Brief Report

Cross-neutralizing activity against Omicron could be obtained in SARS-CoV-2 convalescent patients who received two doses of mRNA vaccination

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Abstract

The SARS-CoV-2 variant Omicron is now under investigation. We evaluated cross-neutralizing activity against Omicron in COVID-19 convalescent patients (n=23) who had received two doses of an mRNA vaccination (BNT162b2 or mRNA-1273). Intriguingly, after the second vaccination, the neutralizing antibody titers of subjects against SARS-CoV-2 variants, including Omicron, all became seropositive, and significant fold-increases (21.1–52.0) were seen regardless of the disease severity of subjects. Our findings thus demonstrate that two doses of mRNA vaccination to SARS-CoV-2 convalescent patients can induce cross-neutralizing activity against Omicron**.**

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BACKGROUND

The Coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been a major public health problem worldwide since November 2019. As of mid-April 2022, more than 500 million individuals worldwide have been infected with SARS-CoV-2, and more than 6.2 million have died [1]. The threat posed by the SARS-CoV-2 variant Omicron (B.1.1.529) is now under intensive investigation. Omicron was first detected in Botswana on 11 November 2021 and in South Africa on 14 November 2021, and the rate of increase in the number of individuals infected with this variant has been explosive.

In the efforts to control the spread of Omicron, a major challenge has been the low seroconversion rates of cross-neutralizing antibody against Omicron in the sera of individuals who are fully COVID-19-vaccinated [2]. In order to ensure the acquisition of sufficient cross-neutralizing activity in vaccinated individuals, several investigators [2-4] and our research group [5] have recommended an mRNA vaccine booster dose, but as of this writing, data on the cross-neutralizing activity against Omicron achieved by two doses or a booster dose in COVID-19 convalescent individuals are lacking [4,6,7].

Like most nations, Japan has been working to overcome the COVID-19 pandemic. Japan has faced six COVID-19 waves (i.e., surges in new COVID cases followed by decreases) so far (**Supplementary Figure 1**) [8]. The 1st, 2nd and 3rd waves were shown to be caused by B.1.1 and its sub-lineages [9], all of which have D614G mutation (hereinafter D614G). The 4th, 5th, and 6th waves were shown to be caused by the Alpha, Delta, and Omicron variants, respectively (**Supplementary Table 1**) [9]. To control this pandemic in Japan, BNT162b2 vaccination (more commonly known as the Pfizer COVID-19 vaccine) was started in mid-February 2021, and mRNA-1273 vaccination (the Moderna COVID-19 vaccine) was started in mid-May 2021. As of

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mid-April 2022, 80.2% of the Japanese population is fully vaccinated, but more than 50% of Japanese have not yet received a third (booster) dose [10].

It is important to determine whether COVID-19 convalescent patients have cross-neutralizing activity against Omicron, because cross-neutralizing activity is the key to protection against reinfection. We thus conducted the present study to evaluate the neutralizing activity against the Delta and Omicron variants in the sera of COVID-19 convalescent patients. We also evaluated the efficacy of two doses of mRNA vaccine by analyzing the neutralizing antibody titer against Omicron in the same individuals.

METHODS

Diagnosis of COVID-19 and definition of severities

A positive SARS-CoV-2 antigen test or positive polymerase chain reaction (PCR) detection of the SARS-CoV-2 genome in nasal, nasopharyngeal, oropharyngeal, or saliva samples was used to confirm the diagnosis of COVID-19. We used the same definitions of severities and groups as in our previous reports (**Supplementary Table 2**) [11-13].

Study site and patient recruitment

At Hyogo Prefectural Kakogawa Medical Center (Kakogawa, Hyogo, Japan), serial sera samples have been collected from COVID-19 patients at various timepoints post-onset: 1–3, 3–6, and 6–9 months post-onset, and approximately 12 months post-onset.

According to the epidemiological data [9], patients with onset between 10 March 2020 and 5 January 2021, corresponding to the 1st–3rd waves, were mainly infected with D614G; those with onset between 28 April 2021 and 26 May 2021, corresponding to the 4th wave, were mainly infected with Alpha; and those with onset between 26 July 2021 and 5 September 2021, corresponding to the 5th wave, were mainly infected with Delta.

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Patients who were given therapeutic monoclonal antibody drugs or re-infected with SARS-CoV-2 were excluded.

Virus strains

The SARS-CoV-2 Biken-2 (B2) strain and the D614G reference variant (accession no. LC644163) were provided by BIKEN Innovative Vaccine Research Alliance Laboratories. The SARS-CoV-2 variants B.1.1.7 (Alpha) (GISAID ID: EPI_ISL_804007), B.1.167.2 (Delta) (GISAID ID: EPI_ISL_2158617), and B.1.1.529.1 (Omicron) (GISAID ID: EPI_ISL_7418017) were obtained from the National Institute of Infectious Diseases (NIID), Tokyo.

Neutralization assay

The virus neutralization assay against the four SARS-CoV-2 variants D614G, Alpha, Delta, and Omicron was conducted using each authentic virus as described previously [11] [12] at Biosafety Level 3.

Statistical analyses

Continuous variables are described using medians and interquartile ranges (IQRs) defined by the 25th and 75th percentiles. Categorical factors are reported as counts and percentages. Neutralizing antibody titers below 2 were assigned a titer of 1 for the geometric mean titer (GMT) calculations. The Wilcoxon signed-rank test or Friedman's test and Bonferroni correction were performed to compare the neutralizing antibody titers. The level of statistical significance in all analyses was set at $P < .05$. Statistical analyses were performed using STATA (ver. 14.2). Sample size calculation was not performed.

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Ethics

Our study was approved by the Ethics Committees of Kobe University Graduate School of Medicine (ID: B200200) and Hyogo Prefectural Kakogawa Medical Center. Written consent or opt-out consent for our observational study was obtained.

RESULTS

Patient characteristics

We assessed 40 sera samples from the 1st –3rd wave patients, 12 from the 4th wave patients, and 16 from the 5th wave patients (**Supplementary Tables 3 and 4**). Sera samples were categorized into four groups based on the four time periods of blood sampling: 1–3, 3–6, 6–9, and 12 months post-onset. The number of patients with pneumonia was 22 for the 1st–3rd waves, 12 for the 4th wave (n=12), and 16 for the 5th wave. The number of convalescent patients who had received two doses of a vaccine (BNT162b2 or mRNA-1273) was 19 in the 1st–3rd waves and 4 in the 4th wave.

Comparison of the neutralizing antibody titers against variants in the sera samples

We compared the neutralizing antibody titers against SARS-CoV-2 variants from the sera of the COVID-19 convalescent patients. These sera samples were collected at 1–3 months post-onset **(Figure 1A, 1B, Supplementary Figure 2, and Supplementary Table 5**). The seropositive rates of neutralizing antibodies were 97.5% for D614G, 87.5% for Delta, and 37.5% for Omicron (**Figure 1A**). The GMTs of neutralizing antibodies were 18.7 against D614G, 7.7 against Delta, and 1.5 against Omicron (**Supplementary Table 5**).

The results of our comparison of the neutralizing antibody titers against D614G, Delta, and Omicron in the patients with or without pneumonia in the 1st–3rd waves are provided in **Supplementary Figure 2 and Supplementary Table 5**. Although the GMTs of neutralizing

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antibody in the patients with pneumonia in the 1st–3rd waves were higher than those of the patients without pneumonia, the seropositive rate of the 1st–3rd waves patients with pneumonia was only 59.1% and the GMT of neutralizing antibody against Omicron was 2.0, which was identical to the cut-off point.

We next compared the neutralizing antibody titers against D614G, Alpha, or Delta with those against Omicron in the patients with pneumonia in the 1st–3rd waves, the 4th wave, and the 5th wave (**Figure 1B**). We aligned the severity among the patient groups because there were no sera samples from the 4th wave or the 5th wave patients without pneumonia. Significant fold-decreases of the neutralizing antibody titer against Omicron relative to those against the other variants were observed: 17.6 ($P < .0001$) for the 1st–3rd waves, 12.0 ($P < .0001$) for the 4th wave, and 13.5 ($P < .0001$) for the 5th wave.

Longitudinal analysis of neutralizing antibody titers against D614G, Alpha, Delta, and Omicron in patients infected in the 1st–3rd waves or the 4th wave after two vaccine doses

We next examined the trends of neutralizing antibody titers in the patients infected during the 1st–3rd waves after their two doses of vaccination at 1–3, 3–6, 6–9, and 12 months post-onset (n=19) (**Figure 2A and Supplementary Table 6**). Two doses of mRNA vaccination were completed before the sera were collected at 12 months post-onset. The GMTs of the neutralizing antibodies against D614G and Delta tended to decline from 1–3 months post-onset to 6–9 months post-onset. The GMTs of the neutralizing antibody against Omicron were below 2 at three of the sampling points. Intriguingly, after the two doses of vaccination, all neutralizing antibody titers including that against Omicron became seropositive and showed significant fold-increases (21.1 to 52.0) regardless of the disease severity of patients (**Figures 2A and 2C, Supplementary Figures 2A and 2B**).

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We then evaluated the trend of neutralizing antibody titers in the patients infected during the 4th wave and subsequently vaccinated at 1–3, 3–6, and 6–9 months post-onset (n=4) (**Figures 2B and 2D, and Supplementary Table 6**). Two doses of the vaccination were completed before the sera were collected at 6–9 months post-onset. The GMTs of the neutralizing antibodies against Alpha, Delta, and Omicron tended to decline from 1–3 months post-onset to 3–6 months post-onset. After two doses of the vaccination, a significant fold-increase of the neutralizing antibody titer against Omicron was also observed (**Figures 2B and 2D**), indicating that the neutralizing antibody against Omicron was boosted by the vaccination; however, the neutralizing antibody titer against Omicron was significantly lower than those against the variants that were suspected to have infected patients (D614G or Alpha) even after vaccination (**Figure 2C and 2D, Supplementary Figures 3C and 3D**).

DISCUSSION

The results of our analyses revealed that the neutralizing antibody titer against Omicron was remarkably decreased in the patients infected in the 1st–3rd waves (**Figure 1A, Supplementary Figure 2 and Supplementary Table 4**). Notably, the seropositive rate of neutralizing antibody titer against Omicron in the patients without pneumonia in the 1st–3rd waves was only 11.1%. These data are similar to those of earlier studies [6,7,14]. The seropositive rate against Omicron of the patients with pneumonia was higher than that of the patients without pneumonia because a stronger immune response was elicited in the severe COVID-19 patients compared to those with mild disease (**Supplementary Figure 2**) [11].

Our findings also demonstrated significant fold-decreases against Omicron relative to D614G (×17.6), Alpha (×12.0), and Delta (×13.5) (**Figure 1B**). Another study showed clear fold-decreases against Omicron compared to Victoria (an early pandemic strain; ×16.9), Alpha

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(×18.4), and Delta (×25.9) [6]. In the present study, the proportion of patients whose condition was considered "critical" in the 5th wave (75%) was much higher than those in the 1st–3rd waves (9%) and the 4th wave (58%), and this might have contributed to the lesser-than-expected fold-decrease against Omicron relative to Delta. Our results suggest that patients who have been infected with any SARS-CoV-2 variant could possibly be re-infected with Omicron.

We also observed that the neutralizing antibody titer against Omicron increased after two doses of mRNA vaccine in convalescent patients of the 1st–3rd waves and the 4th wave (**Figures 2A and 2B, Supplementary Figures 3A and 3B and Supplementary Table 5**). This finding was in agreement with those of other studies which reported that the neutralizing antibody activity against Omicron in convalescent patients who had been vaccinated twice was slightly lower than that against D614G, whereas the neutralizing antibody activity against Omicron in uninfected individuals who had been vaccinated twice was either greatly decreased or undetectable [4,14]. In the present investigation, the ratios of the neutralizing antibody titer against Omicron relative to that of other variants (D614G or Alpha) in vaccinated patients after their recovery (**Figures 2C and 2D**) were almost the same as the previously reported ratios of the antibody titer against Omicron relative to that of D614G in uninfected individuals who received three doses of COVID-19 mRNA vaccine [2,7,14]. Although it remains unknown whether one dose is sufficient for convalescent patients with severe symptoms and whether a third dose is necessary for convalescent patients with mild symptoms, our results show that two doses of mRNA vaccination to SARS-CoV-2 convalescent patients could induce cross-neutralizing activity against Omicron comparably to three doses of mRNA vaccination to uninfected individuals. Based on our finding that neutralizing antibodies against several variants, including Omicron, were induced in the infected individuals after two doses of vaccination, it is

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possible that mRNA vaccination of infected individuals stimulates and expands memory B cells that recognize common neutralizing epitopes among SARS-CoV-2 variants.

Footnotes

Conflict of interest

The authors declare no conflicts of interest associated with this manuscript.

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Meeting

This study has never been presented anywhere.

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Author Contributions

All authors contributed to the concept of this article. YK drafted the manuscript; YM provided revisions; YK, JA, MN, and YM analyzed the data; YK, KF, SS[1], and LT did the experiments; YM supervised the experiments; SI, YT, SS[3], SN, TK, MY, YN and TN collected the samples; YM conducted the project; All authors approved the final version of the manuscript.

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Figure legends

Figure 1. Comparison of the neutralizing antibody titers against D614G, Alpha, Delta, and Omicron from the sera of convalescent COVID-19 patients. *Vertical bar:* The neutralizing antibody titer (log2 scale). *Horizontal bar:* The SARS-CoV-2 variants. All samples in panels **A** and **B** were collected at 1–3 months post-onset. **A:** The neutralizing antibody titers in the patients infected during the 1^{st}–3^{rd} waves. **B:** The neutralizing antibody titers in the patients with pneumonia infected during the 1^{st}–3^{rd}, 4^{th}, and 5^{th} waves. Wilcoxon signed-rank test or Friedman's test and Bonferroni correction were performed to compare the neutralizing antibody titers. Fold-decreases of neutralizing antibody titer (Delta relative to D614G or Alpha, and Omicron relative to D614G, Alpha, or Delta) are above each graph; seropositive rates are below each graph. The *gray dashed line* in each graph indicates the cut-off titer. D614G, Alpha, and Delta are suspected to have infected the individuals in the 1^{st}–3^{rd}, 4^{th} and 5^{th} waves, respectively.

Figure 2. Comparison of the neutralizing antibody titers against D614G/Alpha, Delta, and Omicron in the patients infected during the 1^{st}–3^{rd} waves or 4^{th} wave and subsequently vaccinated. The neutralizing antibody titers against Delta and Omicron in the patients infected during the 1^{st}–3^{rd} waves and subsequently vaccinated **(A)** and the patients infected during the 4^{th} wave and subsequently vaccinated **(B)**. **C:** Comparison of the neutralizing antibody titers against D614G, Delta, and Omicron from the same sera samples as in panel A collected at 12 months post-onset. **D:** Comparison of the neutralizing antibody titers against Alpha, Delta, and Omicron in the same sera samples as in panel **B** collected at 6–9 months post-onset. *Vertical bar:* The neutralizing antibody titer (log2 scale). *Horizontal bar:* The timing of sampling (months post-onset) (**A, B**) or the SARS-CoV-2 variants used in the virus neutralizing assay (**C, D**). *Boxes:* The SARS-CoV-2 variants. Friedman's test and Bonferroni correction were performed to

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compare the neutralizing antibody titers. Fold-increases of neutralizing antibody titer (12 months post-onset relative to 6–9 months post-onset in **A**, and 6–9 months post-onset relative to 3–6 months post-onset in **B**) are shown above each graph. Fold-decreases of neutralizing antibody titer against Delta and Omicron relative to D614G are indicated in **C**, and those against Delta and Omicron relative to Alpha are indicated in **D**. Seropositive rates are shown below each graph. The *gray dashed line* in each graph indicates the cut-off titer. One serum sample at 6–9 months post-onset was missing in the group of patients infected during the 1^{st}–3^{rd} waves and subsequently vaccinated. An *arrow* in each graph shows the timepoint of the second mRNA vaccination. D614G and Alpha are suspected to have infected the patients during the 1^{st}–3^{rd} waves and 4^{th} wave, respectively.

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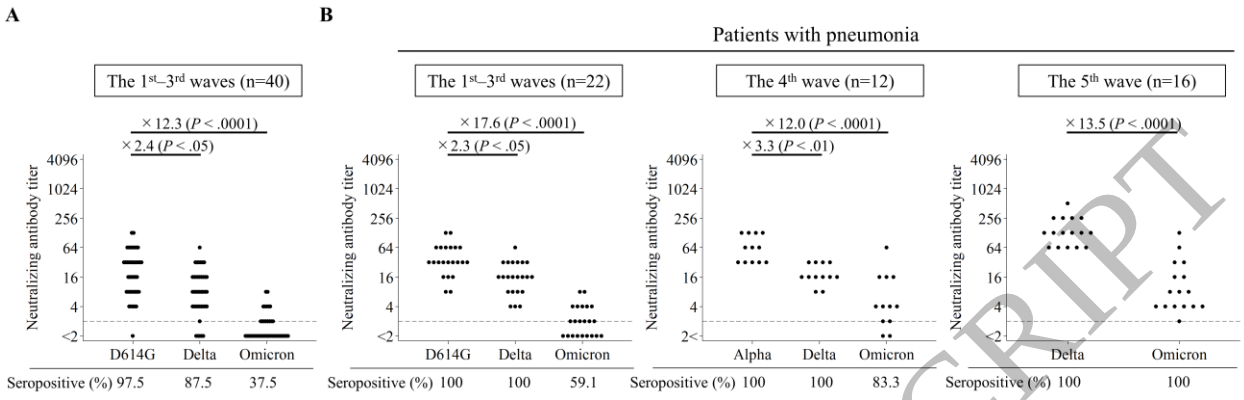

Figure 1
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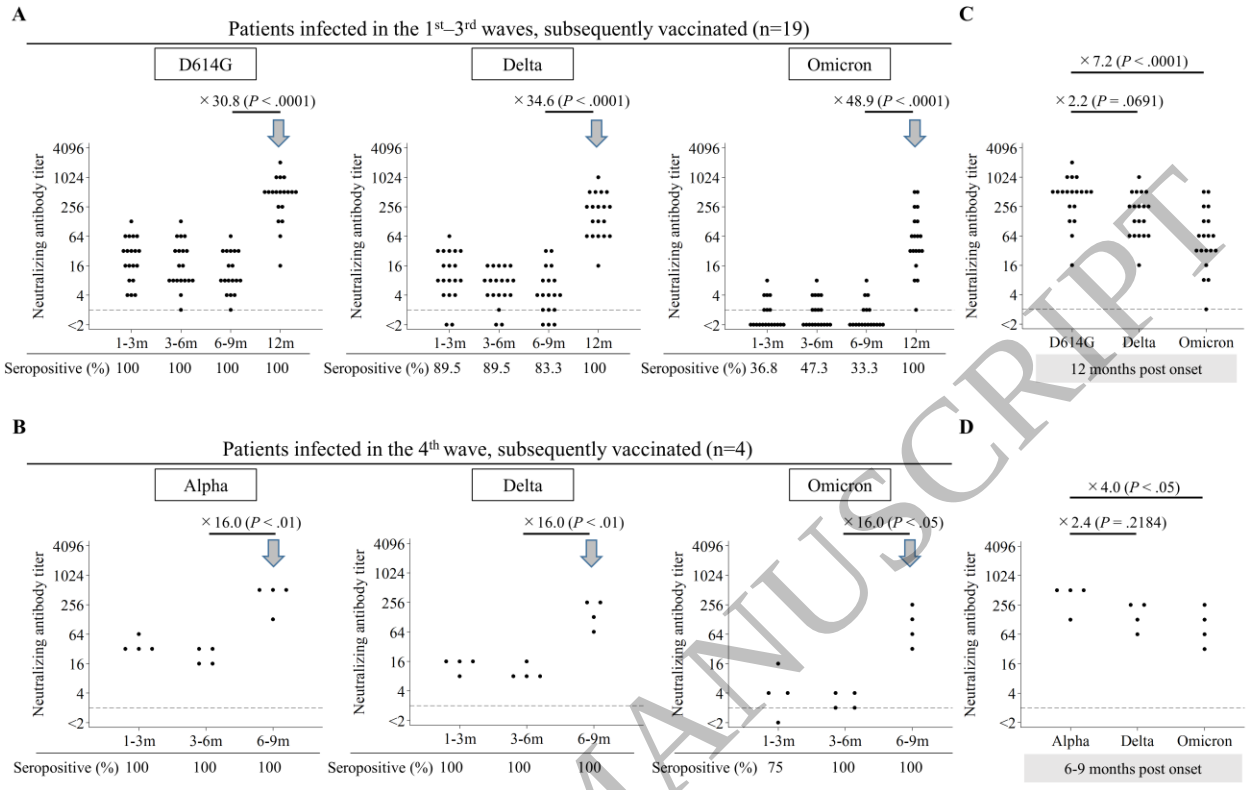

Figure 2

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