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Comparison of Four T-cell Assays and Two Binding Antibody Assays in SARS-CoV-2 Vaccinees With or Without Omicron Breakthrough Infection

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Background: Several T-cell response assays for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are available; however, their comparability and correlations with antibody responses remain unclear. We compared four SARS-CoV-2 T-cell response assays and two anti-SARS-CoV-2 spike antibody assays.

Methods: We enrolled 89 participants who had received a booster dose of the BNT162b2 vaccine after two doses of the ChAdOx1 or BNT162b2 vaccine. Fifty-six participants without breakthrough infection (BI) (ChAdOx1/BNT162b2 group: N=27; BNT162b2 group: N=29) and 33 with BI were included. We evaluated two whole-blood interferon-gamma release assays (IGRAs) (QuantiFERON and Euroimmun), T-SPOT.COVID, an in-house enzyme-linked immunospot (ELISPOT) assay (targeting the spike and nucleocapsid peptides of wild-type and Omicron SARS-CoV-2), Abbott IgG II Quant, and Elecsys Anti-S, using Mann–Whitney U, Wilcoxon signed-rank, and Spearman's correlation tests.

Results: The correlations between the IGRAs and between the ELISPOT assays (ρ=0.60–0.70) were stronger than those between the IGRAs and ELISPOT assays (ρ=0.33–0.57). T-SPOT.COVID showed a strong correlation with Omicron ELISPOT (ρ=0.70). The anti-spike antibody assays showed moderate correlations with T-SPOT.COVID, Euroimmun IGRA, and ELISPOT (ρ=0.43–0.62). Correlations tended to be higher in the BI than in the noninfected group, indicating that infection induces a stronger immune response.

Conclusions: T-cell response assays show moderate to strong correlations, particularly when using the same platform. T-SPOT.COVID exhibits potential for estimating immune responses to the Omicron variant. To accurately define SARS-CoV-2 immune status, both T-cell and B-cell response measurements are necessary.

Key Words: SARS-CoV-2, Omicron variant, Cellular immunity, Interferon-gamma release tests, Enzyme-linked immunospot assay, Humoral immunity

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INTRODUCTION

The ongoing coronavirus disease (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) still poses a significant public health threat worldwide. Vaccination is an important strategy in the management of viral transmission, and more than 13 billion doses of COVID-19 vaccines have been administered to more than 5 billion individuals glob-

ally in just 21 months, with 10 vaccines granted emergency use listing by the WHO to date [1].

The Omicron variant (B.1.1.529), possessing an unprecedented number of mutations, especially in the spike (S) protein, has caused a new surge of infections since the end of 2021 and currently is the dominant strain. SARS-CoV-2 vaccines widely used in Korea, including BNT162b2 (Pfizer/BioNTech) and ChAdOx1 nCoV-19 (AstraZeneca), induce immune responses to S peptides of wild-type SARS-CoV-2 [2]. Individuals who have received only two vaccine doses do not have sufficient neutralizing antibody levels against the Omicron variant [3, 4]. Although booster immunization partially restores neutralizing ability against the Omicron variant, it is still limited [5, 6]. However, the T-cell responses induced by vaccines or naturally acquired by wild-type SARS-CoV-2 infection do not significantly decline even for the Omicron variant, and it is estimated that they may contribute to the defense against the Omicron variant [7, 8]. Therefore, to evaluate immunity against SARS-CoV-2, it is important to assess the T-cell response as well as the humoral response.

Commercial whole-blood interferon-gamma (IFN-γ) release assays (IGRAs) or enzyme-linked immunospot (ELISPOT) assays, such as QuantiFERON SARS-CoV-2, Euroimmun SARS-CoV-2 IGRA, and T-SPOT.COVID, have been evaluated in vaccinees with or without breakthrough infection (BI) [9-24]. However, because there are no standardization materials or harmonization protocols for SARS-CoV-2 T-cell response assays, the comparability of those assays cannot be assumed. In addition, these assays were developed for the wild-type virus and their usefulness for the Omicron variant has not been validated.

We investigated the correlations among four T-cell response assays and two SARS-CoV-2 IgG assays in participants who had completed booster vaccination, with or without subsequent Omicron BI. We also investigated the correlation of each assay with an in-house SARS-CoV-2 ELISPOT assay targeting peptides from the wild-type virus and the Omicron variant.

MATERIALS AND METHODS

Study participants

In this retrospective study, a total of 89 healthcare workers at Seoul National University Hospital, Seoul, Korea, who had received three doses of vaccine against SARS-CoV-2 were enrolled. Among the 89 participants, 46 participants had received two doses of the ChAdOx1 vaccine and a booster dose of the BNT162b2 vaccine six months after the 2nd dose in November–December 2021, and 43 participants had received a primary series of BNT162b2 vaccines and a 3rd dose of BNT162b2 in October–November 2021, approximately seven months after the 2nd dose. After booster vaccination, 17 participants vaccinated with ChAdOx1 and 16 participants vaccinated with BNT162b2 had a BI in February–April 2022. Therefore, we classified the participants into three groups: 33 participants with BI (BI group), 29 participants with heterologous vaccination without BI (ChAdOx1/BNT162b2 group), and 27 participants with homologous vaccination without BI (BNT162b2 group).

After obtaining written informed consent, blood samples and medical questionnaires were collected between April 14 and May 10, 2022. The questionnaire included the medical history, history of SARS-CoV-2 vaccination and infection, and any adverse events (AEs) following vaccination. This study was approved by the Institutional Review Board (IRB) of Seoul National University Hospital (IRB No. H-2203-040-1304).

Peripheral blood mononuclear cells (PBMCs)

PBMCs were isolated from 10 mL of heparinized blood samples by density gradient centrifugation using Lymphoprep (1.077 g/mL; Stem Cell Technologies, Vancouver, Canada) within 5 hours of blood collection. After washing with phosphate-buffered saline, performing red blood cell lysis, and washing with Roswell Park Memorial Institute 1640 medium, the cells were suspended with AIM-V medium (Thermo Fisher, Waltham, MA, USA) at a concentration of 2.5×10^6 cells/mL.

T-SPOT.COVID assay

The characteristics of the four T-cell response assays and two S antibody assays assessed in this study are shown in Table 1. T-SPOT.COVID (Oxford Immunotec, Oxford, UK) was conducted according to the manufacturer's instructions. Briefly, 100 μL of cell suspension (2.5×10^5 cells/well) was aliquoted. Then, 50 μL of panel A (S peptide) and panel B (nucleocapsid [N] peptide), phytohemagglutinin as a positive control, and AIM-V medium as a negative control were added to the corresponding wells. After overnight incubation at 37°C in humidified air with 5% CO_2, the wells were washed and developed using 50 μL of secondary antibody. After washing, 50 μL of BCIP/NBT-plus was added to produce spots indicating IFN-γ secretion from T cells. Spot-forming cells (SFCs) were counted using EliScan+ v.3.0 and related software v.6.1 (A.EL.VIS GmbH, Hannover, Germany). After subtracting the number of spots counted in the negative control well from the number of spots in each antigen well, >8 SFCs was considered reactive, <4 SFCs nonreactive, and 5–7 SFCs borderline.

Table 1. Characteristics of four SARS-CoV-2 T-cell response assays and two anti-SARS-CoV-2 S antibody assays

Assay	Platform	Stimulating agents for T-cell response assays and target epitope for antibody assays	Valid results	Cutoff
QuantiFERON SARS-CoV-2 IGRA	Whole blood IGRA	Ag1–$CD4^+$ epitopes derived from the S1 subunit (WT) Ag2–$CD4^+$ and $CD8^+$ epitopes from the S1 and S2 subunits (WT)	Nil tube ≤8 IU/mL	0.15 IU/mL
Euroimmun SARS-CoV-2 IGRA	Whole blood IGRA	S protein (WT)	Blank ≤0.5 mIU/mL	200 mIU/mL
T-SPOT.COVID	ELISPOT	Panel A–S protein (WT) Panel B–N protein (WT)	1) NC ≤10 SFCs, 2) PC ≥20 or reactive antigen well	8 SFCs
In-house SARS-CoV-2 ELISPOT assay	ELISPOT	Peptide pools of S or N protein of WT or Omicron variant*	NC ≤10 SFCs	12 SFCs
Elecsys Anti-SARS-CoV-2 S Immunoassay	ECLIA	RBD of S protein	0.40–250 U/mL	0.80 U/mL
Abbott SARS-CoV-2 IgG II Quantitative Antibody Assay	CMIA	RBD of S protein	21.0–40,000 AU/mL	50 AU/mL

*15-Mer peptide pools spanning the entire S or N protein of wild-type virus or the Omicron variant.
Abbreviations: SARS-CoV-2; severe acute respiratory syndrome coronavirus 2; NC, negative control; PC, positive control; IGRA, interferon-gamma release assay; ELISPOT, enzyme-linked immunospot; ECLIA, electro-chemiluminescence immunoassay; CMIA, chemiluminescent microparticle immunoassay; S, spike; N, nucleocapsid; WT, wild-type; RBD, receptor-binding domain; SFCs, spot-forming cells.

In-house SARS-CoV-2 ELISPOT assay

The in-house SARS-CoV-2 ELISPOT assay (hereafter abbreviated as in-house ELISPOT) was performed using the human IFN-γ ELISpot PRO kit (Mabtech Ab, Stockholm, Sweden). The plate was washed and blocked with 200 µL/well of AIM-V containing 10% fetal bovine serum at room temperature for 30 minutes. Then, 50 µL of each stimulant solution and 100 µL of cell suspension (2.5×10^5 cells/well) were added. The stimulants were 15-mer peptides spanning the full length of the S or N protein: the wild-type S protein (SW; Cat. No.: RP30020), the S protein of the Omicron variant (B.1.1.529) (SO; Cat. No.: RP30121) (GenScript, Piscataway, NJ, USA), the wild-type N protein (NW; Cat. No.: PM-WCPV-NCAP-2), and the N protein of the Omicron variant (NO; Cat. No.: PM-SARS2-NCAPMUT08-1) (JPT, Berlin, Germany). AIM-V medium was used as a negative control, and monoclonal antibody CD3-2 was used as a positive control. After overnight incubation at 37°C in humidified air with 5% CO_2, 50 µL of 7-B6-ALP was conjugated at 2–8°C for 1 hour. After washing, 50 µL of BCIP/NBT-plus was added to the plate, followed by incubation at room temperature (18–26°C) for 7 minutes. Then, the plate was washed and dried. Spots were counted using EliScan+ v.3.0 and software v.6.1. The number of spots in the negative control well was subtracted from the number of spots in each antigen well, and the cutoff value was determined by calculating the mean plus three-fold the SD value, which was 12 SFCs/2.5×10^5 PBMCs.

QuantiFERON SARS-CoV-2 IGRA

The QuantiFERON SARS-CoV-2 IGRA (hereafter abbreviated as QuantiFERON IGRA) (Qiagen, Germantown, MC, USA) was performed according to the manufacturer's recommendations. Briefly, 1 mL of heparinized blood was placed into each tube, including a negative (Nil) tube, two tubes containing antigens (SARS-CoV-2 Ag1 and Ag2), and a positive (mitogen) control tube, and incubated within 6 hours of sample collection. Following 20 hours of incubation at 37°C, the plasma was harvested from each tube and stored at –20°C until analyzed by QuantiFERON ELISA. The nil value was subtracted from the IFN-γ concentration obtained from each tube. We used a cutoff value of 0.15 IU/mL to determine positivity.

Euroimmun SARS-CoV-2 IGRA

The Euroimmun SARS-CoV-2 IGRA (hereafter abbreviated as Euroimmun IGRA) (Euroimmun, Lubeck, Germany) was performed according to the manufacturer's recommendations. Briefly, 500 µL of heparinized blood was placed into each tube, including CoV-2 IGRA BLANK, CoV-2 IGRA TUBE (S protein S1 domain), and CoV-2 IGRA STIM (mitogen), and incubated within 6 hours of sample collection. After 20 hours of incubation at 37°C, plasma was collected and stored at –20°C until analyzed by Euroimmun IFN-γ ELISA. The IFN-γ concentration measured in BLANK from that measured in TUBE. We used a cutoff value of 200 mIU/mL to determine positivity.

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Elecsys Anti-SARS-CoV-2 Immunoassay

We measured serum anti-S antibodies using the Elecsys Anti-SARS-CoV-2 S Immunoassay (hereafter abbreviated as Elecsys Anti-S) and anti-N antibodies using the Elecsys Anti-SARS-CoV-2 Immunoassay (hereafter abbreviated as Elecsys Anti-N) (Roche Diagnostics International Ltd., Rotkreuz, Switzerland) on a fully automated Roche Cobas 8000 e602 analyzer. According to the manufacturer's instructions, anti-S antibody values >0.8 U/mL and anti-N antibody values >1.0 U/mL were considered positive.

Abbott SARS-CoV-2 IgG II Quantitative Antibody Assay

We detected serum IgG using the Abbott SARS-CoV-2 IgG II Quantitative antibody assay (hereafter abbreviated as Abbott IgG II Quant) (Abbott Laboratories, Abbott Park, IL, USA) on an Abbott ARCHITECT i2000SR analyzer. According to the manufacturer's instructions, values >50 AU/mL were defined positive.

Statistical analysis

We used the Mann–Whitney *U* test for unpaired comparisons and the Wilcoxon signed-rank test for paired comparisons of responses to wild-type and Omicron antigens. For comparisons among the three groups, the Kruskal–Wallis test and chi-square test were used. The agreement between the qualitative results was evaluated based on overall percent agreement and Gwet's first-order agreement coefficient (AC1 statistics) [25, 26]. The coefficient was interpreted based on a report by Altman: very good (0.8–1.0), good (0.6–0.8), moderate (0.4–0.6), fair (0.2–0.4), and poor (≤0.2) [27]. The correlation was evaluated based on Spearman's correlation coefficient (Spearman's rho, ρ) and the *P* value. Correlation was classified as very strong (ρ≥0.8), strong (0.6≤ρ<0.8), moderate (0.4≤ρ<0.6), weak (0.2≤ρ<0.4), or weak to no (ρ<0.2) [28]. Statistical analysis was performed using IBM SPSS v.25.0 (IBM Corp., Armonk, NY, USA), MedCalc v.22.003 (MedCalc Software, Ostend, Belgium), and Microsoft Excel 2013 for Windows 10. *P*-values<0.05 were considered significant.

RESULTS

Characteristics of the study population

The study population characteristics are presented in Table 2. The total participants included 12 men and 77 women, and the median age was 35 years. BI was more frequent among female than among male participants (41.6% vs. 8.3%, *P*=0.028) and showed no difference according to the primary vaccine regimen or AEs. The median interval from the 3rd dose of vaccination to blood collection was 152.0 days, with significant differences in descending order among the BNT162b2, BI, and ChAdOx1/BNT162b2 groups (176.0 vs. 150.0 vs. 141.0 days, *P*<0.001). AEs after vaccination were more frequent in female than in male participants (94.8% vs. 58.3%, *P*=0.002).

Table 2. Characteristics of the study participants

Characteristic	BI group (N=33)	ChAdOx1/BNT162b2 group (N=29)	BNT162b2 group (N=27)	Total (N=89)	*P**	*P*†
Age, yr	33 (29-40)	40 (36-50)	32 (30-36)	35 (30-42)	0.002	NS
Male‡	1 (3.0%)	4 (13.8%)	7 (25.9%)	12 (13.5%)	0.035	0.028
Comorbidities§	5 (15.2%)	8 (27.6%)	5 (18.5%)	18 (20.2%)	NS	NS
Primary vaccine series‖						
ChAdOx1	17 (51.5%)	29 (100%)	-	46 (51.7%)	NA	NA
BNT162b2	16 (48.5%)	-	27 (100%)	43 (48.3%)	NA	NA
Interval between 3rd dose of vaccination and blood collection, day	150 (143.0-182.0)	141 (139.0-147.0)	176 (158.5-188.0)	152 (143.0-181.0)	<0.001	NS
AE following vaccination¶	30 (90.9%)	26 (89.7%)	24 (88.9%)	80 (89.9%)	NS	NS
Interval between BI and blood collection, day	42 (32.3-53.0)	-	-	-	NA	NA

Data are presented as N (%) or median (interquartile range).
*Comparison among three groups (BI, ChAdOx1/BNT162b2, and BNT162b2) using chi-square and Kruskal-Wallis tests. †Comparison between the noninfected and BI groups using chi-square and Mann–Whitney *U* tests. ‡Significantly higher frequency of BI in women than in men (41.6% vs. 8.3%, *P*=0.028). §Comorbidities include hypertension, diabetes mellitus, liver disease, malignancy, autoimmune disorders, polycystic ovary syndrome, allergy, latent tuberculosis, and hypothyroidism. ‖Vaccine for primary vaccination series consisting of two doses. There was no difference in BI according to the type of primary vaccine regimen. ¶Significantly higher frequency of AEs in women than in men (94.8% vs. 58.3%, *P*=0.002).
Abbreviations: BI, breakthrough infection; AE, adverse event; NS, not significant; NA, not available.

Positive rates and agreement among qualitative results of T-cell response assays for SARS-CoV-2 and anti-SARS-CoV-2 S antibody assays

The positive rates of T-cell response assays against the SARS-CoV-2 S protein were the highest for Euroimmun IGRA (97.7%), followed by in-house ELISPOT (75.9%–90.4%), QuantiFERON IGRA (65.5%–79.3%), and T-SPOT.COVID panel A (70.8%). The positive rates of T-cell response assays targeting the N protein in the BI group were 75.0%, 62.5%, and 51.5% for the in-house ELISPOT assays against wild-type SARS-CoV-2 and the Omicron variant and T-SPOT.COVID panel B, respectively. The positive rates of the T-cell response assays were higher in the BI group than in the noninfected group except for Euroimmun IGRA, although direct comparison was not appropriate. The positive rates of the two anti-SARS-CoV-2 S antibody assays were 100% (Supplemental Data Table S1).

Moderate to very good agreement was observed among the four T-cell response assays. Euroimmun IGRA showed good agreement with Ag2 of QuantiFERON IGRA (AC1=0.76), in-house ELISPOT targeting the S protein (AC1=0.68–0.89), and T-SPOT.COVID panel A (AC1=0.62). T-SPOT.COVID showed moderate to very good agreement with in-house ELISPOT (AC1=0.58–0.83) (Table 3).

Correlations among T-cell response assays for SARS-CoV-2 and anti-SARS-CoV-2 S antibody assays

Based on data from all 89 participants, in terms of the response against wild-type protein, we found strong correlation between the two IGRA assays (QuantiFERON and Euroimmun) and the two ELISPOT assays (T-SPOT.COVID and in-house ELISPOT) (ρ=0.61–0.71) and weak to moderate correlation between the whole-blood IGRAs and ELISPOT assays (ρ=0.33–0.58). The two SARS-CoV-2 IgG assays showed very strong correlation (ρ=0.93) with each other and moderate correlation with T-SPOT.COVID, Euroimmun IGRA, and in-house ELISPOT (ρ=0.46–0.62) but weak correlation with QuantiFERON IGRA (ρ=0.26–0.32). Regarding the correlation between the response to the Omicron variant and that to the wild-type virus, the in-house ELISPOT targeting SO showed strong correlation with T-SPOT.COVID panel A (ρ=0.70) and moderate correlation with Euroimmun IGRA and the SARS-CoV-2 IgG assays (ρ=0.43–0.52) (Table 4).

Correlations among T-cell response assays against SARS-CoV-2 and anti-SARS-CoV-2 S antibody assays in the BI and noninfected groups

Regarding the responses against wild-type SARS-CoV-2 in the

Table 3. Agreement of qualitative results of T-cell response assays and S antibody assays in the total participants (N=89)

Assay		In-house ELISPOT				T-SPOT.COVID		QuantiFERON IGRA		Euroimmun IGRA	Abbott IgG II Quant
		SW	SO	NW	NO	A	B	Ag1	Ag2		
In-house ELISPOT	SO	**78.3% (0.70)**									
	NW	48.2% (–0.03)	38.6% (–0.17)								
	NO	48.2% (–0.04)	33.7% (–0.29)	**90.8% (0.85)**							
T-SPOT.COVID	A	75.9% (0.61)	71.1% (0.58)	49.4% (0.01)	49.4% (0.01)						
	B	41.0% (0.12)	28.9% (0.41)	**88.5% (0.82)**	**88.5% (0.83)**	46.1% (0.07)					
QuantiFERON IGRA	Ag1	63.0% (0.38)	65.4% (0.48)	54.1% (0.08)	49.4% (0.00)	71.3% (0.49)	49.4% (0.01)				
	Ag2	70.4% (0.55)	**75.3% (0.67)**	48.2% (0.03)	43.5% (0.13)	71.3% (0.54)	40.2% (0.20)	**83.9% (0.73)**			
Euroimmun IGRA	S1	**75.3% (0.68)**	**90.1% (0.89)**	33.3% (0.23)	26.2% (0.40)	**72.1% (0.62)**	22.1% (0.51)	66.7% (0.52)	**81.0% (0.76)**		
Abbott IgG II Quant		**75.9% (0.69)**	**90.4% (0.89)**	29.9% (0.29)	25.3% (0.40)	**70.8% (0.61)**	19.1% (0.56)	65.5% (0.52)	**79.3% (0.75)**	**97.7% (0.98)**	
Elecsys Anti-S		**75.9% (0.69)**	**90.4% (0.89)**	29.9% (0.29)	25.3% (0.40)	**70.8% (0.61)**	19.1% (0.56)	65.5% (0.52)	**79.3% (0.75)**	**97.7% (0.98)**	**100.0% (1.00)**

Each cell presents the overall percentage agreement among the total participants, and Gwet's AC1 value is shown in parentheses. Higher agreement (AC1 >0.6) is shown in bold.
Abbreviations: S, spike; ELISPOT, enzyme-linked immunospot; IGRA, interferon-gamma release assay; NS, not significant; NA, not applicable; SW, S protein of wild-type virus; SO, S protein of the Omicron variant; NW, nucleocapsid protein of wild-type virus; NO, nucleocapsid protein of the Omicron variant.

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Table 4. Correlations among T-cell response assays and S antibody assays in the total participants (N=89)

Assay		In-house ELISPOT				T-SPOT.COVID		QuantiFERON IGRA		Euroimmun IGRA	Abbott IgG II Quant
		SW	SO	NW	NO	A	B	Ag1	Ag2		
In-house ELISPOT	SO	**0.65*** **									
	NW	0.41***	0.46**								
	NO	0.43***	0.48**	**0.85****							
T-SPOT.COVID	A	**0.61*** **	**0.70****	0.38**	0.44**						
	B	0.42***	0.38**	**0.64****	**0.70****	0.44***					
QuantiFERON IGRA	Ag1	0.35**	0.39***	0.23*	0.27*	0.43***	0.14				
	Ag2	0.33**	0.35**	0.25*	0.25*	0.40***	0.14	**0.89*****			
Euroimmun IGRA	S1	0.55***	0.52***	0.50***	0.55***	0.58***	0.41***	**0.71*****	**0.63*****		
Abbott IgG II Quant		0.52***	0.43***	**0.62*****	**0.61*****	0.46***	0.58***	0.26*	0.30**	0.56***	
Elecsys Anti-S		0.53***	0.44***	**0.60*****	**0.61*****	0.52***	0.59***	0.32**	0.30*	0.59***	**0.93*****

Each cell presents the correlation coefficient (ρ) and associated *P* value obtained using Spearman's correlation test. **P*<0.05; ***P*<0.01; ****P*<0.001. Significantly strong correlation (ρ≥0.6) is shown in bold.
Abbreviations: S, spike; ELISPOT, enzyme-linked immunospot; IGRA, interferon-gamma release assay; NS, not significant; SW, S protein of wild-type virus; SO, S protein of the Omicron variant; NW, nucleocapsid protein of wild-type virus; NO, nucleocapsid protein of the Omicron variant.

BI group, we found moderate to strong correlation between the two whole blood IGRAs (ρ=0.62–0.72) and between the two ELISPOT assays (ρ=0.52–0.78). Between the whole-blood IGRAs and ELISPOT assays, only Euroimmun IGRA correlated with T-SPOT.COVID (ρ=0.45) and in-house ELISPOT targeting NW (ρ=0.36). Regarding the anti-SARS-CoV-2 S antibody assays, Abbott IgG II Quant showed moderate correlation only with T-SPOT.COVID panel B (ρ=0.47). Regarding the response to the Omicron variant, in-house ELISPOT targeting SO was strongly correlated with T-SPOT.COVID panel A (ρ=0.76) and in-house ELISPOT targeting NO showed strong correlation with T-SPOT.COVID panel B (ρ=0.69) (Supplemental Data Table S2).

In the analysis of the responses to the wild-type virus in the noninfected group, we found strong correlation between the two whole-blood IGRAs (ρ=0.60–0.75) and moderate correlation between the two ELISPOT assays (ρ=0.34). The correlation between the whole-blood IGRAs and ELISPOT assays ranged from none to moderate. Both SARS-CoV-2 IgG assays showed either no or weak correlation with the whole-blood IGRAs and ELISPOT assays. Regarding the response to the Omicron variant, only T-SPOT.COVID panel A showed moderate correlation with in-house ELISPOT targeting SO (ρ=0.46) (Supplemental Data Table S2).

Magnitude of T-cell responses against SARS-CoV-2 and anti-SARS-CoV-2 S antibody assays in the BI and noninfected groups

The magnitudes of the T-cell responses against SARS-CoV-2 and anti-SARS-CoV-2 S antibody titers were higher in the BI group than in the noninfected group; however, we could not compare them directly because the interval from events (3rd dose of vaccination or BI) to the sampling time varied (median 42 days for the BI group vs. 153 days for the noninfected group). The T-cell responses obtained using in-house ELISPOT targeting SW and the antibody titers obtained using Abbott IgG II Quant were significantly higher in the BNT162b2 group than in the ChAdOx1/BNT162b2 group (*P*=0.004 and 0.006, respectively) (Supplemental Data Fig. S1).

In the BI group, ELISPOT responses to SO and SW did not differ in magnitude, and the response to NO was slightly lower than that to NW (*P*=0.01). In the ChAdOx1/BNT162b2 group, the response to SW was lower than that to SO (*P*=0.001). In the BNT162b2 group, the responses to wild-type virus (SW, NW) and the Omicron variant (SO, NO) did not differ in magnitude (Supplemental Data Fig. S2).

DISCUSSION

We evaluated the performance of four T-cell response assays for SARS-CoV-2 and two anti-SARS-CoV-2 S antibody assays in booster-vaccinated participants with or without BI. All BI cases that occurred during March–April 2022 can be presumed to have been caused by the Omicron variant considering its prevalence in Korea at that time.

We observed strong T-cell and antibody responses in the BI group. This is in agreement with previous findings of strong hybrid immunity after BI [3, 15, 29]. In addition, the T-cell responses

to the Omicron variant were not significantly lower than those to the wild-type virus in in-house ELISPOT in the BI and noninfected groups. This is in agreement with previous findings that T-cell responses induced by vaccination (wild-type) are relatively effective against the Omicron variant in contrast to antibody responses [8, 21, 30].

The agreement among the T-cell response assays for SARS-CoV-2 was moderate to good. The disagreement may be due to the different nature and different antigen concentrations and cutoff values used. QuantiFERON IGRA showed a lower positive rate, resulting in inferior agreement with the other T-cell response assays, especially for Ag1. Given that Ag1 is a $CD4^+$ epitope derived from the S1 subunit and Ag2 is a $CD4^+$ and $CD8^+$ epitope, the differences in the response degree and inter-assay agreement can be partially explained by different IFN-γ responses depending on the T-cell subpopulation [14, 31].

Regarding the quantitative results, the correlation between the T-cell response assays for SARS-CoV-2 using the same platform (whole-blood IGRA and ELISPOT) was better than that between the different platforms. This may be because whole-blood IGRAs are unable to accurately measure high IFN-γ concentrations because of the relatively narrow measurable range of the ELISA method [18, 19]. ELISPOT may be preferable for measuring the wide range of T-cell responses, especially in participants with BI. For measuring the response to the Omicron variant, T-SPOT.COVID A (S antigen of the wild-type virus) showed a strong correlation with in-house ELISPOT targeting SO (S protein of the Omicron variant) (stronger in the BI group and weaker in the noninfected group), which suggests the usefulness of T-SPOT.COVID to estimate the status of immunity against the Omicron variant, especially in participants with BI.

The noninfected group had lower positive rates in the T-cell response assays and lower agreement of the qualitative results of these assays (data not shown). Moreover, the correlation between in-house ELISPOT targeting SW and T-SPOT.COVID panel A was relatively weak in the noninfected group compared with that in the BI group. This may be attributed to the longer time interval between the 3rd dose of vaccination and blood collection in the noninfected group (median 152.5 days) than between BI and blood collection in the BI group (median 41.5 days), resulting in a lower IFN-γ concentration. Therefore, the correlation and agreement between groups with different time intervals between events and blood sampling should be interpreted cautiously.

The two SARS-CoV-2 IgG assays showed weak to moderate correlation with the T-cell response assays for SARS-CoV-2. Several studies have also suggested that T- and B-cell responses are poorly correlated in vaccinated participants, highlighting that both T- and B-cell response measurements are needed [10, 11, 19, 21]. The anti-SARS-CoV-2 S antibody assay for the Omicron variant has not yet been commercialized, and the Abbott IgG II Quant and Elecsys Anti-S antibody assays targeting the wild-type S protein cannot exactly measure anti-S antibodies against the Omicron variant, especially in vaccinated participants. Therefore, further studies on the correlations between T-cell response assays and S antibody assays for the Omicron variant are needed.

Our results demonstrated that booster vaccination with BNT162b2 induces a sufficient T-cell immune response to the Omicron variant, as the T-cell response magnitudes were not decreased in the subgroups regardless of the primary vaccination regimen. Additionally, the ChAdOx1/BNT162b2-vaccinated participants without BI showed a significantly higher response in in-house ELISPOT targeting SO than in the assay targeting SW, resulting in a higher positive rate. While it remains unclear why the ChAdOx1/BNT162b2 group exhibited a higher T-cell response to the Omicron variant than to the wild-type virus, this finding suggests that the hybrid regimen may confer more effective and stronger immunity to the Omicron variant. However, further evidence is required to confirm this hypothesis.

This study had some limitations. (i) It lacked naive controls to evaluate the specificity of the assays. (ii) Since this was a small-scale cross-sectional study performed shortly after infection or vaccination, immune response dynamics and variability among participants over time could not be observed [10, 24, 32]. (iii) No commercial assays for detecting SARS-CoV-2 IgG antibodies against the Omicron variant were available at the time of this study. (iv) Because of the difference in the interval between events (3rd dose of vaccination or BI) and blood collection, it is not appropriate to directly compare the BI and noninfected groups in this study. (v) Although in-house ELISPOT showed moderate to strong correlation with the validated commercial ELISPOT assay (T-SPOT.COVID), there remains some uncertainty regarding the accuracy and reliability of the in-house ELISPOT assay because it has not been fully harmonized and qualified. To address this issue, it would be necessary to conduct further investigations assaying sample replicates and fractionally diluted samples. Nevertheless, we compared the most used four SARS-CoV-2 T-cell response assays and two anti-SARS-CoV-2 S antibody assays in the participants with or without BI by the Omicron variant. Our results can help laboratory staff and clinicians understand the comparability of commercial SARS-CoV-2 T-cell response assays and anti-SARS-CoV-2 S antibody assays and their useful-

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ness against the Omicron variant.

In conclusion, SARS-CoV-2 T-cell response assays showed moderate to strong correlation, especially when using the same platform. The strong hybrid immunity obtained after booster vaccination and BI resulted in better correlations among assays than those observed in noninfected participants. T-SPOT.COVID appeared to be useful for estimating immune responses to the Omicron variant, particularly in participants with BI. Both T-cell and B-cell responses should be measured to define the status of immunity to SARS-CoV-2 considering the weak correlation between them. Future studies in a larger number of participants covering longer periods can provide more information about the validity of various commercial assays.

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AUTHOR CONTRIBUTIONS

Song EY designed and supervised the study; Seo YJ and Oh I performed the experiments and wrote the manuscript; Nam M and Shin S analyzed the results. Song EY and Roh EY reviewed and revised the manuscript. All authors reviewed and approved the manuscript.

CONFLICTS OF INTEREST

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