Letter to the Editor

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Profound decline of antibody titers 6 months after BNT162b2 vaccination in healthy volunteers

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To the Editor,

As of the beginning of October 2021, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has infected over 219 million individuals worldwide and caused more than 4.55 million deaths. Several kinds of vaccines are now being administered to prevent coronavirus disease 2019 (COVID-19) caused by SARS-CoV-2 infection, including mRNA vaccines [1]. Short durations of humoral responses as well as potential side effects of mRNA vaccines have been reported [2, 3], although the persistence of anti-SARS-CoV-2 antibody titers remains to be clarified. In the current study, we examined levels of SARS-CoV-2 antibodies among healthy volunteers at Tohoku Medical and Pharmaceutical University Hospital 6 months following vaccination with the Pfizer/BioNTech BNT162b2 mRNA vaccine. Antibody titers were evaluated using a newly established, highly sensitive, fully automated chemiluminescent enzyme immunoassay (CLEIA) designed to specifically detect IgG or IgM against the SARS-CoV-2 spike protein receptor-binding domain (RBD). This newly established CLEIA based assay, utilizes antigen (or antibody)-bound magnetic particles and enzyme-labeled antibody, with chemiluminescent substrate for detection. That is different from conventional CLIA assay employing chemiluminescent compound (aclidinium)-labeled antibody, instead of enzyme-linked antibody.

Vaccines (30 μg of BNT162b2/Comirnaty; Pfizer/BioNTech, New York, NY, USA) were administered at Tohoku Medical and Pharmaceutical University Hospital starting on 15 March 2021. A total of 41 volunteer healthcare workers (31 women and 10 men, mean ± standard deviation [SD] age 39.5 ± 12.7 years) were enrolled in the study. Participants received a first dose of BNT162b2 in March or April 2021, followed by a second identical dose 21 days later. Sera were collected at 14, 35, and 180 days after the first vaccination. Results for the 180-day time point are presented in this report. Blood samples were centrifuged at 1,690×*g* for 10 min at room temperature, and sera were separated for storage at −80 °C in two 1-mL aliquots. All serum aliquots were thawed simultaneously for analysis at the time of the study. CLEIAs were conducted using SARS-CoV-2 S-IgG (IB) and SARS-CoV-2 S-IgM (IB) reagents (Fujirebio, Tokyo, Japan) and a Lumipulse L2400 system (Fujirebio). Levels of IgG and IgM were expressed in arbitrary units (AU)/mL and evaluated in relation to a cut-off index calculated by quantification of standard anti-SARS-CoV-2 RBD antibody samples.

Of 41 volunteers who received two doses of BNT162b2 at our hospital, all completed 6 months of follow-up after the first dose. None experienced SARS-CoV-2 infections prior to vaccination or during post-vaccination follow-up. At the time of writing, all 41 participants have completed 6 months of follow up since the first dose of BNT162b2. Serum samples were obtained on average 182.6 days (SD 3.3 days) after the first dose of BNT162b2 (Figure 1A). One hundred and eighty days after the first dose of BNT162b2, mean anti-RBD IgM had decreased by 80.9% (SD 15.9%) and had returned to baseline levels (Figure 1B). Additionally, mean anti-RBD IgG antibodies had also decreased by 88.6% (SD 4.4%) (Figure 1C).

Recently, several groups have investigated the persistence of humoral immune responses 2–3 months after vaccination. Favresse et al. [4] analyzed 200 vaccinees and found mean antibody decreases of 37.9% and 44.7% (among seronegative and seropositive individuals, respectively) 3 months after the first vaccination. Shrotri

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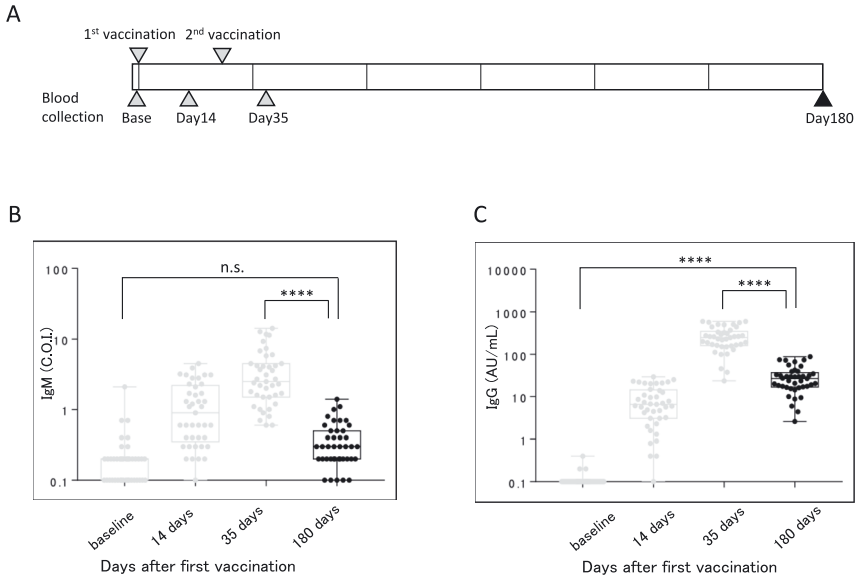

Figure 1: Sampling and vaccination procedure.
(A) Schematic presentation of sampling procedures. (B, C). Box plots of anti-SARS-CoV-2 antibody levels following vaccination with the BNT162b2 vaccine. (B) Anti-SARS-CoV-2 IgM titer 180 days after the first vaccination in all participants. (C) Anti-SARS-CoV-2 IgG 180 days after the first vaccination in all participants. Data are shown as boxplots representing the 25th percentile, 75th percentile, median, and 95% confidence interval using Prism software (version 7.0; GraphPad Software Inc., La Jolla, CA, USA). ****p<0.0001, n.s.: not significant. Data at baseline, day 14 and day 35 are presented for comparison.

et al. [5] identified a significant trend of declining spike protein antibody levels among individuals vaccinated with both ChAdOx1 nCOV-19 (Oxford/AstraZeneca) and BNT162b2: antibody levels were reduced by about five-fold for ChAdOx1 and by about twofold for BNT162b2 70 days after the second dose. Another study in Spain analyzed 258 hospital workers who had completed 3 months of follow-up after a second dose of BNT162b2: the median anti-RBD titers 1.5 months after vaccination were 9,356 AU/mL compared with 3,952 AU/mL 3 months after vaccination [6]. Collectively, these data indicate that 3 months after vaccination with BNT162b2, antibody titers had decreased by approximately twofold compared with the peak after the second dose.

In the current study, 80.9% and 88.6% decreases in IgM and IgG antibody titers, respectively, were observed 6 months after vaccination. This finding indicated that profound declines in anti-SARS-CoV-2 RBD antibodies occurred 3–6 months after vaccination. The significance of this decline in anti-RBD IgG titer has not yet been fully clarified. In addition to specific antibodies generated through B cell responses, another important aspect of BNT162b2 vaccination is elicitation of CD4 and CD8+ T cell responses [7]. However, anti-RBD antibodies are reasonable indicators of antiviral activity, as significant correlations have been identified between anti-RBD antibody titers and viral neutralizing activity [8].

Following vaccination or infection, IgM typically increases before IgG and then decreases before IgG. However, it remains unclear whether IgG and IgM follow these patterns in patients with COVID-19 as well as in individuals receiving mRNA vaccines. Moreover, to the best of our knowledge, no previous reports have investigated the durability of IgM titers [4–6]. In the current study, we found that both anti-SARS-CoV-2 RBD IgG and IgM titers steeply declined over time; for IgM, the decline was to baseline levels. Our findings suggest that the IgM antibody class may have limited importance for evaluating responses to vaccination.

While preparing this letter, a pre-print describing humoral responses 6 months after two doses of BNT162b2 vaccine in 154 patients with solid tumors and 135 healthy controls became available [9]. Although antibody titers were not quantitated, the authors claimed that 15% of both cancer patients and controls became seronegative over the study period [9], suggesting waning of the effects of vaccination. Quite recently, Goel et al. [10] have also observed the similar decline of the antibody titer 6 months after vaccination, and found that mRNA vaccines generated functional memory B cells that increased from 3 to 6 months post-vaccination. mRNA vaccination further induced antigen-specific CD4+ and CD8+ T cells, and early CD4+ T cell responses correlated with long-term humoral immunity. Declining antibody titers over time likely to reduce the potential to prevent infection, however, the durability of cellular immunity, may contribute to rapid recall responses that can limit initial viral replication and dissemination in the host, thereby preventing severe disease. The long-term efficacy of BNT162b2 vaccination remains to be determined. However, based on observations suggesting profound declines in antibody titers and the literature, booster doses of BNT 162b2 may be required to maintain high titers of anti-RBD antibodies.

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Informed consent: Informed consent was obtained from all individuals included in this study.

Ethical approval: Research involving human subjects complied with all relevant national regulations, institutional policies and is in accordance with the tenets of the Helsinki Declaration (as revised in 2013), and has been approved by the Ethics Committee at the Tohoku Medical and Pharmaceutical University Hospital in the 2020 fiscal year (2020-2-256).

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