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Original Article

The humoral and cellular immune responses following booster vaccination with SARS-CoV-2 mRNA in people living with human immunodeficiency virus

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ABSTRACT

Introduction: People living with human immunodeficiency virus (PLWH) have higher mortality rates from COVID-19 than those without HIV. Additionally, the seroconversion rate of antibodies following a second dose of SARS-CoV-2 vaccine is lower in PLWH than non-infected individuals, indicating the need for booster vaccination. Here, we evaluated the humoral and cellular immune responses to booster SARS-CoV-2 vaccination in PLWH.

Methods: The dynamics of anti-spike IgG titers and antigen-specific interferon (IFN)-γ levels to SARS-CoV-2 vaccination were assessed over a 6-month period following a third vaccination of 34 PLWH.

Results: Antibody titers for humoral immunity were 50 % lower at 24 weeks post-vaccination than those at 12 weeks. However, those at 24 weeks after the booster vaccination were approximately eight times higher than before. Regarding cellular immunity, IFN-γ levels at 24 weeks after the third vaccination were lower than those at 12 weeks, but nearly 90 % of participants maintained a cut-off value of ≥0.15 IU/mL. A comparison between two groups with $CD4^+$ T lymphocytes counts of <500/μL or ≥500/μL exhibited no statistically significant differences in antibody or IFN-γ levels. However, in the group with $CD4^+$ T lymphocyte counts of <500/μL, the rate of IFN-γ above the cut-off value at 24 weeks after the booster vaccination was lower than that of ≥500/μL.

Conclusion: An immune response is expected in PLWH given successful antiretroviral therapy with booster SARS-CoV-2 vaccination. However, caution should be exercised for cases with low $CD4^+$ T-lymphocyte counts. (240/250 words)

1. Introduction

While the severity of the coronavirus disease 2019 (COVID-19) pandemic has subdued, it remains a threat to older and immunocompromised hosts. People living with human immunodeficiency virus (PLWH) tend to have severe infections and a high rate of mortality from COVID-19 [1,2]. The mRNA-based severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccines induce robust immune responses and suppress mortality and COVID-19 disease rates [3–5]. However, the immune response declines over time after the first series of vaccinations [6]. Furthermore, antibody titers have been reported to decline more in PLWH than healthy individuals [7], findings that are similar to those noted for influenza and hepatitis B virus [8,9]. Therefore, continuous booster vaccinations are necessary for immunocompromised individuals, including PLWH, and understanding the immune response to such boosters is essential for designing future vaccine strategies. Various observational studies have been conducted on PLWH immune responses following booster vaccination, indicating higher antibody production than in the primary series [10,11]. Understanding the role of cellular as well as humoral immunity in the control of SARS-CoV-2 is essential [12,13]; however, there are few reports on the immune responses at 6 months, especially regarding cellular immunity, to additional vaccinations.

In this study, we examined the responses of humoral and cellular immunity to mRNA booster vaccinations in PLWH.

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2. Methods

2.1. Study design

Between February 2022 and March 2023, we performed an observational study on PLWH outpatients attending the Department of the General Medicine at Kyushu University Hospital. A total of 163 patients who received a primary series dose of mRNA SARS-CoV-2 vaccine (the BNT162b2 or mRNA-1273, NVX-CoV2373) were enrolled. We collected blood samples from PLWH during regular visits at the following three time points, before (Time 0: T0), 3–12 weeks after (Time 1; T1), and 24 weeks after (Time 2; T2) booster vaccination, to assess the continuous immune responses. To further investigate the relationship between human immunodeficiency virus (HIV) status and immune responses to booster vaccination, the patients were divided for comparison into two groups based on the $CD4^+$ T lymphocyte count at the third vaccination: one with a $CD4^+$ T lymphocytes counts of $<500/\mu L$ and one with $CD4^+$ T lymphocytes counts of $\geq 500/\mu L$. Patients who had already received the booster vaccination at the time of study entry were excluded, as were participants who had evidence of current or previous infection with SARS-CoV-2 to eliminate the impact of prior COVID-19 infection. Furthermore, we measured anti-nucleocapsid(N) antibodies at all times from T0 to T2 to eliminate the influence of asymptomatic infections and excluded patients who tested positive at least once (Fig. 1). Patient information and laboratory data regarding HIV status and co-morbidities were extracted from computerized medical records.

The study protocol was approved by the Kyushu University Hospital institutional review board (approval no. 22022-00), and written informed consent was obtained from all participants. Funding for this study was covered by the operating expenses for our department.

2.2. SARS-CoV-2 humoral immune response

We evaluated the amount of serum anti-spike SARS-CoV-2 IgG to assess the humoral immune response to the SARS-CoV-2 vaccine. Titers of the anti-spike (S) IgG were quantified using the SARS-CoV-2 IgG II Quant assay (Abbott Diagnostics, Chicago, IL, USA). The results for anti-spike IgG are expressed as arbitrary units per milliliter (AU/mL) (positive threshold: 50 AU/mL). We also performed qualitative tests of IgG antibodies against the SARS-CoV-2 N protein (positive thresholds: 1.40 index [S/C]) to evaluate the effects of past SARS-CoV-2 infections.

2.3. SARS-CoV-2 cellular immune response

To assess cellular immune responses to the SARS-CoV-2 vaccine, SARS-CoV-2 stimulation of interferon (IFN)-γ was measured using the QuantiFERON SARS-CoV-2 assay kit (QuantiFERON SARS-CoV-2 Starter Set Blood Collection Tubes; Qiagen, Germantown, MD, USA). This assay contains two SARS-CoV-2-specific antigens (Antigen 1 [Ag1] and Antigen 2 [Ag2]) of the S protein. Ag1 is a $CD4^+$ epitope derived from the S1 subunit that is recognized by $CD4^+$ T lymphocytes. Ag2 is a $CD4^+$ and $CD8^+$ epitope derived from the S1 and S2 subunits that is recognized by $CD4^+$ and $CD8^+$ T lymphocytes. The IFN-γ produced was measured after reacting these antigens *in vitro* with whole blood collected from the participants. Moreover, background levels of IFN-γ produced in the negative control and positive control samples were assessed. Finally, a cellular immune response to the SARS-CoV-2 vaccine was defined as an IFN-γ value at least 0.15 IU/mL greater than that of the negative control.

2.4. Statistical analysis

All analyses were performed using JMP pro version 16.0.0 (2021 SAS Institute Inc.). Continuous variables are presented as means and standard deviations or as geometric means and 95 % confidence intervals (CIs). Categorical variables are expressed as counts and percentages. Differences between groups were assessed using a χ^2 test or the Mann–Whitney *U* test. A *P* value less than 0.05 is considered to indicate statistical significance.

3. Results

3.1. Characteristics of participants

As a total of 129 patients met the exclusion criteria, 34 patients were enrolled in the present study (Fig. 1). The patients were divided into two groups according to the number of $CD4^+$ T lymphocytes at the third vaccination: Group 1 (G1) comprised patients with $CD4^+$ T lymphocyte counts of 500/μL or higher, and Group 2 (G2) were patients with counts of less than 500/μL. In total, 19 PLWH were included in G1 and 15 in G2. The baseline characteristics of the two groups are shown in Table 1. Although no differences in age, sex, or co-morbidities were found among the two groups, the body mass index and $CD4^+/CD8^+$ T lymphocyte ratio were higher in G1 than G2, and patients in G2 were more likely to

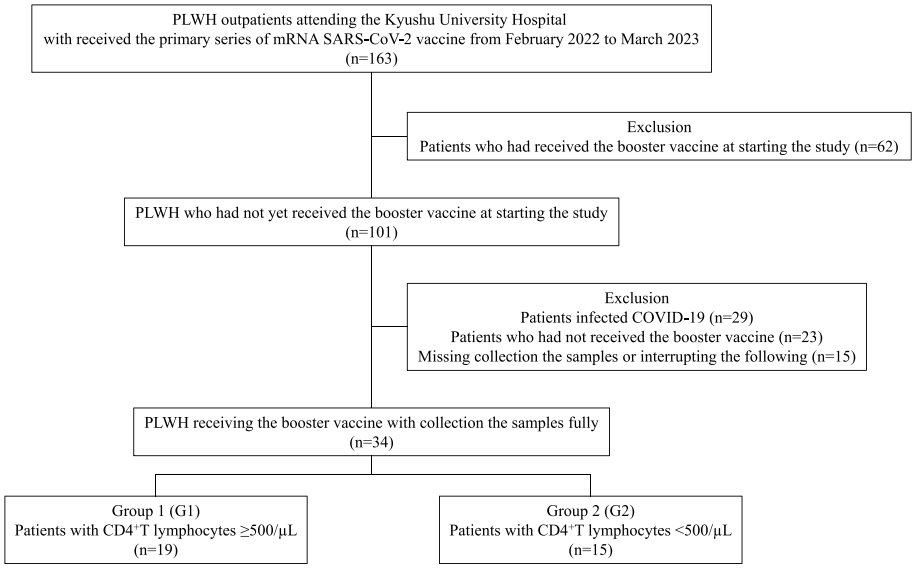

Fig. 1. Study flowchart
The figure shows the study flowchart for the enrollment and exclusion of participants. Abbreviations: PLWH, people living with human immunodeficiency virus; COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Table 1
Clinical characteristics of people living with human immunodeficiency virus between the two groups with CD4$^+$ T lymphocytes counts of ≥500/μL or <500/μL.

	Overall	CD4$^+$ T lymphocytes		*p*-value
		Group 1 (≥500/μL)	Group 2 (<500/μL)	
	N = 34	N = 19	N = 15	
Age, median (years, IQR)	45 (40–51)	45 (41–47)	51 (38–54)	0.25
Male (n, %)	34 (100)	19 (100)	15 (100)	-
Body mass index, median (kg/m^2, IQR)	25.5 (22.8–28.1)	26.8 (23.0–28.9)	24.3 (19.5–25.9)	0.02
CD4$^+$ T lymphocytes (counts/μL, IQR)[a]	521 (405–708)	694 (529–839)	390 (270–457)	<0.01
CD8$^+$ T lymphocytes (counts/μL, IQR)[a]	634 (490–968)	703 (591–1020)	617 (378–861)	0.19
CD4+/CD8+T lymphocytes ratio (counts, IQR)	0.74 (0.41–1.17)	0.85 (0.67–1.45)	0.56 (0.34–0.76)	0.02
Undergoing anti-retroviral therapy (n, %)	34 (100)	19 (100)	15 (100)	-
Duration of anti-retroviral therapy (days, IQR)[b]	2645 (1557–4055)	2740 (1826–4254)	2543 (1050–3545)	0.46
Past history of acquired immunodeficiency syndrome (n, %)	13 (38)	4 (21)	9 (60)	0.02
History of smoking (n, %)	13 (38)	7 (36)	6 (40)	0.85
Co-morbidities				
Diabetes militus (n, %)	3 (8)	1 (5)	2 (13)	0.41
Dyslipidemia (n, %)	8 (23)	5 (26)	3 (20)	0.67
Hypertension (n, %)	5 (14)	1 (5)	4 (26)	0.08
Chronic kidney disease (n, %)	5 (14)	1 (5)	4 (26)	0.08
Current history of malignant tumor (n, %)	0 (0)	0 (0)	0 (0)	-
Use of immunosuppressive agent (n, %)	1 (2)	1 (5)	0 (0)	0.38
HIV-RNA >50 copies/mL (n, %)	5 (14)	2 (10)	3 (20)	0.44
Type of the booster vaccine				
BNT162b2 (n, %)	16 (47)	8 (42)	8 (53)	0.51
mRNA-1273 (n,%)	16 (47)	9 (47)	7 (46)	0.9
NVX-CoV2373 (n, %)	2 (5)	2 (5)	0 (0)	0.20
Median time to T0 from 2nd vaccination, median (days, IQR)	181 (169–201)	176 (166–200)	181 (176–212)	0.13
Median time to T1 from 3rd vaccination, median (days, IQR)	53 (42–66)	53 (43–66)	49 (33–66)	0.65
Median time to T2 from 3rd vaccination, median (days, IQR)	171 (152–209)	167 (150–220)	172 (164–203)	0.91

HIV, human immunodeficiency virus.
[a] These counts were measured at the 3rd vaccination.
[b] The duration indicates the median time to 3rd vaccination from the start of anti-retroviral therapy.

have a history of acquired immunodeficiency syndrome (AIDS). There was only one case each of viral blip (HIV-RNA 50–200 copies/mL) or virologic failure (HIV-RNA>200 copies/mL) during the follow up period. Only two cases in the G2 reached CD4$^+$ T lymphocyte counts of 500/μL or higher at either T1 or T2. There was no significant difference in the median times from second shot to T0, or time to T1 and T2 after the booster shot between G1 and G2 respectively (Table 1).

3.2. Vaccine-induced humoral immune response

The anti-S antibody titers following a booster shot at T1 and T2 are shown in Fig. 2. Overall, the third vaccination resulted in an approximate 14-fold increase in antibody titers at T1, and the antibody titers were halved at T2. However, high titers of 4160 AU/mL were maintained in about 70 % of cases at T2. A comparison of the two groups (G1, G2) showed no significant difference at either T1 or T2.

3.3. Vaccine-induced cellular immune response

The levels of IFN-γ produced by SARS-CoV-2-specific antigen (Ag1 and Ag2) stimulation *in vitro* are shown in Fig. 3. The changes in IFN-γ levels at T1 were +0.46 IU/mL for Ag1 and +0.8 IU/mL for Ag2, and those at T2 were +0.29 IU/mL for Ag1 and +0.36 IU/mL for Ag2 compared with those at T0. There were no significant differences in IFN-γ values between G1 and G2 at any time from T0 to T2. Despite this, the rates of patients who maintained IFN-γ levels above 0.15 IU/mL at T2 were higher in G1 (100 %) than G2 (73 %). Univariate analysis of the maintenance of IFN-γ above 0.15 IU/mL at T2 showed no correlation with respect to factors such as age, co-morbidities, past history of AIDS, type of mRNA SARS-CoV-2 vaccine, or anti-S antibody titer, and only the factor, CD4$^+$ T lymphocyte counts above 500/μL, showed a significant association (p-value <0.05) (Table 2). To examine the relationship between humoral and cellular immunity, Spearman's rank correlation coefficient was calculated for anti-S antibody titers and IFN-γ production (Ag1 and Ag2); however, no significant correlations were found. Furthermore, since the collection time set at T1 varied from 3 to 12 weeks in this study, we also examined the correlation between the time to collect samples after vaccination and anti-S antibody titers or levels of IFN-γ (Fig. 4). The r-values were all found to be low (≤0.3), therefore, there was no correlation between the time from T0 to T1 and the anti-S antibody or IFN-γ levels. Similar correlations are shown for G1 and G2 in Fig. 5. In G2, levels of IFN-γ (Ag1 and Ag2) decreased with the later collection period with significant correlation.

4. Discussion

The present study revealed the characteristics of the immune response to booster SARS-CoV-2 vaccination in PLWH. Although there have been several previous reports on the immune responses to mRNA SARS-CoV-2 vaccination in PLWH, it is important to evaluate not only humoral immunity but also cellular immunity to understand the immune responses to mRNA SARS-CoV-2 vaccination in PLWH with impaired cellular immunity. Furthermore, continuous evaluation of the effect is necessary to determine the need and optimum schedules for additional future vaccinations. In the present study, evaluation was conducted up to 6 months after booster vaccination to examine the sustained immune responses, including those related to cellular immunity.

With regard to the humoral immune response to the SARS-CoV-2 vaccine, booster vaccination increased anti-S antibody titers up to 30-fold before the vaccination and halved them at six months after vaccination; however, they remained eight times higher than levels before vaccination. Similar to the present study, a report examining the effects of booster vaccination in PLWH found that neutralizing antibodies were still significantly higher 6 months after booster vaccination than at the time of the initial vaccination [14]. Although there was no clear correlation between CD4$^+$ T lymphocyte counts and antibody titers in the present study, it has been reported that PLWH with CD4$^+$ T lymphocyte counts of less than 200/μL had lower levels of anti-S antibodies and neutralizing antibodies to the SARS-CoV-2 vaccine compared with those of healthy controls [15].

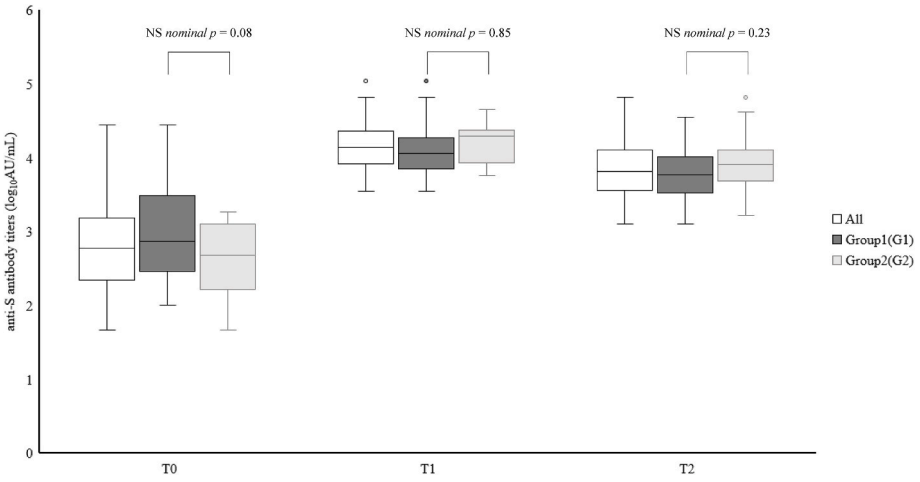

Fig. 2. Anti-S antibody titers at three time points
The figure shows the anti-S antibody titers at the three time points after severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccination; T0 is before the booster vaccination, T1 is 3–12 weeks after the booster vaccination, T2 is 24 weeks after the booster vaccination. Each of the three graphs shows the three groups: all patients (white), $CD4^+$ T cells ≥500/μL (black), and $CD4^+$ T cells <500/μL (gray).

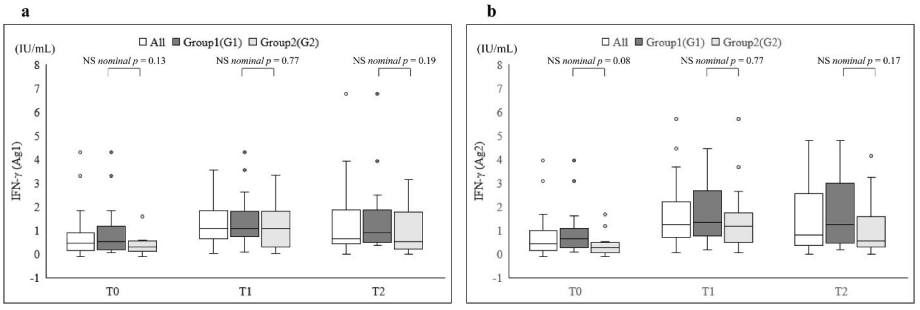

Fig. 3. Levels of IFN-γ produced against SARS-CoV-2-specific antigens (Ag1 and Ag2) at three time points
The figure shows the levels of interferon (IFN)-γ produced against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-specific antigens at the three time points after SARS-CoV-2 vaccination; T0 is before the booster vaccination, T1 is 3–12 weeks after the booster vaccination, T2 is 24 weeks after the booster vaccination. Each of the three graphs shows the three groups: all patients (white), $CD4^+$ T cells ≥500/μL (black), and $CD4^+$ T cells <500/μL (gray).
a: Levels of IFN-γ produced against SARS-CoV-2-specific antigen 1 (Ag1).
b: Levels of IFN-γ produced against SARS-CoV-2-specific antigen 2 (Ag2).

Table 2
Comparison between the rates of IFN-γ ≥ 0.15 IU/mL produced against SARS-CoV-2-specific antigen Ag1 or Ag2 at T2 time points.[a].

	Overall	IFN-γ level against Ag1			IFN-γ level against Ag2		
		≥0.15 IU/mL	<0.15 IU/mL	*p*-value	≥0.15 IU/mL	<0.15 IU/mL	*p*-value
	N = 34	N = 30	N = 4		N = 31	N = 3	
Age ≥65 years	2 (5)	1 (3)	1 (25)	0.08	1 (3)	1 (33)	**0.03**
BMI ≥28 kg/m^2	9 (26)	8 (26)	1 (25)	0.94	8 (25)	1 (33)	0.77
History of smoking	13 (38)	11 (36)	2 (50)	0.6	12 (38)	1 (33)	0.85
Use of immunosuppressive agent	1 (2)	1 (3)	0 (0)	0.71	1 (3)	0 (0)	0.75
Comorbidities							
Diabetes	3 (8)	3 (10)	0 (0)	0.5	3 (9)	0 (0)	0.57
Chronic kidney disease	5 (14)	4 (13)	1 (25)	0.53	4 (12)	1 (33)	0.34
Hypertension	5 (14)	4 (13)	1 (25)	0.53	4 (12)	1 (33)	0.34
Past history of acquired immunodeficiency syndrome	13 (38)	12 (40)	1 (25)	0.56	12 (38)	1 (33)	0.85
HIV-RNA ≥50 copies/mL	5 (14)	4 (13)	1 (25)	0.53	4 (12)	1 (33)	0.34
$CD4^+$T lymphocytes ≥500/μL	19 (55)	19 (63)	0 (0)	**0.01**	19 (61)	0 (0)	**0.04**
Type of the booster vaccine							
BNT162b2	16 (47)	15 (50)	1 (25)	0.34	15 (48)	1 (33)	0.61
mRNA-1273	16 (47)	13 (43)	3 (75)	0.23	14 (45)	2 (66)	0.47
NVX-CoV2373	2 (5)	2 (6)	0 (0)	0.59	2 (6)	0 (0)	0.65

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; HIV, human immunodeficiency virus.
Statistics; number (%).
[a] T2 time point indicates 24 weeks after the booster SARS-CoV-2 vaccination.

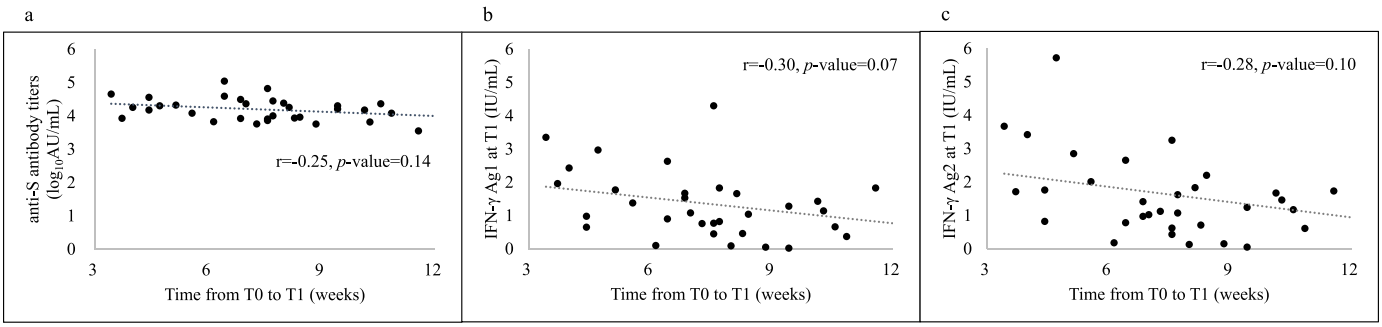

Fig. 4. The correlation between anti-S antibody titers or IFN-γ produced against SARS-CoV-2-specific antigens (Ag1 and Ag2) and the time from T0 to T1
The figure shows the correlation between anti-S antibody titers or IFN-**γ** and the time from T0 to T1; T0 is before the booster vaccination, T1 is 3–12 weeks after the booster vaccination.
a: The correlation between anti-S antibody titers at T1 and the time from T0 to T1
b: The correlation between levels of IFN-γ Ag1 at T1 and the time from T0 to T1
c: The correlation between levels of IFN-γ Ag2 at T1 and the time from T0 to T1.

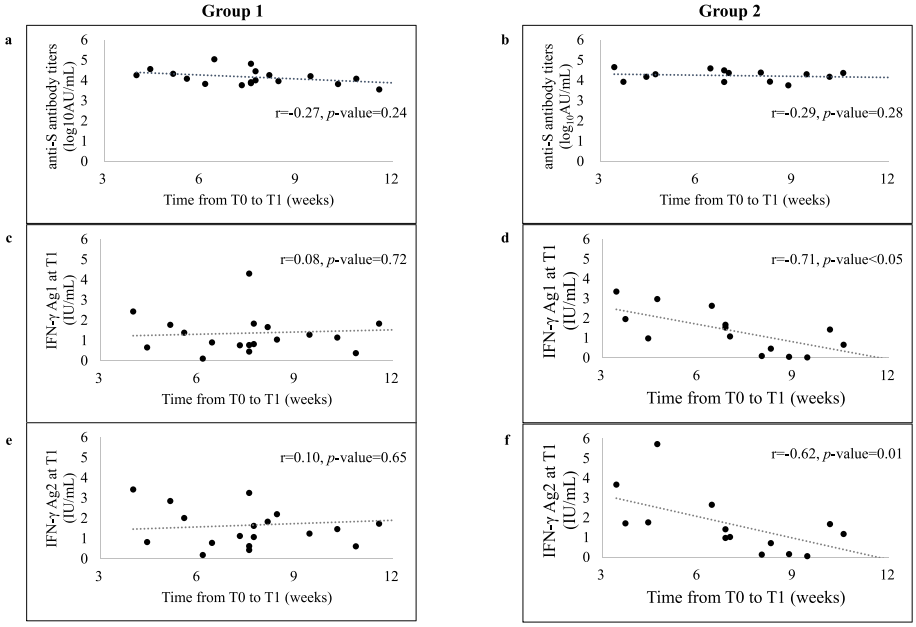

Fig. 5. The correlation between anti-S antibody titers or IFN-γ produced against SARS-CoV-2-specific antigens (Ag1 and Ag2) and the time from T0 to T1 for Group 1 (G1) and Group 2 (G2)
The figure shows the correlation between anti-S antibody titers or IFN-**γ** and the time from T0 to T1 for G1 and G2; T0 is before the booster vaccination, T1 is 3–12 weeks after the booster vaccination. Group 1 (G1) comprised patients with $CD4^+$ T lymphocyte counts of 500/μL or higher, and Group 2 (G2) comprised patients with counts of less than 500/μL.
a: The correlation between anti-S antibody titers at T1 and the time from T0 to T1 in G1.
b: The correlation between anti-S antibody titers at T1 and the time from T0 to T1 in G2.
c: The correlation between the levels of IFN-γ Ag1 at T1 and the time from T0 to T1 in G1.
d: The correlation between the levels of IFN-γ Ag1 at T1 and the time from T0 to T1 in G2.
e: The correlation between the levels of IFN-γ Ag2 at T1 and the time from T0 to T1 in G1.
f: The correlation between the levels of IFN-γ Ag2 at T1 and the time from T0 to T1 in G2.

As for cellular immunity to the SARS-CoV-2 vaccine, the present study showed that booster vaccination resulted in maintenance of the immune response. At the time point before the booster vaccination, the percentage of IFN-γ produced in response to the SARS-CoV-2-specific antigen was about 75 % at 0.15 IU/mL or higher; whereas, at six months after the third vaccination, 90 % of the patients had maintained levels of 0.15 IU/mL or higher. Although there are few studies similar to the present study on cellular immunity to the SARS-CoV-2 vaccine in PLWH, observational studies of other immunocompromised individuals have been reported. For example, there is a report of a study examining immune responses after booster SARS-CoV-2 vaccination in patients with inflammatory bowel disease (IBD) receiving anti-TNF-α inhibitors [16]. Similar to the present study, the QuantiFERON SARS-CoV-2 assay kit was used to evaluate cellular immune responses, and IFN-γ production in IBD patients was sustained at 5 months after booster vaccination, not significantly differing from that in healthy subjects. Similar results have also been reported for maintenance dialysis patients [17].

By contrast, a comparison between the two groups of patients with $CD4^+$ T lymphocyte counts of <500/μL or ≥ 500/μL in the present study showed no significant difference in S antibody titers, but the percentage of patients who maintained IFN-γ production above 0.15 IU/mL was lower in the group with low $CD4^+$ T lymphocyte counts, suggesting that the maintenance of a continuous cellular immune response is related to the number of $CD4^+$ T lymphocytes. This suggests that the number of

$CD4^+$ T lymphocytes is also related to the maintenance of cellular immune responses to the SARS-CoV-2 vaccine.

The limitations of the present study include the small number of cases, the lack of comparison with healthy subjects, and the fact that specimens were not collected at the same time from the different participants. In general, it is reasonable to define cellular immunodeficiency as a $CD4^+$ T lymphocyte count of 200/μL or less, and the immune response after vaccination should be compared at counts of approximately 200/μL. However, in this study, the number of participants with $CD4^+$ T lymphocyte counts below 200/μL was extremely limited, preventing meaningful comparative analysis. Therefore, we used a CD4 count of approximately 500/μL. It is difficult to apply the results of this study to patients who have severe immunodeficiency. Regarding the timing of sample collection, t-tests were performed to compare S antibody titers and IFN-γ levels for different periods from the booster vaccination date to T1 and T2, and no significant associations were found. Moreover, there is no significant difference in the time to T1 measurements between the two groups with $CD4^+$ T lymphocyte counts, so it is unlikely that the discrepancy in sample collection dates caused any bias. Nevertheless, as shown in Fig. 5, the analyzing by G1 and G2 showed that levels of IFN-γ Ag1 and Ag2 in the G2 group were negatively correlated with the time to collecting samples. Thus, it is possible that a bias due to the date of measurement may have occurred. However, since no such correlation was observed in G1, this result may support the final conclusion that cellular immunity cannot be expected to be maintained by vaccination for the group with low $CD4^+$ T lymphocyte counts.

In conclusion, the present study demonstrated that a continuous immune response occurs after mRNA SARS-CoV-2 vaccination in PLWH. For PLWH being provided appropriate treatment, a booster vaccination can be expected to maintain the immune response, but caution should be exercised for groups with low $CD4^+$ T lymphocyte counts.

Authorship statement

All authors meet the ICMJE authorship criteria. Y.M. and M.M. conceived and designed the study. Y.M. and M.M. collected the data. Y. M., M.M., A.O., S.Y., H.I. and K.T. analyzed and interpreted the data. Y. M. wrote the manuscript draft. M.M. and N.S. revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

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Consent for publication

Written informed consent was obtained from the patients in accordance with the Declaration of Helsinki for publication of this case report.

Declaration of competing interest

None.

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Corrigendum to "The humoral and cellular immune responses following booster vaccination with SARS-CoV-2 mRNA in people living with human immunodeficiency virus" [J. Infect. Chemother. 30 (2024) 417–422]

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The authors regret the incorrect sentence currently read as:

A total of 163 patients who received a primary series dose of mRNA SARS-CoV-2 vaccine (the BNT162b2 or mRNA-1273, NVX-CoV2373) were enrolled.

The correct sentence should read as:

A total of 163 patients who received a primary series dose of mRNA SARS-CoV-2 vaccine (the BNT162b2 or mRNA-1273) or a protein subunit vaccine (NVX-CoV2373) were enrolled.

The authors would like to apologise for any inconvenience caused.

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