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Long-term observation of antibody titers against SARS-CoV-2 following vaccination

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ABSTRACT

Objectives: We aimed to understand how SARS-CoV-2 antibody titer decrease following SARS-CoV-2 mRNA vaccination and to estimate the timing of booster vaccination.
Study design: Six hundred sixty-two healthcare workers were administered with total of three doses of SARS-CoV-2 mRNA vaccine during the same short period. Of them, three volunteers were enrolled to measure anti-receptor binding domain (RBD) antibody titers (IgG) monthly following the second and the third doses.
Methods: Serum anti-RBD antibody titers were measured monthly and the decay curve of the antibody was analyzed. We estimate the timing of the third and fourth vaccine based on the observed antibody titer decrease and the period of breakthrough infections in the vaccine recipients.
Results: Anti-RBD antibody decreased exponentially following the 2nd dose. Between 108 and 117 days following the second dose, breakthrough infection of SARS-CoV-2 occurred in 11 out of the 662 vaccine recipients. Based on the decrease in anti-RBD antibody and the timing of the breakthrough infections, we estimate that the optimal timing of a third dose would be at earliest 108 days after the second dose, when anti-RBD antibody titers are less than 338 BAU/mL. The anti-RBD antibody titers were sustained relatively higher for 161 days following the third dose (416 days following the second dose).
Conclusions: We estimate that the optimal timing of a third dose would be at earliest 108 days after the second dose, or anti-RBD antibody titers are less than 338 BAU/mL. We suggest that a fourth dose should be administered later than 161 days following the third dose.

1. Introduction

SARS-CoV-2, a virus that causes the acute respiratory disease COVID-19, has prevailed worldwide since late 2019. SARS-CoV-2 mRNA vaccines, which induce neutralizing antibodies against the spike protein of SARS-CoV-2, were developed rapidly [1]. In spite of their high efficacy of about 95%, breakthrough infections following full vaccination with two doses of the vaccine have occurred worldwide [2,3], leading to the approval of a third dose (booster) in some countries [4]. However, information regarding the durability of their efficacy and the timing of the booster vaccination is limited.

Multiple studies have reported that anti-RBD antibodies are correlated with neutralizing activity against SARS-CoV-2 [5]. In this study, we measured anti-RBD antibody (IgG) titers of vaccinated volunteers each month following their second and third doses of a SARS-CoV-2 mRNA vaccine. Based on the observed antibody titer decrease and the period of breakthrough infections in the vaccine recipients, we aimed to understand how antibody levels decrease over time and to estimate the timing of the third and fourth vaccine doses. To the best of our

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Abbreviations

SARS-CoV-2 severe acute respiratory syndrome coronavirus
RBD receptor-binding domain
IgG immunoglobulin G

knowledge, this is the first report to describe long-term monthly observations of serological responses spaning 416 days following the second dose of a SARS-CoV-2 mRNA vaccine.

2. Methods

2.1. Study design

To confirm anti-RBD antibody titer elevation following SARS-CoV-2 mRNA vaccination, 17 volunteers were enrolled retrospectively from July to November 2021; all volunteers were non-healthcare-workers, > 20 years old, with no evidence of previous SARS-CoV-2 infection. Of these volunteers, 10 had been vaccinated with two doses of mRNA vaccine, BNT162b2 (Pfizer/BioNTech) or mRNA-1273 (Moderna), within 30 days of the measurement, whereas seven volunteers had not been vaccinated with any SARS-CoV-2 vaccine.

Six hundred sixty-two healthcare workers at Gyotoku General Hospital were administered with two doses of BNT162b2; the first dose between March 15th and 19th and the second dose between April 5th and 10th in 2021. A prospective study was planned to measure anti-RBD antibody titers each month following two doses of vaccination for three volunteers. Anti-RBD antibody titers were measured shortly (10 days) after the second dose and followed by monthly measurements (30 ± 7 days). The third dose of BNT162b2 was administered to almost the same healthcare workers, including the three volunteers from this study, between December 21st and 25th in 2021. Observation of anti-RBD antibody titers continued for the volunteers following the third dose of BNT162b2 as following the second dose.

The former study was conducted with the option for workers to opt out, in accordance with ethical guidelines from the Japanese

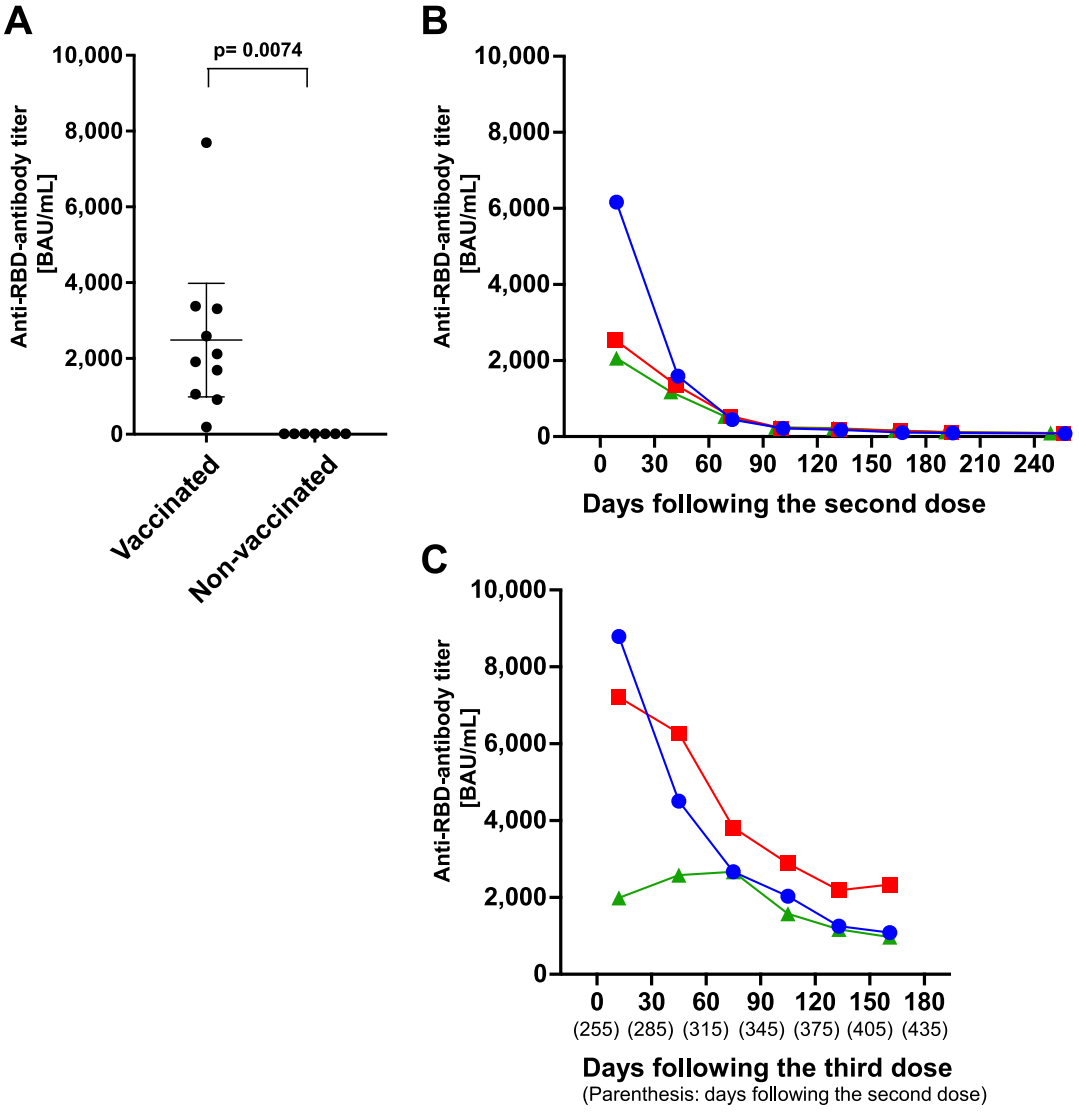

Fig. 1. Anti-RBD antibody titers following the second dose of BNT162b2. (A) Anti-RBD antibody titer was compared between volunteers vaccinated with a SARS-CoV-2 mRNA vaccine (either BNT162b2 or mRNA-1273) within 30 days following their second dose (n = 10) and non-vaccinated volunteers (n = 7). Vertical lines indicate the average with a 95% confidence interval. The level of statistical significance was expressed as a p-value. The time course of anti-RBD antibody titers were shown for three volunteers (V1: blue circle, V2: red square, V3: green triangle) each month following the second dose (B) and the third dose (C) of BNT162b2. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

government. These studies were approved by the ethics committee of Gyotoku General Hospital (#2021-03 and #2021-05). Written informed consent was obtained from the volunteers for the latter study.

2.2. Serological test and statistical analyses

Serum anti-RBD antibody (IgG) titers were measured using a chemiluminescent immunoassay (CLIA) (Siemens Healthcare Diagnostics Co., Tokyo, Japan). Original values were obtained as U/mL and later transformed to the WHO international standard unit (BAU/mL) with the detection limit of 10.9 BAU/mL. Statistical analyses were performed using Prism 9 (GraphPad Software, Inc.). The statistical significance was determined with the Student's t-test. The decay curve of anti-RBD antibody was fitted to the one-phase decay model to confirm the exponential decay and to calculate the half-life of the antibody.

3. Results

To confirm whether anti-RBD antibody titers actually increase following SARS-CoV-2 vaccination, measurements were taken within 30 days following the second dose of a SARS-CoV-2 mRNA vaccine (either BNT162b2 or mRNA-1273) for non-healthcare-worker volunteers without evidence of previous SARS-CoV-2 infection. Titers in vaccinated volunteers were significantly higher, having a mean value of 2484 BAU/mL (95% confidence interval [769, 4178]), while that of the non-vaccinated volunteers were below the detection limit (Fig. 1A). Thus, it was confirmed that anti-RBD antibody titers were elevated by SARS-CoV-2 mRNA vaccine.

We then measured anti-RBD antibody titers each month following the second dose of BNT162b2 to understand how antibody titers decreased. Three volunteers were enrolled: a female in her twenties (V1) and two males in their forties and sixties (V2 and V3, respectively). None of the volunteers had known immune disorders or infectious diseases. While the anti-RBD antibody titers of these volunteers were highest shortly (10 days) following the second dose of the vaccine, the titers decreased gradually and reached their lowest levels around day 257 (Fig. 1B). In all three volunteers, the antibody titer decreases were well fit to the one-phase decay model with $R^2 > 0.99$ indicating the decreases were exactly exponential with a half-life of 16.6–29.9 days.

Between 108 days and 117 days following the second dose, cases of breakthrough infection occurred in 11 out of 662 health care workers, who had been administered with two doses of BNT162b2 during the same short vaccination windows as the three volunteers (data not shown). Taking these breakthrough infections into account, we speculate that the risk of breakthrough infection increased at earliest 108 days following the second dose; this time point corresponds to a range of 97–338 BAU/mL in anti-RBD antibody titers, which was calculated by the previous model generated from the volunteers.

All three volunteers were administered with a third dose of BNT162b2 around 255 days following the second dose, and anti-RBD antibody titers were measured each month (Fig. 1C). Shortly (12 days) following the third dose, the anti-RBD antibody titers of V1 and V2 (in their twenties and forties) were higher compared with those measured immediately following the second dose, whereas it was not the case for V3 (in his sixties). The antibody titer decrease was well fit to the one-phase decay model with $R^2 = 0.998$ indicating exponential decrease in V1, but not V2 and V3. For all three volunteers, the anti-RBD antibody titers were sustained at an adequate level for at least 161 days following the third dose (416 days after the second dose). Overall, the observed elevation of anti-RBD antibody titers following the third dose was more potent compared with that following the second dose.

4. Discussion

The RBD is a key site on the spike subunit S1 of SARS-CoV-2 that serves not only as the receptor binging site for viral entry but also as the target for neutralizing antibodies. In addition, recent reports illustrate that RBD-specific IgG correlates with neutralizing activity against SARS-CoV-2 [5]. Here, we confirmed that the anti-RBD antibody titers elevated following SARS-CoV-2 mRNA vaccination indicating a serological assay for the anti-RBD antibody is available to evaluate immunity following vaccination.

Clinical trials demonstrated a gradual decline of vaccine efficacy over the course of six months following a second dose of BNT162b2, although vaccine efficacy was very high with 91.3% through six months of follow up period [6]. Our study illustrated that anti-RBD antibody titers decreased exactly exponentially following the second doses of BNT162b2, with a determined antibody half-life ranging 16.6–29.9 days.

Breakthrough infection following two doses of the vaccine have been reported worldwide [2,3]. This was partly due to the emergence of new variants of SARS-CoV-2 such as the Delta (B.1.617.2) and the latest Omicron (B.1.1.529), but the waning antibody response may also play an important role [7,8]. In Gyotoku General Hospital, breakthrough infection occurred in 11 cases out of 662 healthcare workers, all of whom had received two doses of BNT162b2 during the same short vaccination windows as the three volunteers. These cases occurred between 108 and 117 days following the second dose; corresponding to 97–338 BAU/mL in anti-RBD antibody titers, as calculated on the basis of the data from the volunteers. Accounting for the breakthrough infection among the vaccine recipients, we estimate that a third dose of vaccination (booster) should be administered at earliest 108 days following the second dose, or when anti-RBD antibody titers are less than 338 BAU/mL. This value was higher enough than detection limit and such discrepancy could be explained by the poor negative percent agreement of the serological assays with neutralization [9].

Recent clinical trial demonstrated that a third dose of BNT162b2 provided 95.3% of vaccine efficacy against COVID-19, even though the Delta variant was predominant when the trial was conducted. According to the present data, the third dose of the vaccine enhanced serological response, with a more potent and sustained impact than the second dose, possibly due to reactivation of the immunity acquired through the second dose, although the early reaction was relatively weak in the elder volunteer (V3).

Recently, a new issue raises regarding the necessity, timing and eligibility criteria of a fourth vaccination. In Israel, clinical trials on fourth dose of the vaccine were conducted for people over the age of 60 years. This trial demonstrated the effectiveness of a fourth dose of BNT162b2 in the prevention of COVID-19-related outcomes including hospitalization, disease severity, and death [10]. The present study showed that anti-RBD antibody titers were sustained at an adequate level for at least 161 days following the third dose. Therefore, we suggest that a fourth vaccination should be administered roughly later than 161 days following the third dose.

5. Conclusions

Anti-RBD antibody levels decreased exactly exponentially after the 2nd dose of BNT162b2. Based on the observed decrease in anti-RBD antibody levels and the timing of breakthrough infections of SARS-CoV-2 among vaccine recipients, we estimate that the optimal timing of a third dose would be at earliest 108 days after the second dose, or anti-RBD antibody titers are less than 338 BAU/mL. The elevation of SARS-CoV-2 antibody titers following the third dose was more potent than that following the second dose. So far, we suggest that a fourth dose should be administered later than 161 days following the third dose.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that would have influenced the work reported in this paper.

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