Exploring the Utility of Enzyme-Linked Immunospot Assays in COVID-19

Kenji SAWAKI[1], Tokio HOSHINA[1], Tomomi MIYAMOTO[1], Yohei SAKAMOTO[1], Makiko MIYAJIMA[1], Kwangyeol LEE[1], Kazuhiko NAKAHARAI[1], Tetsuya HORINO[1], Yukie MISHIMA[2], Yasushi NAKAZAWA[1,2], and Masaki YOSHIDA[1]

[1]Department of Infection Control, The Jikei University School of Medicine
[2]Infection Prevention and Control Department, The Jikei University Hospital

ABSTRACT

Most studies of immunocompetence against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have focused on humoral immunity. Therefore, cellular immunity after coronavirus disease 2019 (COVID-19) infection or vaccination is still unknown. We conducted a single-center cross-sectional observational study in December 2021 to investigate the differences between SARS-CoV-2 serological assays : the enzyme-linked immunospot (ELISPOT) assay for cellular immunity and the enzyme-linked immunosorbent assay (ELISA) for humoral immunity. Thirty participants were enrolled : 5 who had had only 1 vaccination after COVID-19, 5 who had had 2 vaccinations after COVID-19, and 20 without COVID-19 who had received 2 vaccinations. The median interval from when blood samples were obtained was 244 days for the last vaccination and 396 days for infection. Spike-protein T-cell responses remained positive in all participants in both assays. The degree of spike-protein T-cell responses did not differ according to the presence or absence of postvaccination fever in the ELISPOT assay, and spike antibody titer also did not differ according to postvaccination fever. On the other hand, nucleocapsid- and membrane-specific T-cell responses lasted for a longer time (maximum, > 600 days) in participants with a history of COVID-19 compared to the ELISA. The results of this study indicate that after COVID-19 infection and vaccination, cellular immunity persists longer than humoral immunity. Therefore, previously infected persons can be identified more accurately with the ELISPOT assay than with ELISA.

(Jikeikai Med J 2023 ; 70 : 53-60)

Key words : COVID-19, enzyme-linked immunospot (ELISPOT) assay, antibody assay

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), first identified in 2019, has spread worldwide and has significantly affected healthcare and the economy. Various vaccines for coronavirus disease 2019 (COVID-19) have been developed and administered and have effectively controlled the spread of COVID-19. However, the vaccines are not completely effective, and breakthrough infections have occurred in persons who have been vaccinated[1,2].

Therefore, evaluating the immunity acquired with COVID-19 vaccines is crucial to determine the possibility of infection and countermeasures. The method most commonly used worldwide to assess immunity against SARS-CoV-2 is the enzyme-linked immunosorbent assay (ELISA), which measures neutralizing antibodies against SARS-CoV-2[3,4]. This assay has the advantages of being inexpensive and

Received : February 27, 2023 / Accepted : June 28, 2023
澤木 賢司，保科 斉生，宮本 智美，坂本 洋平，宮島真希子，李 広烈，中拂 一彦，堀野 哲也，美島 路恵，中澤 靖，吉田 正樹
Mailing address : Kenji SAWAKI, Department of Infection Control, The Jikei University School of Medicine, 3-25-8, Nishi-Shimbashi, Minato-ku, Tokyo 105-8461, Japan
E-mail : k_sawaki@jikei.ac.jp

easy to perform and is considered useful for evaluating immunity. In contrast, a disadvantage of ELISA is that it measures only humoral immunity, which is only one aspect of the immune response to vaccines. In contrast, the enzyme-linked immunospot (ELISPOT) assay measures cellular immunity, which acts upstream of humoral immunity in the immune response to vaccines. Although the number of studies of the ELISPOT assay has increased, not enough is known about its usefulness in measuring immunity against COVID-19.

The aim of the present study was to examine the strength and durability of cellular immunity to SARS-CoV-2, assessed with the ELISPOT assay, in persons who have either had COVID-19 or been vaccinated against SARS-CoV-2. Many studies have assessed immunity to SARS-CoV-2 by measuring humoral immunity, that is, antibody levels, by means of ELISA. However, the immune response to SARS-CoV-2 is a concerted effort of both cellular immunity and humoral immunity, and evaluating both is important for accurately assessing immunity. To obtain new insights into how infection immunity and cellular immunity are related, we evaluated cellular immunity with the ELISPOT assay and humoral immunity with the ELISA to comprehensively assess the immune response following COVID-19 infection or reception of a SARS-CoV-2 vaccine.

Material and Methods

1. *Study design and participants*

We conducted a single-center cross-sectional observational study in December 2021 with 10 participants who had a history of COVID-19 and 20 participants who did not. The participants, all of whom were healthcare workers, including physicians, nurses, and clinical engineers, were recruited from The Jikei University Hospital (Tokyo, Japan). A history of COVID-19 was defined on the basis of a positive SARS-CoV-2 polymerase chain reaction record. The participants were as follows : Group 1, 5 participants who had recovered from COVID-19 and had later received only 1 dose of a COVID-19 messenger (m) RNA vaccine ; Group 2, 5 participants who had recovered from COVID-19 and had later received 2 doses of a vaccine ; and Group 3, 20 participants who had no history of COVID-19 and had received 2 doses of a vaccine. All participants in this study were older than 20 years.

This study was approved by the Ethics Committee of The Jikei University School of Medicine for Biomedical Research 33-168(10785). All participants provided written informed consent to be included in the study. The procedures were performed in compliance with the principles of the Declaration of Helsinki (2008). After informed consent had been obtained, blood samples and completed questionnaires were collected from the participants.

2. *Measurement of cellular immunity*

Cellular immunity against SARS-CoV-2 was analyzed with the T-SPOT® *Discovery* SARS-CoV-2 ELISPOT assay kit (Oxford Immunotec, Oxford, UK). Blood samples were drawn into sodium heparin tubes, and peripheral blood mononuclear cells (PBMCs) were isolated with density gradient centrifugation. Isolated PBMCs were processed on the basis of the package insert and were incubated under 6 conditions : COVID-19 Panel 1, detecting T-cell immune responses induced by SARS-CoV-2 spike proteins (S1) ; COVID-19 Panel 3, detecting T-cell immune responses induced by SARS-CoV-2 nucleocapsid proteins ; COVID-19 Panel 4, detecting T-cell immune responses induced by SARS-CoV-2 membrane proteins ; and COVID-19 Panel 13, panel containing SARS-CoV-2 epitopes with a high degree of homology with endemic coronaviruses ; and negative and positive controls. Panel 13 allowed the cross-reactivity with endemic strains of coronaviruses to be investigated. A secreted cytokine, interferon-gamma, was captured with specific antibodies on the membrane. A secondary antibody was added and bound to the cytokine captured on the membrane surface. Finally, a soluble substrate was added to each well, which was cleaved by the bound enzyme to form an insoluble precipitate at the reaction site. Each spot represents the footprint of an individual cytokine-secreting T-cell.

The number of spot-forming cells (SFCs) obtained showed the abundance of antigen-specific effector T-cells in the peripheral blood. The number of SFCs in the negative control wells was subtracted from the antigen stimulation wells to quantify antigen-specific responses.

3. *Measurement of humoral immunity*

Humoral SARS-CoV-2 immunity was analyzed with the quantity of antibodies, measured with ELISA, against the SARS-CoV-2 nucleocapsid and the receptor-binding

domain (RBD) of the S1 subunit of the spike protein. Immunoglobulins specific for the SARS-CoV-2 spike protein (spike antibody) and nucleocapsid protein (nucleocapsid antibody) were quantified with the Abbott SARS-CoV-2 IgG II quantification assay (Abbott SARS-CoV-2 IgG II Quant assay; Abbott, Lake Bluff, IL, USA). Blood samples were centrifuged at 3,000 rpm for 5 minutes and run on the Abbott Alinity instrument following the manufacturer's instructions. The cut-off values (1.40 index value) for the nucleocapsid protein–specific immunoglobulin index were determined according to the manufacturer's instructions.

4. *Questionnaire*

The survey asked the participants the following questions: age, sex, underlying medical conditions, medications, adverse reactions to COVID-19 vaccination, history of COVID-19 infection, and symptoms during infection in those with a history of COVID-19. A "fever" was defined as a body temperature ≥ 38°C after vaccination and during COVID-19 infection. Adverse reactions were defined as symptoms occurring within 48 hours after vaccination.

5. *Statistical analysis*

Continuous data are expressed as medians with interquartile ranges (IQRs). Categorical data are presented as numbers and percentages. Continuous variables between groups were compared via the Mann–Whitney U test, and variables of Groups 1, 2, and 3 were compared via the Kruskal-Wallis test and Dunn's post hoc analysis. Categorical data were compared via Fisher's exact test. The correlation between 2 continuous numbers was calculated with Spearman's correlation analysis. All statistical analyses were performed with EZR[5], a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). More precisely, EZR is a modified version of the R commander designed to add statistical functions frequently used in biostatistics. A P value < 0.05 was considered to indicate statistical significance.

Results

1. *Cohort of participants*

The 30 participants had a median age of 25.5 years (range, 22-58 years) when blood samples were collected and consisted of 4 (13.3%) men and 26 (86.7%) women. One participant who had not had COVID-19 had received 2 doses of the mRNA-1273 vaccine (Moderna, Inc., Cambridge, MA, USA), and the other 29 participants received 1 or 2 doses of the BNT162b2 mRNA vaccine (BioNTech SE, Mainz, Germany). The median time until the collection of blood specimens was 244 days (IQR, 215-250.75 days) after the last COVID-19 vaccination and 395.5 days (IQR, 319.3-568.3 days) after the diagnosis of COVID-19 infection.

2. *Questionnaire*

Of the 30 participants, 4 (13.3%) had an underlying disease; 1 had bronchial asthma and was being treated with a medication (an oral glucocorticoid) that might affect immune acquisition. Of the 10 participants who had been infected with COVID-19 (Groups 1 and 2), 6 (60%) had a fever at the time of illness. After receiving the first dose of a COVID-19 vaccine, fever was reported by 7 participants (23.3%). Furthermore, after receiving a second dose of a vaccine (Groups 2 and 3), 16 (64.0%) of 24 participants reported having a fever.

3. *Cellular and humoral immune responses against SARS-CoV-2*

(1) Cellular immune response: S1-specific T-cell response

All participants showed S1-specific T-cell responses (range, 12-532 SFCs/10^6 PBMCs), and the median number of S1-specific T-cells was 84 SFCs/10^6 PBMCs (IQR, 56-242 SFCs/10^6 PBMCs) (Table 1). The S1-specific T-cell responses did not differ significantly among the 3 groups with a different history of COVID-19 infection or vaccination. Furthermore, the responses did not differ significantly between the groups with a history of COVID-19 infection (Groups 1 and 2) and the group without (Group 3). In addition, responses did not differ significantly between participants with or without a fever after COVID-19 vaccination (Fig. 1). Also, no significant difference was noted within the group with no history of COVID-19 (Group 3) between those who had or did not have a fever after COVID-19 vaccination. The T-cell responses specific to S1 were still present in several participants even more than 250 days after their last vaccination. No correlation was found between the decrease in S1-specific T-cell responses and the number of days after vaccination (Fig. 2). Even after the number of vaccinations and history of COVID-19 infection were

Table 1. Characteristics of participants included in the analysis

Variable	Values of participants				*P* value*
	All	Group 1	Group 2	Group 3	
Number of participants	30	5	5	20	
Age, year (range)	25.5 (22-58)	36 (25-58)	33 (28-36)	24 (22-48)	0.006
Sex (male/female)	4/26	1/4	2/3	1/19	0.095
History of COVID-19		Yes	Yes	No	
Time after COVID-19, days	395.5 (319.3-568.3)	324 (295-500)	467 (320-591)	NA	0.69
Number of COVID-19 vaccines received		1	2	2	
Fever after first vaccine dose	7 (23.3%)	2 (40%)	1 (20%)	4 (20%)	0.81
Fever after second vaccine dose	16 (64%)	NA	3 (60%)	13 (65%)	1
Time after last vaccination, days	244 (215-250.8)	235 (169-267)	238 (234-246)	245 (215-250.5)	0.986
S1-specific T-cell response, SFCs/10^6 PBMCs	84 (56-242)	56 (28-212)	208 (164-292)	84 (59-198)	0.438
Nucleocapsid-specific T-cell response, SFCs/10^6 PBMCs	4 (0-27)	56 (28-112)	28 (4-72)	4 (0-8)	0.024
Membrane-specific T-cell response, SFCs/10^6 PBMCs	2 (0-8)	20 (8-24)	4 (0-16)	0 (0-4)	0.064
Spike antibody, AU/mL	915 (658.4-2145)	2,545.5 (628.2-3556.1)	1,937.5 (1093.6-1983.9)	837.3 (619.5-1464.5)	0.310
Nucleocapsid antibody, AU/mL	0.085 (0.0425-0.155)	0.35 (0.08-0.46)	0.14 (0.07-0.27)	0.055 (0.0275-0.0925)	0.048

Continuous variables are expressed as median (interquartile range) unless indicated.
COVID-19, coronavirus disease 2019 ; SFCs, spot-forming cells ; PBMCs, peripheral blood mononuclear cells ; S1, S1 subtype of spike protein ; AU, antibody units ; NA, not available
*Comparisons among groups were conducted using the Mann-Whitney U test, the Kruskal-Wallis test, or Fisher's exact test.

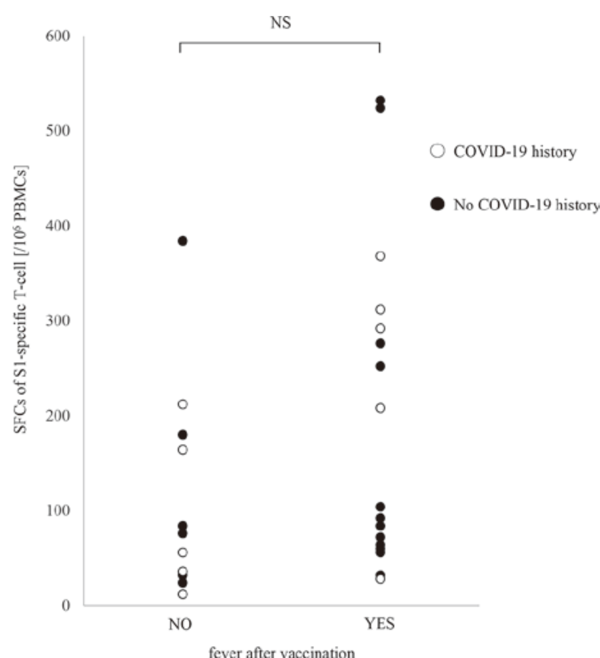

Fig. 1. Relationship between S1-specific T-cell responses and the presence or absence of postvaccination fever and a history of COVID-19.
The number of spot-forming cells of S1-specific T-cell did not differ significantly between participants with or without a fever after COVID-19 vaccination.
SFCs, spot-forming cells ; PBMCs, peripheral blood mononuclear cells ; NS, not significant

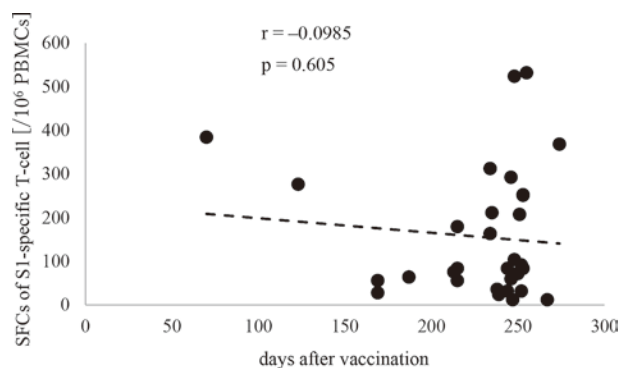

Fig. 2. Relationship between S1-specific T-cell response levels and the number of days since vaccination.
The dashed line is a regression line for the number of spot-forming cells of S1-specific T-cells and the number of days after the last vaccination.
A decrease in the number of spot-forming cells of S1-specific T-cells was not correlated with the number of days after vaccination ($r = -0.0985$, $p = 0.605$).
SFCs, spot-forming cells ; PBMCs, peripheral blood mononuclear cells

considered, no correlation was found.

(2) Cellular immune response : nucleocapsid-specific and membrane-specific T-cell responses

Both nucleocapsid-specific and membrane-specific T-cell responses were significantly higher in participants with

a history of COVID-19 than in those without (nucleocapsid-specific T-cell response : $p = 0.0038$; membrane-specific T-cell response : $p = 0.0169$). In addition, a moderate correlation was noted between nucleocapsid-specific and membrane-specific T-cell responses ($r = 0.679$, $P < 0.00005$). Although the strength of N-specific and membrane-specific T-cell responses in participants with a history of COVID-19 (Groups 1 and 2) were expected to decrease with the time after the infection, no correlation was found between the strength of the response and the number of days after infection (Fig. 3). No correlation was observed in nucleocapsid-specific and membrane-specific T-cell responses, except for those in participants with extremely low numbers of SFCs (< 4).

(3) Humoral immune response : spike antibody and nucleocapsid antibody

The median value of the spike antibody in all participants was 914.95 antibody units (AU)/mL (IQR, 658.375-2,145 AU/mL). The spike antibody titer did not differ significantly among the 3 groups. Furthermore, the S1-specific T-cell responses did not differ significantly between participants who had or did not have a fever after being vaccinated for COVID-19 (Fig. 4), even among participants with no history of COVID-19 infection (Group 3). On the other hand, the spike antibody titer and the number of days after vaccination were negatively correlated (Fig. 5).

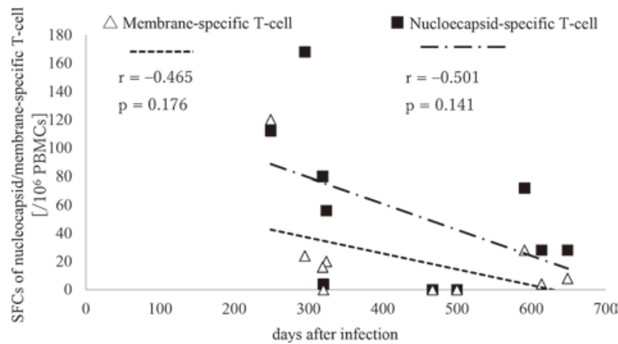

Fig. 3. Relationship among nucleocapsid-specific/membrane-specific T-cell response levels and the number of days since COVID-19 infection.
One dot-dashed line is a regression line for spot-forming cells (SFCs) of nucleocapsid-specific T-cells and the number of days after COVID-19 infection, and the dashed line is a regression line for SFCs of membrane-specific T-cells and the number of days after COVID-19 infection.
The number of days after COVID-19 infection was not correlated with the number of SFCs of nucleocapsid-specific T-cells ($p = 0.141$) or of membrane-specific T-cells ($p = 0.176$).
SFCs, spot-forming cells ; PBMCs, peripheral blood mononuclear cells

Only 1 participant in Group 3 was positive for the nucleocapsid antibody, while the remaining 29 participants, in-

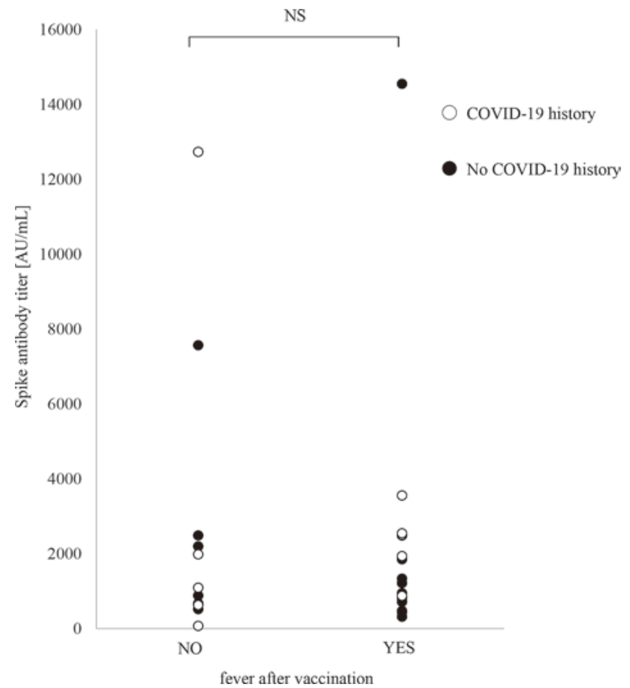

Fig. 4. Relationships of spike antibody titers and the presence or absence of postvaccination fever and the history of COVID-19 disease.
Spike antibody titers did not differ significantly between participants with or without fever after vaccination.
NS, not significant

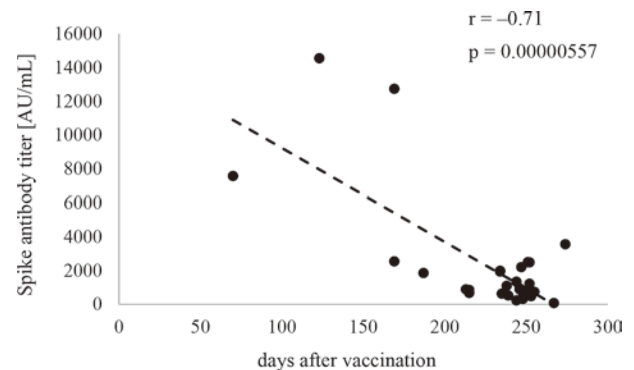

Fig. 5. Relation between spike antibody titer and the number of days since vaccination.
Dashed line is a regression line for the spike antibody titer and the number of days after the last COVID-19 vaccination.
A negative correlation was found between the spike antibody titer and the number of days after vaccination ($r = -0.71$, $p = 0.00000557$).

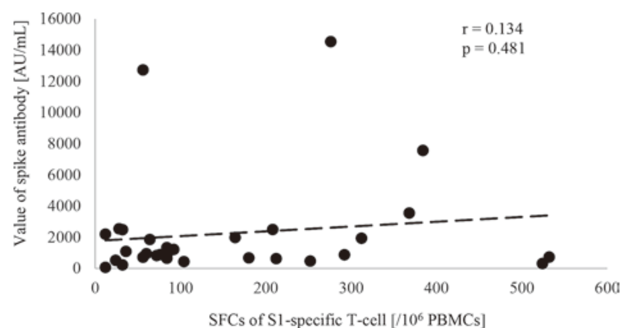

Fig. 6. Relationship between S1-specific T-cell response levels and the spike antibody titer.
The dashed line is a regression line for the number of spot-forming cells of S1-specific T-cells and the spike antibody titer.
No correlation was found between the number of spot-forming cells of S1-specific T-cells and the spike antibody titer ($r = 0.134$, $p = 0.481$).
SFCs, spot-forming cells ; PBMCs, peripheral blood mononuclear cells

cluding those with a history of COVID-19, had negative results. The S1-specific T-cell responses and the value of the spike antibody were not correlated (Fig. 6).

DISCUSSION

In the present study, cellular immunity obtained by COVID-19 infection and vaccination, represented by S1-specific T-cell responses, were similar in participants who had received 1 dose of a vaccine after infection and in participants who had received 2 doses after infection. Previous studies have found that S1-specific T-cell responses and spike protein-neutralizing antibodies are equivalent between 1 and 2 vaccinations in COVID-19–infected persons[6-9]. Additionally, as in a previous study[10], participants of the present study with no history of infection showed similar responses to the 2 vaccine doses. Several studies indicate that spike antibody titers are higher in persons who receive 2 doses of vaccine after COVID-19 infected than in persons who have not been infected[11,12].

Another finding of the present study was that postvaccination fever was not correlated with the strength of the S1-specific T-cell response but was correlated with the spike antibody titer. In contrast, a previous study has found that adverse reactions after the second dose of a COVID-19 vaccine is correlated with acquired cellular immunity examined with the QuantiFERON assay[13]. There are several possible reasons for the differences between the present study and the previous study in the correlation of cellular immunity and postvaccination fever. First, the tests used in the 2 studies differed between the ELISPOT assay and the QuantiFERON assay. A second possible reason is that cellular immunity was measured in the present study at a median time of about 35 weeks after vaccination but was measured in the previous study 8 weeks after vaccination. A third possible reason for the difference in correlation is that the previous study found that cellular immunity was stronger in persons with a history of COVID-19 infection but that the present study found that the strength of cellular immunity was not affected by the history of COVID-19 infection. Regarding the population, the median S1-specific T-cell response measured 6 months after 2 doses of a vaccine in a previous Japanese study that used the ELISPOT assay was 84 SFCs/10^6 PBMCs (IQR, 43-188 SFCs/10^6 PBMCs)[14], similar to our result. Therefore, that the population of the present study differed significantly from those of previous studies is unlikely. In humoral immunity, several studies have found a correlation between the spike antibody titer and adverse reactions after vaccination[15,16], while others have not[17]. Further studies of the relationship between immunocompetence and adverse reactions are warranted.

Another feature of the present study is that 26 of 30 participants had been vaccinated more than 6 months before the study began. The participants' immune status was evaluated as long as 274 days after the last vaccination. Although humoral immunity acquired with vaccination is known to decrease after approximately 6 months[18-20], less is known about the durability of cellular immunity. Several previous studies have found that T-cell responses 6 to 9 months after vaccination decrease to a lesser extent then do spike antibody titers, suggesting that T-cellular immunity is somewhat maintained over the long term[14,21]. Two doses of vaccine against infection have been reported to have a peak effect 1 month after vaccination and gradually decline. However, the effectiveness against any severe, critical, or fatal case of COVID-19 remains high even 6 months after vaccination[22]. In the present study, as in previous studies, S1-specific T-cell responses were maintained even in participants who had been vaccinated more than 9 months earlier. This finding suggests that T-cellular immunity is a major contributing factor in vaccination decreasing the risk of a severe disease. This finding also suggests that a possible reason for not being infected again with SARS-CoV-2, de-

spite low spike antibody titers, is that T-cellular immunity is preserved. Several previous studies have also found that the stronger strength of the T-cell response is associated with a milder outcome[23,24].

Another issue investigated by the present study was the possible contribution to epidemiological information of nucleocapsid-specific T-cell responses and membrane-specific T-cell responses via the ELISPOT assay. The nucleocapsid antibody titer, generally used to determine whether a person has ever had COVID-19, is known to decrease quickly and fall below the cut-off value. Previous studies have found that the half-life of nucleocapsid antibodies is 47 to 59 days[25,26] and that only 43.8% of infected persons have detectable nucleocapsid antibodies after 1 year of infection. In the present study, none of the 10 participants with a history of COVID-19 had detectable levels of nucleocapsid antibodies. In contrast, the ELISPOT assay showed that the nucleocapsid and membrane panels remained positive even in participants more than 600 days after infection, were young, and had no underlying immunocompromising diseases. These findings suggest that the ELISPOT assay is superior to tests of specific immunoglobulin G antibodies for determining the cumulative number of infected persons in a population that includes vaccinated and asymptomatically infected persons and, especially, young and healthy persons, such as healthcare workers.

The present study has several limitations. First, this was a cross-sectional study with a small sample size, and the number of days between COVID-19 infection and vaccination in the blood test was inconsistent. A second limitation is that individuals who had previously contracted COVID-19 might have mistakenly been classified as COVID-19–negative during analysis. For example, several participants in Group 3 were positive for both nucleocapsid-specific and membrane-specific T-cell responses. This result suggests that several participants in Group 3 had had an asymptomatic SARS-CoV-2 infection. However, the quality of infection assessment was not particularly poor in this study. Previous studies have also been limited by their ability to accurately assess the history of COVID-19. In this context, studies relying solely on nucleocapsid antibodies to evaluate past infections may have underestimated these results. A third limitation of the study is that the type of vaccine given to the participants was not standardized : one participant received mRNA-1273. This may have affected the S1-specific T-cell response and the spike antibody titer. However, the analysis excluding 1 mRNA-1273 vaccination did not show any change in trend from the present results. A fourth and final limitation of the present study is that the cellular and humoral immunities were measured only after 1 or 2 doses of the COVID-19 vaccine had been given.

Conclusion

This study shows that cellular immunity persists longer than humoral immunity after COVID-19 infection and vaccination. Therefore, previously infected persons can be identified more accurately with the ELISPOT assay than with ELISA. Further evaluation of immunity after the third or fourth dose, which started after this study recruitment, is needed.

Author contributions

Author KS contributed to the study design, data collection, statistical analysis, interpretation of data, and drafting and editing of the manuscript. TH contributed to the study design, data collection, and interpretation of the data. TM, YS, MM, KL, KN, YM, TH, YN, and MY contributed to data collection and supervision of the analysis. All authors critically revised the manuscript for important intellectual content and approved the final version. All the authors met the ICMJE authorship criteria.

Authors have no conflict of interest.

References

1. Bergwerk M, Gonen T, Lustig Y, Amit S, Lipsitch M, Cohen C, et al. Covid-19 breakthrough infections in vaccinated health care workers. N Engl J Med. 2021 ; 385 : 1474-84.
2. Brosh-Nissimov T, Orenbuch-Harroch E, Chowers M, Elbaz M, Nesher L, Stein M, et al. BNT162b2 vaccine breakthrough : clinical characteristics of 152 fully vaccinated hospitalized COVID-19 patients in Israel. Clin Microbiol Infect. 2021 ; 27 : 1652-7.
3. Ejazi SA, Ghosh S, Ali N. Antibody detection assays for COVID-19 diagnosis : an early overview. Immunol Cell Biol. 2021 ; 99 : 21-33.
4. West R, Kobokovich A, Connell N, Gronvall GK. COVID-19 antibody tests : a valuable public health tool with limited relevance to individuals. Trends Microbiol. 2021 ; 29 : 214-23.
5. Kanda Y. Investigation of the freely available easy-to-use soft-

ware 'EZR' for medical statistics. Bone Marrow Transplant. 2013 ; 48 : 452-8.
6. Hwang J-Y, Kim Y, Lee K-M, Jang E-J, Woo C-H, Hong C-U, et al. Humoral and cellular responses to COVID-19 vaccines in SARS-CoV-2 infection-naïve and -recovered Korean individuals. Vaccines (Basel). 2022 ; 10 : 332.
7. Favresse J, Bayart JL, Mullier F, Dogné JM, Closset M, Douxfils J. Early antibody response in health-care professionals after two doses of SARS-CoV-2 mRNA vaccine (BNT162b2). Clin Microbiol Infect. 2021 ; 27 : 1351.e5-7.
8. Ontañón J, Blas J, de Cabo C, Santos C, Ruiz-Escribano E, García A, et al. Influence of past infection with SARS-CoV-2 on the response to the BNT162b2 mRNA vaccine in health care workers : kinetics and durability of the humoral immune response. EBiomedicine. 2021 ; 73 : 103656.
9. van Gils MJ, van Willigen HDG, Wynberg E, Han AX, van der Straten K, Burger JA, et al. A single mRNA vaccine dose in COVID-19 patients boosts neutralizing antibodies against SARS-CoV-2 and variants of concern. Cell Rep Med. 2021 ; 3 : 100486.
10. Phillips E, Adele S, Malone T, Deeks A, Stafford L, Dobson SL, et al. Comparison of two T-cell assays to evaluate T-cell responses to SARS-CoV-2 following vaccination in naïve and convalescent healthcare workers. Clin Exp Immunol. 2022 ; 209 : 90-8.
11. Milazzo L, Pezzati L, Oreni L, Kullmann C, Lai A, Gabrieli A, et al. Impact of prior infection status on antibody response to the BNT162b2 mRNA COVID-19 vaccine in healthcare workers at a COVID-19 referral hospital in Milan, Italy. Hum Vaccin Immunother. 2021 ; 17 : 4747-54.
12. Desmecht S, Tashkeev A, El Moussaoui M, Marechal N, Perée H, Tokunaga Y, et al. Kinetics and persistence of the cellular and humoral immune responses to BNT162b2 mRNA vaccine in SARS-CoV-2-naive and -experienced subjects : impact of booster dose and breakthrough infections. Front Immunol. 2022 ; 13 : 863554.
13. Wakui M, Uwamino Y, Yatabe Y, Nakagawa T, Sakai A, Kurafuji T, et al. Assessing anti-SARS-CoV-2 cellular immunity in 571 vaccines by using an IFN-γ release assay. Eur J Immunol. 2022 ; 52 : 1961-71.
14. Kato H, Miyakawa K, Ohtake N, Yamaoka Y, Yajima S, Yamazaki E, et al. Vaccine-induced humoral response against SARS-CoV-2 dramatically declined but cellular immunity possibly remained at 6 months post BNT162b2 vaccination. Vaccine. 2022 ; 40 : 2652-5.
15. Yamamoto S, Fukunaga A, Tanaka A, Takeuchi JS, Inoue Y, Kimura M, et al. Association between reactogenicity and SARS-CoV-2 antibodies after the second dose of the BNT162b2 COVID-19 vaccine. Vaccine. 2022 ; 40 : 1924-7.
16. Uwamino Y, Kurafuji T, Sato Y, Tomita Y, Shibata A, Tanabe A, et al. Young age, female sex, and presence of systemic adverse reactions are associated with high post-vaccination antibody titer after two doses of BNT162b2 mRNA SARS-CoV-2 vaccination : an observational study of 646 Japanese healthcare workers and university staff. Vaccine. 2022 ; 40 : 1019-25.
17. Coggins SA, Laing ED, Olsen CH, Goguet E, Moser M, Jackson-Thompson BM, et al. Adverse effects and antibody titers in response to the BNT162b2 mRNA COVID-19 vaccine in a prospective study of healthcare workers. Open Forum Infect Dis. 2021 ; 9 : ofab575.
18. Levin EG, Lustig Y, Cohen C, Fluss R, Indenbaum V, Amit S, et al. Waning immune humoral response to BNT162b2 Covid-19 vaccine over 6 months. N Engl J Med. 2021 ; 385 : e84.
19. Ponticelli D, Antonazzo IC, Caci G, Vitale A, Della Ragione G, Romano ML, et al. Dynamics of antibody response to BNT162b2 mRNA COVID-19 vaccine after 6 months. J Travel Med. 2021 ; 28 : taab173.
20. Khoury J, Najjar-Debbiny R, Hanna A, Jabbour A, Abu Ahmad Y, Saffuri A, et al. COVID-19 vaccine – Long term immune decline and breakthrough infections. Vaccine. 2021 ; 39 : 6984-9.
21. Herzberg J, Fischer B, Lindenkamp C, Becher H, Becker AK, Honarpisheh H, et al. Persistence of immune response in health care workers after two doses BNT162b2 in a longitudinal observational study. Front Immunol. 2022 ; 13 : 839922.
22. Chemaitelly H, Tang P, Hasan MR, AlMukdad S, Yassine HM, Benslimane FM, et al. Waning of BNT162b2 vaccine protection against SARS-CoV-2 infection in Qatar. N Engl J Med. 2021 ; 385 : e83.
23. Rydyznski Moderbacher C, Ramirez SI, Dan JM, Grifoni A, Hastie KM, Weiskopf D, et al. Antigen-specific adaptive immunity to SARS-CoV-2 in acute COVID-19 and associations with age and disease severity. Cell. 2020 ; 183 : 996-1012. e19
24. Moss P. The T cell immune response against SARS-CoV-2. Nat Immunol. 2022 ; 23 : 186-93.
25. Terpos E, Stellas D, Rosati M, Sergentanis TN, Hu X, Politou M, et al. SARS-CoV-2 antibody kinetics eight months from COVID-19 onset : persistence of spike antibodies but loss of neutralizing antibodies in 24% of convalescent plasma donors. Eur J Intern Med. 2021 ; 89 : 87-96.
26. Pradenas E, Trinité B, Urrea V, Marfil S, Ávila-Nieto C, Rodríguez de la Concepción ML, et al. Stable neutralizing antibody levels 6 months after mild and severe COVID-19 episodes. Med (N Y). 2021 ; 2 : 313-20.e4.