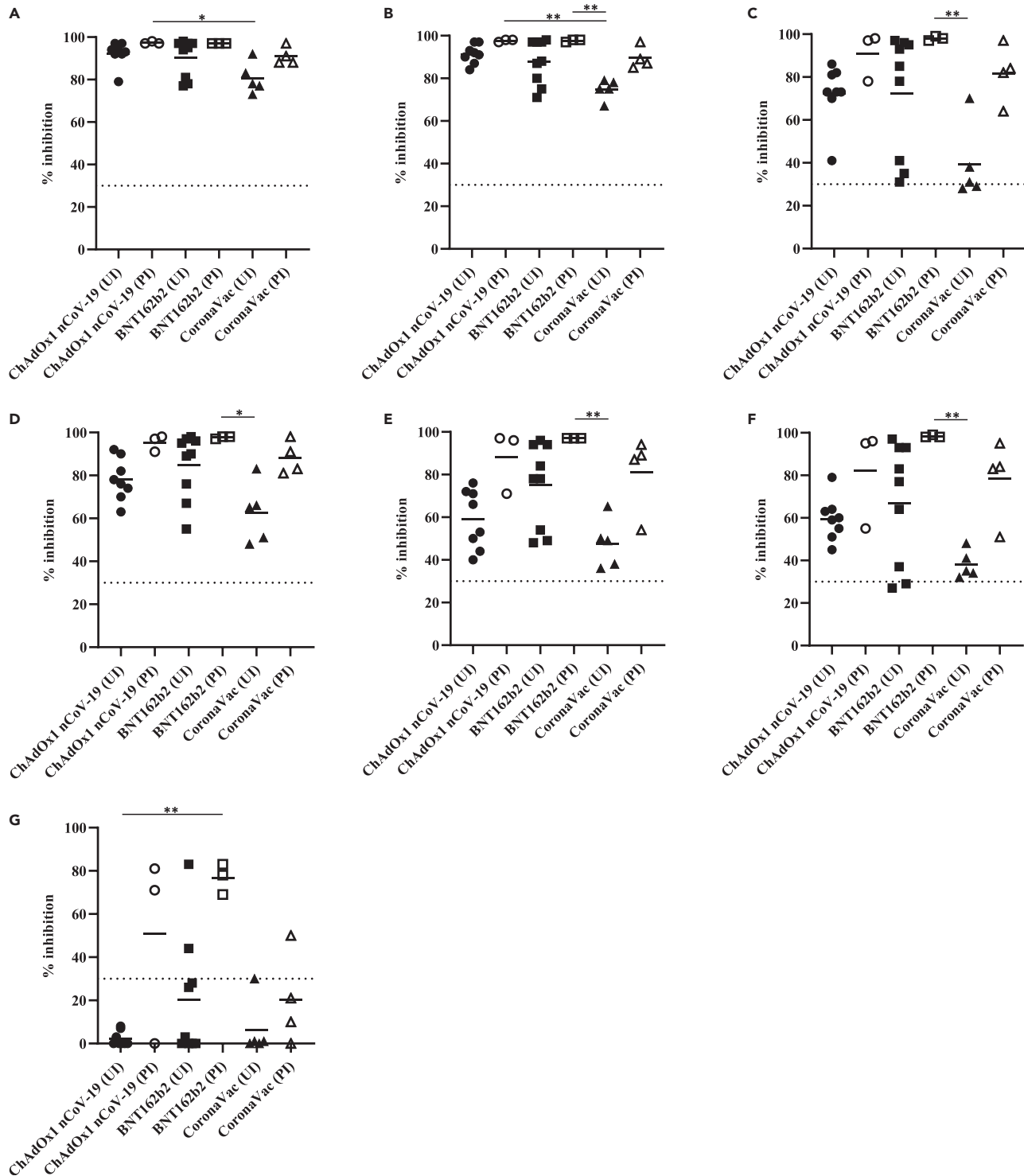


Figure 4. Sera neutralizing activity against SARS-CoV-2 variant RBDs

(A) The sera (1:10 dilution) were pre-incubated with HRPO-conjugated WT-RBD.
 (B) The sera (1:10 dilution) were pre-incubated with HRPO-conjugated Delta-RBD.
 (C) The sera (1:10 dilution) were pre-incubated with HRPO-conjugated Alpha-RBD.
 (D) The sera (1:10 dilution) were pre-incubated with HRPO-conjugated Epsilon-RBD.
 (E) The sera (1:10 dilution) were pre-incubated with HRPO-conjugated Beta-RBD.

Figure 4. Continued

(F) The sera (1:10 dilution) were pre-incubated with HRPO-conjugated Gamma-RBD.

(G) The sera (1:10 dilution) were pre-incubated with HRPO-conjugated Omicron-RBD. Each data point represents a different serum sample from a vaccinee. The dotted line indicates the inhibition of RBD-hACE-2 interaction by sera cut-off at 30% and the mean inhibition shown as line. Comparative analysis of the ELISA results was established by ANOVA followed by unpaired Kruskal-Wallis test with values of *P* calculated accordingly, using the GraphPad Prism 8 software. (*, *p*<0.05; **, *p*<0.005; ***, *p*<0.005). Annotations: *UI*, uninfected; *PI*, previously infected.

Importantly, not only did we observed improved protection against all variants, but protection against Omicron variant was also seen in the ChAdOx1 nCoV-19 [(WT: 97%; 1.0-fold), (Delta: 98%; 1.0-fold), (Alpha: 91%; 1.3-fold), (Epsilon: 95%; 1.2-fold), (Beta: 88%; 1.5-fold), (Gamma: 82%; 1.2-fold), and (Omicron: 51%; 25.5-fold)] and BNT162b2 [(WT: 97%; 1.0-fold), (Delta: 98%; 1.1-fold), (Alpha: 98%; 1.4-fold), (Epsilon: 98%; 1.2-fold), (Beta: 97%; 1.3-fold), (Gamma: 98%; 1.5-fold), and (Omicron: 77%; 3.9-fold)] vaccinees. Although prior infection improved protection against all other variants in CoronaVac vaccinees, Omicron remained unprotected [(WT: 91%; 1.1-fold), (Delta: 90%; 1.2-fold), (Alpha: 82%; 2.1-fold), (Epsilon: 88%; 1.4-fold), (Beta: 81%; 1.7-fold), (Gamma: 78%; 2.1-fold), and (Omicron: 20%; 3.3-fold)].

DISCUSSION

In Malaysia, the ChAdOx1 nCoV-19, BNT162b2 and CoronaVac vaccines were the first three vaccines given emergency use authorization (EUA). Both of the ChAdOx1 nCoV-19 (Watanabe et al., 2021) and BNT162b2 (Anderson et al., 2020) vaccines encode for the full-length WT SARS-CoV-2 S glycoprotein, using a chimpanzee-based non-replicating adenovirus vaccine vector and mRNA incorporated in lipid nanoparticles technologies, respectively; the CoronaVac is an inactivated SARS-CoV-2 viral vaccine, of which the virus is chemically inactivated using β -propiolactone (Gao et al., 2020).

In this study, we observed that two doses of the ChAdOx1 nCoV-19 and BNT162b2 vaccines induced effective blocking antibody activity against WT- and Delta-variant S protein RBD in uninfected vaccinees, whereas CoronaVac was significantly less effective. Further investigation revealed that the antibody durability of CoronaVac uninfected vaccinees was shorter compared to ChAdOx1 nCoV-19 and BNT162b2 uninfected vaccinees. For BNT162b2 uninfected vaccinees, high antibody blocking activity was still detected even six months after second dose vaccination; three months for the ChAdOx1 nCoV-19 uninfected vaccinees; but for CoronaVac uninfected vaccinees, the majority of the sera showed low level antibody blocking activity within two months after second dose vaccination, with a minority as early as three weeks after second dose vaccination. The generation of high neutralizing antibody titers after COVID-19 infection and/or vaccination are associated with protection (Khoury et al., 2021). However, previous studies on other human coronavirus infections revealed that humoral immunity was not long-lasting, dropping below the detection limit two to three years after infection (Callow et al., 1990; Cao et al., 2007; Holmes, 2003; McIntosh et al., 1974), compared to SARS-CoV-specific T cells, which could still be detected 11 years after infection (Ng et al., 2016). The high vaccine effectiveness of the ChAdOx1 nCoV-19 (Sette and Crotty, 2021; van Doremalen et al., 2020) and BNT162b2 (Sahin et al., 2021) vaccines are associated with their ability to induce both humoral and cellular responses. Earlier studies on mRNA and viral vector vaccines demonstrated that once mRNA and viral vector are taken up by antigen-presenting cells at the injection site or lymph nodes, not only was there mass production of target viral proteins, the intracellular innate sensors will be triggered to release high level of type I interferon and pro-inflammatory cytokines and chemokines, contributing to the induction of potent humoral and cellular responses (Pardi et al., 2018; Sayedahmed et al., 2020). Both the humoral and cellular immune responses have complementary effects in fighting viral infection, of which humoral immunity generates antibodies that neutralise viruses whereas cellular immunity involves killing virus-infected cells by CD8 T cells and CD4 T cells provide cognate help for B cell maturation, differentiation and memory. In addition, the BNT162b2 vaccine was observed to induce germinal center (GC) reactions in the spleen and the draining lymph nodes of mice (Lederer et al., 2020; Tam et al., 2016; Walsh et al., 2020), which generated SARS-CoV-2 specific long-lived plasma cells and memory B cells that produce efficient neutralizing antibody over an extended time (Lederer et al., 2020). Unlike the ChAdOx1 nCoV-19 and BNT162b2 vaccines, the CoronaVac vaccine is an inactivated virus vaccine, known to have lower immunogenicity. In addition, as the vaccine is not live and does not replicate itself, this vaccine type usually presented lower antigen levels to the immune system, which may explain why we observed lower neutralizing antibody response in CoronaVac naïve vaccinees in this study. Although the inactivated virus vaccines were known to stimulate predominately humoral responses (Tlaxca et al., 2015), recent studies showed that the CoronaVac vaccine (Chen et al., 2021) and other inactivated

COVID-19 virus vaccines (Deng et al., 2021) induced both humoral and cellular (CD4 and CD8) responses, excluding the possibility that the low antibody response seen in the CoronaVac uninfected vaccinees was due to the absence of cellular immune response.

Mutations occur in the S glycoproteins may disrupt the structural integrity of the S glycoproteins that lead to the reduction of vaccine effectiveness. We demonstrated that the BNT162b2 uninfected vaccinee sera cross-protected against Alpha, Beta, Epsilon, Gamma, and Delta variants, with minimal reduction of antibody activity seen against the Beta and Gamma variants. Multiple studies showed similar results, of which only marginal decrease in the BNT162b2 vaccine efficacy seen against the Alpha, Beta, Gamma, and Delta variants (Abu-Raddad et al., 2021; Wang et al., 2021b). Sahin and coworkers demonstrated that the BNT162b2 vaccinee sera induced neutralizing activity against all pseudoviruses expressing SARS-CoV-2 S glycoproteins with different single point mutations that are conserved among different SARS-CoV-2 variants (Sahin et al., 2021). Similarly, the ChAdOx1 nCoV-19 vaccine cross-protected against the Alpha, Beta, Gamma, and Delta variants. Our result is consistent with previous report that the ChAdOx1 nCoV-19 vaccine has lower vaccine efficacy compared to the BNT162b2 vaccine (Calzetta et al., 2021). The ChAdOx1 nCoV-19 vaccine is an adenovirus-based vaccine, designed to deliver the recombinant genome to the host nucleus, where the desired gene of interest is expressed. Although majority of the viral genes responsible for viral replication are removed, remaining viral genes and promoters have been shown to lead to the production of adenoviral antigens in the ChAdOx1 nCoV-19 and other adenovirus-based vaccines (Almuqrin et al., 2021; Gorziglia et al., 1996; Rittner et al., 1997; Saha and Parks, 2017; Shimizu et al., 2011), which may have restricted the breadth of the humoral response to COVID-19 by presenting competing adenovirus epitopes. Despite the theoretical potential of the inactivated virus vaccines to offer a lead over other COVID-19 vaccine technologies against SARS-CoV-2 variants, we observed significantly lower level of cross-protection across all variants among the CoronaVac uninfected vaccinees, as compared to the ChAdOx1 nCoV-19 and BNT162b2 uninfected vaccinees.

Currently, although the Delta variant is found predominantly worldwide, but the novel Omicron variant, which emerged in November 2021 has caused steep increase in infection due to fast doubling time (Torjesen, 2021). The Omicron variant has been reported to exhibit unusually high mutations that are different from other known SARS-CoV-2 variants, which could be attributed to extensive and independent evolution in isolated human populations, immunocompromised patients or unknown animal species (Kupferschmidt, 2021). The Omicron S glycoprotein contains 37 mutations, of which the G339D, S371L, S373P, and S375F unique mutations found in the RBD domain are shown to modulate ACE-2 binding and/or antibody evasion (Harvey et al., 2021; Li et al., 2021; Liu et al., 2021; Wang et al., 2021b; Wheatley et al., 2021). Changes in the main antigenic target of antibodies generated by majority of COVID-19 vaccines raise concerns about of the potential of reinfection, increase in disease severity, vaccine efficacy and therapeutic monoclonal antibody treatment. In this study, we observed that all three vaccine platforms failed to induce neutralizing antibody response against Omicron variant in all uninfected vaccinees. Recently, multiple studies have shown that the Omicron variant not only evade antibodies induced on infection (Hoffmann et al., 2022) and BNT162b2 vaccination (Chemaitelly et al., 2021), but also can be fully (imdevimab, etesevimab, bamlanivimab) or partially resistance (casirivimab and sotrovimab) against the recombinant monoclonal antibodies used for COVID-19 treatment (Hoffmann et al., 2022).

We performed this study during the WT and Delta infection waves in Malaysia. We discovered that natural infection followed by 2 regimes of vaccination across three vaccine platforms, improved neutralizing antibody activity against all SARS-CoV-2 variants, including Omicron in the ChAdOx1 nCoV-19 and BNT162b2 vaccines. Although enhancement in antibody activity was seen against all other variants, CoronaVac vaccinee with prior infection failed to show protection against Omicron. Our results aligned with recent studies, which showed that the SARS-CoV-2 infection generated memory B cells that continue to undergo somatic mutation, memory B cell clonal turnover for at least a year, which generated antibodies increase in potency and breadth, targeting SARS-CoV-2 variants (Wang et al., 2021a). In contrast, although memory B cells found in BNT162b2 and mRNA-1273 (Moderna) fully vaccinated healthy individuals produce antibodies that evolve increased neutralizing activity, but there was no further increase in potency or breadth thereafter (Cho et al., 2021). Furthermore, natural infection has shown to induce and/or boost SARS-CoV-2 specific T cell responses (Tarke et al., 2021), which are crucial in promoting CD4 T cell help in enhancing neutralizing antibody responses (Zollner et al., 2021). It remains unclear why protection against Omicron

variant is seen in the ChAdOx1 nCoV-19 and BNT162b2 vaccinees but not CoronaVac vaccinees with prior infection. It is possible that multiple exposures to SARS-CoV-2 S protein in the context of natural infection first expand the neutralizing breadth of the antibody responses to the SARS-CoV-2 variants, and subsequent introduction of S protein specific vaccines (ChAdOx1 nCoV-19 and BNT162b2) further boost the S protein specific antibody responses. Given that the inactivated virus vaccines (CoronaVac) do not induce S glycoprotein specific response and generally stimulate a much weaker immune response (Tlaxca et al., 2015), this may explain why CoronaVac vaccine is unable to boost S glycoprotein specific antibody activity against the highly mutated Omicron variant.

To counteract the fast-emerging SARS-CoV-2 variants, several aspects have to be taken into consideration to develop highly effective next-generation COVID-19 vaccines. Firstly, due to natural selection, the SARS-CoV-2 virus enhances its evolutionary advantages at the RBD of S glycoprotein via mutations to increase binding affinity to hACE-2 receptor or to evade the host immunity. Thus, it is essential to explore alternative COVID-19 vaccine antigenic targets, rather than just focusing on the S glycoprotein. Secondly, cellular immunity has gained tremendous attention as SARS-CoV-2-specific CD4 and CD8 T cells were found in patients suffering from moderate, severe and critical COVID-19 (Braun et al., 2020) and associated with reduced disease severity (Grifoni et al., 2020; Rydzynski Moderbacher et al., 2020). Furthermore, the vast majority of the SARS-CoV-2 CD4 and CD8 T cell epitopes found in the COVID-19 patients were not substantially affected by the mutations found in the SARS-CoV-2 variants (Tarke et al., 2021).

Multiple studies have reported the detection of Nucleocapsid (N)-specific humoral and cellular responses in most COVID-19 patients (Grifoni et al., 2020; Long et al., 2020; Okba et al., 2020), suggesting that N glycoprotein could be an alternative target for next-generation COVID-19 vaccines. Rice et al. generated a bivalent human adenovirus serotype 5 (hAd5) vaccine, comprising sequence for both S and N proteins, generated enhanced *de novo* antigen-specific humoral and cellular responses in antigen-naïve preclinical models (Rice et al., 2020). Similarly, Brentville et al. generated a DNA vaccine encoding both the RBD of S and N glycoproteins, elicited strong humoral response coupled with proinflammatory CD4 Th1 and CD8 T cell responses, which provided effective cross-protection against the Alpha, Beta and Delta variants (Brentville et al., 2021).

The emergence of the Omicron variant is a wake-up call for us that new-generation vaccines are needed to fight against COVID-19 pandemic. Current available data with SARS-CoV-2 and past experience with SARS-CoV and MERS-CoV human infections would suggest that vaccines that target the more conserved N glycoproteins by inducing both humoral and cellular immunity could be an effective way to overcome the viral immune escape due to mutations in the S glycoprotein.

Limitations of study

In this study, the vaccinee sera were sampled between August 2021 to November 2021, when Delta variant was prevalence in Malaysia. Our study provides evidence that regardless of the platform type of the vaccines, prolonged target antigen exposure to the immune system as well as diversified the target of interest rather than just focusing on the easily mutated S glycoprotein are important considerations for developing future COVID-19 vaccines. The limitations of this study were the limited number of study participants, vaccinee sera were not sampled at the same day, and lack of phenotypical characterization of the T cell response.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.isci.2022.105379>.

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AUTHOR CONTRIBUTIONS

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DECLARATION OF INTERESTS

We declare no competing interests.

INCLUSION AND DIVERSITY

We support inclusive, diverse and equitable conduct of research.

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STAR★METHODS

KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|--|---------------------------|---------------------------|
| Biological samples | | |
| COVID vaccinee sera | MymAb Biologics Pvt. Ltd. | N/A |
| Chemicals, peptides, and recombinant proteins | | |
| Delta (B.1.617.2) | Genscript, USA | Z03608 |
| Alpha (B.1.1.7) | Genscript, USA | Z03595 |
| Beta (B.1.351) | Genscript, USA | Z03596 |
| Gamma (P.1) | Genscript, USA | Z03601 |
| Epsilon (B.1.429) | Genscript, USA | Z03603 |
| Omicron variants (B.1.1.529) | Genscript, USA | Z03730 |
| Critical commercial assays | | |
| SARS-CoV-2 Surrogate Virus Neutralisation Test Kit | Genscript, USA | EUA version, RES-L00847-B |
| Software and algorithms | | |
| GraphPad Prism 8 software | GraphPad Software Inc. | N/A |

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Jia Xin, Chua (jjaxin.mymab@gmail.com).

Materials availability

This study did not generate new unique reagents.

Data and code availability

All data reported in this manuscript will be shared by the [lead contact](#) on reasonable request. The article does not report original code. Any additional information required to reanalyze the data reported in this article is available from the [lead contact](#) on request.

EXPERIMENTAL SUBJECT DETAILS

We enrolled ChAdOx1 nCoV-19 (n = 33), BNT162b2 (n = 17), and CoronaVac (n = 34) vaccinees in Malaysia, who had completed two doses of vaccination ([Tables 1](#) and [S1](#)). All volunteers provided informed consent for the research work to be carried out. All vaccinees with previous infection, took 2 doses of vaccination after disease recovery. The ChAdOx1 nCoV-19 (n = 30), BNT162b2 (n = 14), and CoronaVac (n = 27) uninfected vaccinee sera were collected, on average of 72, 70 and 40 days; the previously infected ChAdOx1 nCoV-19 (n = 3), BNT162b2 (n = 3), and CoronaVac (n = 7) vaccinee sera were collected, on average of 27, 30, and 33 days, respectively after second immunization. Two unvaccinated individuals with no history of COVID-19 infection or vaccination were included as controls. Antibody neutralizing activity was measured at two time points: within and more than 30 days after second dose vaccination. We considered >30% of inhibition of SARS-CoV-2 S protein RBD binding to hACE-2 receptors as sufficient, based on manufacturer's protocol and on a previously established cut-off value ([Tan et al., 2020](#)). We determined prior SARS-CoV-2 infection status based on concordance of data documented in health records and self-reported survey information (based on evidence of positive SARS-CoV-2 reverse time polymerase chain reaction).

METHOD DETAILS

Horseradish peroxidase (HRPO)-conjugated SARS-CoV-2 spike (S) protein receptor binding domains (RBD)

All of the HRPO-conjugated RBDs were obtained from GenScript, USA. The wild type HRPO-conjugated RBD is one of the components in the SARS-CoV-2 Surrogate Virus Neutralization Test Kit (GenScript, FDA approved EUA version, RES-L00847-B). The variants contained amino acid mutations in their RBD, including L452R, and T478K in the Delta (B.1.617.2; Z03608); N501Y in the Alpha (B.1.1.7; Z03595); E484K, K417N, and N501Y in the Beta (B.1.351; Z03596); E484K, K417T, and N501Y in the Gamma (P.1; Z03601), L452R in the Epsilon (B.1.429; Z03603) and G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, and Y505H in the Omicron variants (B.1.1.529; Z03730).

RBD-hACE-2 interaction inhibition ELISA

ELISA assays were performed using C-Pass kit according to manufacturer's instructions (GenScript). In brief, pre-diluted sera (1:10, 1:30, 1:100, 1:300 and 1:1000 dilutions) were mixed with HRPO-conjugated RBD (1:1000 dilution) of the SARS-CoV-2 S protein of the WT or variants and incubated at 37°C for 30 min. Then, sera-RBD mixtures were added to the wells pre-coated with hACE-2 receptors and incubated at 37°C for 15 min. After incubation, HRPO-conjugated RBD bound to the hACE-2 receptors were detected and developed using 3,3'-5,5'-Tetramethylbenzidine. Plates were read at 450 nm.

QUANTIFICATION AND STATISTICAL ANALYSIS

Comparative analysis of the ELISA results was performed by one- or two-way ANOVA followed by unpaired Kruskal-Wallis test with values of P calculated accordingly, using the GraphPad Prism 8 software. $p < 0.05$ (*) and $p < 0.01$ (**) values were considered statistically significant and $p < 0.001$ (***) and $p < 0.0001$ (****) values were considered highly significant.