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Dynamics of antibody titers and cellular immunity among Japanese healthcare workers during the 6 months after receiving two doses of BNT162b2 mRNA vaccine

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Abstract

Background: The antibody titer is known to wane within months after receiving two doses of the Pfizer-BioNTech BNT162b2 mRNA SARS-CoV-2 vaccine. However, knowledge of the cellular immune response dynamics following vaccination is limited. This study aimed to determine antibody and cellular immune responses following vaccination, and the incidence and determinants of breakthrough infection.

Methods: This prospective cohort study a 6-month follow-up period was conducted among Japanese healthcare workers. All participants received two doses of BNT162b2 vaccine. Anti-SARS-CoV-2 antibody titers and T-cell immune responses were measured in serum samples collected at several timepoints before and after vaccination.

Results: A total of 608 participants were included in the analysis. Antibody titers were elevated 3 weeks after vaccination and waned over the remainder of the study period. T-cell immune responses showed similar dynamics. Six participants without predisposing medical conditions seroconverted from negative to positive on the IgG assay for nucleocapsid proteins, indicating breakthrough SARS-CoV-2 infection. Five of the six breakthrough infections were asymptomatic.

Conclusions: Both humoral and cellular immunity waned within 6 months after BNT162b2 vaccination. The incidence of asymptomatic breakthrough infection within 6 months after vaccination was approximately one percent.

UMIN Clinical Trials Registry ID: UMIN000043340.
antibody titer and the cellular immune response during the 6 months after the initial two doses of BNT162b2 vaccine. Additionally, we describe cases of suspected breakthrough infection based on the development of antibodies to SARS-CoV-2 nucleocapsid proteins.

2. Methods

2.1. Participants

Healthcare workers working on the Keio University Shinanomachi Campus (Tokyo, Japan) who were vaccinated against SARS-CoV-2 between February 16 and March 9, 2021 were recruited for the study. The campus has a university hospital with 960 beds and a medical school. Written informed consent was obtained from all participants before mass vaccination. Mass vaccination was carried out using the BNT162b2 vaccine (Comirnaty intramuscular injection, Pfizer, New York, USA), which was stored and prepared according to the instructions given in the package insert. Each participant received two doses of vaccine, administered 3 weeks apart.

2.2. Study registration and ethics approval

The study protocol was registered with UMIN Clinical Trials Registry on February 16, 2021 (UMIN ID: UMIN000043340). The study was approved by the ethics committee of the Keio University School of Medicine (approval no. 2020-0330).

2.3. Sample collection

Serial serum samples were collected from each participant at five timepoints. The first sample collection timepoint was before or on the day of the first vaccination. The second was between April 15 and 28, 2021, approximately 3 weeks after the second dose. The third was between May 20 and June 2, 2021, approximately 8 weeks after the second dose. The fourth was between June 28 and July 9, 2021, approximately 3 months after the second dose. The final sample collection timepoint was between September 28 and October 8, 2021, approximately 6 months after the second dose. Each participant completed a structured questionnaire at the time of each sample collection. At the time of the first sample collection, participants provided information on their age, sex, height, body weight, use of systemic steroids or other immunosuppressants, ongoing cancer chemotherapy, and history of immunodeficiency, cancers, autoimmune diseases, diabetes, and COVID-19. At the time of subsequent sample collections participants provided information on history of a COVID-19 episode, COVID-19-like illness, SARS-CoV-2 polymerase chain reaction (PCR) testing, and close contact with COVID-19 patients since the preceding sample collection. Participants who did not receive two doses of vaccine and those who did not who did not provide samples at all five timepoints were excluded from the analysis.

2.4. Measurement of antibody titers

Antibody titers to SARS-CoV-2 spike protein (S-IgG) and anti-SARS-CoV-2 neutralizing antibody titers were measured in all samples using three commercially available chemiluminescence enzyme immunoassays (CLEIA). First, IgG antibody titers against the SARS-CoV-2 spike protein S1 subunit receptor-binding domain (RBD) were measured using SARS-CoV-2 IgG II Quant reagents (Abbott Laboratories, Abbott Park, IL, USA) and an Anility Analyzer (Abbott Laboratories, Abbott Park, IL, USA) (Anility RBD-IgG) according to the manufacturer’s instructions. Second, the IgG antibody titers against the anti-SARS-CoV-2 spike protein were measured using HISCL SARS-CoV-2 S-IgG reagents (Sysmex Corporation, Kobe, Japan) and HISCL Analyzer (Sysmex Corporation, Kobe, Japan) and (HISCL S-IgG) according to the manufacturer’s instructions. Third, SARS-CoV-2 neutralizing antibodies were measured using the STACIA SARS-CoV-2 Neutralization Antibody Test (MBL Corporation, Nagoya, Japan) and STACIA Analyzer (LSI Medience Corporation, Tokyo, Japan) and the STACIA Analyzer (Sysmex Corporation, Kobe, Japan) and the HISCL Analyzer (HISCL Analyzer, Kobe, Japan) according to the manufacturer’s instructions. We defined suspected breakthrough infections as follows: A negative N-IgG test result before and after vaccination, followed by a positive N-IgG test result during the 6 months after vaccination. The N-IgG test positivity was determined according to the manufacturer’s cut-off (10 SU/mL). Among participants with suspected breakthrough infection, their clinical characteristics, and S-IgG, neutralizing antibody titers, and IFN for Ag1 and Ag2 before the suspected breakthrough infection were compared with those of participants without suspected breakthrough infection.

2.5. Measurement of cellular immunity

T-cell immunity against SARS-CoV-2 was assessed using an interferon gamma release assay (IGRA). Based on a limited reagent supply, samples were only collected from the first 600 participants to enroll in the study, and whole blood samples were collected in lithium heparin tubes at the first, third, and final sample collection timepoints. Samples were transferred to four QuantIFERON SARS-CoV-2 tubes (Qiagen, Hilden, Germany): coated with antigen 1, antigen 2, phytohemagglutinin (positive control), and no peptide (negative control). Antigen 1 is an epitope of CD4+ T cells derived from the S1 subunit, and antigen 2 is an epitope of CD4+ and CD8+ T cells derived from the S1 and S2 subunits. The tubes were incubated at 37 °C for 16–24 h and enzyme-linked immunosorbent assays were performed using the QuantIFERON SARS-CoV-2 ELISIA kit (Qiagen, Hilden, Germany) according to the package insert using an AP-96 auto microplate enzyme immunoassay reader (Kyowa Medex, Tokyo, Japan). Quality control was conducted daily. The dynamics of the interferon gamma levels for antigen 1 (IFN for Ag1) and antigen 2 (IFN for Ag2) were investigated after correction for the negative control.

2.6. Detection of suspected breakthrough infections among participants

In order to detect all the breakthrough infections including asymptomatic or undiagnosed cases during the study period, we measured the IgG antibody titer against the nucleocapsid protein of SARS-CoV-2, which is not affected by the vaccine which codes for the spike protein regions of SARS-CoV-2. In order to measure the immunoglobulin G (IgG) antibody titer against the nucleocapsid protein (N-IgG), we used HISCL SARS-CoV-2 N-IgG reagents (Sysmex Corporation, Kobe, Japan) and the HISCL Analyzer (Sysmex Corporation, Kobe, Japan) according to the manufacturer’s instructions. We defined suspected breakthrough infections as follows: A negative N-IgG test result before and after vaccination, followed by a positive N-IgG test result during the 6 months after vaccination. The N-IgG test positivity was determined according to the manufacturer’s cut-off (10 SU/mL). Among participants with suspected breakthrough infection, their clinical characteristics, and S-IgG, neutralizing antibody titers, and IFN for Ag1 and Ag2 before the suspected breakthrough infection were compared with those of participants without suspected breakthrough infection.

2.7. Statistical analysis

Summary statistics of the participants were constructed using frequencies and proportions for categorical variables and means and standard deviations (SD) for continuous variables. We compared antibody titers and interferon gamma levels at each sample collection timepoint using paired t-tests. The characteristics of participants with and without suspected breakthrough infections were compared using Fisher exact tests for categorical variables, and Mann-Whitney U tests for continuous variables because of
the small number of participants with suspected breakthrough infections. All statistical analyses were performed using JMP, version 15 and SAS software, version 9.4 (SAS Institute, Cary, NC, USA). Statistical significance was set at \( p < 0.05 \).

3. Results

Of the 673 healthcare workers and university staff members who enrolled in the study, three who did not receive two doses of vaccination and 62 who did not provide samples at all five time-points were excluded from the analysis, leaving a total of 608 participants in the analysis (Table 1).

| Variable                             | Value (N = 608) |
|--------------------------------------|-----------------|
| Age, years, mean ± SD                | 44.4 ± 10.7     |
| Sex, n (%)                           | Male 170 (28.0) |
|                                      | Female 438 (72.0) |
| Body mass index (kg/m²), mean ± SD   | 21.9 ± 3.2      |
| History, n (%)                       | COVID-19 7 (1.1) |
|                                      | Diabetes 3 (0.5) |
|                                      | Malignancy 10 (1.6) |
|                                      | Autoimmune disease 17 (2.8) |
|                                      | Immunosuppressant use, n (%) | Systemic steroids 7 (1.2) |
|                                      | Other 10 (1.6)   |

Before vaccination, the mean Alinity RBD-IgG, HISCL S-IgG, and STACIA Neut-Ab titers were 9.8 ± 109.1 AU/ml, 0.2 ± 3.6 SU/ml, and 1.0 ± 0.6 U/ml, respectively. Three weeks after vaccination the mean Alinity RBD-IgG, HISCL S-IgG, and STACIA Neut-Ab titers increased to 15,443.5 ± 9,655.2 AU/ml, 406.0 ± 242.7 SU/ml, and 23.6 ± 14.1 U/ml, respectively. The antibody titers waned and 6 months after vaccination the mean Alinity RBD-IgG, HISCL S-IgG, and STACIA Neut-Ab titers had decreased to 1,576.8 ± 5080.2 AU/ml, 63.9 ± 195.9 SU/ml, and 3.3 ± 4.9 U/ml, respectively (Fig. 1). Correlation analysis between the Alinity RBD-IgG, HISCL S-IgG, and STACIA Neut-Ab test results of all samples collected in the study demonstrated high correlations (Supplement Fig. S1).

Samples from 536 of the 608 participants were tested using the QuantiFERON SARS-CoV-2 assay. The QuantiFERON SARS-CoV-2 assay results showed similar time dynamics to the SARS-CoV-2 antibody assays. Before vaccination, the mean IFN for Ag1 and IFN for Ag2 was 0.00 ± 0.08 IU/ml 0.01 ± 0.07 IU/ml, respectively. Eight weeks after vaccination, IFN for Ag1 and IFN for Ag2 increased to 0.66 ± 0.87 IU/ml and 1.06 ± 1.25 IU/ml, respectively. However, by 6 months after vaccination, IFN for Ag1 and IFN for Ag2 had decreased to 0.37 ± 0.63 IU/ml and 0.62 ± 0.99 IU/ml, respectively, (Fig. 2).

The mean N-IgG titer was 0.6 ± 9.5 SU/ml before vaccination. The titer was not affected by the vaccination and was 0.7 ± 8.4 SU/ml, 0.4 ± 4.7 SU/ml, and 0.3 ± 3.5 SU/ml 3 weeks, 8 weeks, and 3 months after vaccination, respectively (Supplement Fig. S2). Six months after vaccination, the mean titer was 0.9 ± 8.9 SU/ml. Six participants turned positive serologically and were thus defined as having suspected breakthrough infection.
All six participants with suspected breakthrough infection were female, without pre-existing illnesses or immunosuppressant use. One participant was diagnosed with COVID-19 according to PCR test results performed 4 months after vaccination. One participant had close contact with COVID-19 patients 5 months after vaccination, but her PCR test result was negative. The other four participants were asymptomatic and had no history of close contact with patients with COVID-19 (Table 2). Compared to participants without suspected breakthrough, the antibody titers before the breakthrough infections (measured 3 months after vaccination) were similar, but titers after the breakthrough infections (measured 6 months after vaccination) were significantly higher. However, the IFN for Ag 1 and Ag 2 results were not affected by the breakthrough infections (Table 3).

Table 2
Antibody titers in participants with suspected breakthrough SARS-CoV-2 infection.

| Age (years) | Sex | Antibody titer 3 months after vaccination | Antibody titer 6 months after vaccination | History of possible SARS-CoV-2 infection 3 to 6 months after vaccination
|-------------|-----|------------------------------------------|------------------------------------------|---------------------------------|
|             |     | STACIA Neut-Ab, U/mL | N-IgG, SU/mL | STACIA Neut-Ab, U/mL | N-IgG, SU/mL | COVID-19 | COVID-19-like illness | SARS-CoV-2 PCR testing | Close contact with COVID-19 patients |
| 44          | F   | 6.45 | 0.5 | 51.38 | 19.8 | – | – | – | – |
| 27          | F   | 9.86 | 0.1 | 52.92 | 22.3 | – | – | + | – |
| 47          | F   | 10.73 | 0 | 42.21 | 79.9 | – | – | – | – |
| 31          | F   | 9.92 | 5.4 | 20.55 | 173.3 | – | – | – | – |
| 50          | F   | 7.68 | 0.1 | 53.73 | 55.3 | + | + | – | + |
| 45          | F   | 5.17 | 0.1 | 53.24 | 83.0 | – | – | + | + |

COVID-19, coronavirus disease; N-IgG, immunoglobulin G antibody against the SARS-CoV-2 nucleocapsid antigen; Neut-Ab, neutralization antibody test; PCR, polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Based on answers provided in the questionnaire.
immunoglobulin G; SARS-CoV-2, severe acute respiratory syndrome coronavirus. Ag, antigen; IFN, interferon; IQR, interquartile range; Neut-Ab, neutralization antibody test; RBD-IgG, receptor-binding domain immunoglobulin G; S-IgG, anti-spike protein

tions and miss diagnostic opportunities[13]. The measurement of antibody activity to neutralize SARS-CoV-2 infection. The negativity may be attributed to the lower sensitivity of PCR tests compared with antibody tests, as previously reported[14], with COVID-19 patients, but the result was negative. Although the negativity may be attributed to the lower sensitivity of PCR tests compared with antibody tests, as previously reported[14], the negative result might have been attributable to the faster mean rate of viral load decline and viral clearance in vaccinated individuals compared to unvaccinated individuals[15,16].

Among the participants with suspected breakthrough infection, the median antibody titer 3 months after vaccination was similar to that of those without breakthrough infections. This suggests that breakthrough infections may occur not only in poor responders but also in good responders within 6 months after BNT162b2 vaccination. Among the participants with suspected breakthrough infections, the higher median antibody titer using STACIA Neut-Ab compared to that of those without breakthrough infections. This suggests that antibody titers and IGRA had discrepant results after breakthrough infection. As the reason for the discrepancy between the antibody titers and the IGRA results has not been determined, further studies are warranted.

This study had several limitations. First, STACIA Neut-Ab, which was used as the neutralization assay, could only detect the antibody activity to inhibit RBD binding to human ACE 2. Although the STACIA Neut-Ab results for monoclonal antibodies correlated well with the neutralization assay results using in vitro SARS-CoV-2 infection in cells, neutralization is thought to incompletely represent in vivo humoral immunity against SARS-CoV-2 with polyclonality[18]. Therefore, ideally the presence of neutralizing antibody should be verified using SARS-CoV-2 infected cells or animals. Second, cross-reactivity of the N-IgG antibody test has been reported. False-positive results might be obtained due to cross-reactivity to coronavirus NL63 and 229E infections; therefore, N-IgG-positive conversion does not always signify breakthrough infection. As the reason for the discrepancy between the antibody titers and the IGRA results has not been determined, further studies are warranted.

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breakthrough infections in our study was in the setting of an epidemic of the Delta variant. Currently, the Omicron (B.1.1.529) variant, which can escape the immunity of BNT162b2 vaccination, is dominant worldwide, including in Japan. Thus, the incidence of breakthrough infections may have increased.

In conclusion, the study showed waning of both humoral and cellular immunity within 6 months after two doses of BNT162b2 vaccine among Japanese healthcare workers and the occurrence of suspected asymptomatic breakthrough infection in approximately one percent of participants within 6 months after vaccination with two doses of vaccine. These results support the need for booster vaccination to prevent infection.

5. Authors’ contributions

YU conceived and designed the study. YU, AT, TA, AO, AyS, WY, and MW recruited the participants. TK, YT, Aks, MN, YY, TA, AO, YT and AyS collected the data. YU, YS, NH and MW MM analyzed and interpreted the data; YU wrote the manuscript. HY, HN, YS, NH, and MW MM discussed the data and critically reviewed and revised the manuscript. All authors approved the final version of the manuscript for publication.

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Declaration of Competing Interest

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2022.06.016.

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