Brief Communication
Diagnostic Immunology

Ann Lab Med 2023;43:290-294
https://doi.org/10.3343/alm.2023.43.3.290
ISSN 2234-3806 • eISSN 2234-3814

ANNALS OF LABORATORY MEDICINE

Evaluation of Cellular Responses to ChAdOx1-nCoV-19 and BNT162b2 Vaccinations

Tae Hwan Lee, M.D.[1], Minjeong Nam, M.D., Ph.D.[2], Jong Do Seo, M.D.[1], Hanah Kim, M.D., Ph.D.[1], Hae-Rim Kim, M.D., Ph.D.[3], Mina Hur, M.D., Ph.D.[1], Yeo-Min Yun, M.D., Ph.D.[1], and Hee-Won Moon, M.D., Ph.D.[1]

[1]Department of Laboratory Medicine, Konkuk University School of Medicine, Seoul, Korea; [2]Department of Laboratory Medicine, Korea University Anam Hospital, Seoul, Korea; [3]Department of Internal Medicine, Konkuk University School of Medicine, Seoul, Korea

While numerous studies have evaluated humoral responses to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccines, data on the cellular responses to these vaccines remain sparse. We evaluated T cell responses to ChAdOx1-nCoV-19 and BNT162b2 vaccinations using an interferon gamma (IFN-γ) release assay (IGRA). ChAdOx1-nCoV-19- and BNT162b2-vaccinated participants initially showed stronger T cell responses than unvaccinated controls. The T cell response decreased over time and increased substantially after the administration of a BNT162b2 booster dose. Changes in the T cell response were less significant than those in the anti-receptor-binding domain IgG antibody titer. The study results can serve as baseline data for T cell responses after SARS-CoV-2 vaccination and suggest that the IGRA can be useful in monitoring immunogenicity.

Key Words: Cellular responses, Vaccination, Immunogenicity, Humoral responses, Severe acute respiratory syndrome coronavirus 2

Received: June 14, 2022
Revision received: September 22, 2022
Accepted: October 24, 2022

Corresponding author: Hee-Won Moon, M.D.
Department of Laboratory Medicine, Konkuk University School of Medicine, 120-1 Neungdong-ro, Gwangjin-gu, Seoul 05030, Korea
Tel: +82-2-2030-5583
Fax: +82-2-2030-5587
E-mail: hannasis@hanmail.net

Several vaccines have been developed since the beginning of the coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. However, studies evaluating the humoral responses against SARS-CoV-2 have concluded that vaccine efficacy substantially declines over months [2-4]. The emergence of SARS-CoV-2 variants, such as the omicron variant, further adds to the concern about waning antibody levels despite the administration of multiple vaccine doses [5]. Recent animal studies have revealed a host-protective role of T cells against SARS-CoV-2, particularly when the humoral immune response is insufficient [6, 7]. SARS-CoV-2-specific T cell responses in vaccinated and convalescent humans have been shown to be conserved across variants of concern, even when the humoral responses were insufficient [8, 9]. Jung, *et al.* [10] found that interferon gamma (IFN-γ), tumor necrosis factor, and interleukin 2 production by T cells is important for protection against SARS-CoV-2. IFN-γ is secreted by $CD4^+$ and $CD8^+$ T cells following activation. The IFN-γ release assay (IGRA) is well established for measuring T cell-dependent IFN-γ production during tuberculosis. Given that monitoring cellular responses may be as important as assessing antibody titers in vaccinated populations, we evaluated T cell-dependent IFN-γ production in response to the ChAdOx1-nCoV-19 and BNT162b2 vaccines—two major vaccines administered to the Korean population.

The present study was performed at Konkuk University Medical Center (KUMC), Seoul, Korea, from March to December 2021. We used data from 91 healthy study participants who provided written informed consent. Among them, 36 participants received two doses of the ChAdOx1-nCoV-19 vaccine and 30 received two doses of the BNT162b2 vaccine according to their respective vaccination schedules. For the ChAdOx1-nCoV-19 group, T cell responses were measured three weeks and three and five months after the administration of the second dose. In the BNT-162b2 group, T cell responses were measured three and six months after the second dose. T cell responses were also measured two weeks after a third dose of BNT162b2 in the ChAdOx1-nCoV-19 group (heterologous booster) and three weeks after a third dose of BNT162b2 in the BNT162b2 group (homologous booster). Twenty-five unvaccinated participants with SARS-CoV-2 anti-nucleocapsid IgG-negative results (SARS-CoV-2 IgG, Abbott Laboratories, Sligo, Ireland), indicating no past infections, were assigned as controls.

For each group, T cell responses against SARS-CoV-2 were measured using Covi-FERON ELISA (SD Biosensor, Suwon, Korea). Briefly, 1 mL of whole blood was collected into each of the three assay tubes: Nil, original spike protein (SP) antigen, and mitogen. The Nil tube was used as a negative control to adjust for the background noise, with an upper limit of 0.80 international units (IU)/mL. The original SP antigen tube was coated with a specific SP antigen derived from SARS-CoV-2 20I/501Y.V1 variant (lineage B.1.1.7), with an upper limit of 10.00 IU/mL. The mitogen tube was used as a positive control. All tubes were gently mixed, incubated at 37°C for 16 hours, and centrifuged at 2,300×*g* for 15 minutes to extract plasma. A human IFN-γ ELISA was used to determine the IFN-γ levels in the plasma samples. According to the manufacturer's instructions, the final IFN-γ response was defined as positive when the value of the IFN-γ level in the original SP tube minus that in the Nil tube was ≥0.25 IU/mL. A result with a Nil tube value higher than the upper limit was interpreted as indeterminate. In addition to the IFN-γ response, the anti-receptor-binding domain (RBD) IgG titer (SARS-CoV-2 IgG II Quant, Abbott Laboratories; manufacturer's cut-off, 50 arbitrary units [AU]/mL) was measured in each group at the aforementioned time points. The upper limit for SARS-CoV-2 IgG II Quant was 40,000 AU/mL, and results above this limit were retested according to the dilution protocol. The Mann–Whitney *U* test was used to compare the median age among the three groups. The Wilcoxon test was used to compare the median IFN-γ response and anti-RBD IgG titer between the two vaccinated groups. The independent samples *t*-test was used to compare the IFN-γ response between the vaccinated groups. The Pearson correlation coefficient (*r*) between the IFN-γ level and anti-RBD IgG titer for each group was interpreted according to the standards established by Mukaka [11]: 0.90–1.00 as very high, 0.70–0.90 as high, 0.50–0.70 as moderate, 0.30–0.50 as low, and 0.00–0.30 as negligible correlation. MedCalc Statistical Software (version 20.112; MedCalc Software, Ostend, Belgium) was used for all statistical analyses. This study was approved by the Institutional Review Board of KUMC (file No. 2021-06-022-006).

The median age of all participants was 37 years (range, 19–58 years), and 73% of the participants were female. The median age of the participants in the control group (33 years, 19–45 years) was lower than that of the participants in the ChAdOx1-nCoV-19 (40 years, 22–58 years) (P=0.06) and BNT162b2 groups (40.5 years, 24–58 years) (P=0.06). There was no significant difference in median age between the vaccinated groups (P=0.65). All individuals in the control group showed a negative IFN-γ response and anti-RBD IgG titer according to the manufacturer's cut-off. The median IFN-γ response and anti-RBD IgG titer in the control group were significantly lower than those in the vaccinated groups at any time point (P<0.001). Initially, the IFN-γ response was significantly higher in the vaccinated groups than in the control group. The IFN-γ response gradually decreased over time and increased steeply after the administration of a booster dose (Table 1, Fig. 1A, B). The BNT162b2 vaccine induced a higher IFN-γ response than the ChAdOx1-nCoV-19 vaccine at three months after administration (P=0.007). The post-booster

Table 1. IFN-γ responses and anti-RBD IgG titers after BNT162b2 and ChAdOx1-nCoV-19 vaccinations

Variable	Control	ChAdOx1-nCoV-19-vaccinated				BNT162b2-vaccinated		
		3 weeks after 2nd dose	3 months after 2nd dose	5 months after 2nd dose	2 weeks after booster	3 months after 2nd dose	6 months after 2nd dose	3 weeks after booster
N	25	36	32	31	25	30	29	26
IFN-γ response, IU/mL	0 (0–0)	0.31 (0.12–0.69)	0.14 (0.04–0.24)	0.14 (0.06–0.41)	1.92 (0.73–5.01)	0.54 (0.36–1.01)	0.31 (0.16–0.86)	1.93 (1.13–4.00)
Anti-RBD IgG, AU/mL	1 (0–4)	1,097 (592–1,581)	500 (295–760)	278 (160–454)	17,824 (10,763–22,525)	3,236 (2,382–4,578)	1,080 (758–1,515)	20,277 (14,790–31,932)

Data are shown as median (interquartile range).
Abbreviations: AU, arbitrary units; IFN-γ, interferon gamma; IU, international units; N, number; RBD, receptor-binding domain.

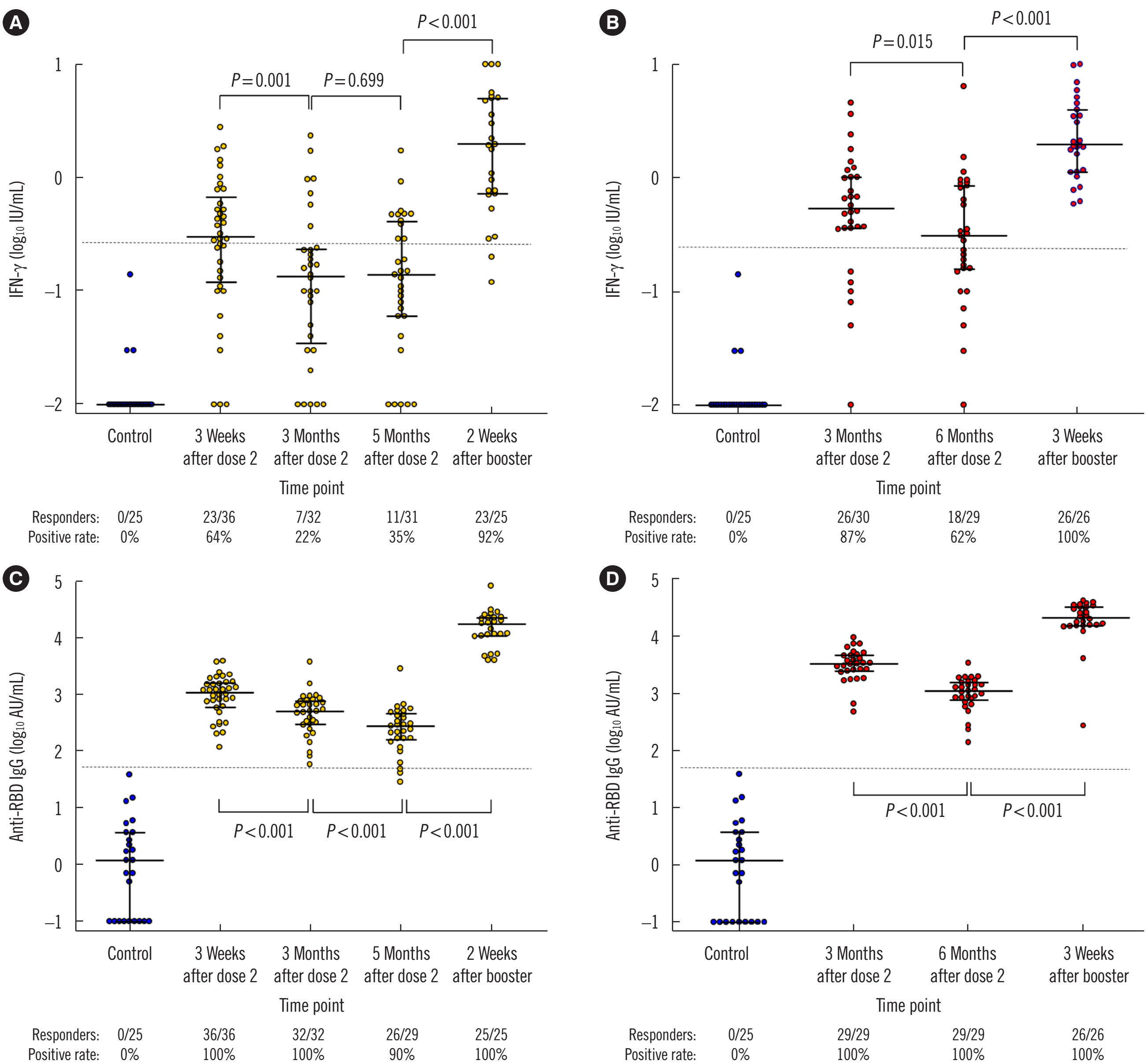

Fig. 1. IGRA and antibody assay results at the indicated time points after vaccination, with responder portions and percentages defined according to the manufacturers' cut-offs. (A) IFN-γ response in the ChAdOx1-nCoV-19-vaccinated (yellow dots) and control groups (blue dots). (B) IFN-γ response in the BNT162b2-vaccinated (red dots) and control groups. (C) Anti-RBD IgG titer in the ChAdOx1-nCoV-19-vaccinated and control groups. (D) Anti-RBD IgG titer in the BNT162b2-vaccinated and control groups. The horizontal dotted lines indicate the cut-off for each assay.

Abbreviations: AU, arbitrary units; IFN-γ, interferon gamma; IGRA, IFN-γ release assay; IU, international units; RBD, receptor-binding domain.

results did not differ significantly between the vaccinated groups ($P=0.967$). The anti-RBD IgG titer showed a similar trend (Table 1, Fig. 1C, D), except that it decreased significantly between three and five months after the second dose in the ChAdOx1-nCoV-19 group ($P<0.001$) unlike the IFN-γ response, which did not change significantly over this period ($P=0.699$). The correlation between the IFN-γ response and anti-RBD IgG titer was low overall ($r=0.46$, $P<0.001$) and was negligible in each group at each time point (r range, –0.02–0.19).

This study was the first to concurrently assess cellular and humoral responses against SARS-CoV-2 in a Korean population following homologous and heterologous vaccination regimens. Our study results were consistent with those of previous similar studies [12, 13] in that the cellular and humoral responses increased after vaccination but then gradually decreased over time and increased again after the administration of a booster dose. In the context of the high vaccination rate in Korea, the IFN-γ values obtained from 25 pre-vaccinated subjects may provide valuable data for future studies. Interestingly, in the ChAdOx1-nCoV-19 group, the median IFN-γ response did not change significantly ($P=0.699$) (Fig. 1A), although the median anti-RBD IgG titer decreased significantly between three and five months after administration ($P<0.001$) (Fig. 1C). This is partially in line with the data of Shaw, *et al.* [14], who found that the decrease in the humoral response was greater than that in the cellular response from one to six months after homologous ChAdOx1-nCoV-19 vaccination (mean [95% confidence interval] fold change; 0.23 [0.21–0.26] vs. 0.62 [0.49–0.79]). Furthermore, three weeks after booster administration, we identified a participant in the BNT162b2 group who had an anti-RBD IgG titer (267.7 AU/mL) below the median value (20,277 AU/mL), but an IFN-γ response (1.16 IU/mL) within the interquartile range (1.13–4.00 IU/mL). Importantly, the participant was on treatment with methotrexate, an immune-modifying drug. Therefore, we speculate that humoral and cellular immune responses play complementary roles. Qui, *et al.* [15] also demonstrated that patients undergoing immune-modifying therapy show a reduced humoral response but a robust T cell response to vaccination. Our study indicates that the two immune responses have a low correlation, at least numerically. Yao, *et al.* [16] also found a lack of significant correlation between IFN-γ levels and antibody responses in convalescent individuals ($r=0.70$, $P=0.593$).

Interestingly, Le Bert, *et al.* [17] reported on the presence of long-lasting SARS-CoV-2-specific cross-reactive memory T cells in patients recovered from SARS 17 years after its outbreak in 2003. Given the high mutation rate of SARS-CoV-2, it is plausible that the effectiveness of the antibodies induced by the current vaccines diminishes over time [18]. However, reluctance to vaccination is undesirable because cellular immunity still plays an important role in preventing severe infection [19]. It would be interesting to measure IFN-γ responses after booster administration, when antibody titers are expected to be low. Notably, the positive rates of the IGRA were low in the vaccinated groups; however, these were determined according to the manufacturer's cut-off (0.25 IU/mL). Given the low IFN-γ levels in the control group, further verification of a qualitative cut-off is necessary.

In summary, ChAdOx1-nCoV-19 and BNT162b2 vaccines elicited a substantial cellular response after the second dose, which increased after the administration of a BNT162b2 booster dose. T cell responses must be monitored when assessing the immunogenicity of COVID-19 vaccines. Our results serve as baseline data for future research and development of COVID-19 vaccines for the Korean population.

ACKNOWLEDGEMENTS

The SARS-CoV-2 IgG kits were provided by Abbott. Abbott had no role in the study design or data analysis.

AUTHOR CONTRIBUTIONS

All authors accept responsibility for the entire contents of this manuscript. Lee TH analyzed the data and wrote the draft; Moon H-W designed the study and finalized the draft; Nam M and Seo JD participated in data collection; and Kim H, Kim H-R, Hur M, and Yun Y-M participated in data analysis and reviewed the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

RESEARCH FUNDING

This research was supported by a MD-PhD/Medical Scientist Training Program grant through Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Korea.

ORCID

Tae Hwan Lee https://orcid.org/0000-0002-3912-4698
Minjeong Nam https://orcid.org/0000-0003-3542-3487
Jong Do Seo https://orcid.org/0000-0001-7449-7978
Hanah Kim https://orcid.org/0000-0002-3266-638X
Hae-Rim Kim https://orcid.org/0000-0002-1911-6236
Mina Hur https://orcid.org/0000-0002-4429-9978
Yeo-Min Yun https://orcid.org/0000-0002-5485-8331
Hee-Won Moon https://orcid.org/0000-0001-9509-6073

REFERENCES

1. Wu F, Zhao S, Yu B, Chen YM, Wang W, Song ZG, et al. A new coronavirus associated with human respiratory disease in China. Nature 2020; 579:265-9.
2. Cham J, Pandey AC, New J, Huynh T, Hong L, Orendain N, et al. 6 month serologic response to the Pfizer-BioNTech COVID-19 vaccine

among healthcare workers. PLoS One 2022;17:e0266781.
3. Robertson LJ, Price R, Moore JS, Curry G, Farnan J, Black A, et al. IgG antibody production and persistence to 6 months following SARS-CoV-2 vaccination: a Northern Ireland observational study. Vaccine 2022;40:2535-9.
4. Kim JA, Bang HI, Shin JW, Park Y, Kim S, Kim MY, et al. Immunogenicity of third-dose BNT162b2 mRNA vaccine following two doses of ChAdOx1 in health care workers: a prospective longitudinal study. Ann Lab Med 2022;42:688-92.
5. Cameroni E, Bowen JE, Rosen LE, Saliba C, Zepeda SK, Culap K, et al. Broadly neutralizing antibodies overcome SARS-CoV-2 Omicron antigenic shift. Nature 2022;602:664-70.
6. Zhuang Z, Lai X, Sun J, Chen Z, Zhang Z, Dai J, et al. Mapping and role of T cell response in SARS-CoV-2-infected mice. J Exp Med 2021;218:e20202187.
7. McMahan K, Yu J, Mercado NB, Loos C, Tostanoski LH, Chandrashekar A, et al. Correlates of protection against SARS-CoV-2 in rhesus macaques. Nature 2021;590:630-4.
8. Woldemeskel BA, Garliss CC, Blankson JN. SARS-CoV-2 mRNA vaccines induce broad CD4+ T cell responses that recognize SARS-CoV-2 variants and HCoV-NL63. J Clin Invest 2021;131:e149335.
9. Geers D, Shamier MC, Bogers S, den Hartog G, Gommers L, Nieuwkoop NN, et al. SARS-CoV-2 variants of concern partially escape humoral but not T-cell responses in COVID-19 convalescent donors and vaccinees. Sci Immunol 2021;6:eabj1750.
10. Jung MK, Jeong SD, Noh JY, Kim DU, Jung S, Song JY, et al. BNT162b2-induced memory T cells respond to the Omicron variant with preserved polyfunctionality. Nat Microbiol 2022;7:909-17.
11. Mukaka MM. Statistics corner: a guide to appropriate use of correlation coefficient in medical research. Malawi Med J 2012;24:69-71.
12. Busà R, Sorrentino MC, Russelli G, Amico G, Miceli V, Miele M, et al. Specific anti-SARS-CoV-2 humoral and cellular immune responses after booster dose of BNT162b2 Pfizer-BioNTech mRNA-based vaccine: integrated study of adaptive immune system components. Front Immunol 2022;13:856657.
13. Groß R, Zanoni M, Seidel A, Conzelmann C, Gilg A, Krnavek D, et al. Heterologous ChAdOx1 nCoV-19 and BNT162b2 prime-boost vaccination elicits potent neutralizing antibody responses and T cell reactivity against prevalent SARS-CoV-2 variants. EBioMedicine 2022;75:103761.
14. Shaw RH, Liu X, Stuart ASV, Greenland M, Aley PK, Andrews NJ, et al. Effect of priming interval on reactogenicity, peak immunological response, and waning after homologous and heterologous COVID-19 vaccine schedules: exploratory analyses of Com-COV, a randomised control trial. Lancet Respir Med 2022;S2213-2600(22)00163-1.
15. Qui M, Le Bert N, Chan WPW, Tan M, Hang SK, Hariharaputran S, et al. Favorable vaccine-induced SARS-CoV-2-specific T cell response profile in patients undergoing immune-modifying therapies. J Clin Invest 2022;132:e159500.
16. Yao L, Wang GL, Shen Y, Wang ZY, Zhan BD, Duan LJ, et al. Persistence of antibody and cellular immune responses in coronavirus disease 2019 patients over nine months after infection. J Infect Dis 2021;224:586-94.
17. Le Bert N, Tan AT, Kunasegaran K, Tham CYL, Hafezi M, Chia A, et al. SARS-CoV-2-specific T cell immunity in cases of COVID-19 and SARS, and uninfected controls. Nature 2020;584:457-62.
18. Chen J, Wang R, Gilby NB, Wei GW. Omicron variant (B.1.1.529): infectivity, vaccine breakthrough, and antibody resistance. J Chem Inf Model 2022;62:412-22.
19. Tarke A, Coelho CH, Zhang Z, Dan JM, Yu ED, Methot N, et al. SARS-CoV-2 vaccination induces immunological T cell memory able to cross-recognize variants from Alpha to Omicron. Cell 2022;185:847-59.e11.