

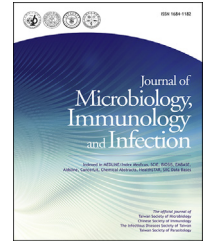


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Original Article

Evaluation of the T cell and B cell response following the administration of COVID-19 vaccines in Korea

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KEYWORDS

COVID-19;
Vaccine;
Booster;
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Neutralizing antibody

Abstract *Background:* The coronavirus disease (COVID-19) has been a worldwide concern since 2019. Vaccines are predicted to be crucial in preventing further outbreaks. The development and kinetics of immune responses determine the efficacy of COVID-19 vaccines.

Methods: We measured interferon-gamma (IFN- γ) levels upon administering homologous adenovirus vector-based (ChAdOx1-S [AZ], Ad26.COV2.S [JAN]), mRNA-based (BNT162b2 [PF]; mRNA-1273 [MO]), and heterologous (AZ/PF) vaccines in healthy Korean individuals using two IFN- γ release assays: the Covi-FERON ELISA and T-SPOT Discovery SARS-CoV-2 assay. B cell responses were evaluated by assessing the production of neutralizing antibodies by surrogate virus neutralization assay. The immune response among the vaccine groups was compared after adjusting the vaccination dose and interactions between each group.

Results: AZ triggered the highest T cell response after the first dose but showed instability after the second. PF and MO yielded stable and higher increments of T and B cell responses compared to AZ. MO yielded a higher immune response than PF. JAN yielded T and B cell responses at lower levels than the other vaccines. The booster dose triggered significant increases in the T and B cell responses and is therefore needed to protect against SARS-CoV-2 given the possibility of waning immune responses.

Conclusion: Administering two doses of mRNA vaccines provides the most effective results among the administered vaccines in triggering the immune response specific to SARS-CoV-2

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in healthy Korean individuals. Administration of booster doses demonstrated a significant increase in the immune response and may provide longer protection against SARS-CoV-2.

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Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes the acute respiratory coronavirus disease (COVID-19), is a zoonotic virus that is highly pathogenic.¹ SARS-CoV-2 is highly transmissible and can be transmitted naturally from vertebrate animals to humans or between humans.² As of May 10, 2022, the cumulative confirmed cases in South Korea have reached 17 million with over 10,000 deaths.³ Great efforts have been made to stop the transmission of SARS-CoV-2 and control infections,^{4–9} including the development of the COVID-19 vaccine.⁹

Diverse platforms have been reported for use in developing COVID-19 vaccines, but only a few have received approval from the U.S. Food and Drug Administration. Several COVID-19 vaccines have been authorized for emergency use. These include BNT162b2 (PF), mRNA-1273 (MO), ChAdOx1-S (AZ), and Ad26.COVS.2.S (JAN).¹⁰ The AZ and JAN vaccines, even though they were widely administered in the early stages of the outbreak, are recently causing some dispute due to the possibility of their side effects.^{11–13} Nevertheless, the administration of vaccines, regardless of the type and brand, has been highly effective in preventing the most severe consequences of COVID-19.^{14,15} In South Korea, the ministry of food and drug safety (MFDS) granted authorization for AZ by February 10, 2021; for PF by March 5, 2021; for JAN by April 7, 2021; and for MO by May 21, 2021.¹⁶ The nationwide COVID-19 vaccination program began on February 26, 2021¹⁷, with AZ being used for the first priority group including employees under the age of 65 in nursing hospitals or facilities and high-risk medical institutions.¹⁸ Individuals aged 18 and older, including older adults (aged 65 and older) are eligible to choose one among the available vaccines to complete their COVID-19 vaccination. By following this policy, in this study, we observed the kinetics of cellular (T cell) and humoral (B cell) immune responses as the hallmarks of adaptive immunity against SARS-CoV-2 in fully vaccinated Korean individuals that received full homologous vaccinations with AZ, PF, MO, JAN, or the heterologous COVID-19 vaccine AZ/PF.

The T and B cells are critical for controlling viral infections and the survival of the host; therefore, monitoring their levels upon administering COVID-19 vaccines is crucial for discerning the efficacy of the vaccines. Few studies have reported comparative data on the T and B cell's responses to the COVID-19 vaccine in South Korea.^{19,20} However, comparisons have only been made between two vaccines,²⁰ or between vaccinated and naturally infected populations.¹⁹ To the best of our knowledge, direct

comparisons of the immunogenicity of several different vaccines, or between the doses of vaccines in healthy individuals, are limited. We, therefore, compared the response of T and B cells to five groups of vaccines in South Korea according to the dose of each vaccine (first, second, and booster doses).

Methods

Study participants

A total of 178 participants were enrolled in this study. All participants are Korean and were confirmed negative for COVID-19 infection by real-time polymerase chain reaction (STANDARD M nCoV Real-Time Detection Kit, SD Biosensor, Suwon, Korea). Participants, with a range of ages from 18 to 65 years (median age of 37 years) were fully vaccinated and defined as healthy persons who received two doses of PF, MO, or AZ; one dose of JAN; or one dose of AZ for the first vaccination and one dose of PF for the second vaccination. Participants made the final decision on which vaccine to get by selecting from a list of COVID-19 vaccines that have been approved by the Korean government. Participants were categorized according to the five groups of vaccines that were administered [homologous: AZ/AZ (n = 48), PF/PF (n = 50), MO/MO (n = 32), JAN (n = 18), and the heterologous vaccine AZ/PF (n = 30)] (Table 1). The interval

Table 1 Characteristics of study subjects and vaccines inoculated.

Characteristics	N = 178
Gender	
Female, N (%)	114 (64.0)
Male, N (%)	64 (36.0)
Nationality	
Korean, N (%)	178 (100.0)
Age, years	
Median (range)	37 (22–59)
Vaccines^a	
ChAdOx1-S/ChAdOx1-S (AZ/AZ), N (%)	48 (27.0)
ChAdOx1-S/BNT162b2 (AZ/PF), N (%)	30 (16.8)
BNT162b2/BNT162b2 (PF/PF), N (%)	50 (28.1)
mRNA-1273/mRNA-1273 (MO/MO), N (%)	32 (18.0)
Ad26.COVS.2.S (JAN), N (%)	18 (10.1)
Booster ChAdOx1-S (AZ), N (%)	20 (52.6)
Booster BNT162b2 (PF), N (%)	18 (47.4)

^a AZ (ChAdOx1-S); PF (BNT162b2); MO (mRNA-1273); JAN (Ad26.COVS.2.S).

between the first and second doses of vaccination is three months, and between the second and booster doses is three to five months (Fig. S1). Participants with a history of specific allergies, pregnant women, or someone receiving immunosuppressants were excluded from the study. Informed consent was obtained from all participants involved in the study.

Sample collection

Blood samples were collected at two time points from participants who received JAN (1 day before receiving the vaccine and 4 weeks following its administration); three time points from participants who received a homologous MO (1 day before receiving the vaccine and 4 weeks after the first and the second dose); three time points from participants who received a heterologous (AZ/PF) vaccination (1 day before receiving the vaccine, 9 weeks after the first dose of AZ, and 4 weeks after the second dose with PF); and four time points from participants who received a homologous AZ and PF (1 day before receiving the vaccine and 4 weeks after the first, second, and booster doses) (Fig. S1). The sample collection was conducted between June 2021 and February 2022.

Covi-FERON ELISA assay

The first IFN- γ release assay (IGRA) was performed using a SARS-CoV-2-specific IGRA kit based on ELISA (Covi-FERON ELISA) (SD Biosensor, Suwon, Korea). The assay was performed according to the manufacturer's instructions. In brief, whole blood specimens were collected from the participants, and 1 mL was distributed into each Covi-FERON tube (Nil tube [negative control], SARS-CoV-2 original S protein antigen [OS] tube, SARS-CoV-2 variant S antigen [VS] tube and mitogen-stimulated T lymphocytes tube [positive control]). The OS tube contained S proteins derived from SARS-CoV-2 (wild type) and the 20I/501Y.V1 variant (B.1.1.7, alpha strain), whereas the VS tube contained those S proteins derived from the 20H/501Y.V2 (B.1.351, beta strain) and 20J/501Y.V3 variants (P.1, gamma strain). After blood stimulation in each tube, plasma cells were collected and subjected to ELISA for IFN- γ detection. The cut-off value, which was determined according to the manufacturer's instructions, was 0.25 IU/mL. The results were defined as "invalid" when the IFN- γ concentration of Nil is > 8 IU/mL or when mitogen minus Nil control is < 0.5 IU/mL, with the OS/VS minus Nil control, is < 0.25 IU/mL or $< 25\%$ of Nil value. The results were defined as 'reactive' when the IFN- γ concentration of Nil is ≤ 8 IU/mL, and of the OS/VS minus Nil control is ≥ 0.25 IU/mL and $\geq 25\%$ of Nil value. The results were defined as "non-reactive" when the concentration of Nil is ≤ 8 IU/mL, the OS/VS minus Nil control, is < 0.25 IU/mL or $< 25\%$ of Nil value, with mitogen minus Nil control, is ≥ 0.5 IU/mL.²¹

T-SPOT discovery SARS-CoV-2 assay

The second IGRA test used in this study was the T-SPOT Discovery SARS-CoV-2 assay (Oxford Immunotec,

Oxfordshire, UK), which is a standardized enzyme-linked immuno-spot (ELISPOT) assay. The T-SPOT Discovery SARS-CoV-2 assay, hereafter referred to as the T-SPOT assay, was performed according to the manufacturer's instructions. In brief, peripheral blood mononuclear cells (PBMCs) were isolated from 5 mL of whole blood samples and washed to remove any sources of interfering background signals. Six wells were prepared for each sample: one nil control to identify non-specific cell activation, three wells to assess the SARS-CoV-2-specific antigens (panel 1 against SARS-CoV-2 spike protein, panel 3 against SARS-CoV-2 nucleocapsid protein, and panel 4 against SARS-CoV-2 membrane protein), one well to investigate cross-reactivity with endemic strains of coronaviruses (panel 13), and one positive control, which was a mitogen solution containing phytohemagglutinin to confirm the functionality of PBMCs. Reactivity to panels 1, 3, and 4 indicated the response of T cells to SARS-CoV-2-specific antigens, whereas reactivity to well 13 indicated cross-reactivity with other coronaviruses. The predetermined cut-off value was ≥ 8 SFCs/250,000 PBMCs. The results were interpreted by subtracting the spot count in the negative control well from the spot count (SFCs) in the SARS-CoV-2 antigen panels.²² The results were defined as "invalid" when the SFCs of Nil control, are > 10 spots or when the SFCs of positive control, are < 20 spots. The results were defined as 'reactive' when the SFCs in at least one of the three SARS-CoV-2 antigen wells (panels 1, 3 and 4) minus the SFCs of Nil control are ≥ 8 and defined as 'non-reactive' when the SFCs in antigen wells minus SFCs of Nil control are < 8 .²³

cPass sVNT assay

The NABs in the sera were detected using a cPass SARS-CoV-2 surrogate virus neutralization (sVNT) test kit (GenScript, Piscataway, New Jersey, USA), hereafter referred to as the cPass sVNT assay. To assess the NABs, the cPass sVNT assay was performed according to the manufacturer's instructions. In our study, the sera of the participants were diluted to a ratio of 1:20 before being subjected to the cPass sVNT assay. This was because we found that at the ratio of 1:1, sera from most of the vaccine groups showed an exceedingly high percentage of inhibition (close to 100%). The results were interpreted as positive according to the manufacturer's recommendations when the inhibition value was \geq the cut-off value (30%), indicating the presence of an anti-SARS-CoV-2 NAB.^{24,25}

Statistical analysis

The differences in the T and B cell levels among the vaccine groups after adjusting the vaccination dose and interactions between the groups and doses were assessed based on a repeated measures analysis of variance (RM-ANOVA). All statistical analyses were two-tailed tests with a type I error of 5% and were performed using the SAS software ver. 9.4 (SAS Institute Inc., Cary, NC, USA). Positive and negative cut-off points were adopted from the manufacturer's package inserts.

Results

Baseline characteristics of the study population

Between June 2021 and February 2022, 178 participants were vaccinated with AZ, PF, MO, or JAN for their first dose; AZ, MO, or PF for their second dose; and AZ or PF for their booster vaccination. None of the participants had been previously infected with SARS-CoV-2. All of the participants enrolled in this study were Korean (100%) and consisted of healthy males ($n = 64$; 36%) and non-pregnant women ($n = 114$; 64%) with a median age of 37 years (range: 22–59 years) (Table 1).

COVID-19 vaccines induce robust T cell responses in healthy individuals

Covi-FERON ELISA assay demonstrated that after the first dose of the vaccines, the homologous AZ group showed the highest positive reactivity (92.9%) compared to other vaccine groups. The MO group showed the highest positive reactivity after the second dose of the vaccine (96.8%) (Table 2).

Upon administering the first dose of the homologous vaccines, the IFN- γ concentration against the original SARS-CoV-2 spike protein (OS) in the group of individuals who received AZ was significantly higher [median 2.57 (0.78–3.81) IU/mL] than in the one who received PF [median 0.66 (0.26–1.28) IU/mL, $p = 0.0001$] or MO [median 0.33 (0.14–0.75) IU/mL, $p \leq 0.0001$]. Correspondingly, the IFN- γ concentration against the variant SARS-CoV-2 spike protein (VS) demonstrated a higher concentration of IFN- γ in the group of individuals who received AZ [median 0.95 (0.31–1.47) IU/mL] than in the one who received PF [median 0.25 (0.08–0.84) IU/mL, $p = 0.03$], and MO [median 0.20 (0.11–0.41) IU/mL, $p = 0.002$] (Fig. 1, Tables S1 and S2).

In the heterologous vaccination group (AZ/PF), the concentration of IFN- γ did not reach the cut-off level of reactivity after the first dose. In the JAN group, the IFN- γ concentration was observed at a moderate level in response to the OS protein [median 0.37 (0.14–0.76) IU/mL] and lower than the cut-off level in response to the VS protein [median 0.15 (0.06–0.33) IU/mL] (Fig. 1, Tables S1 and S2).

The assessment of IFN- γ concentration following the administration of the second dose demonstrated a similar incremental trend in most of the vaccine groups. This increment, however, was not observed in the homologous AZ group, where the median concentration of IFN- γ was decreased to 0.39 (0.11–0.95) IU/mL ($p = 0.01$) and 0.23 (0.10–0.58) IU/mL ($p = 0.01$) in response to the OS and VS proteins, respectively, following the administration of the second dose (Fig. 1, Tables S1 and S2).

Meanwhile, the T-SPOT analysis demonstrated that the homologous AZ group had the highest positive reactivity after the first dose (82.1%) and that the homologous MO group had the highest positive reactivity after the second dose (93.6%) (Table 3).

After the first dose of the vaccine, the median value of the spot counts in the homologous AZ group was significantly higher [median 23.5 (10.5–34.0) SCFs/250,000

Table 2 Covi-FERON ELISA assay positivity according to vaccine and regimen.

	Unvaccinated		AZ ^a 1st		AZ 2nd		AZ booster		AZ/PF 1st		AZ/PF 2nd		PF 1st		PF 2nd		PF booster		MO 1st		MO 2nd		JAN 1st	
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Negative	100 (92.6)	2 (7.1)	18 (37.5)	3 (15.0)	17 (56.7)	2 (6.7)	7 (23.3)	10 (19.6)	1 (5.6)	9 (28.1)	1 (3.2)	9 (27.3)	1 (3.2)	1 (5.6)	10 (19.6)	1 (5.6)	10 (19.6)	1 (5.6)	9 (28.1)	1 (3.2)	1 (3.2)	1 (3.2)	9 (27.3)	9 (27.3)
Positive	8 (7.4)	26 (92.9)	29 (60.4)	17 (85.0)	13 (43.3)	28 (93.3)	23 (76.7)	41 (80.4)	17 (94.4)	23 (71.9)	41 (80.4)	41 (80.4)	17 (94.4)	23 (71.9)	41 (80.4)	41 (80.4)	41 (80.4)	17 (94.4)	23 (71.9)	30 (96.8)	30 (96.8)	30 (96.8)	24 (72.7)	24 (72.7)
Invalid	0 (0.0)	0 (0.0)	1 (2.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

^a AZ (ChAdOx1-S); PF (BNT162b2); MO (mRNA-1273); JAN (Ad26.COV2.S).

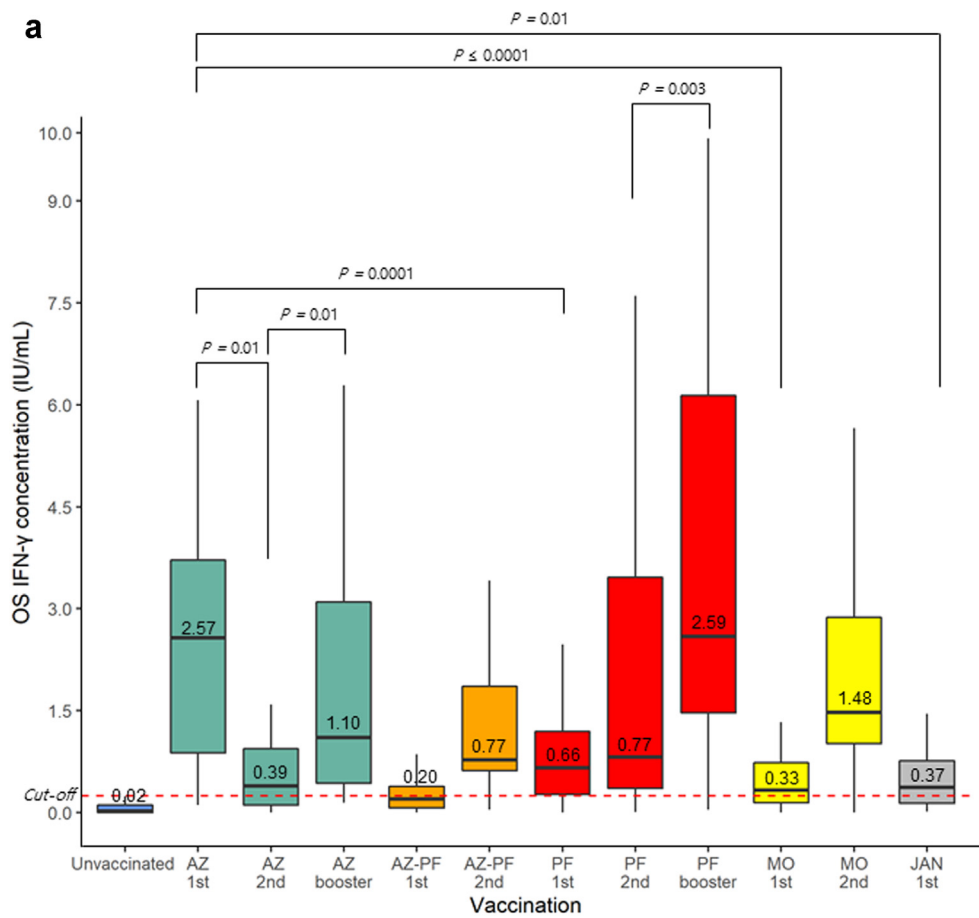


Figure 1. Level of IFN- γ after the first, the second and booster doses of COVID-19 vaccines in response to a) the original SARS-CoV-2 spike protein (OS) and b) the variant SARS-CoV-2 spike protein (VS) in healthy individuals. Dotted line indicates the cut-off value (0.25 IU/mL). Administration of AZ triggered the highest release of IFN- γ after the first dose. The booster dose induced higher production of IFN- γ compared to the second dose. Notes: AZ (ChAdOx1-S); PF (BNT162b2); MO (mRNA-1273); JAN (Ad26.COVS.2.S). Abbreviation: OS, original spike; VS, variant spike; IFN- γ , Interferon-gamma.

PBMCs] than that in the unvaccinated or vaccinated groups, particularly the MO group [median 8.5 (2.0–17.0) SCFs/250,000 PBMCs, $p < 0.0001$] (Table S3). The number of spots, however, decreased in the homologous AZ group after the second dose of the vaccine [median 7.5 (2.0–19.5) SCFs/250,000 PBMCs, $p = 0.30$], whereas in the homologous PF, MO, and heterologous (AZ/PF) groups, the number of spots increased (Fig. 2 and Table S3).

Both mRNA vaccines (PF and MO) showed an increase in the number of spots after the administration of the second dose (Fig. 2). Nevertheless, compared to the PF group [median 17.5 (5.0–27.5) SCFs/250,000 PBMCs], the MO group showed a higher but insignificant increment in spots after the administration of the second dose [median 28.0 (20.0–44.0) SCFs/250,000 PBMCs, $p = 0.95$]. In the JAN group, where the vaccine was only administered once, a moderate number of spots was observed after administering the vaccine [median 10.0 (7.0–17.0) SCFs/250,000 PBMCs] (Fig. 2 and Table S3).

COVID-19 booster vaccines enhance immunogenicity in healthy individuals

The booster doses of PF demonstrated high positive reactivity up to 94.4% and 100% for the Covi-FERON and T-SPOT assays, respectively. These values were higher than those of the AZ group (Tables 2 and 3). Nevertheless, both booster groups showed higher levels of T cell responses than those after the second dose of the vaccines (Figs. 1 and 2).

The concentration of IFN- γ after the booster doses was higher than that after the second dose in response to the S proteins in both the AZ group ($p = 0.01$ and $p = 0.003$, for OS and VS, respectively) and PF group ($p = 0.003$ and $p = 0.01$, for OS and VS, respectively) (Fig. 1, Tables S1 and S2). Subsequently, the evaluation of the number of IFN- γ -producing T cells demonstrated that the booster doses induced a greater number of T cells to produce IFN- γ than the second dose in both the AZ and PF groups (Fig. 2). The median number of T cells that produced IFN- γ after

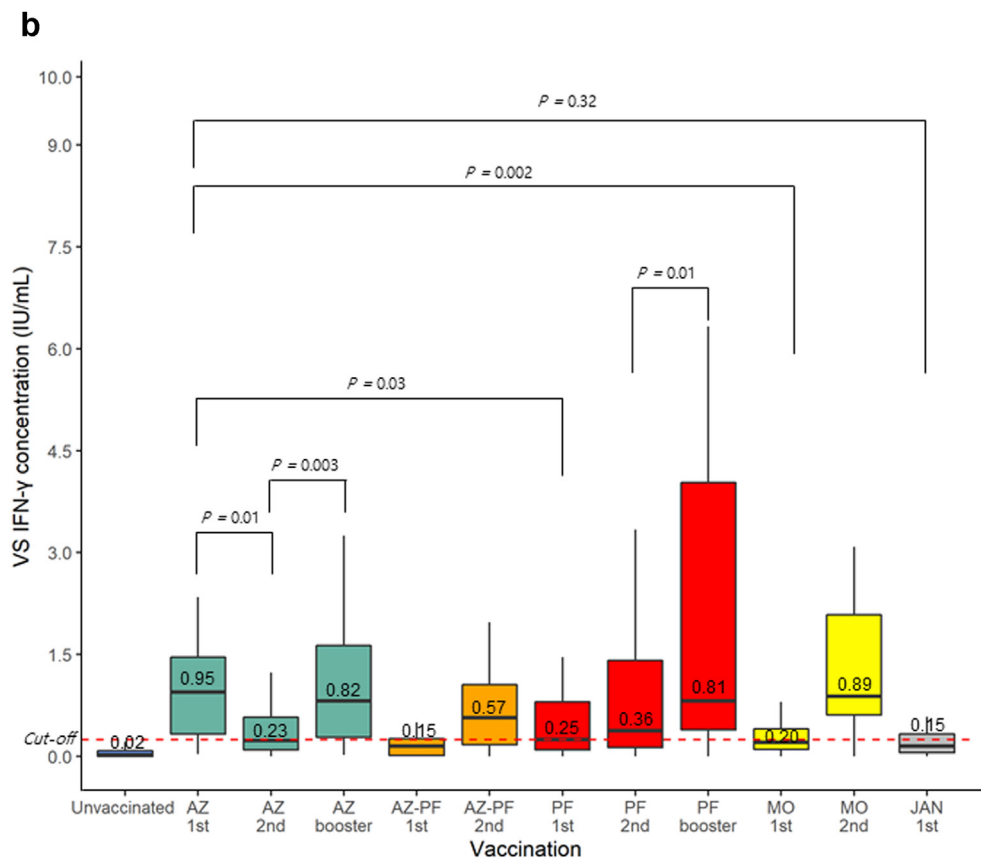


Figure 1. continued

the booster dose in the PF group [median 39.0 (24.0–46.0) SCFs/250,000 PBMCs] was not significantly higher than that after the booster dose by AZ [median 31.5 (14.0–54.0) SCFs/250,000 PBMCs, $p = 0.56$] (Fig. 2 and Table S3).

COVID-19 vaccines significantly boost the neutralizing antibody response in healthy individuals

The MO group demonstrated the highest positive reactivity of cPass sVNT after administering the first and second doses of the vaccine (100%) (Table 4). The PF and AZ/PF groups showed an increased positive reactivity of up to 100% after administering the second dose. The positive reactivity had previously been approximately 93.3% and 46.7% after the first dose of homologous PF and heterologous AZ/PF, respectively. The homologous AZ group, however, showed the lowest reactivity after the first and second doses of the vaccine (53.6% and 71.2%, respectively). The JAN group showed a moderate level of positive reactivity (72.7%). The booster dose with either AZ or PF showed 100% positive reactivity with the sVNT (Table 4). Using samples that were diluted to a ratio of 1:20, we found that in the homologous vaccine groups, the mRNA vaccine, MO, triggered a significantly higher percentage of viral inhibition after the first dose [median 89.16% (81.36–91.61%)] than AZ [median 34.10% (21.66–53.97%), $p < 0.0001$] (Fig. 3, Table S4).

An incremental trend in the percentage of inhibition was observed in all of the vaccination groups after

administering the second dose (Fig. 3). The percentage of inhibition in the MO group [median 98.96% (98.11%–99.22%)] was significantly higher than that in the homologous second dose of AZ group [median 71.03% (32.28%–91.85%), $p < 0.0001$] (Table S4). In the AZ/PF group, the first dose of AZ yielded a low percentage of inhibition [median 27.46% (18.49%–48.30%)]; however, this value increased to a median of 98.73% (97.66%–99.22%) after the second dose with PF. In the JAN group, the vaccine induced moderate levels of NAb with a median for the percentage of inhibition being up to 37.32% (29.84%–54.52%) (Fig. 3 and Table S4). The booster doses with either AZ or PF induced a high production of NAb, with a median for the percentage of inhibition being up to 98.87% (98.62%–98.97%) and 99.02% (98.92%–99.11%) for AZ and PF, respectively (Fig. 3, Table S4).

Discussion

In our study, the T cell responses to the homologous AZ vaccine fluctuated. A similar fluctuation in the T cell response following the administration of COVID-19 vaccines has been reported in individuals who had a SARS-CoV-2 infection 10 months prior to the first vaccination.²⁶ Tormo et al. reported a significant decrease in IFN- γ production 2 weeks after the second dose of the COVID-19 vaccine.²⁶ They hypothesized that the decrease in IFN- γ production may have been related to the activity of regulatory T cells (Tregs) that are stimulated by virus-specific antigens or

Table 3 T-SPOT assay positivity according to vaccine and regimen.

	Unvaccinated		AZ ^a 1st		AZ 2nd		AZ booster		AZ/PF 1st		AZ/PF 2nd		PF 1st		PF 2nd		PF booster		MO 1st		MO 2nd		JAN 1st		
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Negative	108 (100.0)	5 (17.9)	20 (41.7)	2 (10.0)	18 (60.0)	2 (6.7)	6 (20.0)	5 (9.6)	0 (0.0)	18 (60.0)	2 (6.7)	5 (9.6)	0 (0.0)	4 (12.5)	1 (3.2)	7 (21.2)	4 (12.5)	0 (0.0)	18 (60.0)	2 (6.7)	5 (9.6)	0 (0.0)	1 (3.2)	7 (21.2)	4 (12.1)
Positive	0 (0.0)	23 (82.1)	27 (56.3)	18 (90.0)	7 (23.3)	26 (86.7)	24 (80.0)	42 (80.8)	18 (100.0)	7 (23.3)	26 (86.7)	42 (80.8)	18 (100.0)	23 (71.9)	29 (93.6)	22 (66.7)	23 (71.9)	18 (100.0)	23 (71.9)	23 (71.9)	29 (93.6)	29 (93.6)	22 (66.7)	22 (66.7)	22 (66.7)
Invalid	0 (0.0)	0 (0.0)	1 (2.1)	0 (0.0)	5 (16.7)	2 (6.7)	0 (0.0)	5 (9.6)	0 (0.0)	5 (16.7)	2 (6.7)	5 (9.6)	0 (0.0)	5 (15.6)	1 (3.2)	4 (12.1)	5 (15.6)	0 (0.0)	5 (15.6)	5 (15.6)	1 (3.2)	1 (3.2)	4 (12.1)	4 (12.1)	4 (12.1)

^a AZ (ChAdOx1-S); PF (BNT162b2); MO (mRNA-1273); JAN (Ad26.COV2.5).

antiviral vaccines. Tregs enables the control of excessive T cell responses, including the production of IFN- γ , to prevent further damage from uncontrolled inflammation, which is a hallmark of SARS-CoV-2 infection.^{26–28}

The elevation of the anti-inflammatory properties such as interleukin-6 (IL-6), IL-10, IL-18, and the cytokine transforming growth factor beta (TGF- β) was reported in the patient with SARS-CoV-2 infection.^{29,30} Induction of antigen-specific Treg cells was also been observed following the complete influenza vaccination.³¹ Interestingly, the accumulation of these antigen-specific Treg cells was later restricted following the booster immunization with an adjuvanted peptides-containing vaccine and lead to the stimulation of robust T cell immunity.³¹ Moreover, Silva-Cayetano et al. reported that prime immunization with AZ generates the production of Tregs and that the prime-boost strategy of AZ corrected dysregulated CD8⁺ T cell priming and enhance the CD4⁺ T cell in aged mice.³²

Given that: 1. SARS-CoV-2 vaccination mimics the actual infection; 2. The adenovirus-based AZ vaccine may enhance inflammation in healthy individuals, and 3. The majority of those who received homologous AZ in this study were 50 years or older (75%), we assumed that the fluctuation of the IFN- γ after administration of homologous AZ may be related to the Tregs activity.

The differential dosages that were used for each regimen could have also contributed to the fluctuation of the IFN- γ level following the administration of AZ vaccines. It was thought that using half of the recommended dosage for the second dose administration would be a viable way to reduce supply concerns during the initial mass vaccination of COVID-19^{33,34}. The second vaccine regimen which uses a lower dosage than the manufacturer recommends, however, exhibits dose-dependent immunogenicity because it produces lesser cytotoxic T-cells (IFN- γ) compared to regimens that follow the recommended dose size.³³ In this study all AZ-based immunization regimens used the same dosage in accordance with South Korea’s COVID-19 vaccination policy (0.5 mL each).³⁵ Therefore, we conclude that the fluctuation of IFN- γ after administration of AZ in our study is may due to the activity of Tregs to maintain immune homeostasis. Nevertheless, additional investigation is needed to determine the cause of fluctuation in the T cell response following AZ vaccination given that the AZ vaccine has just recently become widely administered.

Following published reports on the occurrence of cerebral venous thrombosis and some other side effects after the administration of AZ,^{36,37} several countries are now advising their citizens who previously received AZ for the first dose of vaccination to take an alternative vaccine for the second dose, with mRNA vaccines being recommended. In our study, the heterologous vaccine group comprised participants who received AZ and PF vaccines. We did not observe significant increases in IFN- γ concentration or in the number of T cell-producing IFN- γ after the first dose of AZ in the heterologous vaccine group, although a plausible increase was observed in the homologous AZ group. We assumed that this discrepancy occurred because the date for sampling following the first dose of the vaccine in the heterologous AZ group was conducted 5 weeks later than that in the homologous AZ group. A previous study has reported that the AZ vaccine induces T cell S-specific

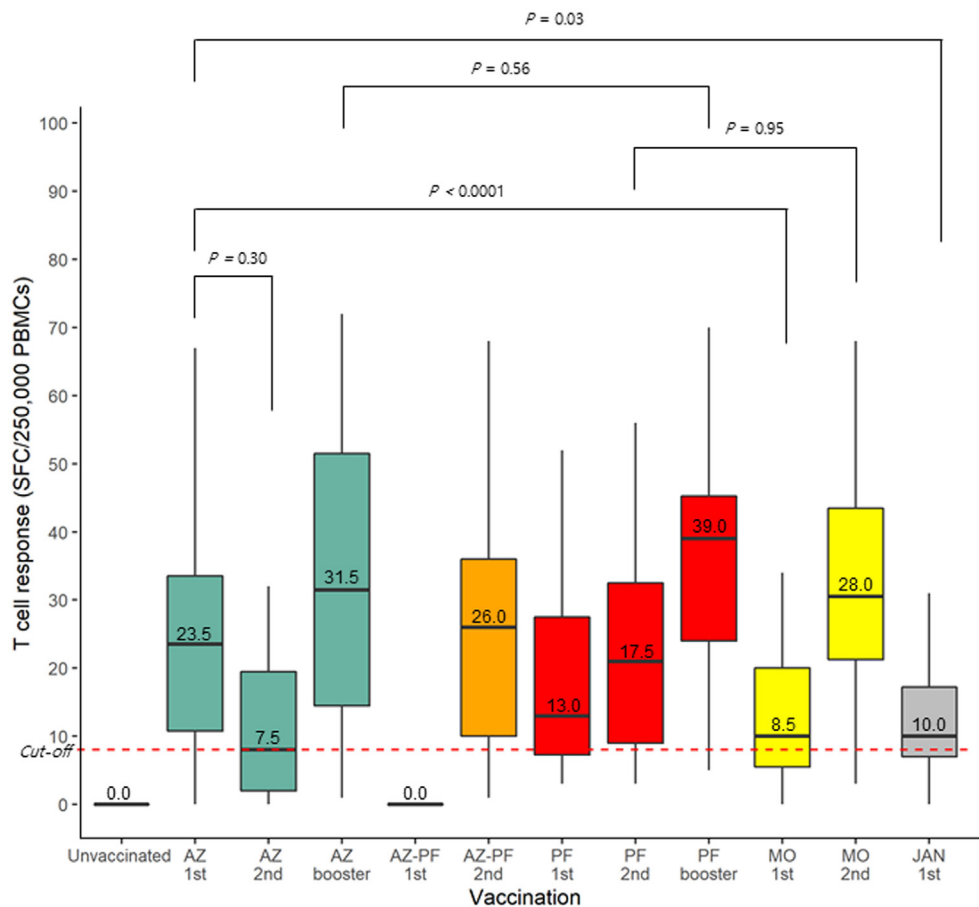


Figure 2. The number of T cells that produce IFN- γ in response to spike proteins of SARS-CoV-2 after the first, the second, and booster doses of COVID-19 vaccines in healthy individuals. Dotted line indicates the cut-off value (8 SFC/250,000 PBMCs). Administration of AZ triggered the highest number of T cells-producing IFN- γ after the first dose, while MO triggered the highest number after administration of the second dose. Administration of PF as the booster dose triggered insignificantly higher T cells-producing IFN- γ compared to the AZ. Notes: AZ (ChAdOx1-S); PF (BNT162b2); MO (mRNA-1273); JAN (Ad26.COVS.2.S). Abbreviations: SFC, spots-forming cells; PBMC, peripheral blood mononuclear cells.

responses approximately 14–22 days after the first dose.³⁸ Hence, at 9 weeks after the first vaccination with AZ in the heterologous group, the immune response had already waned and reached the baseline. In contrast, the administration of PF as the second dose in the heterologous vaccine group yielded a higher production of IFN- γ by T cells compared to the first dose with AZ. This finding is consistent with several studies that have reported that PF as an alternative second dose to AZ yielded an enhanced immune response.^{39,40}

In our study, we also found that the administration of COVID-19 vaccines triggered a high production of NABs, as evidenced by the high percentage of inhibition in the vaccinated groups compared to the unvaccinated group. Furthermore, a single dose of the mRNA vaccine, MO, yields the highest percentage of inhibition of the virus compared to the other vaccine groups. Our data are consistent with a recently published study in which MO vaccination triggered a significant increase in neutralizing potency compared with that in unvaccinated individuals.⁴¹ Although our study used a sVNT to assess the NABs, the results of our study are

still comparable with the results of the live virus-neutralization assay.⁴²

Additionally, our study showed that the T and B cell response was greater in the JAN vaccine recipients than in the unvaccinated group. However, the augmentation of T cell responses is not as strong as in a single dose of MO or PF. The B cells response was also observed to be higher than in the unvaccinated group and insignificantly higher than in a single dose of AZ but lower than in a single dose of MO or PF. Our study is in concordance with other studies on the COVID-19 immunogenicity which reported that administration of a single dose of JAN triggers higher T and B cell responses than in the unvaccinated group but lower than MO or PF recipients.^{43,44}

Our study also demonstrated that the booster dose triggered robust cellular and humoral immune responses. The AZ booster dosage was offered in South Korea along with the PF vaccine despite not being preferred in some nations due to some recorded unfavorable side effects. A total of 20 participants in this study with a median age of 36 who received AZ as the booster dose demonstrated a

Table 4 cPass sVNT assay positivity according to vaccine and regimen.

	Unvaccinated		AZ ^a 1st		AZ 2nd		AZ booster		AZ/PF 1st		AZ/PF 2nd		PF 1st		PF 2nd		PF booster		MO 1st		MO 2nd		JAN 1st	
	N (%)		N (%)		N (%)		N (%)		N (%)		N (%)		N (%)		N (%)		N (%)		N (%)		N (%)		N (%)	
Negative	107 (99.1)		13 (46.4)	9 (18.8)	0 (0.0)		16 (53.3)	0 (0.0)	0 (0.0)		30 (100.0)	0 (0.0)	2 (6.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	9 (27.3)
Positive	1 (0.9)		15 (53.6)	39 (71.2)	20 (100.0)		14 (46.7)		30 (100.0)		28 (93.3)	52 (100.0)	18 (100.0)	52 (100.0)	32 (100.0)	31 (100.0)	31 (100.0)	31 (100.0)	31 (100.0)	31 (100.0)	31 (100.0)	31 (100.0)	24 (72.7)	

^a AZ (ChAdOx1-5); PF (BNT162b2); MO (mRNA-1273); JAN (Ad26.COV2.5).

comparable level of neutralizing antibody to those who receive PF. This finding is consistent with a previously reported study in which the administration of a booster dose of AZ provided comparable protection against SARS-CoV-2 infection with an efficacy of up to 93.1% whereas the efficacy of PF was up to 94.0–95.3%.^{45,46} Additionally, considering the side effect of the COVID-19 vaccine not exclusively occur only in AZ recipients, for some people who had a significant adverse reaction to the mRNA-based vaccine, administration of AZ as the booster dose is necessary. Therefore, given the possibility of waning immunity and the emergence of new viral strains, the administration of a booster dose by either mRNA or adenovirus-based COVID-19 vaccines may be essential for longer and broader protection against SARS-CoV-2.

The main limitation of our study was the lack of measurements of the total immunoglobulins. Nevertheless, we utilized the sVNT assay to evaluate the production of NABs after the COVID-19 vaccines had been administered. The NABs bind to the RBD protein, leading to the inability of SARS-CoV-2 to bind to ACE2 and inhibit the entry of the virus into host cells to undergo replication and cause infection.^{47,48} The higher levels of NABs reported have been associated with increased protection against viral replication in the lungs and nose.⁴⁹ Thus, increments in NABs after vaccination can be used as an indicator of the efficacy of COVID-19 vaccines in protecting healthy individuals from SARS-CoV-2 infection. Another limitation of our study is our inability to include participants who received MO as a booster dosage and the presence of invalid results in the IGRA assays. The invalid results, which occurred when the test does not give either positive or negative results, can be due to a variety of reasons. In our investigation, the Covi-FERON ELISA assay and the T-SPOT assay had invalid results in the range of 0–2.1% and 0–16.7%, respectively. Given the diversity in technical and performance parameters, there will inevitably be heterogeneity between the two IGRA test results in this study. The Covi-FERON ELISA assay is based on the ELISA technique and uses a whole blood sample for analysis. Since whole blood is used in this assay, the test can be performed immediately after sample collection. The T-SPOT assay, on the other hand, is based on the ELISPOT technique and utilized peripheral blood mononuclear cells (PBMCs) for analysis. The T-SPOT assay cannot be carried out immediately since the PBMCs must be separated from the whole blood. Thus, the handling of the blood sample, the PBMC isolation procedure, or blood storage conditions prior to PBMC isolation may have an impact on the PBMCs' condition, which could result in a greater invalid result for the T-SPOT assay than the Covi-FERON ELISA assay. Nevertheless, the T cell response in our study was assessed concurrently by the Covi-FERON ELISA assay and T-SPOT assay, and both IGRAs demonstrated agreement in the overall results, thus the existence of the invalid outcomes of the T-SPOT assay will not weaken the conclusion in our study. Finally, despite these drawbacks, our study offers sufficient information on the T and B cell responses to various COVID-19 vaccine kinds and brands, which might be used as evidence to justify the necessity for additional vaccinations as boosters.

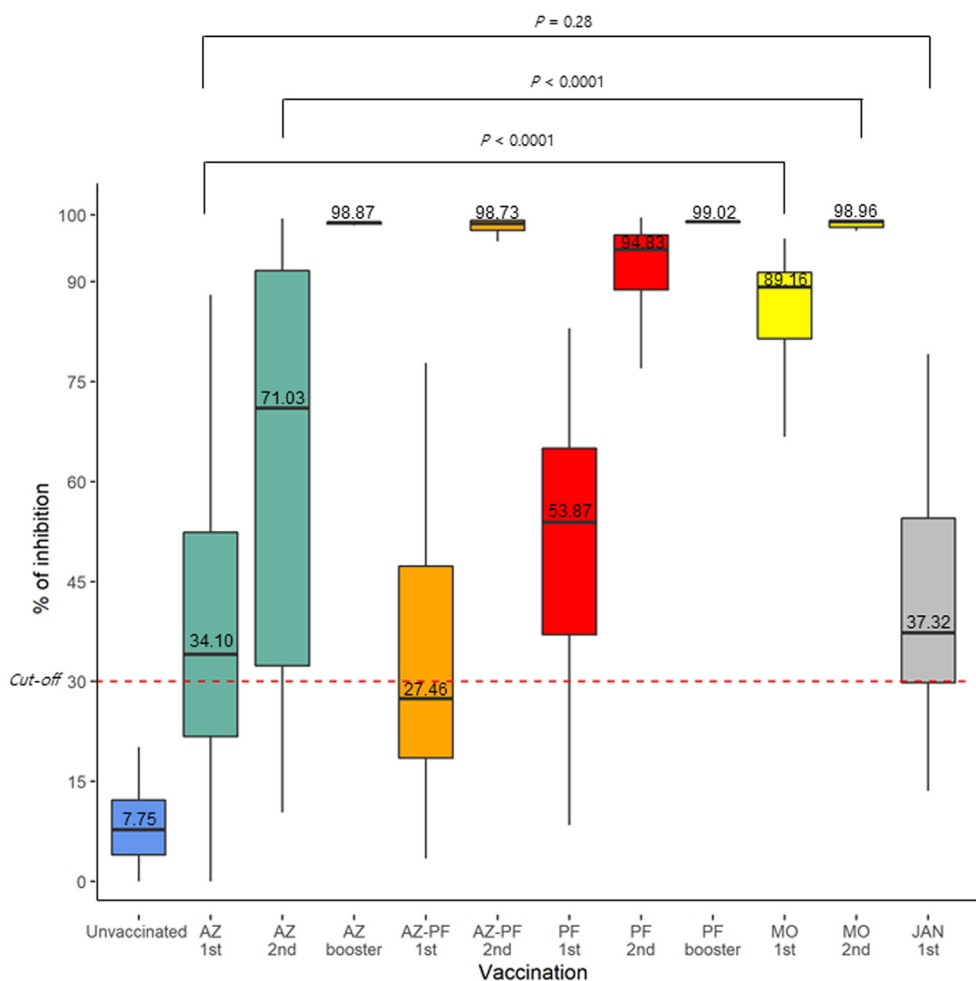


Figure 3. The percentage of inhibition following the first, the second, and booster doses of COVID-19 vaccines in healthy individuals with 1:20 diluted sera. The dotted line indicates the cut-off value (30%). Administration of the MO vaccine triggered a significantly higher percentage of inhibition after the first and the second doses than the AZ. Administration of the booster dose induces a higher percentage of inhibition than the ones in the second dose. Notes: AZ (ChAdOx1-S); PF (BNT162b2); MO (mRNA-1273); JAN (Ad26.COV2.S).

Ethical approval

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board Gyeongsang National University Changwon Hospital (IRB No. 2021-03-20).

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Declaration of competing interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jmii.2022.09.004>.