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Insufficient anti-spike RBD IgA responses after triple vaccination with intramuscular mRNA BNT162b2 vaccine against SARS-CoV-2

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ABSTRACT

Objectives: This study aims to examine whether the parenterally administered mRNA-based COVID-19 vaccines can induce sufficient mucosal-type IgA responses to prevent SARS-CoV-2 transmission.

Methods: We examined the longitudinal kinetics of SARS-CoV-2 spike RBD-specific IgA and IgG responses in sera of Japanese healthcare workers (HCWs) after receiving two doses and the third dose of BNT162b2 mRNA vaccines. During the prospective cohort study, Omicron breakthrough infections occurred in 62 participants among 370 HCWs who had received triple doses of the vaccine. Pre-breakthrough sera of infected HCWs and non-infected HCWs were examined for the levels of anti-RBD IgA and IgG titers.

Results: The seropositivity of anti-RBD IgA at 1 M after the second vaccine (2D-1M) and after the third dose (3D-1M) was 65.4% and 87.4%, respectively, and wanes quickly. The boosting effect on anti-RBD Ab titers following breakthrough infections was more notable for anti-RBD IgA than for IgG. There were partial cause-relationships between the lower anti-RBD IgA or IgG at pre-breakthrough sera and the breakthrough infection.

Conclusions: Parenterally administered COVID-19 vaccines do not generate sufficient mucosal-type IgA responses despite strong systemic IgG responses to SARS-CoV-2. These results demonstrate the necessity and importance of reevaluating vaccine design and scheduling to efficiently increase oral or respiratory mucosal immunity against SARS-CoV-2.

1. Introduction

COVID-19 vaccines were initially highly effective in preventing infection and improving clinical outcomes [1–5]. However, their effectiveness has waned over time with the emergence of variant strains of SARS-CoV-2 [6]. Moreover, the emergence of variants of concern (VOCs), such as Omicron variant BA.1 and BA.2, further reduced the efficacy of vaccine-induced antibodies in preventing infection. Under these circumstances, a third, fourth, and even fifth dose of COVID-19 vaccines have been approved and promoted in

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Japan, but immunogenicity data remain limited, particularly mucosal IgA responses.

Plasma-neutralizing antibodies have been regarded as the best predictors of vaccine-induced protection from infection. Although plasma-neutralizing IgG antibodies prevent viral spread within the body and thus protect against severe disease, mucosal antibodies, particularly secretory IgA in the respiratory tract, may play a more prominent role in preventing airway transmission of SARS-CoV-2. A lower risk of Omicron breakthrough infection was reported in the group with higher levels of wild-type SARS-CoV-2 spike-specific mucosal IgA [7]. Sheikh-Mohamed et al. reported that SARS-CoV-2 mRNA vaccines might induce a durable IgA response [8]. Moreover, Bureerug TC et al. reported heterologous boosters, especially priming with an inactivated vaccine followed by an mRNA-based vaccine induce greater systemic IgA responses [9]. However, it remains unclear whether the mRNA-based COVID-19 vaccines, which are intramuscularly administered, can induce sufficient IgA antibody responses to prevent SARS-CoV-2 infections [10, 11]. Thus, this study aims to examine the longitudinal kinetics of RBD-specific IgA responses and the effectiveness of the responses in preventing SARS-CoV-2 infections.

During the prospective observation period, the sixth wave of COVID-19 (the Omicron variants BA.1 and BA.2) occurred in Japan. In this study, we examined whether the breakthrough infections among triple-vaccinated healthcare workers (HCWs) were associated with lower anti-RBD IgA responses.

2. Materials and methods

2.1. Study design

A prospective observational study was conducted which investigated antibody profiles following BNT162b2 vaccination. The cohort of vaccine recipients was recruited from Fukuoka University Hospital HCWs and was identical to that described in a previous paper [12]. All 431 individuals in the analyses received two doses of the BNT162b2 mRNA vaccine. A large proportion of the cohort

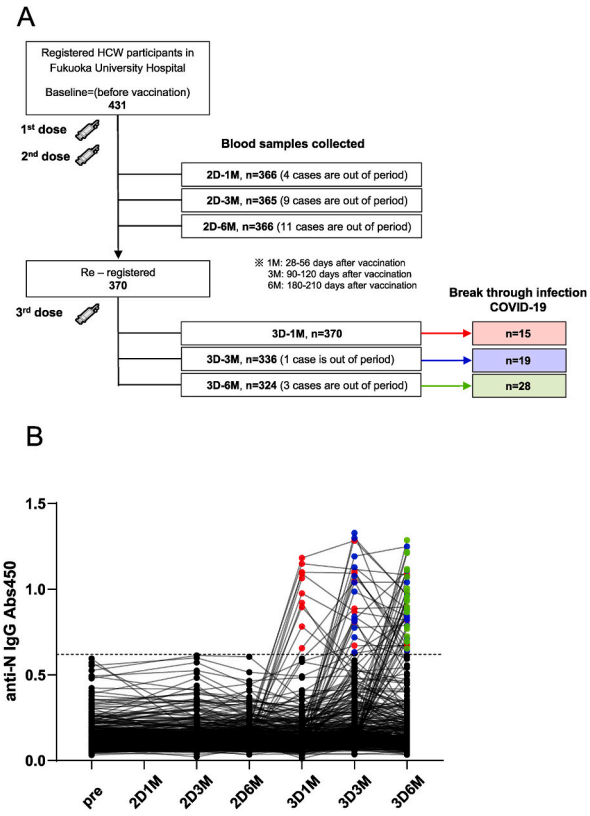

Fig. 1. Study design and time course of breakthrough infection incidence.
Flowchart (A) showing the prospective observational study and case-control studies (Groups A, B, and C) of the health care workers (HCWs) at Fukuoka University Hospital. Cases in the Group A case-control study were infected between the period following the receipt of the third shot of BNT162b2 and 3D-1M. Cases in Group B were infected between 3D-1M and 3D-3M. Cases in Group C were infected between 3D-3M and 3D-6M. Abbreviations: 2D-1M, 2D-3M, and 2D-6M are one month after, three months after, and six months after two doses of vaccine. 3D-1M, 3D-3M, and 3D-6M are one month after, three months later, and six months after the three vaccine doses. Anti-N antibodies of all cohort members over time (B). Colored plots are shown according to when they became positive. (Red: 3D-1M, Blue: 3D-3M, Green: 3D-6M). The dashed line indicates a cutoff value of anti-N IgG antibody.

received the third dose of BNT162b2 vaccination between 11 and January 21, 2022, approximately 8 months (M) after receiving the second dose of the BNT162b2 vaccination. Among them, the 370 participants were re-registered for the further prospective study following a third dose of vaccine. Therefore, these 370 patients were the focus of the analysis in the current study. Blood samples were obtained from the participants as described in Fig. 1A.

During this prospective cohort study, we observed the appearance of many cases of Omicron BA.1/BA.2 breakthrough infection following the third vaccine dose in our cohort as shown in Fig. 1A. Breakthrough infection was diagnosed by PCR, antigen test, or seropositive for anti-SARS-CoV-2 nucleocapsid (N)- total IgG (Fig. 1B). To evaluate the antibody profile in detail, breakthrough infection cases were divided into three groups (Groups A, B, and C) according to the timing of COVID-19 diagnosis. Group A was diagnosed between the third vaccination and 3D-1M, Group B was diagnosed between 3D-1M and 3D-3M, and Group C was diagnosed between 3D-3M and 3D-6M. Two patients in Group A were excluded from the analysis because we could not rule out the possibility that the time of infection was before the third vaccination. Controls for each group were those non-infected participants whose sera were collected at the same time as each diagnosis.

2.2. Detection of anti-spike-RBD antibodies and anti-nucleocapsid (N) antibodies

Enzyme-Linked Immunosorbent Assay (ELISA) detection of anti-RBD and anti-N antibodies was performed as previously described [12]. The following recombinant proteins were used in ELISA: recombinant SARS-CoV-2 Wuhan-Hu-1 spike glycoprotein RBD and full-length SARS-CoV-2 N protein, which was made in-house as previously described [12]. 96-well plates (Thermo Fisher Scientific, Waltham, MA, USA) coated with 50 μL per well of 2 μg/mL recombinant RBD protein (Wuhan-Hu-1) or coated with 50 μL per well of 1 μg/mL recombinant N protein were used for antibody detection. The absorbance 450 (A_{450}) value of serum 1:400 dilution was used to evaluate anti-SARS-CoV-2-N IgG (anti-N IgG). Anti-RBD IgA concentration was assessed by using an Anti-SARS-CoV-2 spike (RBD) fully human mAb (IgA) (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) as standard. The standard curve was generated using a 13-step dilution series of IgA standards ranging from 40 ng/mL to 0.6 ng/mL. First, each serum sample was diluted 200-fold for ELISA, and concentration was calculated from the A_{450} value using the standard curve. Next, the serum dilutions factor was arranged for those outside the range of the standard curve, and concentration was calculated the same as above. For anti-RBD total IgG, we used the arbitrary unit (A.U.) as previously described [12]. Briefly, the standard curve was generated using human anti-SARS-CoV-2-S1

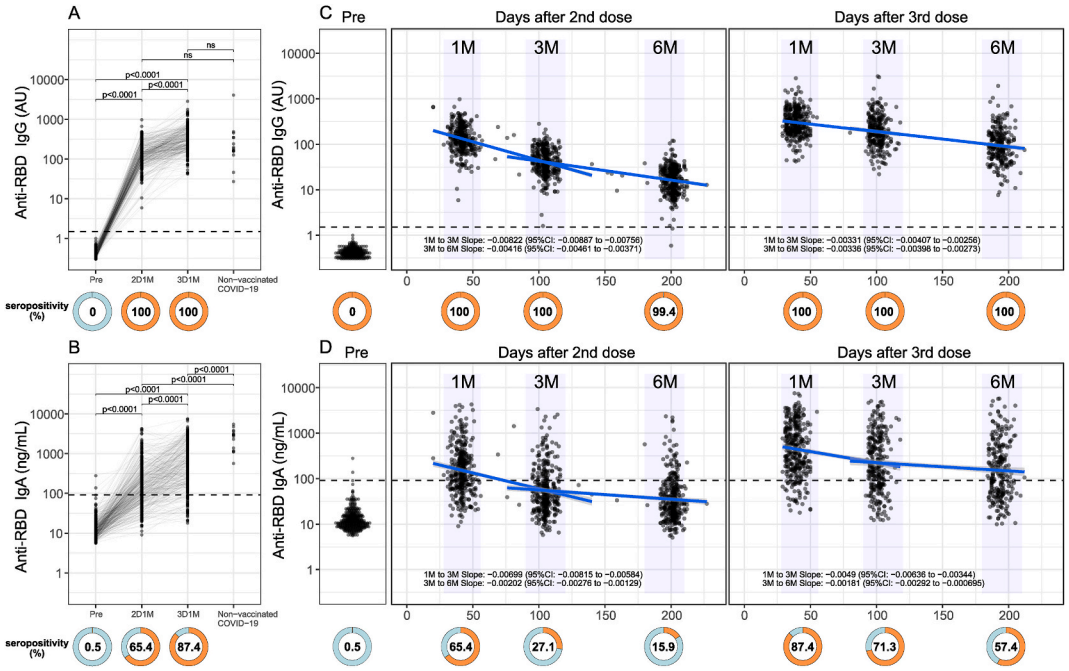

Fig. 2. Longitudinal humoral responses against SARS-CoV-2 following the second and third doses of BNT162b2 mRNA vaccine in HCWs in Japan.

Anti-SARS-CoV-2 spike RBD total IgG (A), -RBD IgA (B) of pre, the peak after the second dose (2D-1M: 28–56 days after the second dose), the peak after the third dose (3D-1M: 28–56 days after the third dose) and previously unvaccinated COVID-19 patients (n = 15, 20–37 days after the onset). Anti-SARS-CoV-2 spike RBD total IgG (C), anti-RBD IgA (D) of serum sample obtained longitudinally after the second dose and after the third dose of vaccine, as determined by ELISA. Statistical significance was determined using Steel-Dwass's multiple comparison tests (A, B). Linear regression models are fitted to the data from the peak humoral response (2D-1M) to 2D-3M, the data from 2D-3M to 2D-6M, the peak humoral response (3D-1M) to 3D-3M, the data from 3D-3M to 3D-6M respectively (C, D). Dashed lines on each graph indicate a cutoff value of anti-RBD IgG (A, C) or anti-RBD IgA antibody (B, D).

monoclonal IgG (Abcam, ab273073, Waltham, MA, USA) at seven different concentrations of 0.5 ng/mL to 8.0 ng/ng/mL. The A_{450} standard between 0.2 and 0.7 was used to calculate the regression line between antibody concentration and A_{450} values. The AU was calculated from the A_{450} values of the ELISA using stepwise diluted sera (AU= (equivalent concentration (ng/mL) of monoclonal antibody deduced from the regression line) * dilution factor/200). The secondary antibodies and their dilutions were used for detection as follows: anti-Human IgG (1:5000 dilution, Jackson ImmunoResearch, West Grove, PA, USA), and anti-Human IgA (1:5000 dilution, Jackson ImmunoResearch, West Grove, PA, USA). Sera collected from unvaccinated COVID-19 patients around 20–37 days after the onset was used as a positive control for anti-RBD antibodies.

2.3. Statistical analyses

No statistical methods were used to predetermine the sample size. In this prospective observational study, the Chi-squared test, Steel's multiple comparison test, and the Wilcoxon rank-sum test were utilized. The Wilcoxon rank sum test was used to compare antibody levels in cases and non-infected individuals in the comparison of pre-breakthrough sera. Linear regression analysis was performed to determine the 95% confidence interval of the slope. These statistical analyses were performed using JMP version 10.0.0 (SAS Institute, Inc., Cary, NC, USA), GraphPad Prism version 10.1.0 (GraphPad Software, San Diego, CA, USA), and R software version 4.1.2 (R Foundation).

3. Results

3.1. Longitudinal dynamics of anti-SARS-CoV-2 spike RBD IgA and IgG antibody responses in sera after triple-dose of BNT162b2 vaccination

We previously reported the longitudinal dynamics of anti-SARS-CoV-2 spike RBD IgG antibody after a two-dose regimen of BNT162b2 vaccination and the effect of a third dose on HCWs in Japan [12]. This study was extended and the longitudinal kinetics of anti-RBD IgA antibodies after the third dose of BNT162b2 vaccinations were examined and compared with those of anti-RBD total IgG responses. As shown in Fig. 1A and B, COVID-19 breakthrough infections occurred in 62 participants among 370 triple-vaccinated HCWs participants and these COVID-19 infected HCWs were excluded from the longitudinal kinetics study of anti-RBD IgA/IgG after triple BNT162b2 mRNA vaccination. We observed that 100% RBD IgG antibody seropositivity was achieved at 1 month (M) after the second vaccine (2D-1M), which contained very high anti-RBD IgG titers and reached the level of SARS-CoV-2 natural infection in vaccine naïve patients and there were no statistically significant differences between RBD IgG titer of 2D-1M and those of non-vaccinated COVID-19 infected patients' sera (Fig. 2A). On the other hand, the seropositivity of anti-RBD IgA response at 2D-1M and 1 M after the third dose (3D-1M) was 64.8% and 87.4%, respectively, and never reached to 100 % seropositivity as observed in IgG Ab response (Fig. 2B). Although anti-RBD IgA titer increased after 2 doses and the third dose of mRNA vaccination, anti-RBD IgA antibody titers at 3D-1M was still significantly lower than those of vaccine-naïve COVID-19 patients (Fig. 2B).

After reaching the peak titer levels after the second (2D-1M) or third dose of mRNA vaccination (3D-1M), anti-RBD IgG titers gradually waned (Fig. 2C). The decrease in IgG antibody titers from 2D-1M to 2D-3M was brisk, but slowed after 2D-3M. The decay rate (declining slope) during 2D-1M to 2D-3M was −0.00822 (95% CI: −0.00887 to −0.00756), but the decay rate during 2D-3M to 2D-6M decreased to −0.00416 (95% CI: −0.00461 to −0.00371). On the other hand, the trajectories of anti-RBD IgG (declining slope) at 1 M, 3 M, and 6 M after the third dose of the BNT162b2 mRNA vaccine revealed the decay rate did not change between the decay rate of 3D-1M to 3D-3M (declining slope: −0.00331, 95% CI: −0.00407 to −0.00256) and that of 3D-3M to 3D-6M (declining slope: −0.00336, 95% CI: −0.00398 to −0.00273) as shown in Fig. 2C.

Similar to the trajectories of anti-RBD IgG titers, anti-RBD IgA titer also waned after the peak (2D-1M, 3D-1M) as shown in Fig. 2D. The declining slope slow-down at 2D-3M was observed not only after the second dose (2D-1M to 2D-3M slope: −0.00699, 95% CI: −0.00815 to −0.00584, and 2D-3M to 2D-6M slope: −0.00202, 95% CI: −0.00276 to −0.00129) but also after the third dose (3D-1M to 3D-3M slope: −0.0049, 95% CI: −0.00636 to −0.00344, and 3D-3M to 3D-6M slope: −0.00181, 95% CI: −0.00292 to −0.000695).

Table 1
Characteristics of the COVID-19 breakthrough-infected or non-infected participants in the BNT162b2-triple-vaccinated Fukuoka University Hospital HCWs cohort.

Characteristic	Overall, N = 326[a]	Infection, N = 62[a]	No infection, N = 264[a]	p-value[b]
Sex				0.6
Female	261 (80%)	51 (82%)	210 (80%)	
Male	65 (20%)	11 (18%)	54 (20%)	
Age	41 (32, 49)	37 (27, 44)	42 (32, 51)	0.004
BMI	20.90 (19.33, 23.05)	20.78 (19.29, 22.64)	20.94 (19.47, 23.14)	0.8
Smoke				0.8
Never	288 (88%)	55 (89%)	233 (88%)	
Ever	17 (5.2%)	4 (6.5%)	13 (4.9%)	
Current	21 (6.4%)	3 (4.8%)	18 (6.8%)	

[a] n (%) or Median (IQR).
[b] Pearson's Chi-squared test (Sex); Wilcoxon rank sum test (Age, BMI); Fisher's exact test (Smoke).

It is worth mentioning that anti-RBD IgA seropositivity also dropped from 65.4 % (2D-1M) to 15.9% (2D-6M), 87.4% (3D-1M) to 57.4% (3D-6M), while anti-RBD IgG seropositivity did not drop and remained 100 % seropositive (2D-6M or 3D-6M). These results are compatible with the short-lived IgA responses compared to IgG responses after COVID-19 mRNA vaccines [13].

3.2. COVID-19 breakthrough infection in triple-vaccinated HCWs

After receiving the third dose of BNT162b2 vaccination, an increase in Omicron BA.1/BA.2 breakthrough infections was observed among the HCWs. Of the 370 participants of the post-third dose survey, 62 breakthrough cases were detected by 3D-6M (Fig. 1). Among these 62 breakthrough-infected participants, 13 participants were infected with SARS-CoV-2 between the third vaccination and 3D-1M (Group A cases), 19 were infected between 3D-1M and 3D-3M (Group B cases), 28 were considered to be infected with SARS-CoV-2 between 3D-3M and 3D-6M (Group C cases), and for two participants, the timing of infection could not be accurately determined. The characteristics of these breakthrough-infected vaccinees and non-infected control vaccinees in HCWs who received triple-dose of the BNT162b2 vaccine and were traceable to 3D-6M are shown in Table 1. All cases were either asymptomatic or mild, if any, and no subjects required hospitalization or oxygen administration. Although there were no significant differences in gender, BMI, or smoking status between the breakthrough-infected cohorts and non-infected cohorts, breakthrough-infected cohorts were significantly younger than non-infected cohorts (median age 37 (IQR, 27–44) vs median age 42 (IQR, 32–51), p = 0.004) (Table 1).

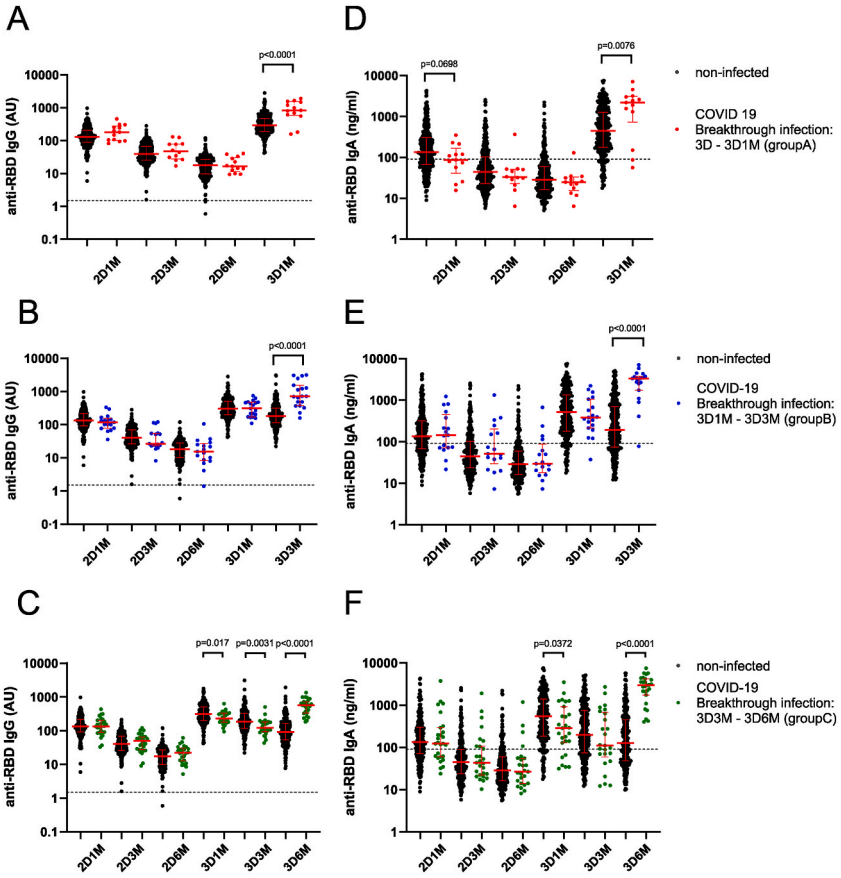

Fig. 3. Comparison of serum anti-RBD IgG and IgA titers at 2D-1M to 3D-6M between breakthrough-infected vaccinees and non-infected vaccinees.

Anti-RBD IgG titers (AU) (A, B, C) and anti-RBD IgA titers (D, E, F) in serum at 2D-1M, 2D-3M, 2D-6M, 3D-1M, 3D-3M and 3D-6M obtained from breakthrough-infected vaccinees and their non-infected vaccinees controls. Breakthrough-infected vaccinees in Group A (n = 13) were diagnosed with COVID-19 during the third vaccination to 3D-1M, breakthrough-infected vaccinees in Group B (n = 19) were diagnosed during 3D-1M to 3D-3M, and Group C (n = 28) diagnosed during 3D-1M to 3D-3M, each non-infected control vaccinees were not experienced breakthrough-infection at that time points. Breakthrough-infected vaccinees in Groups A, B, or C are shown in red (A, D), blue (B, D), or green (C, F), respectively. Medians and IQRs are shown as red lines and statistical significance was determined using the Wilcoxon rank-sum test. Dashed lines on each graph indicate a cutoff value of anti-RBD IgG (A, B, C) or anti-RBD IgA antibody (D, E, F).

3.3. Effects of breakthrough infection on anti-RBD IgG/IgA antibody after COVID-19 mRNA vaccinations

We next examined the boosting effect on anti-RBD IgG/IgA antibody titers following breakthrough infections. COVID-19 breakthrough infections overrode both the anti-RBD IgG and IgA response induced by triple-BNT162b2 vaccinations and the elevated Ab responses persisted resulting in the nearly complete loss of the decay of anti-RBD IgG or IgA compared to those of non-infected triple-vaccinated HCWs. The boosting effect of breakthrough infection on vaccine-induced anti-RBD antibody levels was observed both in anti-RBD IgG (Supplementary Fig. 1) and anti-RBD IgA responses (Supplementary Fig. 2) and these boosting effects continued even 6 months after the third vaccination (3D-6M) as shown in the red line showing breakthrough-infected participants group A. The boosting effect of breakthrough infection on anti-RBD Ab titers was more obvious in anti-RBD IgA responses than in IgG responses. We found that the anti-RBD IgA antibody titers at 3D-6M in breakthrough-infected vaccinees (median, (IQR) in group A: 2286.0, (462.5–4461.4), group B: 2906.4, (1777.8–4623.4), and group C: 3011.4, (1836.0–4334.5)) were significantly higher than IgA titers at 1 M after the fourth dose of vaccination (4D-1M) in non-breakthrough-infected vaccinees (median, (IQR): 421.0 (225.4–1231.9)) (Supplementary Table 1). Anti-RBD IgA responses induced by COVID-19 mRNA vaccines, even after the four doses of vaccinations, did not reach the same level as breakthrough infection after three doses of vaccination. These results suggest that the different routes of antigen exposure between natural infection and parenterally administered COVID-19 vaccine may affect the induction of IgA responses.

3.4. Comparison with pre-breakthrough infection-serum Ab titers of the anti-RBD IgG/IgA of breakthrough-infected vaccinees and non-infected vaccinees

Next, we examined whether breakthrough infections among triple-vaccinated cohorts were associated with lower anti-RBD IgG and/or anti-RBD IgA responses. We compared the anti-RBD IgG and anti-RBD IgA titers of pre-breakthrough sera obtained from breakthrough-infected cohorts (groups A, B, C) and their non-infected cohorts. There were no statistical differences in anti-RBD IgG antibody titers in the pre-breakthrough infection-sera among breakthrough infected cohorts (groups A, and group B) and their non-infected controls (Fig. 3A and B, Supplementary Table 2). Pre-infection-sera of group C which were breakthrough-infected between 3D-3M to 3D-6M showed significantly lower anti-RBD IgG titers at 3D-1M and 3D-3M ($p = 0.017$ and $p = 0.0031$, respectively) (Fig. 3C, Supplementary Table 2). After breakthrough infection, anti-RBD IgG titers were significantly higher than their non-infected controls in all breakthrough infection groups. On the other hand, a comparison of anti-RBD IgA titers in pre-infection-sera of breakthrough-infected group A and its non-infected control revealed that lower IgA antibody titers at 2D-1M in breakthrough-infected group A ($p = 0.0698$) (Fig. 3D). In breakthrough infected group B, anti-IgA titers in pre-infection-sera did not significantly differ between breakthrough-infected cohorts and its non-infected controls (Fig. 3E). However, in group C, anti-RBD IgA at 3D-1M was significantly lower than that of non-infected control ($p = 0.0372$) (Fig. 3F). These results suggest the possible but partial cause-and-effect relationship between the low anti-RBD IgG or IgA antibody responses and breakthrough infection.

Lower anti-RBD IgA titers at 2D-1M of breakthrough-infected cohort (group A) compared to its non-infected cohort (Fig. 3D) in the presence of similar levels of high anti-RBD IgG at 2D-1M of breakthrough infected cohorts and its non-infected cohort (Fig. 3A) prompted us to investigate the correlation of anti-RBD IgG and anti-RBD IgA responses. Scatter plots for anti-RBD total IgG and anti-RBD IgA response in pre-breakthrough infection-sera at 2D-1M were examined in breakthrough-infected HCWs and non-infected HCWs. While anti-RBD IgG titer and anti-RBD IgA titer were positively correlated at the peak after the second immunization (2D-1M) in non-infected controls cohorts (slope 1.73, 95%CI: 1.16 to 2.29, $p = 7.71e\text{-}0.9$), there was no correlation in breakthrough-infected subjects (slope −0.383, 95%CI: −2.37 to 1.6, $p = 0.070$) (Fig. 4). Although the possible mechanism of this dissociation for anti-RBD IgA and IgG response at 2D-1M of breakthrough infected HCWs is unknown, these findings corroborate the importance of IgA responses in preventing COVID-19 breakthrough infection.

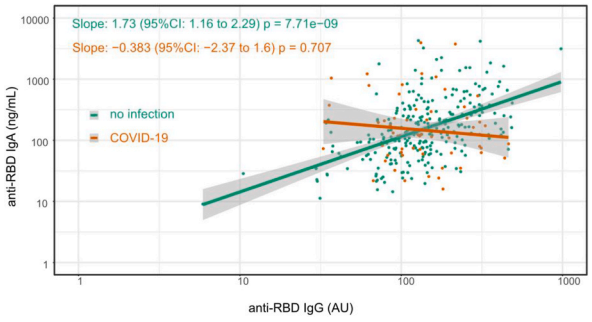

Fig. 4. Correlation between serum anti-RBD IgG and anti-RBD IgA one month after the second vaccination.
The correlation between anti-RBD IgG and anti-RBD IgA antibody titers was assessed using 2D-1M serum samples. Cohorts with breakthrough infection during the observation period (3D to 3D-6M) and those with blood drawn at 3D-6M and non-infected, for whom 2D-1M samples were available, were analyzed (breakthrough-infected cohorts n = 59, and non-infected cohorts n = 303). Each line on the graph represents regression lines of double log-linear models.

4. Discussion

In this study, we demonstrated that triple vaccinations with BNT162b2 do not generate sufficient IgA responses, while high systemic IgG responses were easily achieved by 2 dose immunization. BNT162b2 mRNA vaccine-induced anti-RBD IgA was less than 90% seropositivity even after the third vaccination and anti-RBD IgA titer levels were lower than those of COVID-19 natural infection of previously unvaccinated patients. Furthermore, mRNA vaccine-induced anti-RBD IgA wanes faster than infection-induced anti-RBD IgA. These mRNA vaccine-induced IgA response characteristics were quite different from systemic high IgG responses and suggest the insufficient induction of anti-RBD IgA responses after parenterally administered COVID-19 vaccines.

Comparisons of pre-breakthrough sera suggested that breakthrough infections among triple-vaccinated HCWs were partially associated with lower anti-RBD IgA responses despite high anti-RBD total IgG responses. These results indicate that although the intramuscular administration of the mRNA BNT162b2 vaccine promotes a strong systemic IgG antibody response and protects from severe COVID-19 disease, the BNT162b2 vaccine cannot induce sufficient IgA antibody response to prevent transmission of SARS-CoV-2 at mucosal entry levels in some individuals. These findings concur with the report that vaccinated individuals who experience breakthrough infections have significantly lower serum IgA levels compared with those who do not [8].

Immune responses at the respiratory mucosal interface are critical to prevent respiratory infection, but it is unclear to what extent antigen-specific mucosal secretory IgA antibodies are induced by mRNA vaccination. Sano, Bhavsar et al. recently reported that mRNA vaccination induced a minimal mucosal secretory IgA response in individuals without pre-exposure to SARS-CoV-2, while secretory IgA induction after vaccination was more efficient in patients with a history of COVID-19 [14]. Azzi et al. also reported that the increase in salivary IgA in BNT162b2 vaccine recipients was more pronounced only in previously SARS-CoV-2-exposed individuals [15]. Subsequently, they reported that the BNT162b2-booster vaccination elicits a strong systemic immune response but fails to activate an effective mucosal immunity against the Omicron BA.1 variant [11]. The present study found that boosting after breakthrough infection was most notable for IgA in triple-vaccinated HCWs, implying the different routes of antigen exposure between natural infection and COVID-19 vaccination. Thus, these results demonstrate the importance of IgA response in the prevention of SARS-CoV-2 infection and the necessity for refinement of vaccination strategies to increase mucosal antibody induction.

Limitations of the current study include that the salivary IgA levels of participants were not examined, nor was neutralizing activity measured. However, the correlation between serum IgA and saliva IgA levels has been previously reported [15]. It has also been reported that neutralizing responses were directly correlated with anti-RBD IgG titers [16]. Thus, this study did not examine saliva IgA or neutralizing antibody titers.

This cohort represented mostly young and healthy individuals; all breakthrough infections were asymptomatic or mild, if any, and did not require hospitalization. Thus, the correlations of protection from severe infection, or infections in vulnerable populations of older individuals with coexisting illnesses, could not be determined.

5. Conclusions

Parenterally administered COVID-19 vaccines do not generate sufficient mucosal-type IgA responses despite strong systemic IgG responses to SARS-CoV-2. The observed breakthrough infections among triple-vaccinated individuals were partially associated with lower anti-RBD IgA responses despite high anti-RBD total IgG responses. These results warrant a reevaluation of the current vaccine design and scheduling to efficiently increase oral or respiratory mucosal immunity against SARS-CoV-2.

Ethical approval statement

This research was carried out in accordance with the principles of the Declaration of Helsinki and was approved by the Institutional Ethics Board of Fukuoka University (IRB No. H20-08-003, H20-09-003, H21-02-002, H22-01-009), and all subjects consented.

Data availability statement

The datasets analyzed during the current study are available from the corresponding author upon reasonable request.

CRediT authorship contribution statement

Michinobu Yoshimura: Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Atsuhiko Sakamoto:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation. **Ryo Ozuru:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Funding acquisition, Formal analysis, Data curation. **Yusuke Kurihara:** Writing – review & editing, Investigation, Formal analysis. **Ryota Itoh:** Writing – review & editing, Resources, Methodology, Investigation, Conceptualization. **Kazunari Ishii:** Writing – review & editing, Methodology, Investigation. **Akinori Shimizu:** Writing – review & editing, Investigation. **Bin Chou:** Writing – review & editing, Investigation. **Yusuke Sechi:** Investigation. **Aya Fujikane:** Investigation. **Shigeki Nabeshima:** Writing – review & editing, Supervision, Funding acquisition. **Kenji Hiromatsu:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Funding acquisition, Data curation, Conceptualization.

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.heliyon.2023.e23595.

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