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MAJOR ARTICLE

# Immune Response Kinetics Following a Third Heterologous BNT162b2 Booster Dose After Primary 2-Dose ChAdOx1 Vaccination in Relation to Omicron Breakthrough Infection: A Prospective Nationwide Cohort Study in South Korea

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***Background.*** Immune responses to each vaccine must be investigated to establish effective vaccination strategies for the ongoing coronavirus disease (COVID-19) pandemic. We investigated the long-term kinetics of immune responses after heterologous booster vaccination in relation to Omicron breakthrough infection (BI).

***Methods.*** Our study included 373 healthcare workers who received primary ChAdOx1 vaccine doses and a third BNT162b2 vaccine dose. BIs that occurred after the third vaccine were investigated. Blood specimens were collected before and 3 months after the booster dose from participants without BI and 1, 4, and 6 months after BI from participants who experienced BI. Spike-specific binding and neutralizing antibody levels against the wild-type virus, Omicron BA.1, and Omicron BA.5, as well as cellular responses, were analyzed.

***Results.*** A total of 346 participants (82 in the no BI group; 192 in the BI group during the BA.1/BA.2 period; 72 in the BI group during the BA.5 period) were included in the analysis. Participants without BI exhibited the highest binding and neutralizing antibody concentrations and greatest cellular response 1 month after the third vaccination, which reached a nadir by the ninth month. Antibody and cellular responses in participants who experienced BI substantially increased postinfection. Neutralizing antibody titers in individuals who experienced BI during the BA.1/BA.2 period showed more robust increase against wild-type virus than against BA.1 and BA.5.

***Conclusions.*** Our findings provide evidence of antigenic imprinting in participants who received a heterologous booster vaccination, thereby serving as a foundation for further studies on the impact of BIs on immune responses.

**Keywords.** COVID-19; antigen imprinting; coronavirus disease; heterologous booster vaccination; Omicron breakthrough infection.

The coronavirus disease 2019 (COVID-19) pandemic has presented a significant global threat since its emergence in late 2019 [1]. Effective vaccination regimens are crucial for controlling the spread of the virus. In South Korea, a nationwide COVID-19 vaccination campaign using the BNT162b2 vaccine (Comirnaty, developed by Pfizer/BioNTech, Mainz, Germany) or ChAdOx1 vaccine (Vaxzevria, developed by AstraZeneca, Oxford, United Kingdom) was implemented in early 2021. However, owing to instability in vaccine supply and reports of vaccine-related adverse events, the vaccination strategy in South Korea has been amended multiple times [2–6]. In response to the Delta and Omicron outbreaks in late 2021, individuals were encouraged to receive a third BNT162b2 vaccine

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to boost immunity. Consequently, many young individuals who had the ChAdOx1 virus-vectored vaccine as a primary vaccine were given a booster shot from a different platform.

However, Omicron subvariants have emerged that are particularly adept at causing breakthrough infections (BIs) in individuals who have received a third vaccination dose [7, 8]. Evaluating the kinetics and longevity of humoral and cellular immunity following booster vaccination, particularly in the Omicron outbreak, is essential to establish effective vaccination strategies for the ongoing pandemic and emerging variants. However, we lack research on individuals who have received heterologous booster vaccinations.

In this study, we investigated the long-term kinetics of immune responses and their changes in BI after the third heterologous booster vaccination in individuals who received 2 primary doses of the ChAdOx1 vaccine.

## METHODS

### Study Population

This nationwide, multicenter, prospective cohort study was led by the Korean Disease Control and Prevention Agency and healthcare workers (HCWs) from 10 hospitals in South Korea. Previous analyses of data from this cohort study have been published [5, 6]. In the current study, we conducted a 1-year follow-up analysis of 373 participants who completed 2 primary doses of ChAdOx1 vaccines followed by a BNT162b2 dose as the third. All participants provided written informed consent, and the institutional review board approved the study protocol of each participating hospital.

During the study period, self-reports of COVID-19 diagnosis by polymerase chain reaction (PCR) or antigen test were obtained and overall COVID-19 diagnosis during the study period was retrospectively reconfirmed at the end of the study. In cases of unclear diagnosis (eg, those who experienced COVID-19–associated symptoms but did not have a confirmatory test), an anti-spike antibody titer at each blood sampling point was retrospectively compared, and if it did not decrease, BI was suspected.

When BI was diagnosed or suspected, an anti-nucleocapsid antibody test was additionally performed on the samples from before and after vaccination for the serological diagnosis of BI. Anti-nucleocapsid antibody test was performed on all samples collected at the end of the study, and for participants with positive anti-nucleocapsid antibody seroconversion without history of confirmed or suspected COVID-19, additional anti-nucleocapsid antibody tests were performed on previously collected samples to identify the first positive seroconversion point. A serologic diagnosis of BI was defined as a positive seroconversion of anti-nucleocapsid antibody or an unexplained $\geq$1.5-fold increase in anti-spike antibody titer. When detecting BI through anti-spike antibody titer increase, the use of 1.5-fold cutoff was determined as the most sensitive criterion, because of the analysis of the change in anti-spike antibody titer before and after the anti-nucleocapsid antibody seroconversion in an analysis of 92 participants with PCR-confirmed COVID-19 in this cohort; most participants experienced an average 4.3-fold increase in titer, in contrast, 19.5% had a <2-fold increase.

BIs that occurred after the third vaccination were investigated. When BI was only diagnosed serologically, the date of the BI diagnosis was defined as the median value of the collection dates of the 2 samples from which serologic changes were determined. The BI period was then classified into 3 categories, the "pre-Omicron period," "BA.1/BA.2 outbreak period," and "BA.5 outbreak period," based on the dominant variant of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that accounted for more than half of cases detected in South Korea.

### Data Acquisition and Sample Collection

Data on baseline characteristics of age, sex, height, body weight, and underlying diseases were collected. Reactogenicity data after the third vaccinations were collected for 7 days using an electronic diary format, which was developed based on phase 3 clinical trials of vaccines [2, 9]. A total of 11 side effects as well as the need for acetaminophen to control side effects were investigated. Local side effects included pain, redness, and swelling at the injection site. Systemic side effects were fever, chill, myalgia, arthralgia, fatigue, headache, vomiting, and diarrhea. Participants rated each symptom on a scale of 0–4 every day from day 0 (vaccination day) to day 7. For symptoms, a score of 0 was selected for no symptoms, 1 for mild, 2 for moderate, 3 for severe, and 4 for critical. For acetaminophen, a score of 0 was selected for no need for acetaminophen, 1 for 1–2 tablets per day, 2 for 3–4 tablets, 3 for 5–6 tablets, and 4 for $\geq$7 tablets. Reactogenicity was calculated from the total sum of scores [2]. Blood specimens were collected before and at the 3-month interval after the third booster vaccine. For participants diagnosed with BI after the third vaccine, blood samples collected after the BI were retrospectively categorized into 1 month after BI (up to 75 days postinfection), 4 months after BI (76–150 days), and 6 months after BI (beyond 150 days) based on the time elapsed since BI.

### Immunogenicity Analysis

Sera from all participants were analyzed at each sampling point using the Elecsys Anti-SARS-CoV-2 S assay (Roche Diagnostics, Basel, Switzerland) to estimate the total antibody titers against the receptor-binding domain of the spike protein. The presence of an anti-nucleocapsid antibody was used as a surrogate marker of past SARS-CoV-2 infections. The Elecsys Anti-SARS-CoV-2 N protein assay (Roche Diagnostics) was performed on baseline samples of all participants and samples of participants when breakthrough COVID-19 infection was suspected. The results of the Elecsys antibody test (U/mL)

were converted to World Health Organization international units, defined as binding antibody units per milliliter (BAU/mL), according to the correlation curve provided by the manufacturer.

In addition, plaque reduction neutralization test (PRNT) was performed on the sera from randomly selected 16 participants in the BI group in the BA.1/BA.2 period and the 16 age-matched participants in no infection group at each sampling point for the SARS-CoV-2 wild-type (WT; NCCP43326) and Omicron (NCCP43408) strains. The 50% neutralization dilution ($ND_{50}$) was expressed as the reciprocal of the highest serum dilution, resulting in a 50% reduction in plaque number. The Spearman-Kärber method was used to calculate the $ND_{50}$ titers.

Moreover, we investigated cell-mediated immunity by measuring interferon gamma (IFN-γ) secreted by T cells in response to the SARS-CoV-2 antigen. We used a SARS-CoV-2–specific IFN-γ release assay (IGRA) kit with enzyme-linked immunosorbent assay (Covi-FERON ELISA, SD Biosensor, Suwon, Republic of Korea) in 197 randomly selected participants [2]. Whole blood specimens from participants were collected, and 1 mL was injected into each Covi-FERON tube (nil tube, SARS-CoV-2 spike protein antigen [Sp]1 tube, Sp2 tube, and mitogen tube). The Sp1 tube contained spike protein antigens derived from the original SARS-CoV-2 (Wuhan/Hu-1/2019) and B.1.1.7 variant, whereas the Sp2 tube contained those derived from the B.1.351 and P.1 variants [2]. As the positive cutoff value of the SARS-CoV-2 IGRA kit had not been established, the IFN-γ concentration of the Sp tubes minus that of the nil tube was compared quantitatively between the groups. Details of the laboratory procedures are presented in Supplementary Text 1.

### Statistical Analysis

The Student *t* test, Mann-Whitney *U* test, or 1-way analysis of variance was used for continuous variables. The $\chi^2$ or Fisher exact test was used for categorical variables to compare the characteristics, reactogenicity, and laboratory test results of the vaccinated groups. Continuous variables are presented as means and standard deviations. Categorical variables are presented as numbers and percentages. Antibody levels were analyzed as geometric mean titers (GMTs) with 95% confidence intervals (CIs). All *P* values were 2-tailed, and those <.05 were considered statistically significant. IBM SPSS Statistics version 27 (IBM, Armonk, New York) was used for all statistical analyses, and GraphPad Prism version 8.0 (GraphPad Software, San Diego, California) was used for graph plotting the results.

## RESULTS

### Study Population and Baseline Characteristics

A total of 373 HCWs, who received 2 primary doses of the ChAdOx1 vaccine, were classified based on vaccination status, presence, and period of BI (Figure 1). During follow-up, 360 HCWs finally received the third booster vaccine, including 3 HCWs who received the fourth booster vaccine. Eighty-two HCWs did not experience BI after the third booster vaccine (no BI group), 194 HCWs experienced BI during the BA.1/BA.2 period (BI during BA.1/BA.2 group), and 75 HCWs experienced BI during the BA.5 period (BI during BA.5 group). The antibody kinetics in the 3 groups were compared as major groups. Two HCWs in the BI during BA.1/BA.2 group who experienced a second infection during the BA.5 period and 3 HCWs in the BI during BA.5 group who received the fourth-dose vaccine were excluded from the major group analysis.

Of the total 264 BI cases (192 BI during BA.1/BA.2 outbreak period and 72 BI during BA.5 outbreak period), 216 BIs (81.8%) were diagnosed via PCR or antigen test. The date of BI diagnosis is shown in Supplementary Figure 1 by the type of COVID-19 diagnosis. One hundred eighty-five of 192 (96.4%) of the BI during BA.1/BA.2 group and 66 of 72 (91.7%) of the BI during BA.5 group showed positive anti-nucleocapsid antibody seroconversion. Of the 216 participants with PCR- or antigen test–confirmed COVID-19, 210 (97.2%) had anti-nucleocapsid antibody seroconversion.

The baseline characteristics of the 3 groups are summarized in Table 1. No significant differences in demographics were observed among the 3 groups. The mean time interval between the second and third vaccination dose was 186.7 days, and the GMT of the anti-spike protein antibody 1 month after the third-dose vaccination was not significantly different among the groups ($P = .441$). BIs in the BA.1/BA.2 and BA.5 groups occurred after an average of 113.6 and 243.7 days from the third-dose vaccination, respectively.

### Kinetics of Binding Antibody and IFN-γ Concentrations

Changes in anti-spike binding antibody and IFN-γ concentrations after the third vaccination were measured in the serial blood samples of HCWs in each group. In the no BI group, samples were analyzed before and 1, 3, 6, and 9 months after the third vaccination dose. In the BI during the BA.1/BA.2 period group, samples were analyzed before and at 1 and 3 months after the third dose of vaccination as well as 1, 4, and 6 months after BI. In the BI during the BA.5 period group, samples were analyzed before and 1, 3, and 6 months after the third vaccination dose and 1 month after the BI.

Antibody concentrations in HCWs in the no BI group were the highest 1 month after the third vaccination and gradually waned until 9 months thereafter (Figure 2*A* and Supplementary Table 1). In BI-experienced HCWs, binding antibody concentrations re-increased after BI, and titers were significantly higher than those at 1 month after the third vaccination (11 936 BAU/mL vs 17 168 BAU/mL, $P < .001$ in the BI during BA.1/BA.2 group and 13 169 BAU/mL vs 20 146 BAU/mL, $P < .001$ in the BI during BA.5 group).

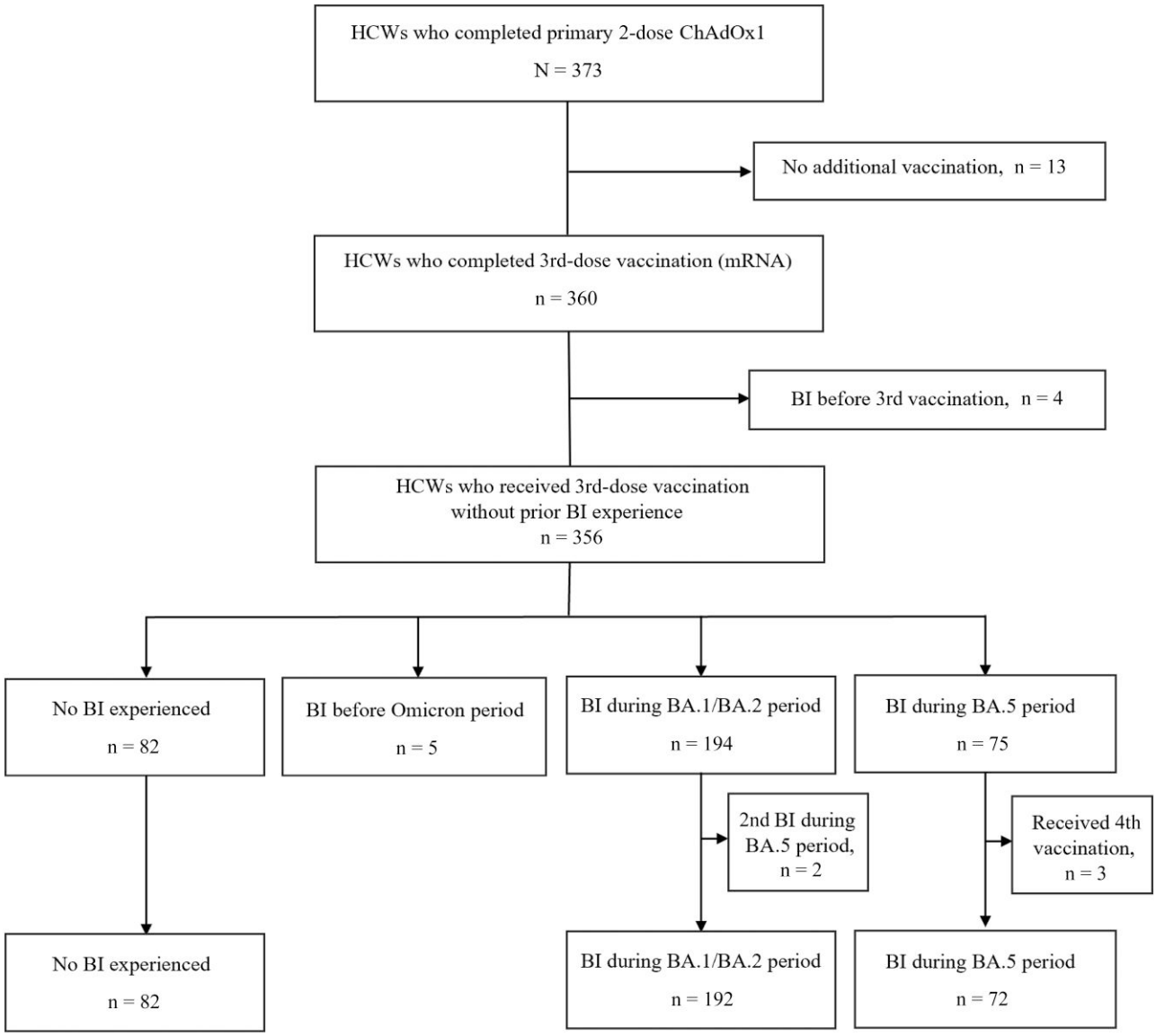


**Figure 1.** Status of additional vaccination and breakthrough infection of the participants who completed primary 2-dose ChAdOx1 vaccination. Abbreviations: BI, breakthrough infection; HCW, healthcare worker; mRNA, messenger RNA (BNT162b2; Pfizer).

A similar trend was observed in SARS-CoV-2–specific cellular immune responses (Figure 2*B* and 2*C* and Supplementary Table 1). HCWs in the no BI group presented peak IFN-γ concentrations 1 month after the third vaccination. They reached a nadir of 0.627 IU/mL and 0.351 IU/mL for Sp1 and Sp2 IGRAs, respectively, at 9 months after the third vaccination. HCWs who experienced BI exhibited a re-increase after BI. IFN-γ concentrations 1 month after the third vaccination and 1 month after the BI were not statistically different.

#### Neutralizing Antibody Response

After the third vaccination, neutralizing antibody titers against the WT virus and Omicron BA.1 and BA.5 variants in HCWs in the no BI group exhibited peak levels 1 month after vaccination and then gradually decreased to a nadir at 9 months. The increase in neutralizing antibody titers against Omicron variants 1 month after the third vaccination was minimal (910.7 against WT virus vs 77.1 against Omicron BA.1 vs 133.2 against Omicron BA.5). The GMT of the PRNT $ND_{50}$ against the WT virus reached a nadir of 182.5 within 9 months after the third vaccination. However, the GMT reached nadirs of 36.1 and 32.7, respectively, for the Omicron BA.1 and BA.5 variants (Figure 3*A* and Supplementary Table 1).

In the BI during the BA.1/BA.2 period group, neutralizing antibody titers against the WT virus, Omicron BA.1, and Omicron BA.5 re-increased after BI at a significantly higher level than those after the third vaccination (905.3 vs 1278, $P = .017$ against WT virus; 70.2 vs 148.1, $P = .003$ against BA.1 variant; 115.8 vs 235.5,

**Table 1. Baseline Characteristics of the Study Population Depending on the Presence and Timing of Breakthrough Infection**

| Characteristic | Total (N = 346) | No BI-Experienced (n = 82) | BI During BA.1/BA.2 Period (n = 192) | BI During BA.5 Period (n = 72) | *P* Value |
|---|---|---|---|---|---|
| Sex, male | 82 (23.7) | 18 (22.0) | 45 (23.4) | 19 (26.4) | .805 |
| Age, y, mean ± SD | 38.8 ± 2.8 | 40.0 ± 9.9 | 38.0 ± 9.3 | 39.8 ± 9.4 | .148 |
| BMI, kg/m$^2$, mean ± SD | 22.3 ± 2.8 | 22.2 ± 2.6 | 22.2 ± 2.9 | 22.7 ± 3.0 | .443 |
| Comorbidity | 47 (13.6) | 15 (18.3) | 22 (11.5) | 10 (13.9) | .318 |
| Hypertension | 10 (2.9) | 3 (3.7) | 5 (2.6) | 2 (2.8) | .891 |
| Diabetes | 8 (2.3) | 3 (3.7) | 3 (1.6) | 2 (2.8) | .491 |
| Asthma | 4 (1.2) | 1 (1.2) | 3 (1.6) | 0 (0) | .816 |
| Dyslipidemia | 6 (1.7) | 3 (3.7) | 2 (1.0) | 1 (1.4) | .281 |
| Thyroid disease | 10 (2.9) | 1 (1.2) | 6 (3.1) | 3 (4.2) | .530 |
| Rheumatic disease | 3 (0.9) | 3 (3.7) | 0 (0) | 0 (0) | .022 |
| Solid tumor | 5 (1.4) | 1 (1.2) | 3 (1.6) | 1 (1.4) | 1.000 |
| Reactogenicity after the third dose, score sum, mean ± SD | 11.7 ± 12.9 | 14.6 ± 15.2 | 10.4 ± 10.8 | 12.1 ± 14.8 | .045 |
| Interval between second and third dose, d, mean ± SD | 186.7 ± 12.4 | 185.4 ± 11.0 | 187.3 ± 13.0 | 186.4 ± 12.2 | .508 |
| Interval between the third dose and BI, d, mean ± SD | … | … | 113.6 ± 29.1 | 243.7 ± 19.2 | <.001 |
| Anti–spike protein antibody titer at 1 mo after the third dose, BAU/mL, GMT (95% CI) | 12 163 (11 281–13 114) | 11 812 (9738–14 328) | 11 936 (10 904–13 066) | 13 169 (11 195–15 492) | .441 |

Data are expressed as No. (%) unless otherwise indicated.

Abbreviations: BAU, binding antibody units; BI, breakthrough infection; BMI, body mass index; CI, confidence interval; GMT, geometric mean titer; SD, standard deviation.

$P < .001$ against BA.5 variant) (Figure 3*B* and Supplementary Table 1). The increase in neutralizing antibody titers 1 month after BI was more robust against WT virus than against Omicron BA.1 and BA.5 (840 vs 107.4 vs 200.4 against WT, BA.1, and BA.5, respectively, $P < .001$) The differences between neutralization titers against BA.1 and BA.5 did not significantly differ at each time point ($P = .08$, .913, .130, and .403 in 1 and 3 months after the third shot and 1 and 4 months after BI, respectively) regardless of the experience of BI during the BA.1/BA.2 period.

The ratio of neutralization titers against Omicron BA.1 or BA.5 to those against the WT virus following the third vaccination is shown in Figure 4 and Supplementary Table 2. Although BI-experienced HCWs underwent BI during the BA.1/BA.2 period, the WT/BA.1 ratio after BI was higher than the WT/BA.5 ratio after BI (12.1 vs 5.5, $P = .037$ in 1 month after BI; 8.8 vs 6.3, $P = .168$ in 4 months after BI; Supplementary Table 2).

## DISCUSSION

We conducted a 1-year follow-up analysis of a nationwide multicenter cohort of HCWs who completed 2 primary doses of ChAdOx1 vaccines, followed by a BNT162b2 dose as the third, to evaluate changes in antibody- and cell-mediated immune responses in relation to Omicron BI.

Our results indicate that additional booster shots may be necessary to ensure adequate immunity in individuals without BI after a third-dose vaccination. In individuals who experienced BI, we observed an enhancement in antibody and cellular responses after BI. We observed that the neutralizing antibody response to both the WT virus and Omicron BA.1 and BA.5 increased after BI during the BA.1/BA.2 period.

In previous reports, neutralizing antibody titers against Omicron variants were high after infection in unvaccinated individuals; however, they did not show cross-reactivity against earlier ancestral strains [10, 11]. On the contrary, samples obtained after 3 mRNA vaccination doses exhibit broad-spectrum, cross-neutralizing antibody responses against the WT virus as well as Omicron variants [10, 12]. Jeong et al reported that a full-length WT spike gene encoded by the vaccine could generate antibodies that may recognize conserved epitopes commonly possessed by a broad range of SARS-CoV-2 variants, and these antibodies could be amplified by a booster vaccine [10].

Similarly, studies suggest that Omicron BI after 3 mRNA vaccination doses results in a robust increase in cross-neutralizing antibodies against pan-SARS-CoV-2, enhancing the magnitude, affinity, and breadth of neutralizing activities [10, 13]. However, we lack reports on the immune response in individuals who primarily received non-mRNA COVID-19 vaccines.

In this study, cross-neutralizing antibody response was elicited after primary ChAdOx1 vaccination and a BNT162b2 booster dose and re-increased after BI. The neutralizing antibody response to WT virus after BI during the BA.1/BA.2 period was more robust than the antibody response to Omicron BA.1 or BA.5. Other studies also showed that the neutralizing antibody response to Omicron variants was similar to or lower than the response to the WT virus after Omicron BI in the 3-dose mRNA-vaccinated individuals [10, 13–16]. These results suggest that vaccine-induced antibody responses may elicit broad-spectrum immunity by initially increasing the titer of neutralizing antibodies against ancestral strains, and could be consistent with the doctrine of "original antigenic sin or antigenic imprinting," which results in a progressively narrowed

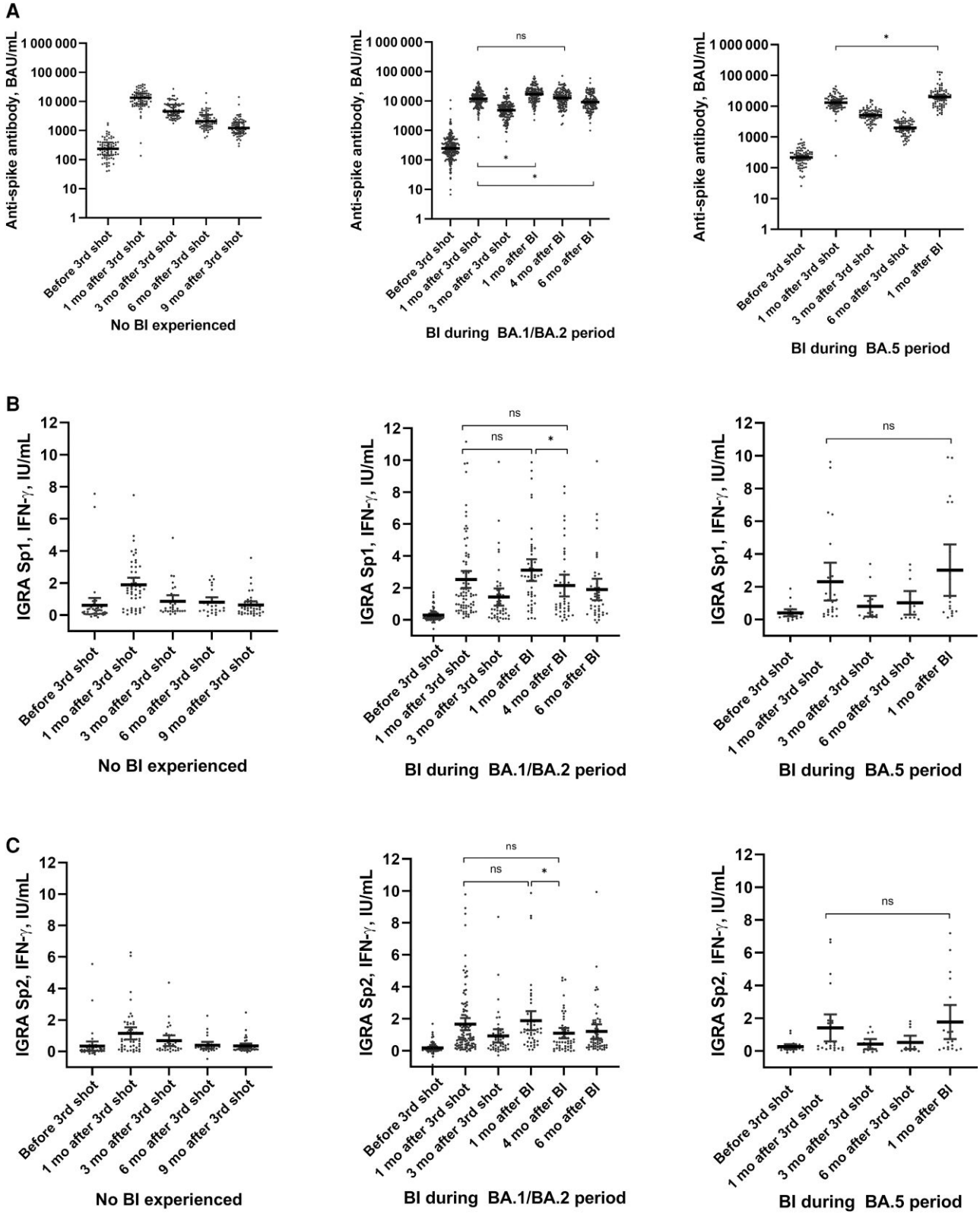


**Figure 2.** Changes in anti-spike binding antibody and interferon gamma (IFN-γ) concentrations after the third heterologous BNT162b2 vaccination dose. Changes in anti-spike binding antibody concentrations (*A*) and IFN-γ concentrations using IFN-γ release assay Sp1 (*B*) and Sp2 (*C*) following the third heterologous vaccination dose, according to the presence of breakthrough infection after the third dose. The Sp1 contained spike protein antigens derived from the original severe acute respiratory syndrome coronavirus 2 (Wuhan/Hu-1/2019) and B.1.1.7 variant, whereas Sp2 contained those derived from the B.1.351 and P.1 variants. Data are presented as geometric mean titer (95% confidence interval [CI]) for anti-spike antibody concentration and mean (95% CI) for IFN-γ concentrations. *Statistically significant ($P < .05$). Abbreviations: BAU, binding antibody units; BI, breakthrough infection; IFN-γ, interferon gamma; IGRA, interferon-γ release assay; ns, not significant; Sp, spike protein antigen.

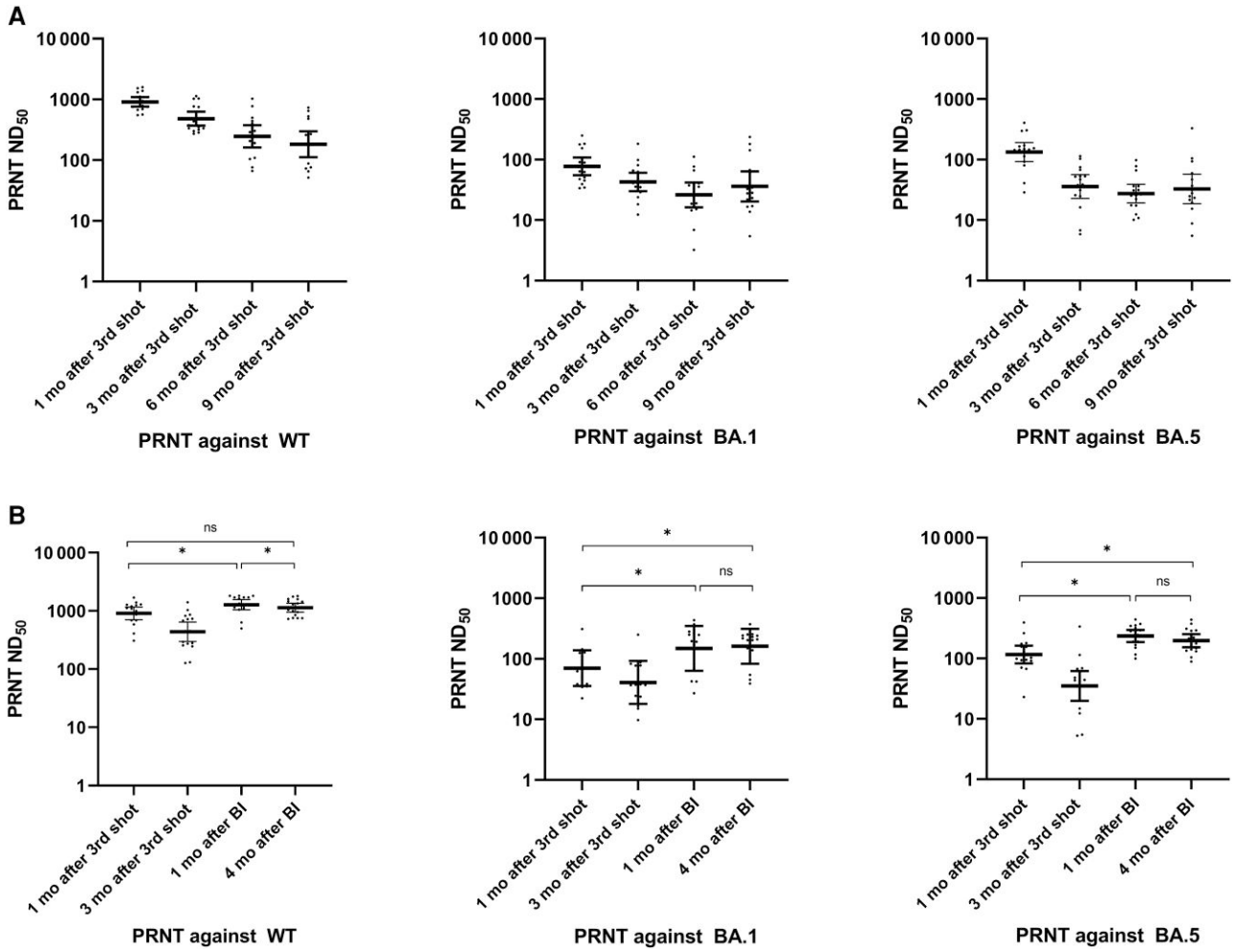


**Figure 3.** Neutralization titers against wild-type virus and Omicron BA.1 and BA.5 variants following the heterologous third BNT162b2 vaccination. Changes in neutralizing antibody concentrations following the third heterologous BNT162b2 vaccination dose in the no breakthrough infection (BI)–experienced group (*A*) and with BI in the BA.1/BA.2 period group (*B*) after the third-dose vaccination. Data are presented as geometric mean titer (95% confidence interval) of neutralizing antibody concentration. *Statistically significant ($P < .05$). Abbreviations: BI, breakthrough infection; $ND_{50}$, 50% neutralization dilution; ns, not significant; PRNT, plaque reduction neutralization test; WT, wild-type virus.

immune response toward a new strain; this was first proposed by Thomas Francis in 1960 [17, 18].

However, hybrid immunity induced by vaccination followed by infection is also known to increase the overall titers with the capacity to bind to and neutralize variants, including Omicron [10]. Therefore, the final status of the hybrid immunity may vary depending on the type of vaccine received, timing, and strain of the BI. A recent study conducted in Korea showed that significantly augmented neutralizing activity against the Omicron variant after the third vaccination dose compared with that of the second dose was observed in only 3 doses of the BNT162b2 vaccination group and not in the ChAdOx1-ChAdOx1-BNT162b2 or ChAdOx1-BNT162b2-BNT162b2 groups [19]. Data on the immune responses for each vaccination strategy and variant strain are essential. In this study, the findings of antigenic imprinting, as previously reported in other studies, were also observed after BI.

Neutralizing antibody titers against Omicron BA.1 after BI during the BA.1/BA.2 period demonstrated statistically similar values compared with that of BA.5 in this study. Omicron BA.1 BI is known to augment neutralizing titers against variants, including BA.2, but not against BA.4/BA.5 [16]. However, studies suggest that BA.2 BI is associated with broad neutralizing activity against variants, including BA.2 and its descendants BA.4/BA.5 [16, 20, 21]. In the current study, we considered the BA.1 and BA.2 outbreak period as 1 period in the analysis. Considering that the BA.2 outbreak period was longer than the BA.1 outbreak period, the BI that occurred in the BA.1/BA.2 outbreak period may not have been caused by BA.1 but rather by BA.2.

In this study, >95% of participants who experienced BI showed anti-nucleocapsid antibody seroconversion, significantly higher than that reported in other studies of vaccine BIs. Follmann et al reported that only 40.4% of patients with

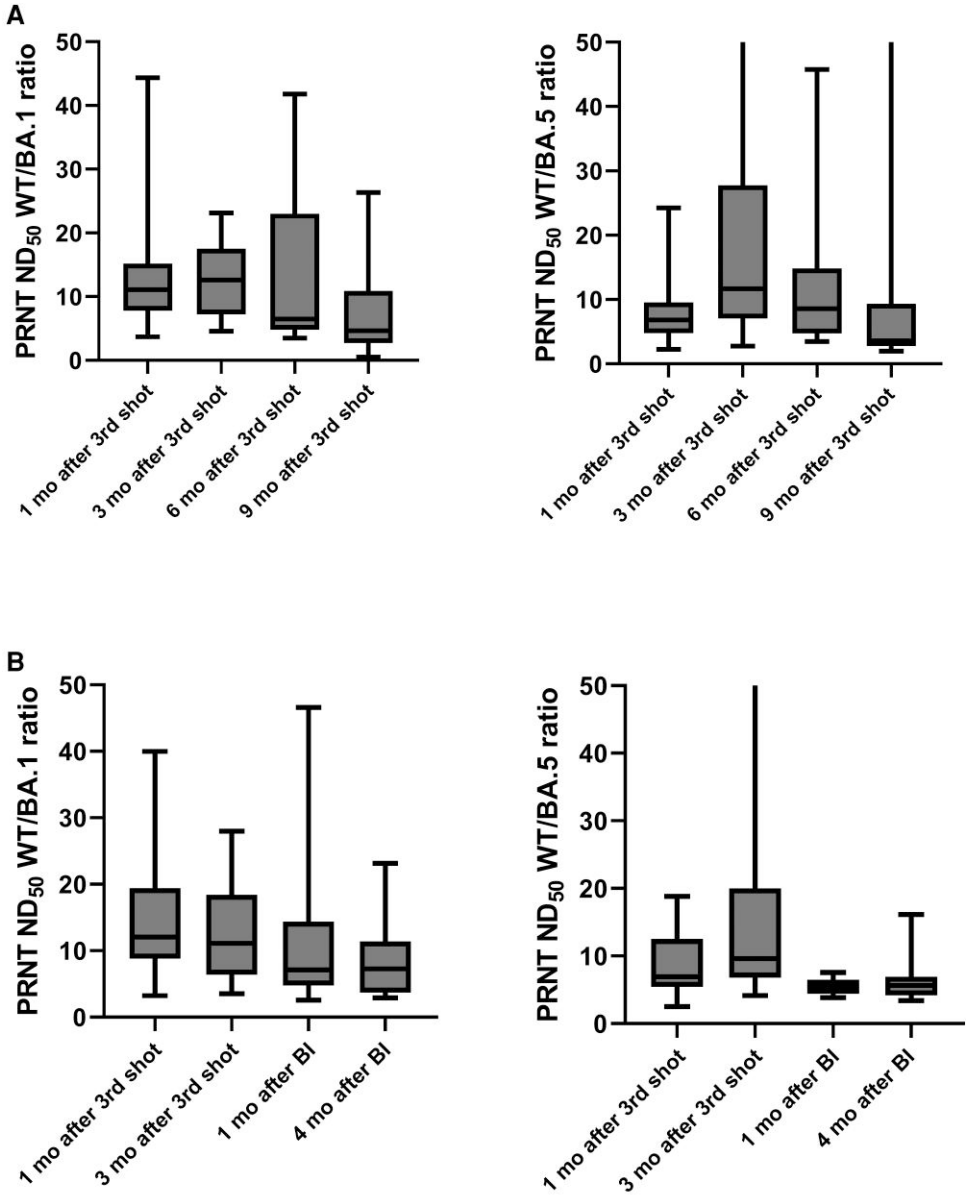


**Figure 4.** Ratio of neutralization titers against Omicron BA.1 or BA.5 to those against wild-type virus following the third heterologous BNT162b2 vaccination dose. Changes in the ratio of plaque reduction neutralization test 50% neutralization dilution against wild-type virus and Omicron BA.1 or BA.5 following the third heterologous BNT162b2 vaccination dose in the no breakthrough infection (BI)–experienced group (*A*) and the BI in the BA.1/BA.2 period group (*B*) after the third-dose vaccination. Abbreviations: BI, breakthrough infection; $ND_{50}$, 50% neutralization dilution; PRNT, plaque reduction neutralization test; WT, wild-type virus.

BI had seroconversion after 2 doses of the mRNA vaccine [22]. Possible reasons for this discrepancy include the different ethnicity and age group of the participants, the different study period and epidemic SARS-CoV-2 strain, and different vaccine protocols. In another Korean study conducted from November to December 2021, more similar to the results of this study, HCWs who received 2 doses of the ChAdOx1 vaccine and an mRNA booster had a 79.3% anti-nucleocapsid antibody seropositivity rate [23].

This study had several limitations. The relatively small sample size, especially for PRNT, may limit the general applicability of the findings. However, while most studies have evaluated neutralizing activity using pseudovirus-neutralizing assays, we used the PRNT method to evaluate the actual protective and cross-neutralizing activities against WT and variant viruses. Furthermore, the participants were relatively young and healthy; these data might not reflect those of older age, with high comorbidity, or with immunocompromised conditions. Furthermore, not all BIs were confirmed by the positive PCR

or antigen test; in the case of BI defined as a serologic diagnosis, there may be a bias toward the serologic responders, and it is difficult to determine the exact date of the BI. Also, the exact SARS-CoV-2 strain causing BI could not be identified. Last, the Omicron antigen was unavailable for use in the analysis of cell-mediated immunity through IGRA, as it has not yet been produced. Therefore, we could not analyze the specific cellular response to the Omicron variant in this study.

In conclusion, Omicron BI in individuals receiving 2 primary doses of the ChAdOx1 vaccine and BNT162b boosters leads to an increase in neutralizing antibody titers against the WT virus, BA.1, and BA.5. The neutralizing activity against the WT virus was particularly prominent. The findings of this study may serve as a basis for establishing future vaccine strategies. Primary vaccination and boosting, as well as the timing and type of BI, can significantly affect antigenic seniority and imprinting-mediated immune responses. Therefore, data specific to each vaccination are essential, and further research is required to analyze the immune response to simultaneous immunization with multiple variant antigens, such as bivalent COVID-19 vaccines.

## Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

## Notes

***Author contributions.*** J. Y. C. and W. S. C. were the co-principal investigators of this study and contributed to the conceptualization. J. Y. A. and J.-H. K. were the co-first authors of this manuscript and contributed equally to the original draft writing. S. B., K. H. L., Y. S. P., and K.-H. S. contributed to the conceptualization of the study protocol. K. R. P., S.-H. K., Y. G. S., Y. C. K., E. S. K., H. W. J., S.-W. K., K. T. K., W. S. C., and J. Y. C. were responsible for the site work, including participant recruitment, data collection, and follow-up. S. B., K. H. L., Y. C. K., and Y. S. P. verified the data. J. Y. A., J.-H. K., K.-H. S., E. S. K., and K. T. K. performed the data analysis and interpretation. K. R. P., S.-H. K., H. W. J., S.-W. K., and J. Y. C. performed the immunogenicity tests. J. Y. A., J.-H. K., W. S. C., and J. Y. C. analyzed the immunogenicity data. All authors contributed to the article preparation, including reviewing and editing, and approved the final version for submission. All authors had full access to all the data in the study and were responsible for the decision to submit for publication.

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