Vaccine: X 20 (2024) 100565

Contents lists available at ScienceDirect

Vaccine: X

journal homepage: www.elsevier.com/locate/jvacx

# Time-course analysis of antibody and cytokine response after the third SARS-CoV-2 vaccine dose

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ARTICLE INFO

*Keywords:*
Antibody response
Cytokine response
SARS-CoV-2
Third dose
JAK/STAT pathway

ABSTRACT

The widespread administration of an additional dose of the SARS-CoV-2 vaccine has been promoted across adult populations, demonstrating a robust immune response against COVID-19. Longitudinal studies provide crucial data on the durability of immune response after the third vaccination. This study aims to explore the antibody response, neutralizing activity, and cytokine response against the SARS-CoV-2 ancestral strain (wild-type) and its variants during the timeline before and after the administration of the third vaccine dose. Anti-spike antibody titers and neutralizing antibodies blocking ACE2 binding to spike antigens were measured in 62 study participants at baseline, and on days 7, 21, and 180 post-vaccination. Cytokine levels were assessed at the same points except for day 180, with an additional measurement on day 3 post-vaccination. The analysis revealed no substantial variation in anti-spike antibody titer against the SARS-CoV-2 ancestral strain between the pre-vaccination phase and three days following the third dose. However, a significant nine-fold increase in these titers was observed by day 7, maintained until day 21. Although a decrease was observed by day 180, all participants still had detectable antibody levels. A similar trend was noted for neutralizing antibodies, with a four-fold rise by day 7 post-vaccination. At day 180, a diminution of neutralizing antibody titers was evident for both wild-type and all variants, including Omicron subvariant. A transient increase in cytokine activity, notably involving components of the Janus kinase (JAK)/signal transducers and activators of transcription (STAT) pathway, such as CXCL10 and IL-10, was observed within three days after the third dose. This study underscores a distinct amplification of humoral immune response seven days following the third SARS-CoV-2 vaccine dose and observes a decline in neutralizing antibody titers 180 days following the third dose, thus indicating the temporal humoral effectiveness of booster vaccination. A short-term cytokine surge, notably involving the JAK/STAT pathway, highlights the dynamic immune modulation post-vaccination.

## Introduction

Since the emergence of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic in 2020, coronavirus disease 2019 (COVID-19) has presented a formidable global health challenge. Multiple guidelines and recommendations for managing and preventing infection have been proposed, with vaccination emerging as a paramount strategy [1]. The preliminary two-dose regimen of vaccination has demonstrated robust immune responses and effectiveness in mitigating COVID-19, but a decline in antibody titers coupled with the advent of new variants, notably Delta and Omicron, have culminated in a significant number of breakthrough infections [2–7]. Consequently, a third vaccine dose has been advocated to amplify attenuated immune responses and diversify immunity against variants of SARS-CoV-2 of

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https://doi.org/10.1016/j.jvacx.2024.100565
Received 1 February 2024; Received in revised form 20 September 2024; Accepted 24 September 2024
Available online 24 September 2024

concern. This additional dose has garnered approval for the general population in South Korea, and existing studies conducted in this region attest to its protective effect against SARS-CoV-2 infections [8–10].

The protective effect of COVID-19 vaccines is predominantly contingent on the humoral immune system, which mobilizes antibodies and neutralizing activity as principal indicators of post-vaccination protection [11]. Furthermore, proinflammatory responses, including cytokine release, play a fundamental role in the host response to viral infections and the ensuing immunopathology [12]. Hence, the assessment of anti-SARS-CoV-2 antibody titers, neutralizing activity, and cytokines could be instrumental in understanding the immune response elicited by the vaccine and forecasting its efficacy. Effectiveness of the third vaccines in reducing infection and severe illness was well documented, and our insight into the immune responses it elicits continues to expand [13,14]. To guide the refinement and development of future vaccines, further data on the sequential immunologic response post-vaccination are warranted. Thus, the current study aimed to examine the immune response to SARS-CoV-2 ancestral strain (hereafter referred to as wild-type) and its variants in healthy volunteers with no prior SARS-CoV-2 infection. This was achieved by measuring anti-spike antibody titers, neutralizing activity, and cytokines at multiple time points before and after the administration of the third vaccine dose and by comparing antibody levels in accordance with infection status following vaccination.

## Methods

### *Participants and study design*

This study involved a total of 62 healthy volunteers, all of whom had not been previously infected with SARS-CoV-2 and had given informed consent. Their infection status was verified by quantifying the anti-nucleocapsid antibody. Within this group, 44 had been part of a former study conducted by the present authors, which probed antibody and cytokine responses post-heterologous vaccination with the ChAdOx1 nCoV-19 vector (AZD1222, AstraZeneca) and BNT162b2 mRNA vaccines (Pfizer/BioNTecn) (hereafter referred to as ChAd and BNT, respectively) [6]. The study spanned from January to September 2022, during which blood samples were drawn on the day of vaccination and subsequently on days 3, 7, 21, and 180 following the third vaccination. Information on SARS-CoV-2 infection status within 180 days following the third dose was also assembled to facilitate a comparison of antibody levels according to infection status. If any participant exhibited COVID-19 symptoms, such as respiratory issues, a SARS-CoV-2 PCR test was performed to assess infection status. In South Korea, due to governmental approval and availability, the ChAd, an adenovirus-based vaccine (Ad-based vaccine), was predominantly used for the initial dose. However, due to sporadic serious adverse events like thrombosis with thrombocytopenia syndrome associated with the Ad-based vaccines, those who received ChAd as a first dose were offered a subsequent mRNA vaccines, either BNT or mRNA-1273 (Moderna).

Additionally, six previously infected but unvaccinated participants (recorded in February 2022) were enrolled to serve as a positive control group, while nine uninfected and unvaccinated individuals constituted the negative control group. Blood samples from the positive control group were procured approximately 19 days following qRT-PCR confirmed COVID-19 infection, with an interquartile range from 18 to 22 days. The Asan Medical Center, Ulsan University College of Medicine's Institutional Review Board (IRB No. 2021-0898) approved the study protocol, and all participants provided their informed consent.

### *SARS-CoV-2 serology and neutralizing response*

Serological assessments for SARS-CoV-2 were performed using two distinctive SARS-CoV-2 antibody kits that leverage the electrochemiluminescence method, which detects antibodies in blood specimens by producing light via an electrical current. The V-PLEX SARS-CoV-2 Panel 5 (IgG) kit, supplied by Meso Scale Discovery (MSD, Maryland, USA), was used on the day of vaccination and again on days 7, and 21 post-vaccination. This kit facilitates the identification of circulating IgG antibodies responsive to a range of SARS-CoV-2 antigens, including the wild-type and variants, Alpha (B.1.1.7), Beta (B.1.351), and Gamma (P.1). Outcomes were quantified in arbitrary units per milliliter (AU/mL). Concurrently, the Elecsys® Anti-SARS-CoV-2 S immunoassay kit, designed by Roche Diagnostics International Ltd, was utilized at day 180 to detect and quantify total antibodies, including IgG, against the receptor-binding domain of the SARS-CoV-2 spike protein. Results were presented in units per milliliter (U/mL), with titers of 5000 U/mL or higher recorded as 5000 U/mL.

Neutralizing responses were assessed at days 0, 7, and 21 using the V-PLEX SARS-CoV-2 Panel 19 (ACE2) kit (MSD), capable of quantifying antibodies that restrict the binding of ACE2 to spike antigens of the wild-type and several SARS-CoV-2 variants. The V-PLEX SARS-CoV-2 Panel 29 (ACE2) kit (MSD) was used to evaluate neutralizing antibody titers at day 180. The resulting data was expressed either in units per milliliter (U/mL) or as a percentage of inhibition, with titers of 200 U/mL or above documented as 200 U/mL.

### *Quantification of cytokines*

To gauge proinflammatory cytokines, serum samples were procured from all study participants and analyzed using the Human XL Cytokine Luminex Performance Base Kit from R&D Systems, utilizing a Bio-Plex Manager 6.1 system, courtesy of Bio-Rad. Cytokines that were assessed included IFN-α, IFN-γ, IL-1Rα, IL-6, IL-10, CXCL10/IP-10, and VEGF.

### *Statistical analysis*

Continuous variables were depicted using median and interquartile range (IQR). Comparative evaluations of antibody and cytokine concentrations were conducted utilizing the Mann-Whitney *U* test for two groups, and Dunn's Kruskal-Wallis multiple comparison test for larger groups. *P*-values for cytokines were computed using the two-stage linear step-up procedure of Benjamini, Krieger, and Yekutieli. All statistical computations and data representation were conducted using GraphPad PRISM, version 9.0 (GraphPad Software, Inc, USA), and IBM SPSS Statistics, version 20.0 (IBM Corporation, Armonk, NY, USA). A *P*-value below 0.05 was deemed statistically significant and denoted as $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$.

## Results

### *Study design and participant characteristics*

The demographic and baseline characteristics of our cohort of 62 participants are detailed in Table 1. The participants had a median age of 38 years (IQR, 34–43), with 82.3 % (n = 51) identifying as female. The median time span between the administration of the second and third vaccine doses was 115 days (IQR, 107–126). Blood samples were procured either on the day of vaccination or the day prior (noted as D0) and then at subsequent intervals post-vaccination on days 1–4 (median 3 days), 7–12 (median 7 days), 20–27 (median 21 days), and 174–183 (median 182 days) (referred to as D3, D7, D21, and D180). The vaccines administered varied among the participants; however, the most commonly received type was an Ad-based-mRNA-mRNA vaccines, reported in 69.8 % of the cases. Of the 62 participants, 24.2 % (n = 15) contracted a SARS-CoV-2 infection within a timeframe of two to five months following the third dose, with the shortest and longest durations from vaccination to infection noted as 44 days and 126 days, respectively. Comparative control groups were constituted for this study. The positive control group was predominantly male (83 %) and had a

**Table 1**
Characteristics of study participants before and after the third dose.

| Characteristics | All (n = 62) |
|---|---|
| Age (years), median (IQR) | 38 (34–43) |
| Gender, n (%) | |
| Female | 51 (82.3) |
| Male | 11 (17.7) |
| Medical condition, n (%) | 5 (8.1) |
| Hypertension | 2 (3.2) |
| Diabetes mellitus | 2 (3.2) |
| Autoimmune disease | 1 (1.6) |
| Interval between the second and third dose (days), median (IQR) | 115 (107–126) |
| Vaccine type (first – second – third) | |
| ChAd-mRNA-mRNA | 44 (71.0) |
| ChAd-ChAd-mRNA | 6 (9.7) |
| mRNA-mRNA-mRNA | 12 (19.4) |
| COVID-19 infection within 180 days of the third dose, n (%) | 15 (24.2) |
| Interval between the third dose and COVID-19 infection (days), median (IQR) | 91 (72–99) |

Data are presented as median [interquartile range], or number (%).
IQR: interquartile range.

median age of 63 years (IQR, 55–65). The negative control group, on the other hand, was comprised of 56 % men, with a median age of 44 years (IQR, 43–45).

*Kinetics of anti-spike and neutralizing antibodies*

First, we measured circulating antibody responses in serum samples from the individuals receiving the third vaccination using enzyme-linked immunosorbent assay (ELISA) (Table 2, Figs. 1–2). A substantial nine-fold increase in anti-spike antibody levels was noted at D7 compared to baseline (D0), a level which persisted to D21. Specifically, the anti-spike titers for the wild-type strain rose from 23,263 AU/mL (interquartile range [IQR], 12,949–61,352) at D0 to 212,792 AU/mL (IQR, 135,088–271,525) at D7 (Table 2). The anti-spike concentrations against the wild-type strain in the positive controls were lower than those observed at D7 and D21, although the difference was not statistically significant ($P = 0.757$ and $P = 0.882$, respectively). A similar trend was observed for the spike proteins of the Alpha, Beta, and Gamma variants (Fig. 1A). The detection of anti-nucleocapsid antibody confirmed individual participant's history of COVID-19 infection. At D180, anti-spike antibodies were detectable for all participants (Fig. 2A).

The third vaccination dose prompted an approximately four-fold surge in the level of neutralizing antibodies against the wild-type strain, increasing from 7 U/mL (IQR, 5–10) at D0 to 31 U/mL at D7 (IQR, 16–47) and D21 (IQR, 21–45) (Table 2). The neutralizing antibody levels against all other variants followed a similar trajectory to that of the wild-type strain (Fig. 1C). At D180, a decrease in neutralizing antibody titers was observed across most variants in uninfected participants, though this reduction was not statistically significant for the wild-type strain ($P = 0.101$) and other variants. However, for BA.2.75, the reduction in neutralizing antibody titers was statistically significant ($P = 0.023$) (Fig. 2B). The level of neutralizing antibodies in the positive control group showed similar to that observed after the second and third vaccination dose (Fig. 1C).

**Table 2**
Anti-spike Ab titers and neutralizing antibody responses against SARS-CoV-2 wild-type before and after the third dose.

| Characteristics | | *P* value |
|---|---|---|
| Anti-spike antibody titer, median (IQR), AU/mL | | <0.001 |
| Before the third dose | 23,263 (12,949–61,352) | |
| At day 7 | 212,792 (135,088–271,525) | |
| At day 21 | 183,345 (130,064–280,569) | |
| Anti-spike antibody titer, median (IQR), U/mL | | |
| At day 180 | 3960 (1559–5000) | |
| Neutralizing antibody titer, median (IQR), U/mL | | <0.001 |
| Before the third dose | 7 (5–10) | |
| At day 7 | 31 (16–47) | |
| At day 21 | 31 (21–45) | |
| At day 180 | 10 (3–32) | |

Data are presented as median [interquartile range].
IQR: interquartile range.

No significant differences were observed in the anti-spike and neutralizing antibodies against both wild-type and variants between participants who contracted SARS-CoV-2 after vaccination and those who remained uninfected up to 21 days post-vaccination. Conversely, at D180, infected participants demonstrated significantly elevated levels of anti-spike and neutralizing antibodies against the wild-type strain compared to uninfected individuals (Fig. 2A). Furthermore, the levels of neutralizing antibodies against various variants, such as Delta, BA.2.75, BA.4, and BA.5, were also significantly higher in infected participants relative to uninfected counterparts. Both infected and uninfected participants exhibited significantly elevated neutralizing antibody titers against wild-type, B.167.2, and BA.2.75, as compared to Beta, BA.2.12.1, and BA.2 strains (Fig. 2B; e-Table 1; e-Fig. 1). When comparing the levels of anti-spike and neutralizing antibodies after the first, second, and third vaccine doses, it was observed that the antibody levels decreased just before administering the third dose and increased once again (e-Fig. 2).

*Kinetics of serum cytokine levels*

Circulating levels of CXCL10, IL-1Rα, and IL-10 showed transient increases within three days post-vaccination, consistent with earlier responses seen after the first (ChAd) and second (BNT) vaccine doses [6]. Levels of seven cytokines were measured at days 3, 7 and 21 following third vaccination. Among the seven cytokines analyzed, CXCL10 and IL-1Rα showed a transient elevation within three days post-vaccination, which returned to baseline at D7. Specifically, IL-1Rα demonstrated a 1.80-fold increase from D0 to D3, followed by a 2-fold decrease from D3 to D7. Similarly, CXCL10 exhibited a 3.25-fold increase from D0 to D3, with a subsequent 61 % reduction from D3 to D7. IL-10 levels also showed a significant rise at D3, with a 2.03-fold increase. Conversely, the levels of IFN-α and IFN-γ remained below the detection threshold of the utilized assay kit in all participants, and no statistically significant alterations were observed in the remaining three circulating cytokines: IL-1Rα, IL-6, and VEGF, following the third vaccine dose (Fig. 3).

Further analysis revealed an augmentation of the levels of CXCL10 and IL-1Rα after each subsequent vaccination, whereas IL-10 was reduced. The circulating levels of VEGF remained consistent across all vaccination doses; notably, even prior to the third vaccination, VEGF values on the day of administration (III-D0) were higher than those recorded on day 7 following the second vaccine dose (II-D7) (e-Fig. 3).

## Discussion

This study evaluated the durability of the immune response elicited by the third COVID-19 vaccination in healthy individuals. Our approach involved monitoring the trajectory of both antibody and cytokine responses over time considering the impact of COVID-19 infection on the antibody response during follow-up. The enrolled healthy individuals received the third vaccine dose four months after their second. Our findings indicated a considerable elevation in the presence of circulating anti-spike and neutralizing antibodies against different SARS-CoV-2 strains within seven days of administration. These augmented antibody levels were maintained for a period of 21 days following

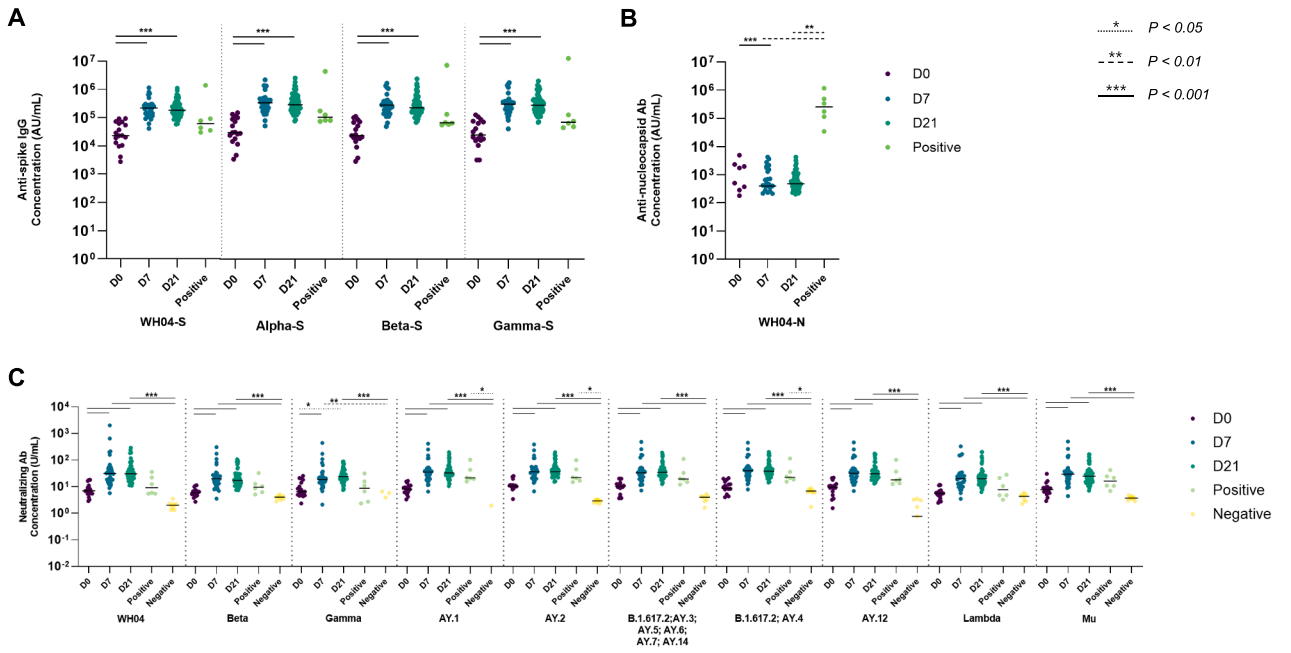


**Fig. 1.** Antibody and neutralizing antibody responses to SARS-CoV-2 following the third dose. (A) Anti-spike antibody responses across different strains. (B) Anti-nucleocapsid antibody response to SARS-CoV-2. (C) Neutralizing antibody responses to viral spike proteins, including levels of neutralizing antibodies in unvaccinated, seronegative study participants and previously infected SARS-CoV-2 study participants. Lines indicate medians. A $P$ value less than 0.05 was considered statistically significant and is presented as $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. In the case of Gamma and AY.1 variants, the median for the Negative group was 0, which is why it is not displayed in the figure.

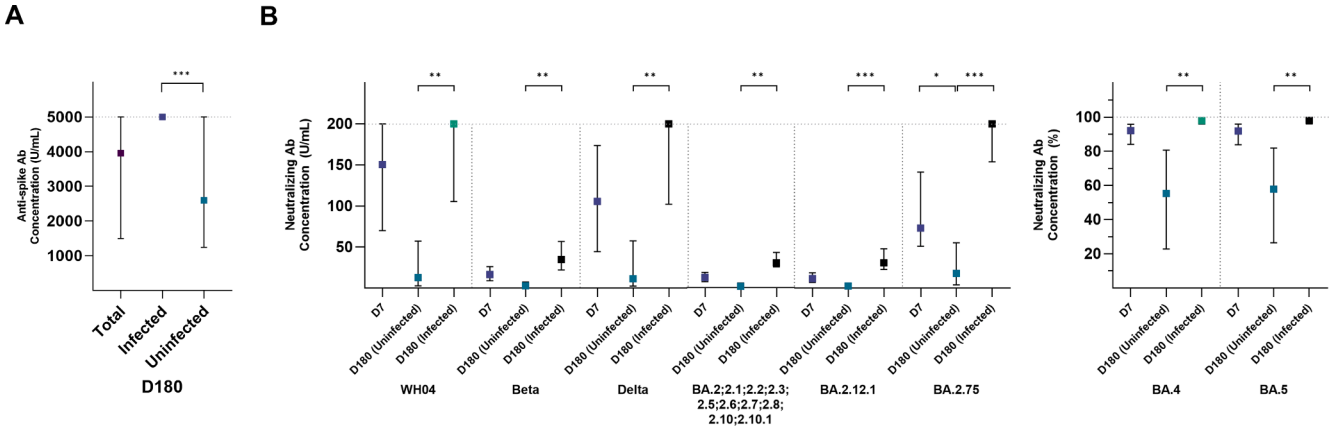


**Fig. 2.** Anti-spike antibody and neutralizing antibody responses 180 days following the third dose and antibody titers in accordance with infection status following vaccination. **(A)** Anti-spike antibody response at 180 days following the third dose. **(B)** Neutralizing antibody response at 180 days following the third dose. Dots indicate medians and lines indicate interquartile ranges. A $P$ value less than 0.05 was considered statistically significant and is presented as $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

vaccination. Nevertheless, a downward trend in neutralizing antibody titers was observed six months post-vaccination. It should be noted, however, that all participants continued to test positive for anti-spike antibodies at D180. Participants who contracted the virus following vaccination demonstrated significantly increased titers of both anti-spike and neutralizing antibodies against an array of variants, including the Omicron variants, when juxtaposed with their counterparts who did not contract infection after the vaccination. Our data also demonstrated transient increases in levels of cytokine CXCL10, IL-1Rα and IL-10, which were observable within three days following the administration of the third dose.

The majority of participants, upon receiving the third vaccine dose, demonstrated the generation of anti-spike and neutralizing antibodies, both of which serve as key indicators of immunity against viral infection. These findings align with previous research studies [15,16]. An important element in the evaluation of the third dose's efficacy lies in its capacity to swiftly trigger immune memory and enhance subsequent immune response. Research on antibody production kinetics has consistently shown that the anti-spike and neutralizing antibody titers peak roughly between the third and fifth weeks post-vaccination, following which they gradually decline [17–19]. Our prior research also evidenced a surge in antibody levels seven days following heterologous vaccination (ChAd followed by BNT) [6]. This study demonstrated a significant increase in antibody levels seven days following the third dose, a level which was maintained through the 21st day. As per a prior report, the neutralizing geometric mean titers against the wild-

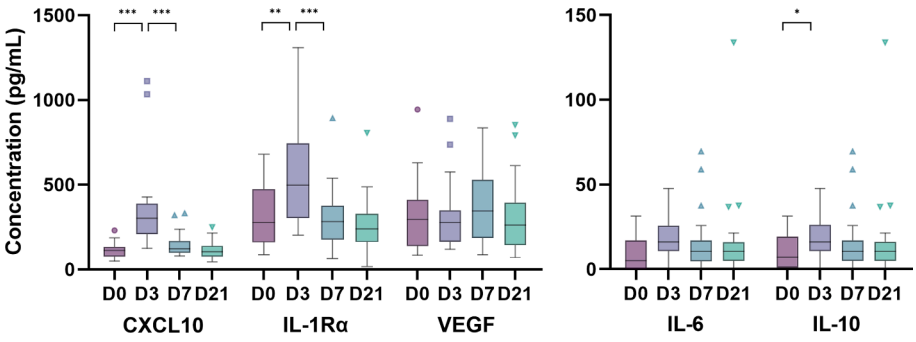


**Fig. 3.** Serum cytokine response following the third dose. Serum concentrations of cytokines prior to and post the third dose (pg/mL). Lines and boxes represent median and 25th and 75th percentiles, respectively, and whiskers indicate the range. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.

type virus were found to be approximately two-fold higher following the third dose when compared to after the second dose [20]. In parallel, the present study confirmed a roughly 1.5-fold increase in the anti-spike antibody level seven days after the third dose, reaching 212,792 AU/mL. This compares to 142,000 AU/mL, the level observed seven days after the second dose in our preceding study [6].

Our study revealed that 24.2 % of participants acquired SARS-CoV-2 infection within a span of two to five months after their third dose administration, coinciding with the dominance of Omicron variant BA.2 and BA.1 in South Korea. Following the second dose, there was an absence of substantial neutralizing inhibition against the Omicron variant (B.1.1.529) in the majority of participants [6]. However, following the third administration, most participants exhibited measurable neutralizing inhibition against Omicron subvariants, including BA.2 [21], BA.2.75, BA.4, and BA.5. Given the occurrence of breakthrough infections, this increase in neutralizing activity may not directly correlate with enhanced protection against Omicron infection, or it may be inadequate to confer robust protection. There were no significant disparities in neutralizing antibody titers based on breakthrough infection, at least within the limitations of the 21 days follow-up time for assessment of neutralizing titers. In line with these findings, a previous research also demonstrated a lack of correlation between neutralizing antibody titers at two weeks and three months after the booster vaccination and subsequent breakthrough Omicron infection [22]. It has been further elucidated by multiple studies that immunization against the wild-type virus tends to attenuate the severity of infection, rather than confer significant protective benefits against novel and highly immune-evasive variants like Omicron [11,23]. Consistent with these earlier reports, those participants in our study who contracted SARS-CoV-2 exhibit only mild symptoms and did not necessitate hospitalization. The fourth vaccination dose might be needed as a precautionary measure because we did observe a consistent trend of decaying neutralizing antibody at D180 compared to D21 for both wild-type and other variants in the uninfected individuals.

Previous research has established that hybrid immunity, a combination of natural infection and vaccination, results in superior humoral immune responses compared to vaccination alone [24–27]. This study concurs, demonstrating that six months following the third vaccine dose, individuals previously infected with COVID-19 exhibited higher levels of both anti-spike and neutralizing antibodies against all variants, including Omicron, than those without prior infection. This finding supports the concept of hybrid immunity.

The Janus kinase (JAK)/signal transducers and activators of transcription (STAT) pathway encompasses diverse cytokine signaling mechanisms, among which IL-6, IL-2, IL-4, IL-15, and IL-10 levels are reported to rise in COVID-19 [28–30]. This pathway is also implicated in the cytokine storm associated with COVID-19 [31,32]. Knabl, L et al. indicates that vaccinated patients manifest a heightened JAK/STAT-mediated immune transcriptome response compared to their unvaccinated counterparts [33]. The present study notes a transient increase in CXCL10, IL-1Rα, and IL-10 levels within three days following the third vaccine dose, similar to the previous findings of an early surge of CXCL10 and IL-1Rα after the second dose [6]. CXCL10, which is swiftly upregulated after vaccination, is regulated by IFN-γ through JAK/STAT pathway [34,35]. This upsurge aligns with research conducted by Cristina et al. who reported that the BNT vaccine led to a systemic cytokine/chemokine signature, including increases in IL-15, IFN-γ, and IP-10/CXCL10, which were interpreted as playing significant roles in eliciting both innate and adaptive immune responses [36]. The IL-1 and IL-1Rα have also been identified as key to activating innate signaling pathways associated with RNA vaccines [37]. However, upsurge of cytokines suggest the possibility of cytokine overproduction by repeated vaccination in immunocompetent patients.

While patients with chronic lymphocytic leukemia may not show an antibody response following COVID-19 vaccination, JAK-STAT signaling is evident within two days post-vaccination [38]. Accordingly, our findings suggest that CXCL10 and IL-1Rα could be utilized to monitor the immune response to vaccination in immunocompromised individuals whose antibody reaction was attenuated.

This study is characterized by several limitations that warrant consideration. First, the participant sample size was relatively constrained due to the investigation being conducted at a single center. Second, our sample predominantly consisted of young females, which introduces potential bias when interpreting results. It is widely recognized that women generally mount stronger inflammatory, antiviral, and antibody-mediated immune responses compared to men. Additionally, as individuals age, their immune system gradually undergoes functional decline [39–42]. These factors limit the generalizability of our findings across different populations. However, despite these limitations, our study provides valuable insights into the dynamics of immune response following the third SARS-CoV-2 vaccine dose, specifically highlighting the early kinetics of antibody production and the transient cytokine surge, which are critical for understanding short-term vaccine efficacy and long-term immune durability. The third notable limitation stems from the diagnostic protocol employed after D21, which relied solely on symptomatic testing for COVID-19 infection. Given that anti-nucleocapsid antibodies were not assessed at D180, the study may have inadvertently overlooked cases of asymptomatic infection.

In conclusion, our research offers a notable observation of the marked enhancement of humoral immune responses against various SARS-CoV-2 strains seven days following the administration of the third vaccine dose, alongside a documented trend of diminishing neutralizing antibody titers at D180. We found significantly elevated levels of anti-spike and neutralizing antibodies in participants who had both been vaccinated and previously infected compared to those who had only been vaccinated. The study also observed transient increases in certain cytokines including the JAK/STAT pathway, a key component in cytokine signaling and immune response modulation. These findings offer valuable insights pertaining to the durability of immunity conferred by a

third dose vaccine strategy.

## CRediT authorship contribution statement

**Hyeon Hwa Kim:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Hye Kyung Lee:** Writing – review & editing, Investigation. **Lothar Hennighausen:** Writing – review & editing, Investigation. **Priscilla A. Furth:** Writing – review & editing. **Heungsup Sung:** Writing – review & editing, Supervision, Software, Investigation. **Jin Won Huh:** Writing – review & editing, Supervision, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jvacx.2024.100565.

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