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Short communication

Vaccine-induced humoral response against SARS-CoV-2 dramatically declined but cellular immunity possibly remained at 6 months post BNT162b2 vaccination

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ABSTRACT

To evaluate vaccine-induced humoral and cell-mediated immunity at 6 months after completion of two doses of BNT162b2 vaccination, immunoglobulin G against SARS-CoV-2 spike protein (SP IgG), 50% neutralizing antibody (NT_{50}), and spot-forming cell (SFC) counts were evaluated by interferon-γ releasing ELISpot assay of 98 healthy subjects (median age, 43 years). The geometric mean titers of SP IgG and NT_{50} decreased from 95.2 (95% confidence interval (CI) 79.8–113.4) to 5.7 (95% CI 4.9–6.7) and from 680.4 (588.0–787.2) to 130.4 (95% CI 104.2–163.1), respectively, at 3 weeks and 6 months after the vaccination. SP IgG titer was negatively correlated with age and alcohol consumption. Spot-forming cell counts at 6 months did not correlate with age, gender, and other parameters of the patients. SP IgG, NT_{50}, and SFC titers were elevated in the breakthrough infected subjects. Although the levels of vaccine-induced antibodies dramatically declined at 6 months after vaccination, a certain degree of cellular immunity was observed irrespective of the age.

1. Introduction

Severe acute respiratory coronavirus-2 (SARS-CoV-2) vaccine is a powerful tool to control the coronavirus disease-2019 (COVID-19) pandemic and the mRNA vaccine BNT162b2 (Pfizer-BioNTech, USA) is reported to have a vaccine efficacy of 95% [1]. However, the long-term decline in efficacy in preventing infection has been a major concern regarding most vaccines, with most vaccines reportedly exhibiting declining efficacy after 6 months after the vaccination [2,3]. However, the efficacy of a vaccine in preventing severe disease is reported to remain at a high level. The titer of immunoglobulins (IgG) against spike proteins (SPs) induced by the vaccine dropped to 7% in 6 months [4]. The decline in the efficacy for preventing the infection can be attributed to the decrease in antibody titer, although the prevention of severe disease cannot be explained by antibody titer alone and cellular immunity may be involved. mRNA vaccines induce not only humoral immunity but also cellular immunity [5]. We previously evaluated IgG against SARS-CoV-2 SP (SP IgG) by chemiluminescent enzyme immunoassay (CLEIA) before and up to 6 weeks after the first dosing with BNT162b2 vaccine [6]. In this study, we analyzed the SP IgG level against the original and Delta strains by CLEIA and 50% neutralizing titer (NT_{50}) at 6 months after vaccination. In addition, SARS-CoV-2-specific T-cell response was evaluated by interferon γ (IFN- γ) releasing ELISpot assay to assess the status of cellular immunity at 6 months after vaccination.

2. Subjects and methods

The healthcare workers at the Yokohama City University Hospital who had completed two doses of the BNT162b2 vaccine

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(Comirnaty 30 μg, Pfizer/BioNTech, USA) given intramuscularly on the deltoid muscle (lot number: EP9605 as a first dose and ER9480 as a second dose) between March and April 2021 at a 3-week interval were recruited in this study. For the 6-month post-vaccination evaluation, the blood samples were drawn at 180 ± 15 days after the second dose of vaccination. The subjects' information, including the date of birth, sex, alcohol consumption (active drinker or non-drinker), smoking status (active smoker or non-smoker/ex-smoker), comorbidities, body height, and body weight at the time of first dose of vaccination were obtained. The participants' age and body mass index (BMI) at the time of first vaccination was then calculated.

Immunoglobulin G titer against SP and nucleocapsid (NP) IgG were determined using the commercial chemiluminescent enzyme immunoassay (AIA-CL SARS-CoV-2 SP IgG antibody detection reagent, Tosoh, Japan). Furthermore, the IgG index values between the original and Delta stain (D614G, T478K, P681R, and L452R) were measured and compared as previously described [6]. The cut-off values (1.0 index value) for NP and the SP IgG index were determined according to the manufacturer's instructions. The neutralizing titer assay was performed using an HIV-based pseudovirus bearing the SARS-CoV-2 spike. Then, 50% neutralizing titer (NT_{50}) values were calculated using the Image J software (NIH). NT_{50} values of $\leq$ 50 were considered negative. All samples were assayed in at least duplicate experiments. SARS-CoV-2-specific T-cell response was measured using interferon (IFN)-γ ELISpot analysis (T-SPOT Discovery SARS-CoV-2, Oxford Immunotec Ltd, UK). The corresponding blood samples were submitted for examination at SRL laboratory (H.U. Frontier Co., Ltd. Tokyo, Japan). As the specific T-cell response against SARS-CoV-2, the number of spots forming cells (SFC) per 250,000 peripheral blood mononuclear cell (PBMC) reported were calculated by subtracting the number of the negative control panel from the number of SFC in Spot 1 (peptide pool for SP). SFC was multiplied by four to express the final results in 10^6 PBMCs.

Continuous data were presented either as means with 95% confidence interval (CI) or medians with interquartile range (IQR). Categorical data were presented as numbers and percentages. Continuous variables between the two groups were compared using the two-tailed Mann–Whitney *U* test. Categorical data were compared using the Fisher's exact test. The correlation between two continuous numbers was calculated by Spearman's correlational analysis. A multivariable regression model was employed to investigate the association between the background variables and antibody titers. The SP IgG index values were log-transformed for analysis to remove positive skewness. Statistical analyses were performed using the Prism 9.1 (GraphPad Software, San Diego, CA, USA) and JMP Pro 15 software (SAS Institute, Cary, NC, USA). $P < 0.05$ was considered to indicate statistical significance. We previously analyzed the SP IgG index titers after 1 and 3 weeks after two doses of BNT162b2 in the other study [6]. In this study, written informed consent was obtained independently from each participant. This study was approved by the relevant institutional review board (Yokohama City University Hospital, approval number: B210800024).

3. Results

After excluding 4 subjects who failed to submit their blood samples at the designated date and 3 subjects who were undergoing antitumor chemotherapy or immunosuppression, 98 subjects were analyzed. The subjects were of median age 43 (IQR 38–49) years, BMI of 21.8 (IQR 19.9–24.2), and included 24 males and 74 females. There were 32 (32.7%) active drinkers and 5 (5.6%) active smokers among the subjects. There were four patients with COVID-19 history before vaccination and four patients with breakthrough infection that occurred after the completion of the second dose of vaccination. The history of COVID-19 and breakthrough infections was considered as medical history of the participants, which was reported to the Infection Prevention and Control Department of our institution and was serologically confirmed by the change to positive in the antibody to nucleocapsid protein of SARS-CoV-2. The breakthrough infections were individual cases. The occupational categories were 18 physicians, 37 nurses, 20 technicians, 6 pharmacists, and 17 others.

The geometric mean titer (GMT) of SP IgG index titer and NT_{50} of all the participants are shown in Fig. 1A and 1B. The GMTs of SP IgG index titer were 48.75 (95% CI 37.93, 62.66), 97.02 (81.58, 115.4), and 6.751 (5.399, 8.443) and of NT_{50} were 727.5 (95% CI 597.3, 886.1), 680.4 (588.0, 787.2), and 130.4 (104.2, 163.1) at 1 week, 3 weeks, and 6 months after the second dose of vaccination, respectively. SP-specific T-cell count for all the participants was 84 (IQR 43–188) (range: 0–700) SFC per million PBMCs.

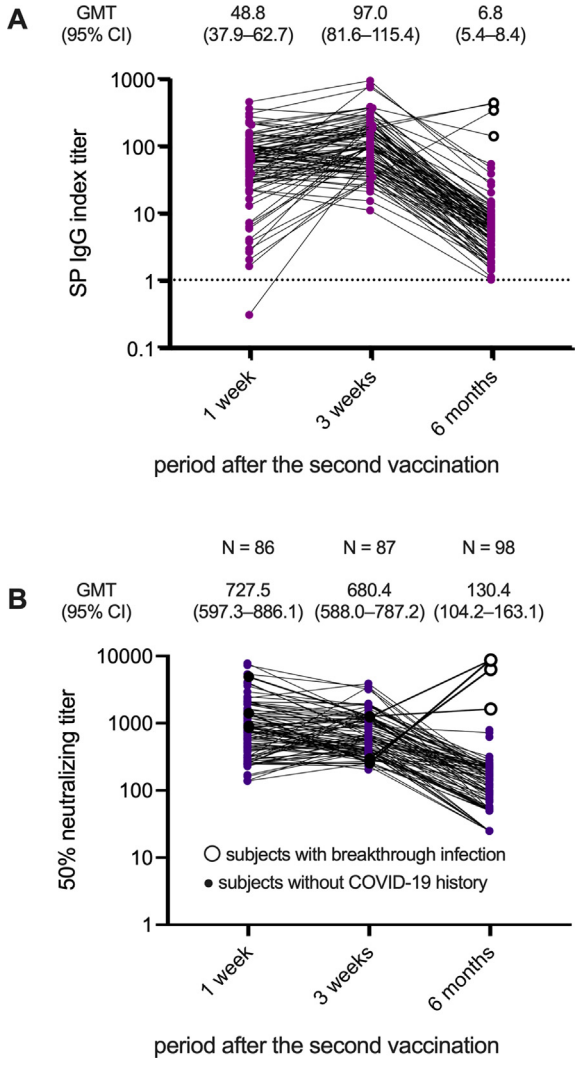

Fig. 1. Spike protein (SP) IgG index titer (A) and the 50% neutralizing titer (NT_{50}) (B) at 1 week, 3 weeks, and 6 months after the second dose of the BNT162b2 vaccination. The white circle represents subjects with breakthrough infection after the completion of vaccination. (A) Geometric mean titers of SP IgG index titer decreased from 3 weeks to 6 months after 2 doses of vaccination (from 97.0 to 6.8). SP IgG index 1.0 was set as the threshold of positive antibody according to the testing kit manufacturer.. (B) NT_{50} decreased from 1 week to 6 months after 2 doses of vaccination (from 727.5 to 130.4).Abbreviations: GMT, geometric mean titer; CI, confidence interval.

In 94 subjects, excluding the 4 subjects with breakthrough infections, the GMT of SP IgG index titer was 47.7 (95% CI 36.8, 61.9), 95.2 (95% CI 79.8, 113.4), and 5.7 (95% CI 4.9, 6.7) at 1 week, 3 weeks, and 6 months after vaccination, respectively. The ratio of SP IgG index titer to the Delta strain to the original strain was 0.605 (IQR 0.515–0.718) after 3 weeks of 2 doses and 0.609 (IQR 0.539–0.693) after 6 months (Fig. 2). The GMT of NT_{50} was 111.4 (95% CI 94.15, 131.7) 6 months after the second dose in 94 subjects without breakthrough infections. A correlation was noted between the SP IgG index titer and age and alcohol consumption. The GMT of SP IgG index titer among non-drinkers was significantly higher than that among active drinker {7.57 (5.89, 9.73) vs 5.34 (95% CI 3.37, 8.44)}. Multivariable regression analysis performed to exclude the confounders revealed that age and alcohol consumption were negatively correlated with the SP IgG index titer (Table 1). The number of SFC in the 94 subjects without breakthrough infection was a median of 80 (IQR 40–176) (range: 0–472) SFC per million PBMCs after 6 months of 2 doses. A weak correlation was noted between the SP-specific T-cell response and the SP IgG index titer (r = 0.216, P = 0.033), albeit no correlation was noted with NT_{50} (r = 0.181, P = 0.076) (Fig. 3A and 3B). The SP-specific T-cell response was observed in 14 subjects who were negative for NT_{50} (48 [IQR 22–113] SFC per million PBMCs). The SP protein-specific T-cell response did not correlate with age, gender, BMI, alcohol consumption, or smoking status (Appendix Table 1).

In the 4 subjects with breakthrough infections, the SP IgG index titers ranged from 146.1 to 459.1, while NT_{50} ranged from 1631 to 8756, and SP protein-specific T-cell response ranged from 80 to 700/ SFC per million PBMCs 6 months after the second dose of BNT162b2 vaccination. These subjects were reported to be infected with SARS-CoV-2 5 months after the second dose (August to early September 2021, when Delta strain was dominant in Japan). The ratio of SP IgG index against the Delta/original strains (0.902 to 0.954) was high in these patients (Fig. 2).

4. Discussions

At 6 months of receiving the second dose of BNT162b2 vaccine, SP-specific IgG decreased markedly, with a mean GMT decreasing from 95.2 at 3 weeks after vaccination to 5.7 at 6 months. A previous report showed a peak at 1 week after 2 doses and a decrease to 7% at 6 months [4]. Our data using the CLEIA method demonstrated a surprising decrease to 1/15 after 3 weeks of vaccination, and the same trend was noted for NT_{50}, which also depicted a marked decrease. However, the correlation between cellular immunity assessed by SP-specific T-cell response and the SP IgG index titer and NT_{50} was weak, suggesting that cellular immunity may have a different dynamic from antibody titer. In this study, the SP-specific T-cell response was 84 SFC per million PBMCs. The previously reported SP-specific T-cell response immediately after vaccination in other study cohort was 184 SFC per million PBMCs after

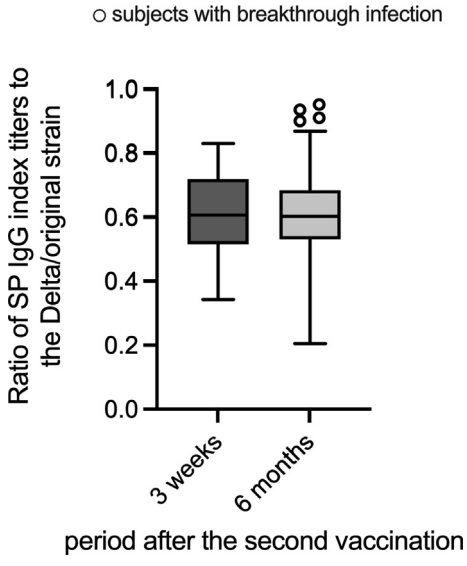

Fig. 2. Ratio of SP IgG index titers to the Delta strain to the original strain. The ratio was 0.605 (IQR 0.515–0.718) after 3 weeks of 2 doses in 98 subjects and 0.609 (IQR 0.539–0.693) after 6 months in 94 subjects. White circles represent the subjects with breakthrough infection. Higher ratio of SP IgG index titers to the Delta to the original strain was noted in these four cases.Abbreviations: SP IgG, immunoglobulin G against spike protein.

Table 1
Multivariable regression analysis of the background characteristics affecting the SP IgG index titers in subjects at 6 months after the second BNT162b2 vaccination in subjects without COVID-19 breakthrough infection.

Background factor	ß	95% CI	*P* value
Age (year)	−0.0099	(−0.018, −0.002)	0.012 *
Male gender	0.0321	(−0.132, 0.196)	0.699
BMI	0.0120	(−0.008, 0.032)	0.232
Current drinker	−0.1839	(−0.332, −0.036)	0.015 *
Current smoker	−0.1179	(−0.420, 0.184)	0.440

Abbreviations: BMI, body mass index; CI, confidence interval.
* statistically significant.

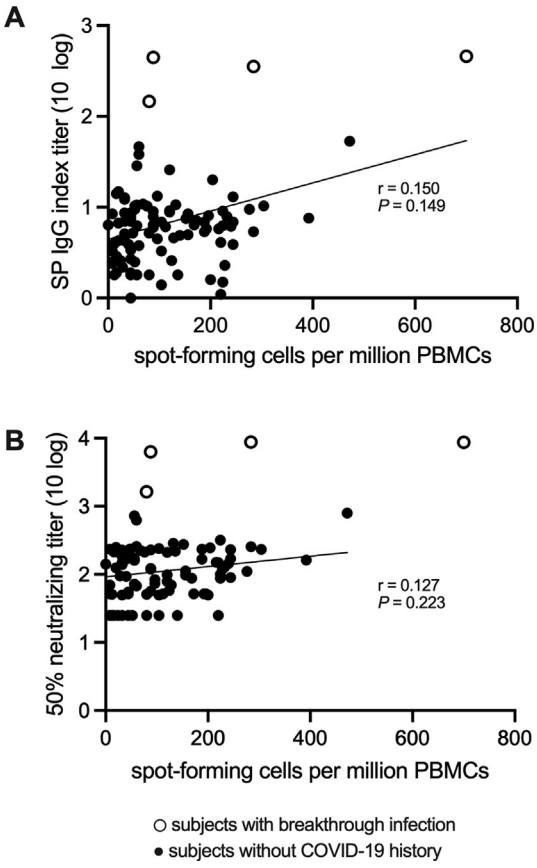

Fig. 3. Association between several spots forming cells (SFC) using T-SPOT SARS-CoV-2 and immunoglobulin G against spike protein (SP IgG) (A), and 50% neutralizing titer (NT_{50}) (B). The white circle represents subjects with breakthrough infection after the completion of vaccination. The number of SFC and SP IgG index titer and NT_{50} showed no significant correlation. Correlation analysis was performed using Spearman's correlation analysis, comparing 10 logged SP IgG indexes, NT_{50}, and the number of SFC.Abbreviations: SP IgG, immunoglobulin G against spike protein; PBMC, peripheral blood mononuclear cell.

two doses of BNT162b2 vaccine [7]. The ELISpot assay at 6 months in naturally infected individuals reported 97 (IQR 38–143) SFC per million PBMCs, and the SP-specific T-cell response observed in the study participants at 6 months post-vaccination was not significantly different from that observed in naturally infected individuals (P = 0.865, calculated using supplemental data from reference [8]). There seemed no difference between cellular immunity conferred by natural infection and the reduction in cellular immunity by vaccines. It has been previously reported that the antibody titer is negatively correlated with age up to 6 months after vaccination [9], but SP-specific T-cell response has not been related to age [10]. Our study showed no correlation among age, gender, BMI, or alcohol and smoking status. While a study reported a strong correlation between cellular immunity and neutralizing antibody titers in convalescent subjects [11], cellular immunity has been observed in antibody negative subjects as well [12]. SARS-CoV-2 specific lymphocytes are maintained between 6 months and 1 year after infection [13], and the dynamics of cellular immunity and antibody titer were observed to be different [14]. The decline in cellular immunity was slower than that of antibody titer, indicating it to be less negatively correlated with age, which possibly explains its long-term effect in protecting from developing the severe form of this disease.

In this study, four cases of breakthrough infection after the second vaccination were investigated. It is believed that a high antibody titer was induced in patients with breakthrough infection as previously reported [15]. Although the titer of antibodies against SP of the Delta strain was 60% of that against the SP of original strain in the patients without breakthrough infection, the ratio of anti-Delta/anti-original antibody titers was as high as 90% in the patients with breakthrough infection. In Japan, the Delta strain has been the predominant strain from April to December 2021 [16]. Furthermore, our study showed that antibodies of the Delta strain are strongly induced in individuals infected with the Delta strain [17]. This study has several limitations. For instance, the data on SP-specific T-cell response immediately after the vaccination was lacking and the sample size of the cohort was small. In addition, generally, cellular immunity is weakened in elderly and immunosuppressed individuals. It is necessary to evaluate the long-term humoral and cellular immunocompetences for evaluating the long-term efficacy of vaccines and to consider the required adaptation for additional vaccinations. Our study demonstrated that antibody titers do not necessarily correlate well with cellular immunity, indicating that the dynamics of cellular immunity are different from those of humoral immunity and suggesting that the evaluation of cellular immunity is warranted for long-term evaluation of the efficacy of SARS-CoV-2 vaccines.

Author contributions

HKato contributed to the study design, data collection, statistical analysis, and interpretation of data, as well as the drafting and editing of the manuscript. SY, EY, and TS contributed to the study design and data collection. KM, NO, and YY performed the laboratory tests. TM, AG, HNakajima, and AR contributed to the study design, data collection, supervision of the analysis, and preparation of the manuscript. All authors made critical revisions to the manuscript for important intellectual content and approved the final manuscript. All authors meet the ICMJE authorship criteria.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2022.03.057.

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