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Short Communication

The appearance of anti-spike receptor binding domain immunoglobulin G4 responses after repetitive immunization with messenger RNA-based COVID-19 vaccines

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ABSTRACT

Objectives: It is crucial to analyze the consequences of repeated messenger RNA (mRNA)-based COVID-19 vaccinations on SARS-CoV-2 spike receptor binding domain (RBD)-specific immunoglobulin (Ig)G subclass and the possible causal relationship with breakthrough infection.
Methods: We examined the longitudinal kinetics of RBD-specific IgG subclass antibodies in sera after receiving the second, third, and fourth doses of mRNA-based COVID-19 vaccines in Japanese healthcare workers. Anti-RBD IgG subclass in sera of patients with COVID-19-infected who had not received the COVID-19 vaccine were also examined. We compared anti-RBD IgG subclass antibody titers in the serum of pre-breakthrough-infected vaccinees and non-infected vaccinees.
Results: The seropositivity of anti-RBD IgG4 after the vaccination was 6.76% at 1 month after the second dose, gradually increased to 50.5% at 6 months after the second dose, and reached 97.2% at 1 month after the third dose. The seropositivity and titers of anti-RBD IgG1/IgG3 quickly reached the maximum at 1 month after the second dose and declined afterward. The elevated anti-RBD IgG4 Ab levels observed after repeated vaccinations were unlikely to increase the risk of breakthrough infection.
Conclusions: Repeated vaccinations induce delayed but drastic increases in anti-RBD IgG4 responses. Further functional investigations are needed to reveal the magnitude of the high contribution of spike-specific IgG4 subclasses after repeated mRNA-based COVID-19 vaccinations.

Introduction

Although it has been reported that repeated boosting with messenger RNA (mRNA)-based COVID-19 vaccines has been protective [1], some concerns about repeated vaccination of mRNA-based COVID-19 vaccines have emerged. Repeated vaccination with COVID-19 vaccines back-boosts previous memory and dampens the immune response to a new antigenically related but distinct virus strain, so-called vaccine-induced immune imprinting or original antigenic sin [2]. It has also been reported that the class switches toward noninflammatory, spike-specific immunoglobulin (Ig)G4 antibodies (Ab) after repeated SARS-CoV-2 mRNA-type vaccination [3–5]. The presence of these antibodies was also linked with a reduction in Fcγ effector function (i.e. complement, antibody-dependent cellular phagocytosis) [4]. However, it is still unknown whether IgG4 responses can be beneficial or detrimental in host defense against SARS-CoV-2 infection. Thus, to validate the safety of mRNA-based vaccination, it is crucial to unravel the consequences of repetitive administration of mRNA-based COVID-19 vaccinations on antigen-specific IgG subclass Ab response in detail and determine whether there exists any causal relationship between breakthrough infection and anti-receptor binding domain

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(RBD) IgG4 responses induced by the mRNA-based COVID-19 vaccination.

Methods

Study design

This is a prospective observational study investigating antibody profiles following COVID-19 mRNA vaccination. Sera were collected sequentially from the time before vaccination up to 3 months after the four doses of vaccine. Anti-SARS-CoV-2-Spike-RBD IgG subclass antibodies were detected by enzyme-linked immunosorbent assay. The details of study cohort recruitment, the complete methods, and statistical analyses are provided in the Supplementary Methods.

Results

The appearance of anti-spike RBD IgG2/IgG4 responses after repetitive immunization with mRNA-based COVID-19 vaccines

The kinetics of anti-spike RBD IgG subclass (IgG1, IgG2. IgG3, and IgG4) after two, three, and four doses of mRNA-type COVID-19 vaccination in 101 non-breakthrough-infected vaccinees are shown in Figure 1. The demographic characteristics of these participants are shown in Supplementary Table 1.

At 1 month after the second dose of vaccination(2D-1M), the seropositivity of anti-RBD IgG1and anti-RBD IgG3 reached 100% and 94.2%, respectively, while the anti-RBD IgG2 or anti-RBD IgG4 remained very low seropositivity (Figure 1a-1d). Anti-RBD IgG1 levels were boosted by the third vaccination (3D-1M) but reached the plateau, and there was no further elevation of anti-RBD IgG1 levels after the fourth dose of vaccination (4D-1M) (Figure 1e). Anti-RBD IgG3 Ab increased after the second vaccination and decayed over (Figure. 1c). The peak antibody titers at 3D-1M and 4D-1M were significantly lower than that of 2D-1M (Figure 1g).

Although there was no induction of anti-RBD IgG2 after the second vaccination, a delayed increase in seropositivity after the third vaccination (3D-1M: 30.1%) and after the fourth vaccination (4D-1M: 55.8%) was observed (Figure 1b). The peak anti-RBD IgG2 levels after vaccinations gradually elevated with the repeat of COVID-19 vaccination (Figure 1f). Anti-RBD IgG4 showed little change in antibody titers from pre-vaccination to 1 month after the second dose of vaccination (Figure 1d, 1h). The antibody levels of anti-RBD IgG4 and the seropositivity gradually increased over 6 months after the second vaccination (Figure 1d). Anti-RBD IgG4 antibody levels increased markedly after the third immunization (Figure 1h), and the seropositivity reached 97.2% at 3D-1M (Figure 1d).

Anti-RBD IgG2 or IgG4 were not detected in the sera of previously unvaccinated patients with COVID-19

We next examined the anti-RBD IgG subclass antibody levels in previously unvaccinated patients with COVID-19. All patients were diagnosed by SARS-CoV-2 RT-PCR and confirmed positive for anti-SARS-CoV-2 nucleocapsid (N) protein total IgG antibody [6]. Anti-RBD IgG1 and -IgG3 antibody titers increased early after the onset of COVID-19, followed by a decrease over time (Figure 2a, c). In most patients, anti-RBD IgG3 returned to the negative range around 200 days after the disease onset, but persistently high levels of anti-RBD IgG1 were observed in some patients. Antibody levels of anti-RBD IgG2 and anti-RBD IgG4 in most patients with COVID-19 were under cutoff value, or very low level, if any, as shown in Figure 2b, d, which corroborates the previous finding [7,8]. Taken together, these results suggest that repeated mRNA-type COVID-19 vaccinations but not natural infection with SARS-CoV-2 induce considerable RBD-specific IgG4/IgG2 responses.

Breakthrough infection and anti-RBD IgG subclass antibodies

We compared the pre-breakthrough infection-serum Ab titers of the anti-RBD IgG subclass of breakthrough-infected vaccinees and those of non-infected vaccinees. Demographic characteristics of breakthrough-infected vaccinees group A, B, C, and D and their non-infected control vaccinees were shown in Supplementary Table 2-5. Comparison of anti-RBD IgG subclass Ab titers at pre-breakthrough-infection serum between breakthrough-infected vaccinees and their non-infected controls were shown in Supplementary Figure 3A-3D and Supplementary Tables 6-9. Breakthrough-infected vaccinees, whose onsets of breakthrough infection were between 3D-3M to 3D-6M, revealed significantly lower anti-RBD IgG1 than those of non-infected control at 3D-1M and 3D-3M before the breakthrough infection. The other anti-RBD IgG subclasses including IgG4 showed no difference between breakthrough-infected vaccinees and non-infected vaccinees at any time points before breakthrough infection (Supplementary Figure 3C). In the analysis of breakthrough infection after receiving the fourth dose of vaccination, a lower anti-RBD IgG4 antibody level was observed in the breakthrough-infected vaccinees at pre-breakthrough sera (3D-3M, 3D-6M) (Supplementary Figure 3D). In our cohort we did not find any evidence to suggest that the increased anti-RBD IgG4 Ab levels observed after repeated mRNA-based COVID-19 vaccination increase the risk of breakthrough infection.

We also found that those breakthrough-infected vaccinees after receiving COVID-19 mRNA vaccinations showed boosting effects in all anti-RBD IgG subclasses including IgG4 (Supplementary Figure 3B and 3C). Our results corroborate the previous observation that hybrid immunity [9], as generated by breakthrough infections after mRNA-type COVID-19 vaccination, can also induce anti-RBD IgG4 [5].

Discussion

This study demonstrated that repeated vaccinations with mRNA-based COVID-19 vaccine induce a delayed augmentation of anti-RBD IgG2 and IgG4 Ab responses while causing the decrement of the peak titer of anti-RBD IgG3 and the loss of further boosting effect on anti-RBD IgG1 Ab. Anti-RBD IgG2 or IgG4 Ab responses in serum were not observed in patients with COVID-19 who had not received previous COVID-19 vaccination. Breakthrough infection after mRNA-type COVID-19 vaccination augments the level of anti-RBD IgG4 and IgG2.

In this study, we showed that the kinetics of anti-RBD IgG4 after the two-dose regimen of BNT162b2 vaccination differed distinctly from the trajectory of anti-RBD IgG1 and IgG3 which showed the initial peak at 2D-1M, followed by a declining slope. In sharp contrast, anti-IgG4 antibody levels increased slowly but persistently up to 2D-6M, and then increased robustly after the third dose of vaccination, which confirmed the previous studies [3–5].

The interaction of antibodies with various FcγRs is influenced by the IgG subclass, with IgG1 and IgG3 binding efficiently to all Fc gamma receptors. On the other hand, effector functions of IgG4 and IgG2 have been reported to be rather anti-inflammatory [10]. Whether there are functional consequences associated with this striking elevation of the RBD IgG4 subclass for host-defense mechanisms against SARS-CoV-2 re-infection has not been determined. In our cohort study, we found no evidence that the increased anti-RBD IgG4 subclass profiles were associated with a higher rate of breakthrough infection, implying that the higher

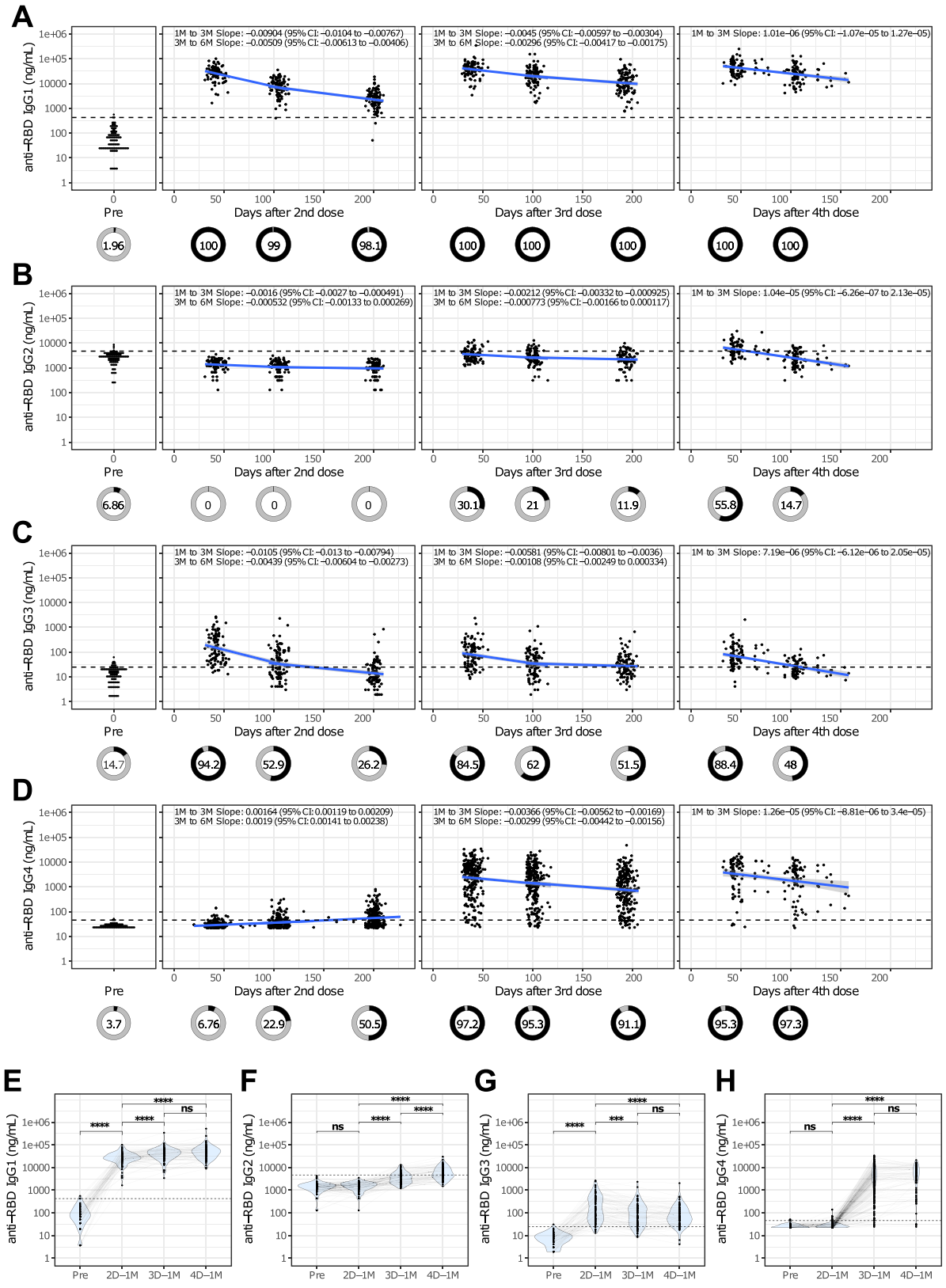

Figure 1. The appearance of anti-spike RBD IgG4 responses after repetitive immunization with messenger RNA-based COVID-19 vaccines. Anti-SARS-CoV-2 spike RBD IgG subclasses (IgG1: a, IgG2: b, IgG3: c, and IgG4: d) of serum samples were examined in these non-breakthrough-infected vaccinees who received four doses of messenger RNA-type COVID-19 vaccination (n = 101). In anti-RBD IgG4 measurement (Figure 1d), we also included a further set of sera from participants who had participated till 3D-6M but did not enter after the fourth vaccination in addition to these 101 individuals. Blue lines on each graph represent the regression lines of a log-linear model. Dotted lines on each graph showed a cutoff value of each subclass. Seropositivity is shown in a pie chart and numbers in circles indicate percentages. For each IgG subclass (IgG1: e, IgG2: f, IgG3: g, and IgG4: h), pre-vaccination antibody titers were compared with peak antibody titers after the second, third, and fourth vaccination. Serum samples used peak titers comparison were obtained before the vaccination (pre), 28-56 days after the second dose (2D-1M), 28-56 days after the third dose of vaccine (3D-1M), and 28-56 days after the fourth dose of vaccine (4D-1M). Statistical significance was determined using Steel-Dwass's multiple comparison test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. CI, confidence interval; Ig, immunoglobulin; RBD, receptor binding domain.

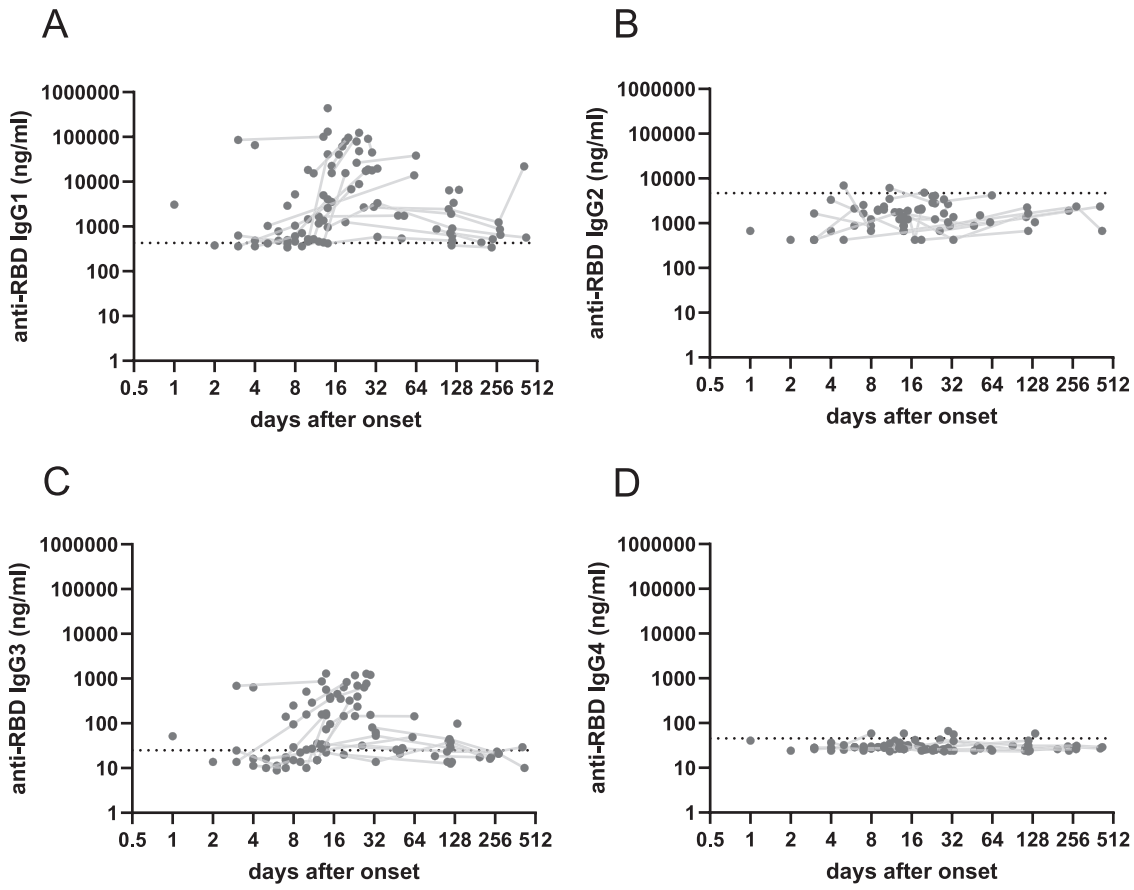

Figure 2. Anti-RBD IgG2 or IgG4 were not detected in the sera of previously unvaccinated patients with COVID-19.
Anti-RBD IgG subclass antibody levels were measured by enzyme-linked immunosorbent assay in the serum of patients with COVID-19, which were collected at the indicated days from the onset of the disease. All patients had not received the COVID-19 vaccination before the SARS-CoV-2 infection and after the infection during the observation period. Ninety-two serum samples from 52 patients were evaluated. In total, 25 samples were under the limit of detection in IgG2. The dots with the same patient origin were connected with the lines.
Ig, immunoglobulin; RBD, receptor binding domain.

prevalence of RBD-specific IgG4 subclass would have minimal impact on protection. However, as the unique properties of IgG4 and its roles in health and disease have been recently summarized by Rispens et al. [11], IgG4 may have an unexplored physiological role in mucosal immunity. Thus, the present results do not exclude the prospect that the IgG4 subclass can participate in the immune responses against SARS-CoV-2. The long-term effect of anti-RBD IgG4 Ab is currently unknown, and careful follow-up will be needed to conclude.

Declaration of competing interests

The authors have no competing interests to declare.

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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.

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Author contributions

All authors have read and approved the final manuscript. MY, A. Sakamoto, and KH designed the study. MY, A. Sakamoto, YK, RI, KI, A. Shimizu, BC, and SN acquired the data, and MY, A. Sakamoto, RO, and KH analyzed the data. MY and KH drafted the manuscript. MY, A. Sakamoto, RO, and KH critically reviewed the manuscript.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2023.11.028.

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