Received: 24 July 2022 | Accepted: 27 July 2022

DOI: 10.1002/jmv.28032

RESEARCH ARTICLE

JOURNAL OF MEDICAL VIROLOGY WILEY

Neutralizing antibodies to SARS-CoV-2 variants of concern including Delta and Omicron in subjects receiving mRNA-1273, BNT162b2, and Ad26.COV2.S vaccines

George Fei Zhang[1,2] | Wen Meng[1,2] | Luping Chen[1,2] | Ling Ding[1,2] | Jian Feng[1,2] | Joseph Perez[1,2] | Abid Ali[1,2] | Shenyu Sun[1,2] | Zhentao Liu[1,3,4] | Yufei Huang[1,3,4] | Haitao Guo[1,2] | Shou-Jiang Gao[1,2]

[1]Cancer Virology Program, UPMC Hillman Cancer Center, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA

[2]Department of Microbiology and Molecular Genetics, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA

[3]Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA

[4]Department of Electrical and Computer Engineering, Swanson School and Engineering, Pittsburgh, Pennsylvania, USA

Correspondence

Shou-Jiang Gao, Cancer Virology Program, UPMC Hillman Cancer Center, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213, USA.
Email: gaos8@upmc.edu

Funding information

Pittsburgh Foundation Endowed Chair in Drug Development for Immunotherapy

Abstract

SARS-CoV-2 vaccines have contributed to the control of COVID-19 in some parts of the world. However, the constant emergence of variants of concern (VOCs) challenges the effectiveness of SARS-CoV-2 vaccines over time. In particular, Omicron contains a high number of mutations in the spike (S) protein gene, on which most vaccines were developed. In this study, we quantitated neutralizing antibodies in vaccine recipients at various times postvaccination using S protein-based pseudoviruses derived from wild type (WT) SARS-CoV-2 and five 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). We found that two-dose mRNA-1273 and BNT162b2 vaccines elicited robust neutralizing antibodies against WT, Alpha, Beta, Gamma, and Delta, but wanned after 6 months with a faster decline observed for BNT162b2. Both mRNA-1273 and BNT162b2 elicited weak neutralizing antibodies against Omicron. One dose of Ad26.COV2.S vaccine induced weaker neutralizing antibodies against WT and most VOCs than mRNA-1273 and BNT162b2 did but moderate neutralizing antibodies against Delta and Omicron, which lasted for 6 months. These results support current recommendations of the Centers for Disease Control and Prevention for a booster 5 months after full immunization with an mRNA-based vaccine and the use of an mRNA-based vaccine 2 months after Ad26.COV2.S vaccination.

KEYWORDS

COVID-19, neutralizing antibodies, SARS-CoV-2, vaccine efficacy, variants of concern (VOCs)

1 | INTRODUCTION

The COVID-19 pandemic caused by SARS-CoV-2 has persisted for more than 2½ years since the report of the first case in December 2019.[1,2] A number of variants of concern (VOCs) have subsequently emerged including Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), and Omicron (B.1.1.529), first reported in the United Kingdom, South Africa, Brazil, India, and Botswana, respectively.[3–12] The most common mutations in these VOCs are in the spike (S) protein including: Δ69, Δ114Y, N501Y, D614G, and P681H for Alpha; L18F, Δ242, K417N, E484K, N501Y, D614G, and A701V for Beta; L18F, K417T, E484K, N501Y, and D614G for Gamma; T95I, L452R, T478K, D614G, and P681R for Delta; and A67V, Δ69-70, T95I, G142A, Δ143-145, Δ211, L212I, ins214EPE, G339A, S371L,

S373P, S375P, L417A, A440L, G446S, S477A, T478L, G484A, G493A, Gly496Ser, G498A, A501T, T505H, T547L, A614G, H655T, A679L, P681H, A764L, A796T, A856L, G954H, A969L, and L981P for Omicron.[9,10,13] In November 2021, the Omicron variant was first reported and has since spread throughout the world.[14,15] SARS-CoV-2 mutations impact virus characteristics, including cell tropism, virus replication and production, transmissibility, and antigenicity. VOCs emerge in response to the changing immune profile of the human population and the selective pressure this puts on viral fitness.[16] The S protein is required for cell entry and hence is an ideal target for vaccine development. The S protein binds with high affinity to ACE2, the receptor for SARS-CoV-2, which is widely distributed in human tissues.[17,18] Incidentally, similar to the SARS-CoV-2 nucleocapsid, the S protein is an ideal biomarker for diagnosis.[19,20]

Vaccination has played a significant role in controlling the COVID-19 pandemic.[21] Numerous SARS-CoV-2 vaccines have been approved or are under development, including mRNA-based vaccines, viral vector-based vaccines, inactivated vaccines, and recombinant subunit vaccines, and so on.[22–29] The mRNA-based vaccines Moderna mRNA-1273 and Pfizer-BioNTech BNT162b2 were granted emergency use authorization by the US Food and Drug Administration (FDA) in late 2020 and have since been widely used all over the world.[30] mRNA-1273 and BNT162b2 are two-dose vaccines based on the S protein gene sequence but with different immunization intervals (28 vs. 21 days, respectively) and different doses (100 vs. 30 μg, respectively).[31–33] Adenovirus serotype 26 (Ad26) vector-based vaccine Janssen Ad26.COV2.S has also played a crucial role in preventing COVID-19 disease.[34,35] Ad26.COV2.S is a single dose vaccine of 5×10^{10} viral particles.[35]

Because of high mutation rates in the S protein in VOCs, there is emerging evidence of reduced neutralizing activity by antibodies produced in vaccinated subjects against some SARS-CoV-2 VOCs.[7,12,36,37] In this study, we used a pseudovirus neutralization assay[18] to evaluate the states of neutralizing antibodies against SARS-CoV-2 wild type (WT) and different VOCs in plasma samples collected at various time points, from study participants who received mRNA-1273, BNT162b2, and Ad26.COV2.S under emergency use authorization.

2 | MATERIALS AND METHODS

2.1 | Study design and samples collection

The study was approved by the Institutional Review Board (IRB) of the University of Pittsburgh (STUDY20090114, STUDY20120181, STUDY20030228, STUDY20060154). All study participants provided written informed consent. All participants received a SARS-CoV-2 vaccine mRNA-1273 (100 μg/dose), BNT162b2 (30 μg/dose), or Ad26.COV2.S (5×10^{10} viral particles/dose) under emergency use authorization. Data on this cohort has been previously described.[38] In this study, a total 281 plasma samples were collected from 74 participants, of which 24 received mRNA-1273, 26 received BNT162b2, and 24 received Ad26.COV2.S (Table 1). Up to four blood samples were collected from each participant: Bleed 1 was collected on the same day before the first dose of vaccine (prevaccination); Bleed 2 was collected at a median of 21–31 days after the first dose of vaccine, which was also the day of the second dose of vaccine for mRNA-1273 and BNT162b2; Bleed 3 was collected at a median of 45–63 days after the first dose of vaccine; and Bleed 4 was collected at a median of 182-184 days after the first dose of vaccine (Figure 1A). In total, 90 samples from 24 mRNA-1273 participants, 103 samples from 26 BNT162b2 participants, and 88 samples from 24 Ad26.COV2.S participants were collected.

2.2 | Cell lines and cell culture

HEK293/hACE2, a cell line derived from HEK293T cells with overexpression of human ACE2 from Dr. Patrick Moore and obtained from BEI Resources, and HEK293T cell line purchased from ATCC were maintained in Dulbecco's modified Eagle medium (DMEM) with

TABLE 1 Demographics of participants

Participant	mRNA-1273	BNT162b2	Ad26.COV2.S	Total
Number of subjects (*n*)	24	26	24	74
Age (median, range)	48 (20–75)	41 (21–71)	49 (25–76)	46 (20–76)
Gender				
Female (%)	16 (67)	23 (88)	7 (29)	46 (62)
Male (%)	8 (33)	3 (12)	17 (71)	28 (38)
Race				
White (%)	21/24 (88)	24/26 (92)	18/24 (75)	63/74 (85)
Asian (%)	2/24 (8)	0/26 (0)	5/24 (21)	7/74 (9)
Black (%)	0/24 (0)	1/26 (4)	1/24 (4)	2/74 (3)
>1 (%)	1/24 (4)	0/26 (0)	0/24 (0)	1/74 (1)
N/A (%)	0/24 (0)	1/26 (4)	0/24 (0)	1/74 (1)

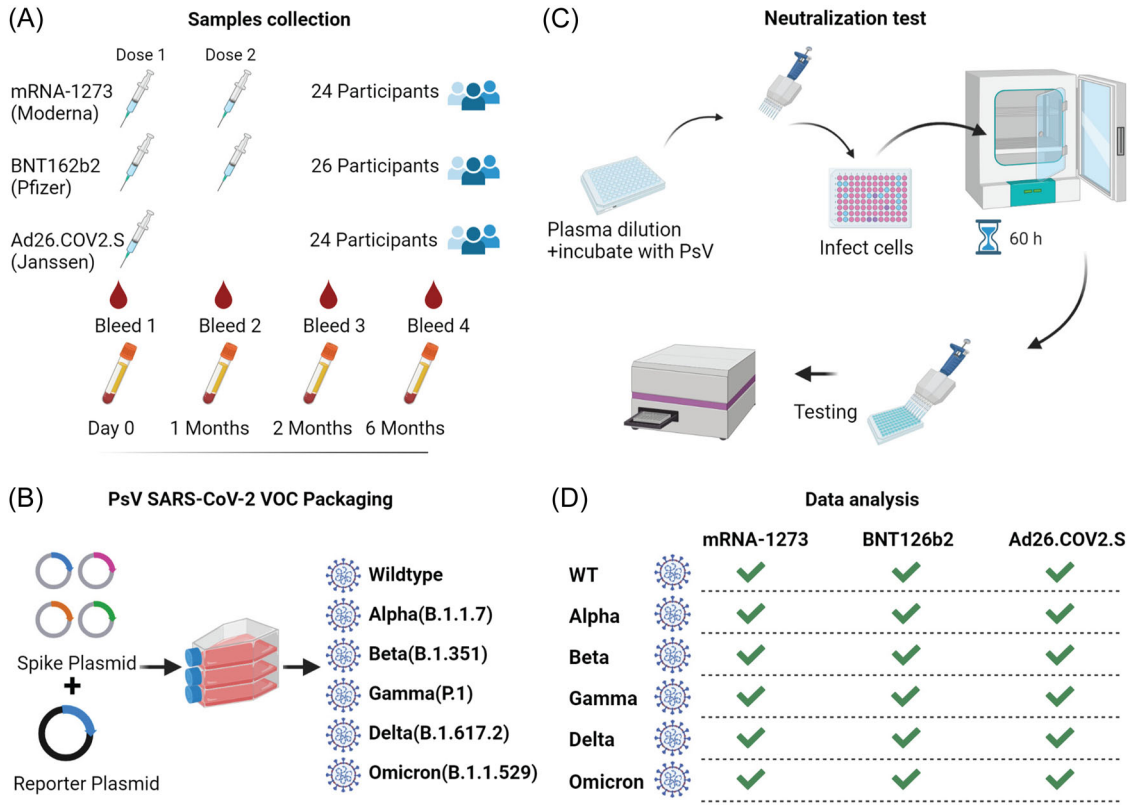

FIGURE 1 Workflow of the study. (A) Sample collection. Plasma samples were collected from 24, 26, and 24 participants immunized with mRNA-1273, BNT162b2, and Ad26.COV2.S, respectively. Participants were administered with two doses of mRNA-1273 (100 μg) or BNT162b2 (30 μg), and plasma samples were collected at four time points: immediately before the first dose (Bleed 1), about 1 month after the first dose, which was also immediately before the second dose (Bleed 2), about 1 month after the second dose, which was after full immunization (Bleed 3), and about 6 months after the full immunization (Bleed 4). Ad.COV2.S was administrated with a single dose (5×10^{10} viral particles), and plasma samples were collected at four time points: immediately before vaccination (Bleed 1), about 1 month after immunization, which was considered as full immunization (Bleed 2), about 1 month after full immunization (Bleed 3), and about 6 months after the full immunization (Bleed 4). (B) The spike proteins of SARS-CoV-2 wild type and variants of concern were packaged as pseudoviruses and used to detect neutralizing antibodies. (C) Assay for detecting neutralizing antibodies. Plasma samples were inactivated, and five-fold serially diluted and tested at final dilutions of 1:50, 1:250, 1:1, 250, 1:6, 250, and 1:31, 250. Pseudoviruses were incubated with diluted plasma samples for 1 h and then used to infect HEK293T/hACE2 cells. Cells were collected at 60 h postinfection and luciferase activity was measured. (D) Data analysis. Neutralizing antibody titers (pVNT50) were calculated and geometric mean titers of the plasma samples from subjects receiving the three vaccines were obtained.

10% heat-inactivated fetal bovine serum (FBS) and 1% Penicillin-Streptomycin 100× Solution. Cells were maintained at 37°C with 5% CO_2. HEK293T cells were used for pseudovirus packaging, while HEK293/hACE2 cells were used for the pseudovirus antibody neutralization assay.

2.3 | Spike (S) proteins of SARS-CoV-2 WT and VOCs

Plasmid expressing S proteins of SARS-CoV-2 WT and VOCs including Alpha, Beta, Gamma, Delta, and Omicron were used in this study.[18] Plasmids encoding Alpha, Beta, Gamma, and Delta S protein genes were gifts from Dr. David Nemazee[39,40] while the Omicron S protein gene plasmid was a gift from Dr. Tongqing Zhou.[41] The S protein genes were cloned from the original plasmids into pcDNA3.1(+) by restriction digestion and T4 ligation. Constructs were verified by restriction analysis and DNA sequencing. The appropriate expression of the S protein by each plasmid was confirmed by Western blotting as previously described.[18]

2.4 | Packaging of pseudoviruses

Pseudoviruses containing the S proteins from SARS-CoV-2 WT and VOCs were packaged as previously described (Figure 1B).[18] Each S protein gene plasmid and reporter backbone plasmid were cotransfected into HEK293T cells with jetOPTIMUS transfection reagent (117-15; Polyplus) according to the manufacturer's instructions. The supernatant was collected at 36 h posttransfection, centrifuged at 1,500 rpm in 4°C, filtered with a 45 μm filter. WT and VOCs

pseudoviruses were titrated and normalized by relative luminescence unit (RLU) and stored at −80°C for further use.

2.5 | Antibody neutralization assay

All manipulations of the blood samples were performed in a BSL-2+ biosafety cabinet. Based on the normalized virus titers, equivalent amounts of SARS-CoV-2 pseudoviruses were used in the infection assay for all variants. Plasma samples were heat-inactivated at 56°C for 30 min. To determine the neutralizing antibody titer of a plasma sample, pseudovirus at 50 µl was incubated with the sample diluted at 1:50; 1:250; 1:1,250; 1:6,250; 1:31,250 in 96-well plate for 1 h at 37°C, followed by infection of HEK293T-ACE2 cells as previously described (Figure 1C).[18] Briefly, cells were seeded one day before infection and infected at a confluence at 70%–90%. Wells infected with untreated pseudoviruses and uninfected cells were set as positive and negative controls, respectively, which were applied to every plate in triplicate. All the infection assays were performed in triplicate. At 60 h postinfection, 65 µl Reporter Lysis Buffer (E397A; Promega) were added to each well for cell lysis. After a 5 min centrifugation, 25 µl lysate supernatant were mixed with 25 µl Luciferase reagent (E1501; Promega) in a LumaPlate-96 white plate, followed by measuring luciferase activity on a Turner BioSystems Instrument. The results were used to calculate the 50% pseudovirus antibody neutralization titer ($pVNT_{50}$) and geometric mean titer (GMT) with 95% confidence intervals (CI) for each sample (Figure 1D).

2.6 | Statistical analysis

GraphPad Prism 9 was used for data analysis. One-way analysis of variance (ANOVA) was applied for grouped analyses followed by Tukey's multiple comparisons between the groups. GMTs with 95% CIs were presented as neutralization titers. $p < 0.05$ was considered as significant.

3 | RESULTS

3.1 | mRNA-1273, BNT162b2, and Ad26.COV2.S vaccines elicited robust neutralizing antibodies against SARS-CoV-2 WT

Consistent with the original study describing the immune responses of the studied participants using a live virus neutralization assay,[38] the mRNA-1273, BNT162b2, and Ad26.COV2.S vaccines elicited neutralizing antibodies against WT pseudovirus in most participants after the first dose of immunization (Figure 2). However, the GMTs of Bleed 2 samples from BNT162b2 subjects after the first dose were lower than those from mRNA-1273 and Ad26.COV2.S subjects (52 vs. 183 and 153, respectively, $p = 0.0246$ by one-way ANOVA test in Figure 3A). After the second dose of vaccine, the GMTs of Bleed 3 samples from both mRNA-1273 and BNT162b2 immunized subjects were significantly increased by 3.8- and 9.0-fold, reaching 698 and 467, respectively, while the GMT from the one-dose Ad26.COV2.S immunized subjects at Bleed 3 had no further increase, remaining at 102 (Figure 2). It was clear that, after the full two-dose vaccination, mRNA-1273 and BNT162b2 immunized subjects had higher titers of neutralizing antibodies than one-dose Ad26.COV2.S immunized subjects had ($p = 0.0033$ by one-way ANOVA test in Figure 3A). These results were consistent with the reported higher protective efficacies of mRNA-1273 and BNT162b2 than that of Ad26.COV2.S.[34,42–44]

3.2 | Duration of neutralizing antibodies against SARS-CoV-2 WT after full mRNA-1273, BNT162b2, or Ad26.COV2.S immunization

To measure the durability of the immune response, we examined neutraliing antibody titers 6 months after mRNA-1273, BNT162b2, or Ad26.COV2.S immunization. There was a slight 1.9-fold drop of GMT to 368 for the mRNA-1273 subjects (Figure 2A). However, a significant 8.8-fold drop of GMT to 53 was observed for the BNT162b2 subjects (Figure 2B). In contrast, no drop of GMT was observed for the Ad26.COV2.S subjects maintaining at 157 (Figure 2C). This is again consistent with the original study describing the immune responses of these participants using a live virus neutralization assay, which also demonstrated a waning of immunity over time in the BNT162b2 immunized subjects.[38]

3.3 | Neutralization against different SARS-CoV-2 VOCs for recipients of mRNA-1273, BNT162b2, and Ad26.COV2.S vaccines

Neutralizing antibodies against five variants including Alpha, Beta, Gamma, Delta, and Omicron were measured in the plasma samples from subjects who received mRNA-1273, BNT162b2, and Ad26.COV2.S vaccines. After the first dose of vaccine (Bleed 2), mRNA-1273 induced robust neutralizing antibodies against Alpha, Beta, and Gamma VOCs similar to WT (GMTs 498, 137, and 662 vs. 183, respectively, in Figure 4A); however, the titers of neutralizing antibodies were slightly lower against Delta (GMT 70 vs. 183) but were significantly lower against Omicron (GMT 12 vs. 183) with a reduction of 15.3-fold (Figure 4A). After the second dose (Bleed 3), mRNA-1273 immunized subjects had high GMTs against Alpha, Beta, and Gamma with titers similar to or higher than the GMT against WT (1,586, 594, and 2,074 vs. 698, respectively, in Figure 4A); however, a 2.1-fold drop against Delta and a sharp 15.2-fold drop against Omicron were observed (698 vs. 325 and 46, respectively) (Figure 4A). At 6 months after mRNA-1273 immunization (Bleed 4), GMTs against Alpha, Beta, and Gamma remained high compared to WT (631, 487, and 950 vs. 368, respectively, in Figure 4A). However,

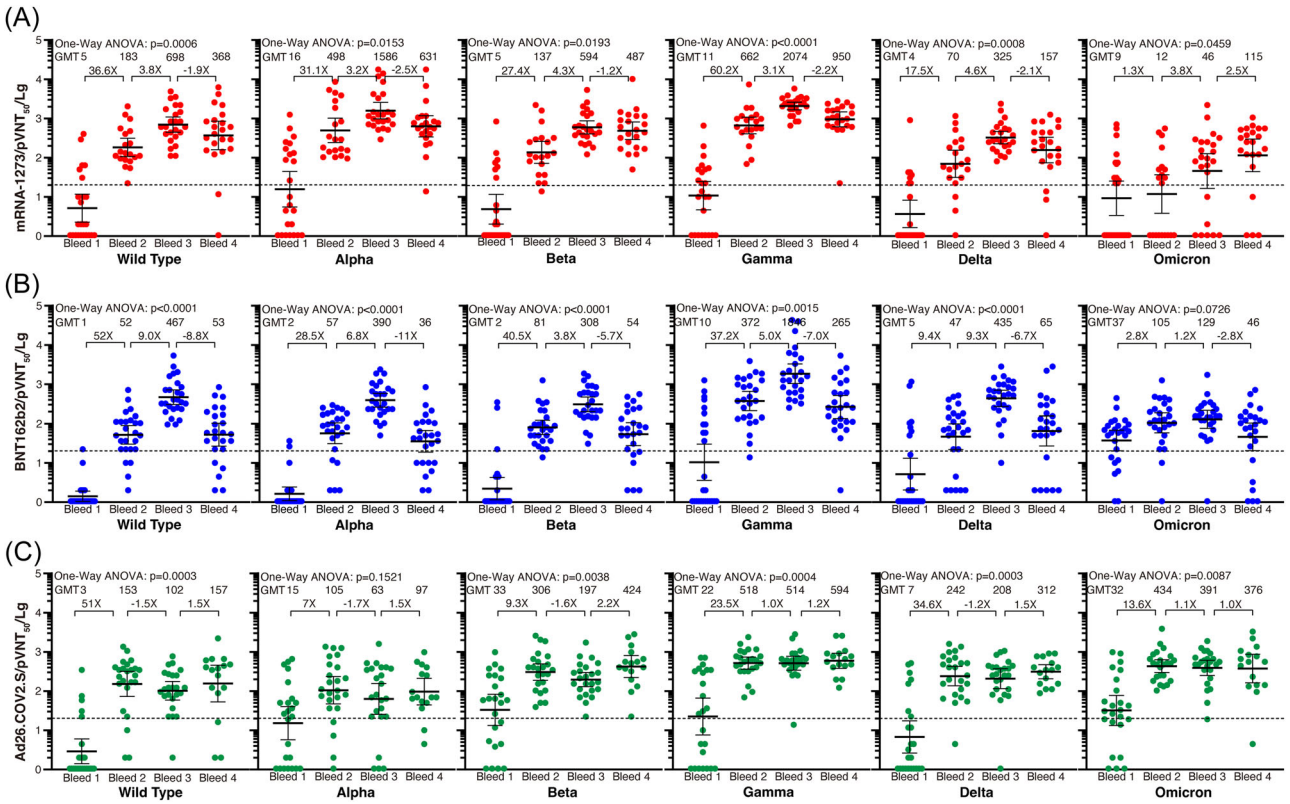

FIGURE 2 Neutralizing antibody titers against SARS-CoV-2 wild type (WT) and variants of concern in samples collected at different times from subjects immunized with mRNA-1273, BNT162b2, and Ab26.COV2.S. (A) mRNA-1273. (B) BNT162b2. (C) Ad26.COV2.S. Plasma samples were collected at four time points (Bleed 1–4) for the participants, and geometric mean titers (GMTs) against WT, Alpha, Beta, Gamma, Delta, and Omicron are presented. Each dot represents a sample. Multiple groups were compared by one-way analysis of variance test. Fold change of geometric mean titers between two bleeds was calculated and listed between two columns. The dashed line represents limitation of detection.

the GMT against Delta at 6 months had a further 2.1-fold drop from 325 to 157, and maintained a low level against Omicron at 115 (Figure 4A). These results indicate that the mRNA-1273 vaccine can induce equivalent neutralizing antibodies against Alpha, Beta, and Gamma as against WT. However, it induced lower levels of neutralizing antibodies against Delta and Omicron as shown in the lower neutralizing antibody titers after the first dose vaccine, full immunization, and 6 months after full immunization (Figure 4A).

The induction of neutralizing antibodies by BNT162b2 against Alpha, Beta, Gamma, and Delta was similar to that of WT manifesting lower neutralizing antibody titers after the first dose of vaccine than the other two vaccines did (Bleed 2), reaching high titers after two-dose immunization with GMTs ranging from 308 to 1846 (Bleed 3) but declined at 6 months after full immunization with GMTs ranging from 36 to 65 except for Gamma (Bleed 4 in Figure 4B). Similar to mRNA-1273, high neutralizing antibody titers against Gamma were observed for BNT162b2 subjects after full immunization reaching a GMT 1846 (Bleed 3) and maintained at 265 at 6 months after immunization (Bleed 4 in Figure 4B). Hence, Gamma appears to be more sensitive to neutralizing antibodies produced by these two mRNA vaccines. Similar to mRNA-1273, neutralizing antibodies against Omicron never reached high titers after vaccination (GMT 129) and declined by 6 months after vaccination (GMT 46 in Figure 4B).

The Ad26.COV2.S vaccine induced moderate neutralizing antibodies against all VOCs similar to WT (Figure 4C). While these single-dose Ad26.COV2.S immunized subjects had lower GMTs against Alpha, Beta, Gamma, and Delta than fully immunized mRNA-1273 and BNT162b2 subjects had (Bleed 3 in Figure 3B–E), their levels were maintained at 6 months after immunization (Bleed 4 in Figure 3B–E). Compared to fully immunized mRNA-1273 and BNT162b2 subjects, Ad26.COV2.S induced moderate but slightly higher neutralizing antibodies against Omicron (GMT 391 vs. 46 and 129, respectively, in Bleed 3, $p = 0.007$ by one-way ANOVA test in Figure 3F). At 6 months after immunization, Ad26.COV2.S subjects had slightly higher GMTs against Omicron than mRNA-1273 and BNT162b2 subjects had (376 vs. 115 and 46, respectively, in Bleed 4, $p = 0.0004$ by one-way ANOVA test in Figure 3F). Hence, while Ad26.COV2.S vaccination did not achieve the highest titers, the response demonstrated longevity.

4 | DISCUSSION

Vaccination plays an important role in reducing the spread, morbidity, and mortality of infectious diseases.[45] The COVID-19 pandemic prompted the unprecedented rapid development of vaccines against

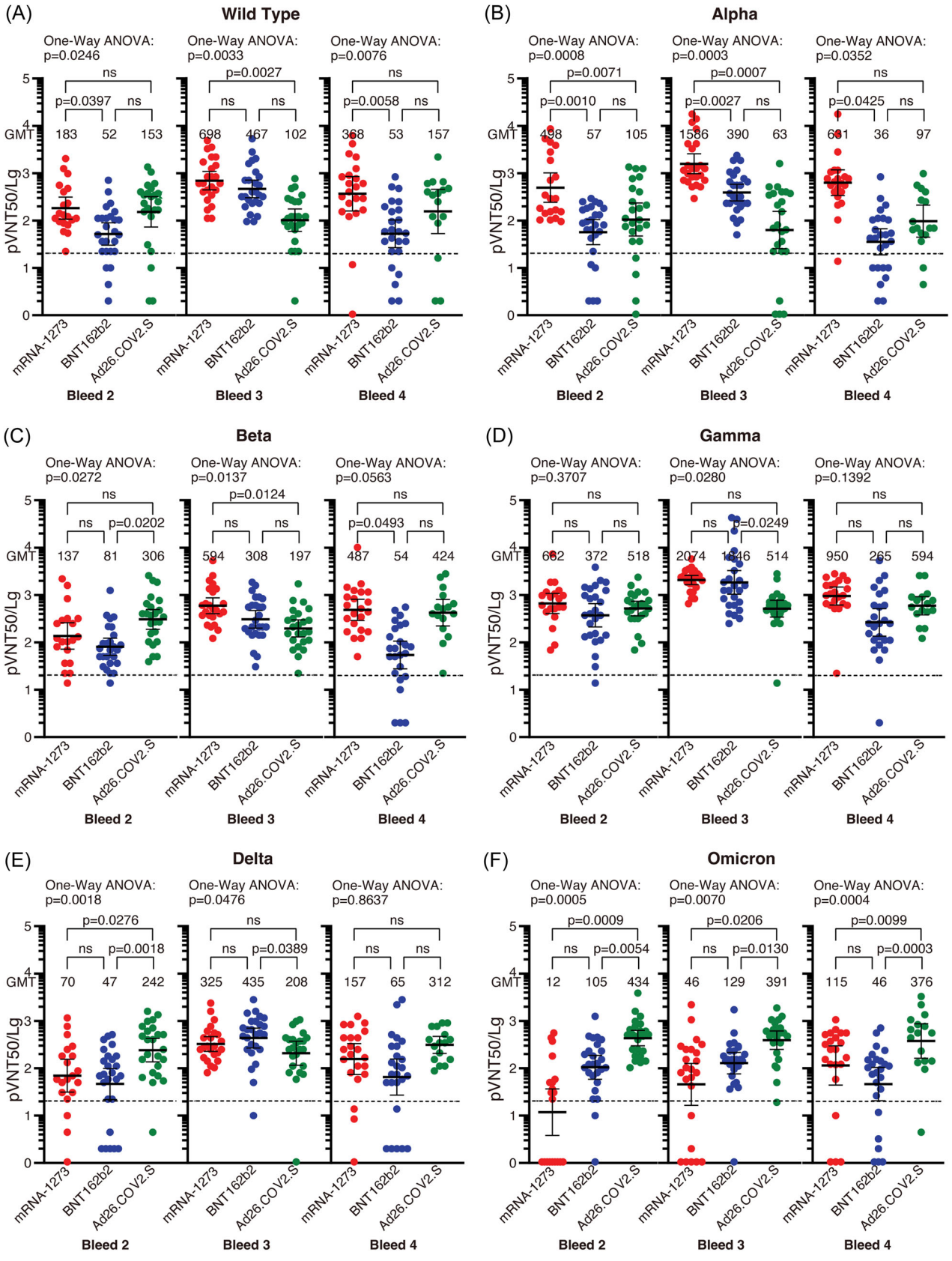

FIGURE 3 (See caption on next page)

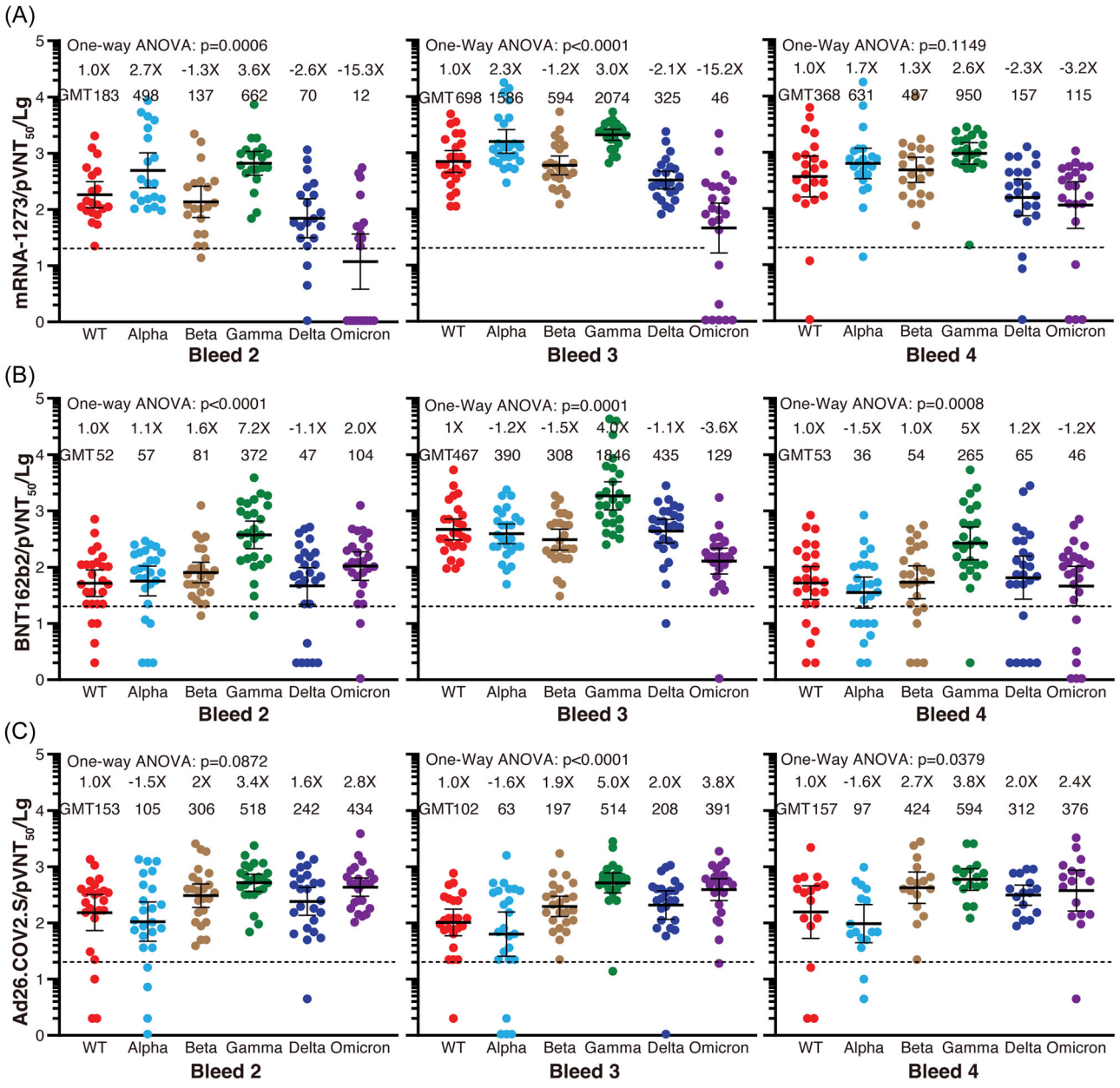

FIGURE 4 Differences of neutralizing antibody titers in subjects immunized with mRNA-1273, BNT162b2, and Ab26.COV2.S against SARS-CoV-2 wild type (WT) and variants of concern (A) mRNA-1273. (B) BNT162b2. (C) Ad26.COV2.S. Plasma samples were examined at different times after immunization (Bleed 2, 3, and 4). Multiple groups were compared by one-way analysis of variance test. geometric mean titers and change folds compared with WT were listed on the top of each group. The dashed line represents limitation of detection.

FIGURE 3 Comparison of neutralizing antibody titers in subjects immunized with mRNA-1273, BNT162b2, and Ab26.COV2.S against SARS-CoV-2 wild type (WT) and variants of concern. (A) WT. (B) Alpha. (C) Beta. (D) Gamma. (E) Delta. (F) Omicron. The geometric mean titers (GMTs) against WT, Alpha, Beta, Gamma, Delta, and Omicron in participants immunized with mRNA-1273, BNT162b2, and Ab26.COV2.S are presented. Each dot represents a sample. Differences of neutralizing antibody titers among mRNA-1273, BNT162b2, and Ad26.COV2.S were compared in each Bleed (Bleed 2, 3, and 4). Multiple groups were compared by one-way analysis of variance test. Tukey's test was performed for post-hoc comparison. Significant *p* values were listed at the top of each panel. No significant difference was labeled as ns. The dashed line represents limitation of detection.

SARS-CoV-2. The first three vaccines against SARS-CoV-2 used in the US were mRNA-1273, BNT162b2, and Ad26.COV2.S, which have since contributed to the control of the pandemic.[32,34,44] While Ad26.COV2.S is a conventional adenovirus vector-based vaccine, both mRNA-1273 and BNT162b2 are novel type of mRNA vaccines.[32,34,44] This is the first time that this RNA-based vaccine technology was successfully applied against infectious disease.[30,46]

Since S protein is essential for SARS-CoV-2 entry into cells, neutralizing antibodies against S protein in immunized subjects are likely to play a role in the effective control of SARS-CoV-2 infection and COVID-19 disease.[47] The emergence of SARS-CoV-2 VOCs has challenged the effectiveness the mRNA-1273, BNT162b2, and Ad26.COV2.S vaccines, which were all developed based on the WT S protein gene sequence. The original SARS-CoV-2 has since evolved to harbor high numbers of mutations in new VOCs.[13] In this study, we used lentiviral pseudoviruses of SARS-CoV-2 WT and 5 other VOCs including Alpha, Beta, Gamma, Delta, and Omicron to quantitate neutralizing antibodies in the plasma samples of mRNA-1273, BNT162b2, and Ad26.COV2.S immunized subjects. Our results showed that all three vaccines elicited neutralizing antibodies against SARS-CoV-2 WT and most of the VOCs tested after full immunization, which are consistent with the success of these vaccines in controlling COVID-19 during the early phase of the pandemic.[48] However, the efficacies of these vaccines against Delta and Omicron VOCs have been challenged. We have compared the results of our study with those of others for their neutralizing antibody titers after full immunization, durability, and effectiveness against the Delta and Omicron VOCs of these vaccines (Table 2).

We observed overall higher levels of neutralizing antibodies in fully immunized mRNA-1273 and BNT162b2 subjects than those immunized with the Ad26.COV2.S vaccine (Table 2). These results are consistent with other studies showing robust neutralizing antibodies against WT and most VOCs following BNT162b2 and mRNA-1273 immunization[72–74] as well as those of another study of the same studied subjects using a live virus neutralization assay.[38] Between the two RNA vaccines, BNT162b2 induced lower levels of neutralizing antibody titers against WT and most of VOCs than mRNA-1273 did after the first and second doses of immunization. Furthermore, neutralizing antibody titers of BNT162b2 subjects declined rapidly 6 months after immunization. The lower neutralizing antibody titers of BNT162b2 subjects could be due to the lower dose used compared to that of mRNA-1273 (30 vs. 100 μg) or the different dosing interval. Indeed, in an early study of mRNA-1273 in which three different doses at 25, 100, and 250 μg were administered twice, subjects immunized with a higher dose developed higher antibody titers than those immunized with a lower dose.[75] Meanwhile, mRNA-1273 produced higher levels of binding and neutralizing antibodies, which declined slightly over time but remained elevated in all participants 3 months after the second dose immunization.[76] Nevertheless, we observed some reduction of neutralizing antibodies in mRNA-1273 vaccinated subjects 6 months after immunization. These results support the current CDC recommendations for a booster dose 5 months after two-dose mRNA-1273 or BNT162b2 immunization (https://www.cdc.gov/coronavirus/2019-ncov/vaccines/booster-shot.html).

Ad26.COV2.S encodes a stabilized S protein.[35] Despite the lower levels of neutralizing antibodies than those of mRNA-1273 and BNT162b2 fully immunized subjects, we found that Ad26.COV2.S induced stable neutralizing antibodies 1 month after immunization, and the titers were sustained up to 6 months. Our results are consistent with those of another study that elevated neutralizing antibodies with GMTs ranging from 212 to 354 against SARS-CoV-2 WT were detected in 90% participants 29 days after the first dose of Ad26.COV2.S.[35] Furthermore, in Ad26.COV2.S phase 1/2a and 2 clinical trials, durable and stable S protein binding and neutralizing antibodies, which lasted for more than 6 months, were detected in 18–55 and >65 years old participants.[68] In another study, durable humoral and cellular immune responses were also observed for at least 8 months following Ad26.COV2.S immunization.[71] However, despite these in vitro findings in vaccine recipients, the effectiveness of Ad26.COV2.S vaccination, even with boosting remains low.[77]

The emergence of Delta and Omicron VOCs has posed significant challenges for the control of COVID-19. Our results showed that mRNA-1273 and BNT162b2 immunized subjects had reduced levels of neutralizing antibodies against Omicron, which further decreased against Delta and Omicron after 6 months of immunization (Table 2, Figure 4). It was reported that compared to WT, neutralizing antibodies against Omicron from BNT162b2 immunized subjects decreased 43 times by 3 months and were not detected in the period of 6–12 months after immunization.[50] In fact, the two-dose series of either mRNA vaccine without boost was not effective at preventing infection with Omicron.[78]

In contrast, low but moderate neutralizing antibodies were detected in Ad26.COV2.S immunized subjects, not only against WT, but also VOCs, including Delta and Omicron, which were sustained for at least 6 months (Table 2). In Janssen's final analysis of efficacy and safety of single-dose Ad26.COV2.S, the results showed that Ad26.COV2.S′ protective effect varied according to the variant.[79] In the 39,185 participants, vaccine efficacy against moderate to severe–critical COVID-19 at least 28 days after administration was 52.9% (433 cases in the vaccine group vs. 883 in the placebo group).[79] One-dose Ad26.COV2.S provided protection against most sequenced variants with the observed vaccine efficacies against WT, Alpha, Beta, Gamma, and Delta at 58.2%, 70.2%, 51.9%, 36.5%, and 5.7%, respectively, after 28 days of administration.[79] In Janssen's another study, it was demonstrated that one-dose of Ad26.COV2.S induced 8-month durable humoral and cellular immune responses with detectable pseudovirus neutralizing antibodies against all the tested pseudoviruses, including but not limited to WT, Alpha, Beta, Gamma, and Delta.[80]

It is unclear why the medium levels of neutralizing antibody titers against Omicron in the Ad26.COV2.S immunized subjects were

TABLE 2 Summaries of performance of mRNA-1273, BNT162b2, and Ad26.COV2.S vaccines in different studies.

Vaccine	Study	Subjects (*n*)	Dose	WT titer[a]	Durability (month)[b]	Delta titer[c]	Omicron titer[d]
mRNA-1273	This study	74	2	High (PsV)	~5–7	High	Low
mRNA-1273	[49]	48	2	High (PsV)	~6–8	High	Med
mRNA-1273	[50]	239	2	High (PsV)	~6	High	Low
mRNA-1273	[51]	54	2	High (LV)	N/A	N/A	Low
mRNA-1273	[52]	120	2	High (PsV)	~9	N/A	Low
mRNA-1273	[53]	46	2	High (PsV)	N/A	High	Low
mRNA-1273	[71]	30	2	High (PsV)	N/A	N/A	Med
mRNA-1273	[72]	30	2	High (PsV)	N/A	N/A	Low
mRNA-1273	[51]	54	3	High (LV)	N/A	N/A	Med
mRNA-1273	[52]	120	3	High (PsV)	~6–8	N/A	High
mRNA-1273	[72]	30	3	High (PsV)	N/A	N/A	High
BNT162b2	This study	74	2	High (PsV)	~4–6	High	Low
BNT162b2	[49]	48	2	High (PsV)	~4–6	High	Low
BNT162b2	[73]	19	2	Med (LV)	N/A	N/A	Low
BNT162b2	[50]	239	2	High (PsV)	~4–6	Med	ND
BNT162b2	[74]	32	2	High (PsV)	~6	N/A	Low
BNT162b2	[58]	39	2	High (PsV)	N/A	N/A	Low
BNT162b2	[59]	40	2	Low (LV)	N/A	Low	ND
BNT162b2	[60]	171	2	High (LV)	~8–9	Med	Low
BNT162b2	[51]	54	2	High (LV)	N/A	N/A	Low
BNT162b2	[61]	234	2	Med (LV)	N/A	N/A	Low
BNT162b2	[62]	22	2	High (rV)	~6–7	N/A	Low
BNT162b2	[53]	46	2	High (PsV)	~8–9	High	Low
BNT162b2	[63]	39	2	High (PsV)	N/A	Med	Low
BNT162b2	[81]	50	2	Med (LV)	N/A	Med	Low
BNT162b2	[82]	48	2	High (PsV)	N/A	Med	Low
BNT162b2	[51]	54	3	High (PsV)	N/A	N/A	Med
BNT162b2	[83]	30	3	High (PsV)	N/A	High	Med
BNT162b2	[58]	39	3	High (PsV)	N/A	N/A	High
BNT162b2	[60]	171	3	High (PsV)	N/A	High	High
BNT162b2	[61]	234	3	High (PsV)	N/A	N/A	Med
BNT162b2	[74]	32	3	High (PsV)	N/A	N/A	High
BNT162b2	[62]	22	3	High (rV)	>4	N/A	High
BNT162b2	[53]	46	3	High (PsV)	N/A	High	Med
BNT162b2	[63]	39	3	High (PsV)	N/A	High	High
BNT162b2	[82]	48	3	High (PsV)	N/A	High	High
BNT162b2	[59]	40	3	High (LV)	N/A	High	Med
Ad26.COV2.S	This study	74	1	Med (PsV)	>6–8	Med	Med
Ad26.COV2.S	[50]	239	1	Low (PsV)	~4–6	Low	Low

(Continues)

TABLE 2 (Continued)

Vaccine	Study	Subjects (*n*)	Dose	WT titer[a]	Durability (month)[b]	Delta titer[c]	Omicron titer[d]
Ad26.COV2.S	[61]	19	1	Med (PsV)	>6	Low	Low
Ad26.COV2.S	[54]	137	1	Med (LV)	>8	N/A	N/A
Ad26.COV2.S	[84]	20	1	Med (LV)	N/A	N/A	N/A
Ad26.COV2.S	[70]	25	1	Med (LV)	N/A	N/A	N/A
Ad26.COV2.S	[71]	20	1	Med (PsV)	>8	Med	N/A

Abbreviations: GMT, geometric mean titer; LV, live virus; N/A, no comparable data; ND, not detected; PsV, pseudovirus; rV, recombinant virus; VOC, variant of concern; WT, wild type.

[a]Neutralizing antibody titer to WT virus after full immunization.

[b]Durability of neutralizing antibody titer to WT after full immunization.

[c]Neutralizing antibody titer to Delta VOC after full immunization.

[d]Neutralizing antibody titer to Omicron VOC after full immunization; Neutralizing titer with GMT > 300, 300 > GMT > 150, and GMT < 150 were considered high, medium (Med) and low, respectively.

observed in this study but not others.[50,67] Similarly, only low neutralizing antibody titers against Delta were detected in a live virus neutralization assay with the same Ad26.COV2.S immunized subjects in a separate study.[38] In those studies, the viruses were incubated with the plasma samples for a short time (1 h) and used to infect cells. Hence, the inhibitory effect of the neutralizing antibodies is primarily due to the blocking effect on virus entry. In the current study, the pseudoviruses were incubated with the plasma samples for a short time but the infection was performed in the presence of the plasma samples. In this case, the inhibitory effect of the neutralizing antibodies could exert not only on virus entry but also on cell-to-cell spread as reported by other studies.[81] More investigations are required to clarify these discrepancies.

It is interesting that we observed low titers of neutralizing antibodies in a small subset of subjects before immunization with any SARS-CoV-2 vaccines (Figure 2). Similar results have also been observed.[82] It is highly possible that these subjects have developed cross-reactive antibodies following prior exposure to other coronaviruses.[82–84] It has also been reported that other infections or prior immunizations with other vaccines could generate cross-reactive antibodies against SARS-CoV-2.[85]

In conclusion, our findings indicate that both mRNA-1273 and BNT162b2 elicited robust neutralizing antibodies against WT, Alpha, Beta, and Gamma after full immunization but wanned after 6 months with a faster decline observed for the BNT162b2 immunized subjects. Both mRNA-1273 and BNT162b2 elicited some but minimal neutralizing antibodies against Omicron. Ad26.COV2.S vaccine induced lower neutralizing antibodies against WT and VOCs, including Delta and Omicron, which lasted for up to 6 months. To prevent moderate to severe COVID-19, mRNA-1273, BNT162b2, and Ad26.COV2.S recipients are advised to receive a booster.

AUTHOR CONTRIBUTIONS

Conceptualization, planning, and management: Shou-Jiang Gao. *Laboratory experimental design*: Shou-Jiang Gao and George Fei Zhang. *Laboratory execution of experiments, and data acquisition, analysis, and interpretation*: George Fei Zhang, Wen Meng, Luping Chen, Ling Ding, Jian Feng, Joseph Perez, Abid Ali, Shenyu Sun, Zhentao Liu, Yufei Huang, Haitao Guo, and Shou-Jiang Gao. *Drafting and revision of the manuscript*: George Fei Zhang and Shou-Jiang Gao. *Manuscript editing and approval*: George Fei Zhang, Wen Meng, Luping Chen, Ling Ding, Jian Feng, Joseph Perez, Abid Ali, Shenyu Sun, Zhentao Liu, Yufei Huang, Haitao Guo, and Shou-Jiang Gao.

ACKNOWLEDGMENTS

Study specimens used in this study were shared with us by Anita K. McElroy, Judith M. Martin, and W. Paul Duprex. Results from the parent study have been published.[38] and were supported by the R. K. Mellon Foundation, University of Pittsburgh Center for Vaccine Research, and Henry L. Hillman Foundation. This study was in part supported by UPMC Hillman Cancer Center Startup Fund and Pittsburgh Foundation Endowed Chair in Drug Development for Immunotherapy to S. J. G. The authors thank all the Gao Lab members for their helpful discussions and technical support.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

George Fei Zhang http://orcid.org/0000-0002-1316-523X
Haitao Guo http://orcid.org/0000-0002-7146-916X
Shou-Jiang Gao http://orcid.org/0000-0001-6194-1742

REFERENCES

1. Lu H, Stratton CW, Tang YW. Outbreak of pneumonia of unknown etiology in Wuhan, China: the mystery and the miracle. *J Med Virol*. 2020;92(4):401-402.

2. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*. 2020;579(7798):270-273.
3. Davies NG, Abbott S, Barnard RC, et al. Estimated transmissibility and impact of SARS-CoV-2 lineage B.1.1.7 in England. *Science*. 2021;372(6538):eabg3055.
4. Dejnirattisai W, Zhou D, Supasa P, et al. Antibody evasion by the P.1 strain of SARS-CoV-2. *Cell*. 2021;184(11):2939-2954.
5. Faria NR, Mellan TA, Whittaker C, et al. Genomics and epidemiology of the P.1 SARS-CoV-2 lineage in Manaus, Brazil. *Science*. 2021;372(6544):815-821.
6. Galloway SE, Paul P, MacCannell DR, et al. Emergence of SARS-CoV-2 B.1.1.7 lineage—United States, December 29, 2020-January 12, 2021. *MMWR Morb Mortal Wkly Rep*. 2021;70(3):95-99.
7. Mlcochova P, Kemp SA, Dhar MS, et al. SARS-CoV-2 B.1.617.2 Delta variant replication and immune evasion. *Nature*. 2021;599(7883):114-119.
8. Poterico JA, Mestanza O. Genetic variants and source of introduction of SARS-CoV-2 in South America. *J Med Virol*. 2020;92(10):2139-2145.
9. Saxena SK, Kumar S, Ansari S, et al. Characterization of the novel SARS-CoV-2 Omicron (B.1.1.529) variant of concern and its global perspective. *J Med Virol*. 2022;94(4):1738-1744.
10. Tao K, Tzou PL, Nouhin J, et al. The biological and clinical significance of emerging SARS-CoV-2 variants. *Nat Rev Genet*. 2021;22(12):757-773.
11. Wang L, Cheng G. Sequence analysis of the emerging SARS-CoV-2 variant Omicron in South Africa. *J Med Virol*. 2022;94(4):1728-1733.
12. Zhou D, Dejnirattisai W, Supasa P, et al. Evidence of escape of SARS-CoV-2 variant B.1.351 from natural and vaccine-induced sera. *Cell*. 2021;184(9):2348-2361.e6.
13. Chavda VP, Patel AB, Vaghasiya DD. SARS-CoV-2 variants and vulnerability at the global level. *J Med Virol*. 2022;94(7):2986-3005.
14. Viana R, Moyo S, Amoako DG, et al. Rapid epidemic expansion of the SARS-CoV-2 Omicron variant in southern Africa. *Nature*. 2022;603(7902):679-686.
15. Gao SJ, Guo H, Luo G. Omicron variant (B.1.1.529) of SARS-CoV-2, a global urgent public health alert! *J Med Virol*. 2022;94(4):1255-1256.
16. Harvey WT, Carabelli AM, Jackson B, et al. SARS-CoV-2 variants, spike mutations and immune escape. *Nat Rev Microbiol*. 2021;19(7):409-424.
17. Ramos da Silva S, Ju E, Meng W, et al. Broad severe acute respiratory syndrome coronavirus 2 cell tropism and immunopathology in lung tissues from fatal coronavirus disease 2019. *J Infect Dis*. 2021;223(11):1842-1854.
18. Zhang F, Li W, Feng J, et al. SARS-CoV-2 pseudovirus infectivity and expression of viral entry-related factors ACE2, TMPRSS2, Kim-1, and NRP-1 in human cells from the respiratory, urinary, digestive, reproductive, and immune systems. *J Med Virol*. 2021;93(12):6671-6685.
19. Ali MA, Hu C, Jahan S, et al. Sensing of COVID-19 antibodies in seconds via aerosol jet nanoprinted reduced-graphene-oxide-coated 3D electrodes. *Adv Mat*. 2021;33(7):e2006647.
20. Ali MA, Hu C, Zhang F, et al. N protein-based ultrasensitive SARS-CoV-2 antibody detection in seconds via 3D nanoprinted, micro-architected array electrodes. *J Med Virol*. 2022;94(5):2067-2078.
21. Rashedi R, Samieefar N, Masoumi N, et al. COVID-19 vaccines mix-and-match: the concept, the efficacy and the doubts. *J Med Virol*. 2022;94(4):1294-1299.
22. Cao Y, Yisimayi A, Bai Y, et al. Humoral immune response to circulating SARS-CoV-2 variants elicited by inactivated and RBD-subunit vaccines. *Cell Res*. 2021;31(7):732-741.
23. Yang S, Li Y, Dai L, et al. Safety and immunogenicity of a recombinant tandem-repeat dimeric RBD-based protein subunit vaccine (ZF2001) against COVID-19 in adults: two randomised, double-blind, placebo-controlled, phase 1 and 2 trials. *Lancet Infect Dis*. 2021;21(8):1107-1119.
24. Wu Y, Huang X, Yuan L, et al. A recombinant spike protein subunit vaccine confers protective immunity against SARS-CoV-2 infection and transmission in hamsters. *Science Transl Med*. 2021;13(606):eabg1143.
25. Bangaru S, Ozorowski G, Turner HL, et al. Structural analysis of full-length SARS-CoV-2 spike protein from an advanced vaccine candidate. *Science*. 2020;370(6520):1089-1094.
26. Shin MD, Shukla S, Chung YH, et al. COVID-19 vaccine development and a potential nanomaterial path forward. *Nat Nano*. 2020;15(8):646-655.
27. Kaur SP, Gupta V. COVID-19 vaccine: a comprehensive status report. *Virus Res*. 2020;288:198114.
28. Wang H, Zhang Y, Huang B, et al. Development of an inactivated vaccine candidate, BBIBP-CorV, with potent protection against SARS-CoV-2. *Cell*. 2020;182(3):713-721 e719.
29. Gao Q, Bao L, Mao H, et al. Development of an inactivated vaccine candidate for SARS-CoV-2. *Science*. 2020;369(6499):77-81.
30. Mullard A. FDA drug approvals. *Nat Rev Drug Discovery*. 2021;20(2):85-90.
31. Ali K, Berman G, Zhou H, et al. Evaluation of mRNA-1273 SARS-CoV-2 vaccine in adolescents. *N Engl J Med*. 2021;385(24):2241-2251.
32. Walsh EE, Frenck RW Jr, Falsey AR, et al. Safety and immunogenicity of two RNA-based Covid-19 vaccine candidates. *N Engl J Med*. 2020;383(25):2439-2450.
33. Falsey AR, Frenck RW Jr, Walsh EE, et al. SARS-CoV-2 neutralization with BNT162b2 vaccine dose 3. *N Engl J Med*. 2021;385(17):1627-1629.
34. Sadoff J, Gray G, Vandebosch A, et al. Safety and efficacy of Single-Dose Ad26.COV2.S vaccine against Covid-19. *N Engl J Med*. 2021;384(23):2187-2201.
35. Sadoff J, Le Gars M, Shukarev G, et al. Interim results of a phase 1-2a trial of Ad26.COV2.S Covid-19 vaccine. *N Engl J Med*. 2021;384(19):1824-1835.
36. Notarte KI, Guerrero-Arguero I, Velasco JV, et al. Characterization of the significant decline in humoral immune response six months post-SARS-CoV-2 mRNA vaccination: a systematic review. *J Med Virol*. 2022;94(7):2939-2961.
37. Planas D, Veyer D, Baidaliuk A, et al. Reduced sensitivity of SARS-CoV-2 variant Delta to antibody neutralization. *Nature*. 2021;596(7871):276-280.
38. Barbeau DJ, Martin JM, Carney E, et al. Comparative analysis of human immune responses following SARS-CoV-2 vaccination with BNT162b2, mRNA-1273, or Ad26.COV2.S. *NPJ Vaccines*. 2022;7(1):77.
39. Cho H, Gonzales-Wartz KK, Huang D, et al. Bispecific antibodies targeting distinct regions of the spike protein potently neutralize SARS-CoV-2 variants of concern. *Sci Transl Med*. 2021;13(616):eabj5413.
40. Yuan M, Huang D, Lee CD, et al. Structural and functional ramifications of antigenic drift in recent SARS-CoV-2 variants. *Science*. 2021;373(6556):818-823.
41. Zhou T, Wang L, Misasi J, et al. Structural basis for potent antibody neutralization of SARS-CoV-2 variants including B.1.1.529. *Science*. 2022;376(6591):eabn8897.
42. Anderson EJ, Rouphael NG, Widge AT, et al. Safety and immunogenicity of SARS-CoV-2 mRNA-1273 vaccine in older adults. *N Engl J Med*. 2020;383(25):2427-2438.
43. Baden LR, El Sahly HM, Essink B, et al. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. *N Engl J Med*. 2021;384(5):403-416.

44. Polack FP, Thomas SJ, Kitchin N, et al. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. *N Engl J Med*. 2020;383(27):2603-2615.
45. Delany I, Rappuoli R, De Gregorio E. Vaccines for the 21st century. *EMBO Mol Med*. 2014;6(6):708-720.
46. Thompson MG, Burgess JL, Naleway AL, et al. Prevention and attenuation of Covid-19 with the BNT162b2 and mRNA-1273 vaccines. *N Engl J Med*. 2021;385(4):320-329.
47. Wan Y, Shang J, Graham R, et al. Receptor recognition by the novel coronavirus from Wuhan: an analysis based on decade-long structural studies of SARS coronavirus. *J Virol*. 2020;94(7):e00127-00120.
48. Qamar N, Rukh G, Khan SN. Vaccines for Covid-19: an insight on their effectiveness and adverse effects. *J Med Virol*. 2022;94(8):3554-3560.
49. Evans JP, Zeng C, Carlin C, et al. Neutralizing antibody responses elicited by SARS-CoV-2 mRNA vaccination wane over time and are boosted by breakthrough infection. *Sci Transl Med*. 2022;14(637):eabn8057.
50. Garcia-Beltran WF, St Denis KJ, Hoelzemer A, et al. mRNA-based COVID-19 vaccine boosters induce neutralizing immunity against SARS-CoV-2 Omicron variant. *Cell*. 2022;185(3):457-466.e454.
51. Carreño JM, Alshammary H, Tcheou J, et al. Activity of convalescent and vaccine serum against SARS-CoV-2 omicron. *Nature*. 2022;602(7898):682-688.
52. Pajon R, Doria-Rose NA, Shen X, et al. SARS-CoV-2 omicron variant neutralization after mRNA-1273 booster vaccination. *N Engl J Med*. 2022;386(11):1088-1091.
53. Tada T, Zhou H, Dcosta BM, et al. Increased resistance of SARS-CoV-2 Omicron variant to neutralization by vaccine-elicited and therapeutic antibodies. *EBioMedicine*. 2022;78:103944.
54. Girard B, Tomassini JE, Deng W, et al. mRNA-1273 vaccine-elicited neutralization of SARS-CoV-2 omicron in adolescents and children. *medRxiv*. 2022. doi:10.1101/2022.01.24.22269666
55. Doria-Rose NA, Shen X, Schmidt SD, et al. Booster of mRNA-1273 strengthens SARS-CoV-2 omicron neutralization. *medRxiv*. 2021. doi:10.1101/2021.12.15.21267805
56. Cele S, Jackson L, Khoury DS, et al. Omicron extensively but incompletely escapes Pfizer BNT162b2 neutralization. *Nature*. 2022;602(7898):654-656.
57. Yu J, Collier AY, Rowe M, et al. Neutralization of the SARS-CoV-2 omicron BA.1 and BA.2 variants. *N Engl J Med*. 2022;386(16):1579-1580.
58. Arora P, Zhang L, Rocha C, et al. Comparable neutralisation evasion of SARS-CoV-2 omicron subvariants BA.1, BA.2, and BA.3. *Lancet Infect Dis*. 2022;22(6):766-767.
59. Nemet I, Kliker L, Lustig Y, et al. Third BNT162b2 vaccination neutralization of SARS-CoV-2 omicron infection. *N Engl J Med*. 2022;386(5):492-494.
60. Wratil PR, Stern M, Priller A, et al. Three exposures to the spike protein of SARS-CoV-2 by either infection or vaccination elicit superior neutralizing immunity to all variants of concern. *Nat Med*. 2022;28(3):496-503.
61. Cheng SMS, Mok CKP, Leung YWY, et al. Neutralizing antibodies against the SARS-CoV-2 Omicron variant BA.1 following homologous and heterologous CoronaVac or BNT162b2 vaccination. *Nat Med*. 2022;28(3):486-489.
62. Xia H, Zou J, Kurhade C, et al. Neutralization and durability of 2 or 3 doses of the BNT162b2 vaccine against Omicron SARS-CoV-2. *Cell Host Microbe*. 2022;30(4):485-488.e483.
63. Lusvarghi S, Pollett SD, Neerukonda SN, et al. SARS-CoV-2 BA.1 variant is neutralized by vaccine booster-elicited serum, but evades most convalescent serum and therapeutic antibodies. *Sci Transl Med*. 2022;14:eabn8543.
64. Lu L, Mok BW, Chen LL, et al. Neutralization of SARS-CoV-2 Omicron variant by sera from BNT162b2 or Coronavac vaccine recipients. *Clin Infect Dis*. 2021. doi:10.1093/cid/ciab1041
65. Hoffmann M, Krüger N, Schulz S, et al. The Omicron variant is highly resistant against antibody-mediated neutralization: implications for control of the COVID-19 pandemic. *Cell*. 2022;185(3):447-456 e411.
66. Ariën KK, Heyndrickx L, Michiels J, et al. Three doses of BNT162b2 vaccine confer neutralising antibody capacity against the SARS-CoV-2 Omicron variant. *NPJ Vaccines*. 2022;7(1):35.
67. Kitchin D, Richardson SI, van der Mescht MA, et al. Ad26.COV2.S breakthrough infections induce high titers of neutralizing antibodies against Omicron and other SARS-CoV-2 variants of concern. *Cell Rep Med*. 2022;3(3):100535.
68. Sadoff J, Le Gars M, Cardenas V, et al. Durability of antibody responses elicited by a single dose of Ad26.COV2.S and substantial increase following late boosting. *medRxiv*. 2021. doi:10.1101/2021.08.25.21262569
69. Alter G, Yu J, Liu J, et al. Immunogenicity of Ad26.COV2.S vaccine against SARS-CoV-2 variants in humans. *Nature*. 2021;596(7871):268-272.
70. Stephenson KE, Le Gars M, Sadoff J, et al. Immunogenicity of the Ad26.COV2.S vaccine for COVID-19. *JAMA*. 2021;325(15):1535-1544.
71. Barouch DH, Stephenson KE, Sadoff J, et al. Durable humoral and cellular immune responses 8 months after Ad26.COV2.S vaccination. *N Engl J Med*. 2021;385(10):951-953.
72. Liu Y, Liu J, Xia H, et al. BNT162b2-Elicited neutralization against new SARS-CoV-2 spike variants. *N Engl J Med*. 2021;385(5):472-474.
73. Liu J, Liu Y, Xia H, et al. BNT162b2-elicited neutralization of B.1.617 and other SARS-CoV-2 variants. *Nature*. 2021;596(7871):273-275.
74. Liu J, Liu Y, Xia H, et al. BNT162b2-elicited neutralization of Delta plus, Lambda, Mu, B.1.1.519, and Theta SARS-CoV-2 variants. *NPJ Vaccines*. 2022;7(1):41.
75. Jackson LA, Anderson EJ, Rouphael NG, et al. An mRNA vaccine against SARS-CoV-2—preliminary report. *N Engl J Med*. 2020;383(20):1920-1931.
76. Widge AT, Rouphael NG, Jackson LA, et al. Durability of responses after SARS-CoV-2 mRNA-1273 vaccination. *N Engl J Med*. 2021;384(1):80-82.
77. Natarajan K, Prasad N, Dascomb K, et al. Effectiveness of homologous and heterologous COVID-19 booster doses following 1 Ad.26.COV2.S (Janssen [Johnson & Johnson]) vaccine dose against COVID-19-associated emergency department and urgent care encounters and hospitalizations among adults—VISION Network, 10 States, December 2021-March 2022. *MMWR Morb Mortal Wkly Rep*. 2022;71(13):495-502.
78. Accorsi EK, Britton A, Fleming-Dutra KE, et al. Association between 3 doses of mRNA COVID-19 vaccine and symptomatic infection caused by the SARS-CoV-2 omicron and delta variants. *JAMA*. 2022;327(7):639-651.
79. Sadoff J, Gray G, Vandebosch A, et al. Final analysis of efficacy and safety of single-dose Ad26.COV2.S. *N Engl J Med*. 2022;386(9):847-860.
80. Barouch DH, Stephenson KE, Sadoff J, et al. Durable humoral and cellular immune responses 8 months after Ad26.COV2.S vaccination. *N Engl J Med*. 2021;385(10):951-953.
81. Zeng C, Evans JP, King T, et al. SARS-CoV-2 spreads through cell-to-cell transmission. *Proc National Acad Sci*. 2022;119(1):e2111400119.
82. Naranbhai V, Garcia-Beltran WF, Chang CC, et al. Comparative immunogenicity and effectiveness of mRNA-1273, BNT162b2, and Ad26.COV2.S COVID-19 vaccines. *J Infect Dis*. 2022;225(7):1141-1150.

83. Lv H, Wu NC, Tsang OT-Y, et al. Cross-reactive antibody response between SARS-CoV-2 and SARS-CoV infections. *Cell Rep*. 2020;31(9):107725.
84. Wu NC, Yuan M, Bangaru S, et al. A natural mutation between SARS-CoV-2 and SARS-CoV determines neutralization by a cross-reactive antibody. *PLoS Pathog*. 2020;16(12):e1009089.
85. Tai W, Zhang X, He Y, et al. Identification of SARS-CoV RBD-targeting monoclonal antibodies with cross-reactive or neutralizing activity against SARS-CoV-2. *Antiviral Res*. 2020;179:104820.

How to cite this article: Zhang GF, Meng W, Chen L, et al. Neutralizing antibodies to SARS-CoV-2 variants of concern including Delta and Omicron in subjects receiving mRNA-1273, BNT162b2, and Ad26.COV2.S vaccines. *J Med Virol*. 2022; 1-13. doi:10.1002/jmv.28032