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Robust neutralizing antibody responses after single-dose BNT162b2 vaccination at long intervals from prior SARS-CoV-2 infection and ceiling effect with repeated vaccination

Dear editors,

In response to the continued emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants and the time-dependent decline in neutralizing antibodies (nAbs) after coronavirus disease 2019 (COVID-19) vaccination, various strategies for repeated vaccination have been adopted (1-3). There may be significant differences in vaccine immune response depending on whether the person is previously infected with SARS-CoV-2, the time elapsed after recovery from infection, and the interval between vaccinations. Those are very important to establish an optimal vaccination strategy in a situation where the COVID-19 transitions to endemic spread and repeated vaccinations must be considered. In the previous study by Mak et al., a second COVID-19 vaccination in prior-infected individuals did not further increase anti-SARS-CoV-2 immunoglobulin G responses in comparison to single-dose vaccination (4). Thus, we evaluated anti-SARS-CoV-2 neutralizing antibody responses in wild-type (WT) SARS-CoV-2-infected individuals who received the primary series and booster dose of BNT162b2.

This prospective cohort study was conducted on 36 individuals infected with the ancestral Wuhan-1 strain of SARS-CoV-2 who received three doses of the BNT162b2 COVID-19 vaccine. We investigated the kinetics of anti-SARS-CoV-2 nAbs by measuring anti-SARS-CoV-2 nAbs 3 weeks after the first dose (V1–3 w), 1 month after the second dose (V2–1 m), 3 months after the second dose (V2–3 m), and 3 weeks after the third dose of BNT162b2 (V3–3 w). The median day (range) from the COVID-19 diagnosis to each dose of BNT162b2 vaccination was 17.2 months (498–536 days) for the first dose, 18.6 months (533–578 days) for the second dose, and 22.5 months (652–696 days) for the third dose. Cross-reactive immunogenicity was also evaluated against delta and omicron variants. The study protocol was approved by the Institutional Review Board of Korea University Guro Hospital (approval no.:2021GR0099), and written informed consent was obtained from all participants.

For the nAb analysis, a plaque reduction neutralization test (PRNT) was performed using WT SARS-CoV-2 (hCoV/Korea/KCDC03/2020), delta variant (B.1.617.2 lineage, hCoV-19/Korea/KDCA229079/2021), and omicron variant (lineage B.1.1.529, hCoV-19/Korea/KDCA447321/2021). The mixture of serum dilution/virus (40 PFU/well) was incubated at 37 °C for 2 h, added to a plate seeded with Vero E6 cells, and incubated at 37 °C for 1 h, followed by the addition of 0.5% agarose (Lonza, Basel, Switzerland). After 2–3 days of incubation, the cells were fixed with 4% paraformaldehyde and stained to visualize plaques. A reduction in plaque count of 50% (PRNT50) was then calculated for the median neutralizing titer (ND50) using the Spearman–Karber formula, and an ND50 > 1:20 was considered positive. As for the comparison of geometric mean titer (GMT) of nAbs from paired sera at each time point, the Wilcoxon signed rank test was performed.

All participants were women, and the median age was 50 years (range, 38–57 years). SARS-CoV-2 infection was diagnosed by real-time polymerase chain reaction. Although the viral sequences of SARS-CoV-2 were not investigated at the time of diagnosis, our study population was assumed to be infected with the ancestral Wuhan-1 strain of SARS-CoV-2 because they were diagnosed with COVID-19 during the early phase of the pandemic in March 2020. The GMT of nAbs of V1–3 w against SARS-CoV-2 WT was the highest among serial time points (112.9 at pre-vaccination, 7324.4 in V1–3 w, 5287.6 in V2–1 m, 2173.2 in V2–3 m and 3409.3 in V3–3 w) (Table S1). Interestingly, in the comparison of paired sera collected longitudinally from each subject, the GMT of V1–3 w nAbs was significantly higher than that of V2–1 m (P = 0.008, Fig. 1A), indicating that the second dose of vaccination at 3-week intervals did not raise the nAb titers sufficiently in individuals infected with SARS-CoV-2 18 months ago. Although the third dose of the vaccine showed a booster effect on the antibodies neutralizing SARS-CoV-2 (P<0.001), the titers in V3–3 w were rather lower than those of V1–3 w and V2–1 m.

As for cross-reactive immunogenicity, the titers of nAbs against the omicron strain were significantly attenuated compared with those against WT (21–30 fold) in individuals previously infected with SARS-CoV-2 who received the primary series and booster dose of BNT162b2, while nAb titers against the delta strain were 1.9–2.5 fold lower than those against WT (Fig. 1B and Table 1). In our study, the fold difference of titers of nAbs between the WT and omicron was larger than the results of other studies (5-7). Multiple factors may be involved in this difference, including the heterogeneity in neutralization assays, characteristics of the study population, and the timing of prior SARS-CoV-2 infection and vaccination. However, the fold difference between the ancestral strain and variants was found to be smaller after the booster dose of BNT162b2, which was consistent with the results of other studies (7, 8).

There has been a lack of information regarding the optimal intervals of vaccination. Determination of the interval required for repeated COVID-19 vaccination is essential to induce an optimal immune response that protects against SARS-CoV-2 (9, 10). When vaccinated at a sufficiently long interval after natural SARS-CoV-2 infection and when memory B-cells were fully mature, single-dose vaccination induced robust neutralizing antibody responses, and cross-reactive immunity was also induced against the omicron variant. However, repeated vaccination at short-term intervals may elicit a limited boosting effect (ceiling effect) on the nAb response.
especially in individuals who have already gained substantial levels of nAbs. It is necessary to investigate whether the same phenomenon will occur in the case of booster vaccinations at longer intervals following the primary vaccination series without prior infection.

In conclusion, although the number of samples is small and a longitudinal analysis was not performed at all time points in all subjects, we found that robust antibody response would be induced even with a single-dose COVID-19 vaccination when vaccinated at long intervals (more than 12 months) after SARS-CoV-2 infection. However, remarkable ceiling effects were observed with repeated vaccination.

Author contributions

JYN and JYS conceived and designed the study. JYN and JYS prepared the manuscript and analyzed the data. All authors contributed to the acquisition of the clinical and laboratory data. JYN, BK and JYS contributed to data interpretation and statistical analysis. All the authors critically reviewed the manuscript for intellectual content and approved the final draft for submission.

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Declaration of Competing Interest

The authors declare no conflict of interest.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2022.07.024.

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Table 1

|                      | Pre-vaccination (n = 11) | V1–3 w (n = 11) | V2–1 m (n = 10) | V2–3 m (n = 10) | V3–3 w (n = 7) |
|----------------------|--------------------------|----------------|----------------|----------------|----------------|
| Wild-type, GMT       | 1962.2                   | 7207.5         | 4861.3         | 2452.7         | 3928.1         |
| (95% CI)             | (84.2–457.2)             | (5469.6–9497.6)| (3404.6–6941.3)| (1495.1–4023.7)| (2448.2–6302.4)|
| Delta variant, GMT   | 37.1                     | 3260.6         | 2039.5         | 987.9          | 2039.7         |
| (95% CI)             | (21.4–64.2)              | (2171.7–4895.3)| (1176.4–3515.7)| (601.7–1622.1)| (1048.6–3967.7)|
| Omicron variant, GMT | 31.3                     | 294.1          | 161.5          | 84.6           | 186.7          |
| (95% CI)             | (20.6–47.6)              | (198.5–435.7)  | (83.9–311.0)   | (48.3–148.1)   | (81.3–428.3)   |

V1–3w: three weeks after the first dose; V2–1m: one month after the second dose; V2–3m: three months after the second dose; V3–3w: three weeks after the third dose of BNT162b2; GMT: geometric mean titer; CI: confidence interval.
References

1. Levin EG, Lustig Y, Cohen C, Fluss R, Indenbaum V, Amit S, et al. Waning Immune Humoral Response to BNT162b2 Covid-19 Vaccine over 6 Months. N Engl J Med 2021;385(24):e84 Dec 9 PubMed PMID: 34614326. Pubmed Central PMCID: PMCP522797. Epub 2021/10/07.

2. Magen O, Waxman JG, Makov-Assif M, Vered R, Dicker D, Hernan MA, et al. Fourth Dose of BNT162b2 mRNA Covid-19 Vaccine in a Nationwide Setting. N Engl J Med 2022;386(17):1603–14 Apr 28 PubMed PMID: 35417631. Pubmed Central PMCID: PMCP9020581. Epub 2022/04/14.

3. Moreira ED Jr, Kitchin N, Xu X, Dychter SS, Lockhart S, Curtman A, et al. Safety and Efficacy of a Third Dose of BNT162b2 Covid-19 Vaccine. N Engl J Med 2022 Mar 23 PubMed PMID: 35320659. Pubmed Central PMCID: PMCP9006787. Epub 2022/03/24.

4. Mak WA, Koellemann GMC, van der Vliet M, Keuren F, Ong DSY. SARS-CoV-2 antibody and T cell responses one year after COVID-19 and the booster effect of vaccination: a prospective cohort study. J Infect 2022;94(2):171–8 Feb PubMed PMID: 34896516. Pubmed Central PMCID: PMCP856569. Epub 2021/12/14.

5. Celé S, Jackson L, Khoury DS, Khan K, Moyo-Gwete T, Tegally H, et al. Omicron extensively but incompletely escapes Pfizer BNT162b2 neutralization. Nature 2022;602(7898):654–6.

6. Carrello JM, Alshammary H, Tcheou J, Singh G, Raskin AJ, Kawabata H, et al. Activity of convalescent and vaccine serum against SARS-CoV-2 Omicron. Nature 2022;602(7898):682–8.

7. Garcia-Beltran WF, St Denis KJ, Hoelzemer A, Lam EC, Nitido AD, Sheehan ML, et al. mRNA-based COVID-19 vaccine boosters induce neutralizing immunity against SARS-CoV-2 Omicron variant. Cell 2022;185(3):457–66.e4.

8. Shen X. Boosting immunity to Omicron. Nat Med 2022;28(3):445–6.

9. Payne RP, Longert S, Austin JA, Shelly DT, Dejnirattisai W, Adele S, et al. Immuno- geneticity of standard and extended dosing intervals of BNT162b2 mRNA vaccine. Cell 2021;184(23):5699–714 Nov 11 PubMed PMID: 34735795. Pubmed Central PMCID: PMCP8519781. Epub 2021/11/05.

10. Zhao X, Li D, Ruan W, Chen Z, Zhang R, Zheng A, et al. Effects of a Prolonged Booster Interval on Neutralization of Omicron Variant. N Engl J Med 2022;386(9):894–6 Mar 3 PubMed PMID: 35081296. Pubmed Central PMCID: PMCP8805906. Epub 2022/01/27.

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