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Vaccine 41 (2023) 7019–7025

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Distinct features of SARS-CoV-2 humoral immunity against Omicron breakthrough infection

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ARTICLE INFO

Keywords:
SARS-CoV-2
Breakthrough infection
Neutralizing activity
Anti-spike antibody
Anti-RBD antibody
Humoral immunity

ABSTRACT

Background: SARS-CoV-2 Omicron breakthrough infection (Omicron-BTI) after vaccination has been frequently observed. A more detailed understanding of the humoral immunity against Omicron-BTI is required.
Methods: We measured strain-specific live-virus based neutralizing activity, anti-spike IgG, and anti-receptor-binding domain (RBD) IgG titers in individuals with Omicron/BA.1-BTI and directly compared them with controls with diverse combinations of wild-type (WT) mRNA vaccination and infection history.
Results: Omicron-BTI individuals showed markedly higher neutralizing titers against all the WT, Delta, and Omicron strains in convalescent sera, compared with unvaccinated Omicron-infection individuals with only Omicron neutralizing activity. Similar tendencies were found in strain-specific anti-spike and anti-RBD IgG titers. The Omicron-specificity (BA.1/WT neutralizing ratio), Omicron-neutralizing efficiency per antibody unit, and anti-Omicron RBD-directivity of anti-spike antibodies in Omicron-BTI individuals were all significantly lower than those in unvaccinated Omicron-infection individuals, but they were equivalent to or higher than those in uninfected vaccinees. The induction of Omicron-specific neutralizing activity after Omicron-BTI was not weakened for eight months from the last vaccination.
Conclusions: These findings suggest that cross-reactive vaccine-induced immunity was intensively stimulated following Omicron breakthrough infection, which contributed to Omicron neutralization. Measuring SARS-CoV-2 variant-specific antibody levels as well as neutralizing activity is useful for evaluating humoral immunity after breakthrough infection in the current situation of antigenic gaps between vaccinated and epidemic (Omicron sub-lineages) strains.

1. Introduction

Vaccine-induced immunity played a crucial role in preventing infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) during the epidemics of the wild-type (WT) and Delta (B.1.617.2) strains [1,2]. However, with the emergence of the Omicron (B.1.1.529) variant, known for its high immune evasion capabilities through possible structural and antigenic changes in the epitope regions [3–5], a marked reduction of vaccine efficacy and an increase in breakthrough cases have been observed [6,7]. Due to these distinct viral structural and epidemiological characteristics, entirely different immunodynamic assessments are required before and after the appearance of the Omicron variant. A key difference between prior strains and the Omicron variant is the extent of mutations in the spike protein, particularly in the receptor-binding domain (RBD) [8]. The RBD, a highly immunogenic region that is crucial for the virus to enter into host cells

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https://doi.org/10.1016/j.vaccine.2023.10.035
Received 4 August 2023; Received in revised form 27 September 2023; Accepted 13 October 2023
Available online 17 October 2023

through the ACE-2 receptor, remained functional in the Omicron variant [9]. With continuous mutations in the spike protein, primarily in the RBD, accompanying the emergence of Omicron sub-lineages, the discrepancy between antigens targeted by vaccine-induced antibodies and those expressed in the circulating Omicron strains is expected to remain an unavoidable challenge in the future. Evaluating the impact of these antigenic gaps on immune responses using convalescent sera of vaccinated individuals who are subsequently infected with the Omicron variant is an important step for estimating the efficacy of vaccines against newly emerging SARS-CoV-2 variants. Although some studies have investigated humoral immunity in breakthrough infected cases of the Omicron variant [10,11], the features have yet to be fully characterized due to limitations in control group selection and measurement methodologies.

In this study, we evaluated and directly compared the humoral immunity of convalescent sera of individuals with Omicron breakthrough infections following vaccination with sera of several control groups: vaccinated individuals with no infection, unvaccinated individuals with Delta variant infection, vaccinated individuals with Delta variant breakthrough infection, and unvaccinated individuals with Omicron variant infection. In our assessment, we investigated not only the neutralizing activity using live viruses, a gold standard for evaluating humoral immunity, but also the strain-specific antibody titers to the whole-spike and RBD protein. We aimed to provide additional insights into humoral immunity against SARS-CoV-2 breakthrough infection through a comprehensive assessment of both the quantitative and qualitative aspects.

2. Methods

2.1. Participants and serum sample collection

Serum samples were collected from staff members and patients at Fukuoka City Hospital and Kyushu University Hospital, respectively. Medical record data, including the number of vaccinations and vaccination dates, of all hospitalized patients diagnosed with COVID-19 by Nucleic acid amplification test or antigen test between August 1, 2021 and March 15, 2022 were retrospectively reviewed. Patients were selected based on the following criteria: availability of a convalescent serum sample collected between 14 and 21 days from symptom onset (if unavailable, a sample collected on the day closest to 14 days was selected), no history of organ or hematopoietic stem cell transplantation, and no ongoing chemotherapy or immunosuppressive medication. Peri-infection serum samples between 0 and 3 days from onset were also collected. This study was approved by the ethics review board of Kyushu University Hospital, Fukuoka City Hospital (approval numbers 22156–00 and 241, respectively), and Hara-doi Hospital (approval date Jun 21, 2022). Information on the study was provided to the participants on the homepages of their hospitals as an opt-out. Among the participants with a history of SARS-CoV-2 vaccination, all had received WT mRNA vaccines, either BNT162b2 (Pfizer-BioNTech) or mRNA-1273 (Moderna).

2.2. Measurement of SARS-CoV-2 strain-specific neutralizing activity

The measurements of SARS-CoV-2 strain-specific neutralizing activity using live virus against the WT, Delta, and Omicron BA.1 strains were conducted following the same procedure as described in Shinkai et al. [12]. In brief, Serum samples were thawed on ice, inactivated at 56 °C for 30 min, and diluted in assay medium. Diluted samples were mixed with equivalent volumes of virus solution and incubated at room temperature for 1 h. Next, 100 μL of the mixture was transferred to a 96-well plate, and 100 μL/well of VeroE6/TMPRSS2 cell suspension was added. The plate was incubated for 3 days at 37 °C and 5 % CO2. Afterward, 100 μL of supernatant was removed, and 100 μL/well of CellTiter-Glo® 2.0 Reagent (Promega, Madison, WI, USA) was added. The mixture was shaken, and the reaction proceeded for 30 min in the dark. Luminescence was measured using an EnSpire 2300 microplate reader (PerkinElmer, Waltham, MA, USA). The SARS-CoV-2 viruses used in this study were provided by the National Institute of Infectious Diseases in Japan, and the virus strains used were wild-type, WK-521; Delta Strain, TY11 927-P1; and Omicron Strain BA.1, TY38-873.

2.3. Measurement of SARS-CoV-2 strain-specific anti-spike IgG antibody titers

The SARS-CoV-2 strain-specific anti-spike (anti-S) IgG titers were measured with in-house Enzyme-linked immunosorbent assay (ELISA) using trimeric full-length spike proteins generated by silkworms, as previously described [13]. Briefly, baculoviruses were produced with transfected recombinant bacmid (QD04 strain) extracted from *E. coli* DH10Bac transformed with the pFastBac-SARS2/SNFPP(1–1208) + CMP + TEV-H8STREPH6 vector. Target proteins of the WT, Delta, and BA.1 strains with His-tag and STREP-tag were extracted and purified from infected silkworm sera. The optical density at 450 nm (OD 450 nm) and 570 nm (OD 570 nm) were read using a microplate reader after ELISA. The anti-S IgG titers of each sample were determined based on an endpoint method with multiple dilutions of serum and calculated by assigning the OD value (OD 450 nm − 570 nm) to a calibration curve established by the dose–response of an anti-His-Tag antibody reacting to His-Tag, which is a contiguous sequence of 6 and 8 histidine residues pre-embedded one per monomer (three per trimer) of each recombinant protein.

2.4. Measurement of SARS-CoV-2 strain-specific anti-RBD IgG antibody titers

The anti-RBD IgG titers for the WT strain were measured using the Abbott SARS-CoV-2 IgG II Quant immunoassay (Abbott, Chicago, IL, USA). The anti-RBD IgG titers for the Delta and BA.1 variants were measured by in-house ELISA with SARS-CoV-2 spike RBD antigens for the Delta (Accession # YP_009724390.1, with mutations L452R, T478K; ACROBiosystems, Newark, DE, USA) and BA.1 variants (Accession # QHD43416.1, with mutations G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H; Sino Biological, Chesterbrook, PA, USA). Horseradish peroxidase conjugated goat-anti-human IgG (H + L) antibody (Invitrogen, A18811) was used as the detection antibody. Sample absorbance was measured as the difference between absorbance values at 405 nm and 490 nm, and the mean and coefficient of variation (CV) of absorbance for each duplicate were determined.

2.5. Sequencing analysis

Viral sequencing was conducted using specimen from nasopharyngeal swabs collected for COVID-19 diagnosis. PCR amplification was conducted with Alt_nCov2019_primers ver. N5(Itokawa. K, https://github.com/ItokawaK/Alt_nCov2019_primers/tree/master/Primers/ver_N5). Libraries were prepared using the QIAseq FX DNA Library UDI Kit (Qiagen, Hilden, Germany) for sequencing with the MiSeq Reagent Kit v2 (Illumina, San Diego, CA, USA). The obtained reads were mapped to the original SARS-CoV-2 reference genome (GISAID, hCoV-19/Wuhan/WIV04/2019/EPI_ISL_402124) using the BWA-MEM algorithm. Variant assignment was conducted with Nextclade (https://clades.nextstrain.org/). The GATK variants were filtered to at least 10x depth. Data analysis was conducted using Python Ver 3.8.8. The sequencing results revealed that all virus genomes obtained from the patients analyzed in this study matched to the circulating Delta and BA.1 variants. These genomes were subsequently registered on GISAID (hCoV-19/Wuhan/WIV04/2019/ EPI_ISL_16970345,347–367).

Table 1
Demographic characteristics and categorization of serum samples according to vaccination and infection history.

Vaccination		Primary (2x)	Booster (3x)	Unvaccinated	Primary (2x)	Unvaccinated	Primary (2x)	Primary (2x)	Booster (3x)	Booster (3x)
Infection		Uninfected	Uninfected	Delta	Delta	BA.1	BA.1	BA.1	BA.1	BA.1
Sample number, n		12	12	8	4	8	9	15	3	6
Age, median yrs (range)		39.5 (22–59)	39.5 (22–59)	46.0 (28–67)	71.8 (45–100)	59.0 (24–82)		71.7 (45–93)		80.5 (69–94)
Sex, n (%)	Female	9 (75.0)	9 (75.0)	4 (50.0)	2 (50.0)	4 (50.0)		7 (46.7)		3 (50.0)
	Male	3 (25.0)	3 (25.0)	4 (50.0)	2 (50.0)	4 (50.0)		8 (53.3)		3 (50.0)
Sampling point from the last vaccination or onset of Infection, median days (range)		33.2 (30–36)	32.5 (29–35)	14.8 (12–17)	16.5 (14–21)	13.8 (9–17)	1.6 (0–3)	13.0 (11–14)	1.0 (0–2)	13.0 (11–14)
Interval between the last vaccination and onset of Infection, median days (range)		N/A	N/A	N/A	54.8 (19–86)	N/A		202.1 (148–245)		11.8 (5–21)
Severity[1], n (%)	mild/ moderate	N/A	N/A	1 (12.5)	0 (0.0)	2 (25.0)		7 (46.7)		5 (83.3)
	severe	N/A	N/A	3 (37.5)	2 (50.0)	6 (75.0)		7 (46.7)		1 (16.7)
	critical	N/A	N/A	4 (50.0)	2 (50.0)	0 (0.0)		1 (6.6)		0 (0.0)

N/A, not applicable.
[1] The definition of disease severity is based on reference no. [14].

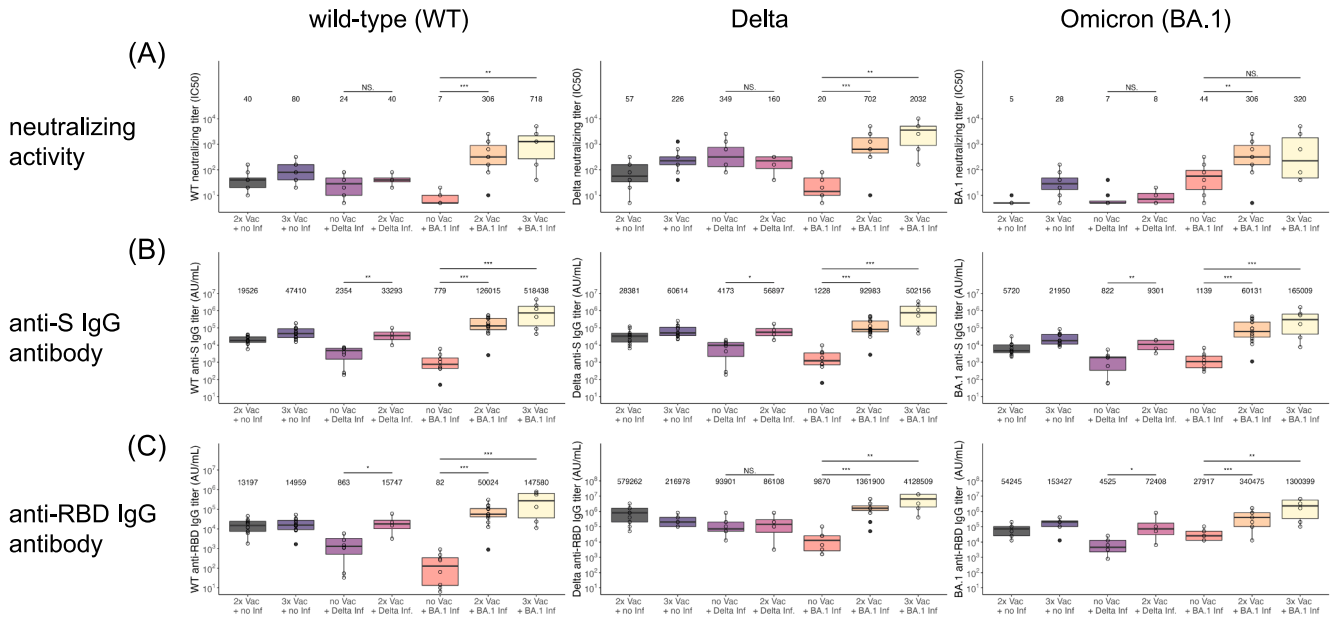

Fig. 1. SARS-CoV-2 strain-specific neutralizing activity, anti-spike IgG antibody titers, and anti-RBD IgG antibody titers. The box plots show the quantified values of humoral immunity, categorized by immunological backgrounds, for neutralizing titers against the live virus (A), anti-spike (anti-S) IgG titers (B), and anti-RBD IgG titers (C); the left graphs represent values from the assay of the wild-type (WT) strain, the middle graphs represent Delta (B.1.617.2), and the right graphs represent Omicron BA.1 (B.1.1.529). The elements of the box plot are represented as follows: The horizontal black lines inside each box signify the median values. The boxes themselves represent the interquartile range, stretching from the 25th percentile to the 75th percentile of each group's distribution of values. Vertical black lines extending from the boxes represent the adjacent values, which are the highest and lowest values within 1.5 times the interquartile range from the 25th and 75th percentiles. Each individual white dot inside these vertical lines or boxes represents a value within 1.5 times the interquartile range from the 25th and 75th percentiles, and black dots outside these vertical lines represent outliers. The upper number on each group shows geometric mean titers (GMT) within the group. The symbols above the bars indicate the statistical difference between the two groups. IC50, 50 % inhibitory concentration; WT, wild-type; anti-S, anti-spike; anti-RBD, anti-receptor binding domain; 2x Vac + no Inf, twice vaccinated and no history of infection of SARS-CoV-2; 3x Vac + no Inf, three times vaccinated and no history of infection of SARS-CoV-2; no Vac + Delta Inf, infected with the Delta variant without vaccination; 2x Vac + Delta Inf, infected with the Delta variant after the second vaccination; no Vac + BA.1 Inf, infected with the BA.1 variant without vaccination; 2x Vac + BA.1 Inf, infected with the BA.1 variant after the second vaccination; 3x Vac + BA.1 Inf, infected with the BA.1 variant after the third vaccination; NS, not significant; *P < 0.05, **P < 0.01, ***P < 0.001.

2.6. Statistical analysis

Comparisons of significant differences between two groups were conducted by Wilcoxon rank-sum test. Pearson's product-moment correlation coefficient was calculated to examine the correlation of different continuous variables. Two-tailed *P* values of < 0.05 were considered statistically significant. All data analysis and plotting were performed on R version 4.1.1 and R studio 2021.09.0.

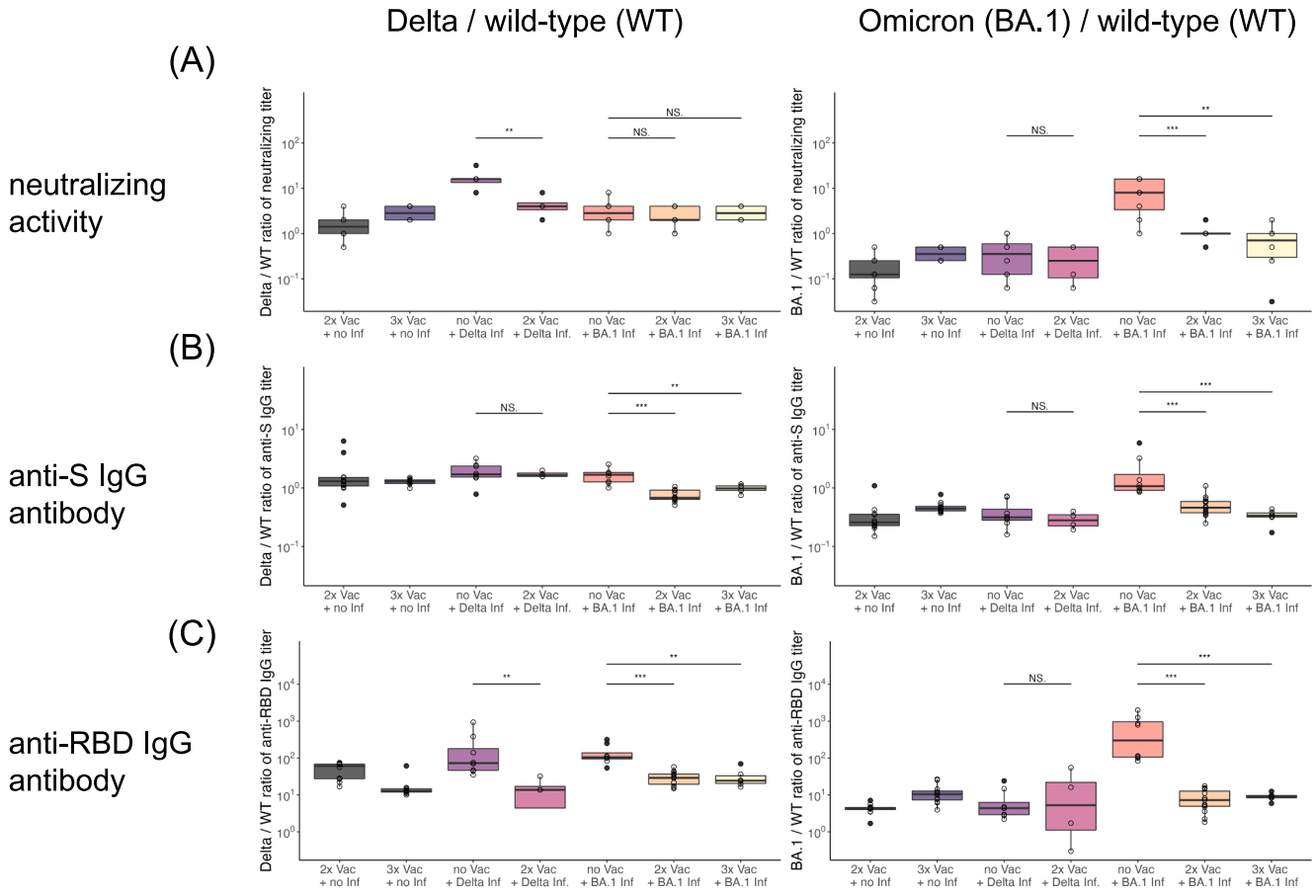

Fig. 2. Strain-specificity of neutralizing activity, anti-spike IgG antibody titers, and anti-RBD IgG antibody titers. The box plots show the ratio of values from the assay of the Delta (B.1.617.2) strain to those of the wild-type (WT) (left side) and values from the assay of the Omicron BA.1(B.1.1.529) strain to those of the WT (right side) for neutralizing titers against the live virus (A), anti-spike (anti-S) IgG titers (B), and anti-RBD IgG titers (C). The elements of the box plot are represented as in Fig. 1. The symbols above the bars indicate the statistical difference between the two groups. WT, wild-type; anti-S, anti-spike; anti-RBD, anti-receptor binding domain; 2x Vac + no Inf, twice vaccinated and no history of infection of SARS-CoV-2; 3x Vac + no Inf, three times vaccinated and no history of infection of SARS-CoV-2; no Vac + Delta Inf, infected with the Delta variant without vaccination; 2x Vac + Delta Inf, infected with the Delta variant after the second vaccination; no Vac + BA.1 Inf, infected with the BA.1 variant without vaccination; 2x Vac + BA.1 Inf, infected with the BA.1 variant after the second vaccination; 3x Vac + BA.1 Inf, infected with the BA.1 variant after the third vaccination; NS, not significant; *P < 0.05, **P < 0.01, ***P < 0.001.

3. Results

3.1. Categorization of serum samples according to vaccination and infection history

Serum samples were obtained and categorized based on vaccination and infection history, as shown in Table 1. The groups included twelve sets of serum samples from uninfected staff members after the primary series (first and second doses) and a booster (third dose) vaccination (uninfected vaccinee group), eight convalescent samples from unvaccinated individuals with Delta variant infection (unvaccinated Delta-infection group), four from individuals with Delta variant breakthrough infection (BTI) after primary series vaccination (Delta-BTI group), eight from unvaccinated individuals with BA.1 variant infection (unvaccinated BA.1-infection group), and twenty-one from individuals with BA.1 variant BTI after vaccination, including fifteen after the primary series and six after the booster dose (BA.1-BTI groups). Peri-infection serum samples were also collected for the BA.1-BTI groups.

3.2. Strain-specific neutralizing activity, anti-S IgG antibody titers, and anti-RBD IgG antibody titers

The strain-specific neutralizing activity for each group is shown in Fig. 1A. Convalescent sera of the unvaccinated BA.1-infection group showed lower neutralizing titers against the WT and Delta strains than those of the uninfected vaccinee group and unvaccinated Delta-infection group. In contrast, this relation was reversed for the BA.1 variant assay. Convalescent sera of the BA.1-BTI groups showed significantly higher neutralizing titers against the BA.1 variant than those of the unvaccinated BA.1-infection group. Convalescent sera of the BA.1-BTI groups also showed significantly higher neutralizing titers against the WT and Delta strains in contrast to those of the unvaccinated BA.1-infection group. The neutralizing titers of convalescent sera after BA.1-BTI were substantially increased from those at the *peri*-infection period (Supplementary Fig. 1). Significant differences for all three strains between the BA.1-BTI groups and the unvaccinated BA.1-infection group were also observed in both anti-S and anti-RBD IgG titers (Fig. 1B and C). The correlation between neutralizing titers and anti-S IgG titers against the same strain was statistically significant in both the BA.1-BTI groups and the unvaccinated BA.1-infection group (Supplementary Fig. 2).

3.3. Strain-specificity of neutralizing activity, anti-S IgG antibody titers, and anti-RBD IgG antibody titers

To evaluate the strain-specificity of neutralizing activity, we compared the ratio of neutralizing titers against the Delta and BA.1

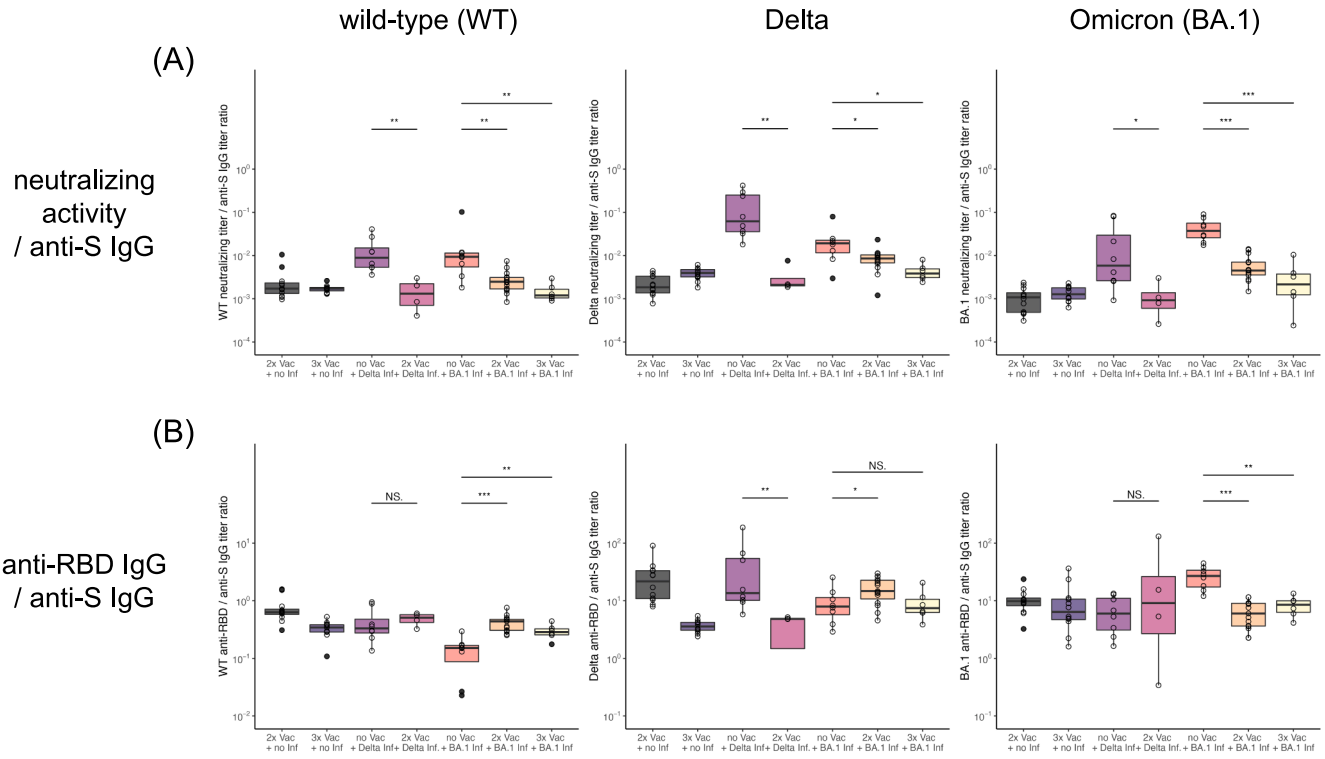

Fig. 3. Strain-specific neutralizing efficiency of anti-S IgG antibodies and Strain-specific RBD-directivity of whole-spike antibodies. The box plots show the ratio of neutralizing titers to anti-spike (anti-S) IgG titers (A) and the ratio of anti-RBD IgG titers to anti-spike IgG titers (B), from the assay of the wild-type (WT) (left side), Delta (B.1.617.2) (middle), and Omicron BA.1 (B.1.1.529) strain (right side). The elements of the box plot are represented as in Fig. 1. The symbols above the bars indicate the statistical difference between the two groups. WT, wild-type; anti-S, anti-spike; anti-RBD, anti-receptor binding domain; 2x Vac + no Inf, twice vaccinated and no history of infection of SARS-CoV-2; 3x Vac + no Inf, three times vaccinated and no history of infection of SARS-CoV-2; no Vac + Delta Inf, infected with the Delta variant without vaccination; 2x Vac + Delta Inf, infected with the Delta variant after the second vaccination; no Vac + BA.1 Inf, infected with the BA.1 variant without vaccination; 2x Vac + BA.1 Inf, infected with the BA.1 variant after the second vaccination; 3x Vac + BA.1 Inf, infected with the BA.1 variant after the third vaccination; NS, not significant; *P < 0.05, **P < 0.01, ***P < 0.001.

variants to those against the WT strain for each group (Fig. 2A). BA.1-specificity in the BA.1-BTI groups was significantly lower than that in the unvaccinated BA.1-infection group, but it was equivalent to or higher than that in the uninfected vaccinee group. Similar relations were observed for the anti-S and anti-RBD IgG titer ratios, with the difference between the BA.1-BTI groups and the unvaccinated BA.1-infection group seeming more distinct in the anti-RBD IgG titer ratio (Fig. 2B and C). Similar to the BA.1-BTI groups, the Delta-BTI group tended to show lower Delta-specificity compared to the unvaccinated Delta-infection group, but it was equivalent to or higher than that in the uninfected vaccinee group.

3.4. Strain-specific neutralizing efficiency of anti-S IgG antibodies

To evaluate the neutralizing efficiency per unit of antibodies for each strain, we compared the ratio of neutralizing titers to anti-S IgG titers for the same strain assay (Fig. 3A). Similar to the observed strain-specificity, BA.1-neutralizing efficiency in the BA.1-BTI groups was significantly lower than that in the unvaccinated BA.1-infection group, but it was equivalent to or higher than that in the uninfected vaccinee group. These relations were maintained for the WT strain as well, although the differences between the groups appeared to be smaller. Similar relations were shown between the Delta-BTI group and the unvaccinated Delta-infection group.

3.5. Strain-specific RBD-directivity of whole-spike IgG antibodies

To evaluate the RBD-directivity of whole-spike IgG antibodies for each strain, we compared the ratio of anti-RBD IgG titers to anti-S IgG titers for each group (Fig. 3B). RBD-directivity to the BA.1 variant in the BA.1-BTI groups was significantly lower than that in the unvaccinated BA.1-infection group, but it was equivalent to or higher than that in the uninfected vaccinee group. Notably, the relation between the unvaccinated BA.1-infection group and the BA.1-BTI groups or the uninfected vaccinee group was reversed when tested for the WT strain. Similar relations were observed in the Delta-BTI and unvaccinated Delta-infection groups.

3.6. Correlation of neutralizing activity, anti-S IgG titers, and anti-RBD IgG titers with intervals from vaccination to breakthrough infection

To investigate whether the interval from vaccination to infection affects neutralizing activity after BTI, we analyzed the negative correlation between the interval from the last vaccination to the onset of infection and the neutralizing titers in convalescent sera of the BTI groups (Fig. 4A). Within eight months, no tendency was observed for the neutralizing titers in convalescent sera against the BA.1 variant to decline over time. Similar results were obtained for the WT and Delta strain assays. Similar trends were observed for the anti-S and RBD IgG titers (Fig. 4B and C).

4. Discussion

In this study, we directly compared SARS-CoV-2 related humoral immunity among individuals with diverse immunological backgrounds. By measuring not only neutralizing activity against live viruses but also strain-specific anti-S and anti-RBD IgG antibody levels, we were able to investigate both the quantitative and qualitative antibody properties of

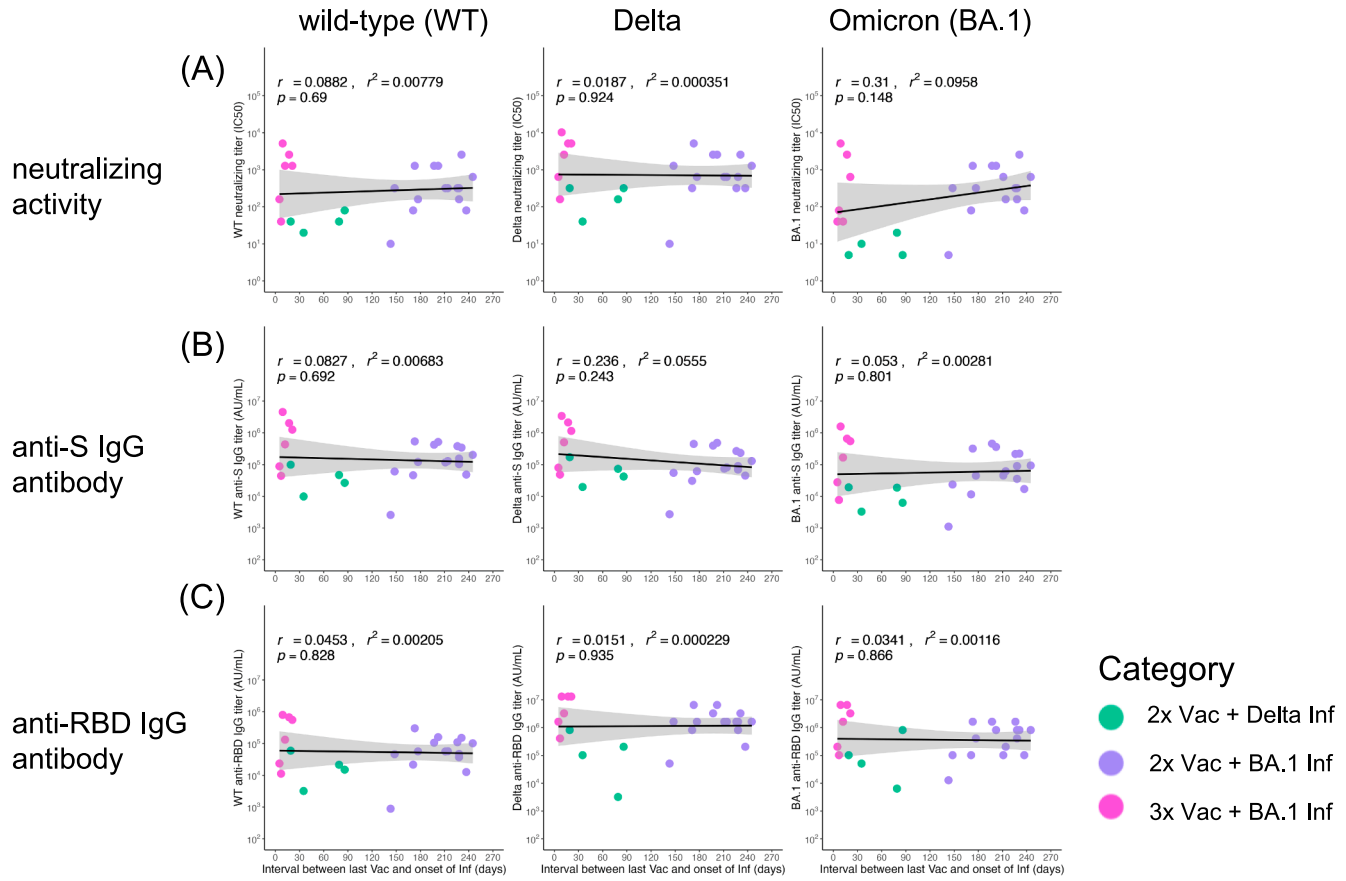

Fig. 4. Correlation of strain-specific neutralizing activity, anti-spike IgG antibody titers, and anti-RBD IgG titers with intervals from vaccination to breakthrough infection. The scatter plots show the quantified values of humoral immunity among individuals with breakthrough infection in relation to the interval between the last vaccination and subsequent infection (Day) for neutralizing titers against the live virus (A), anti-spike (anti-S) IgG titers (B), and anti-RBD IgG titers (C); the left graphs represent values from the assay of the wild-type (WT) strain, the middle graphs represent the Delta (B.1.617.2), and the right graphs represent the Omicron BA.1 (B.1.1.529). Black lines represent the regression line for all plots, with the gray shaded areas indicating the 95 % confidence intervals (95 %CI) for the regression lines. The formulas above the plots provide the coefficient of correlation, coefficient of determination, and p-value. IC50, 50 % inhibitory concentration; WT, wild-type; anti-S, anti-spike; anti-RBD, anti-receptor binding domain; 2x Vac + Delta Inf, infected with the Delta variant after the second vaccination; 2x Vac + BA.1 Inf, infected with the BA.1 variant after the second vaccination; 3x Vac + BA.1 Inf, infected with the BA.1 variant after the third vaccination.

convalescent sera of individuals with Omicron-BTI.

The levels of neutralizing activity between two types of sera after pre-Omicron (WT and Delta) and Omicron antigen exposures were reversed between pre-Omicron and Omicron strain assays. This finding suggests a significant difference in the antigenicity of the Omicron variant from the pre-Omicron strains, potentially reflecting the structural and antigenic changes in the epitope regions of the spike protein in the Omicron variant, as previously reported [3–5].

The markedly higher neutralizing activity in sera of the Omicron-BTI groups than was seen in the unvaccinated BA.1-infection group was not only observed against the Omicron variant but also against the pre-Omicron strains, including WT. These results suggest that humoral immunity after Omicron-BTI exhibited cross-reactivity against both the Omicron and WT strains. Differences between Omicron-BTI and unvaccinated BA.1-infection against all analyzed strains were also observed in assays for anti-spike IgG, with high correlations with neutralizing activity, and those for anti-RBD IgG, which indicates that the cross-reactivity of neutralizing activity in Omicron-BTI could be attributed to anti-spike, including RBD, antibodies.

Analysis of qualitative antibody characteristics showed that strain specificity, neutralizing efficiency per antibody unit, and RBD-directivity of whole-spike antibodies exhibited a similar pattern among each group; sera of the Omicron-BTI groups showed lower levels than the unvaccinated BA.1-infection group and equivalent to or higher levels than the uninfected vaccinee group. These results suggest that the immune properties of sera of Omicron-BTI individuals cannot be explained only by the effect of Omicron variant infection and that vaccine-induced immunity against the WT strain would be stimulated after Omicron-BTI. Similar suggestions have been reported in previous studies discussing cross-reactivity from a distinct perspective on memory B cell kinetics [15,16]. Taken together, the cross-reactive humoral immunity shown in Omicron-BTI should be attributed to anti-spike and RBD antibodies that were derived from vaccine-induced immunity against the WT strain.

In the unvaccinated BA.1-infection group, RBD-directed anti-spike antibodies with high BA.1-specificity and high BA.1-neutralizing efficiency were shown to have been intensively produced. This finding suggests that significantly changed regions in the RBD of the BA.1 variant from the WT strain were recognized as epitopes with high immunogenicity among whole-spike protein regions, contributing to the induction of BA.1-specific antibodies. The next question here is how vaccine-induced antibodies targeting the WT strain can be produced by Omicron infection despite findings that show a significant antigenic gap between vaccinated (WT) and infected (Omicron) strains. It is likely that shared epitopes expressed on both the WT and Omicron strains remain in the spike region and even in the highly mutated RBD region (at least 15 amino acid substitutions in the RBD), which in turn explains why WT vaccine-induced antibodies are cross-reactive to the Omicron variant. The detection of anti-S and RBD antibodies against the Omicron variant in the uninfected vaccinee group supports this scenario.

It is of note that our results suggest that the production of anti-S IgG antibodies against the WT strain among the Omicron-BTI groups, which is likely due to stimulating vaccine-induced immunity, contributed to significantly higher neutralizing activity against the Omicron variant compared to the unvaccinated BA.1-infection group. This phenomenon may be related to the reason why vaccination targeting the WT strain can reduce severe illness after Omicron variant infection, as previously reported [17,18]. Furthermore, the observation that the induction of neutralizing activity generated after Omicron-BTI was not decreased up to eight months after vaccination, possibly supported by a previous report that analyzed the durability of WT vaccine-induced memory B cell function [19], should be clinically meaningful.

In the current SARS-CoV-2 epidemic phase, frequent breakthrough infections caused by an antigenic gap between vaccine and circulating strains are inevitable. Nevertheless, given that efficient vaccine-induced neutralizing activity against epidemic strains, with possible clinical benefits, could be stimulated following breakthrough infections, SARS-CoV-2 vaccination would be significant. The observational stimulation of vaccine-induced humoral immunity against epidemic strains due to breakthrough infections, even at a long period of time after vaccination, could provide beneficial information on subsequent SARS-CoV-2 vaccination strategies, such as an optimal interval.

A limitation of this study is the small sample size in each group, which resulted in diminished statistical power that could potentially affect the generalizability of the findings. The current vaccination targets the BA.5 variant, and circulating variants exhibiting more significant antigenic changes, such as BQ.1.1 and XBB.1.5, have emerged [20]. We have not examined whether serological characteristics similar to the findings of this study will be observed between current SARS-CoV-2 vaccinations and epidemic variant infections. Our findings suggest that antigenic changes in the BA.1 variant have not yet reached the “critical point” where vaccine-induced immunity against the WT strain ceases to be stimulated due to totally differing antigenicity. This argument would be also applicable to the relation between the current vaccine and the epidemic strains. SARS-CoV-2 vaccination will retain its effectiveness as long as the cross-reactivity of antibodies between vaccine and epidemic strains is not lost. In the challenging situation of antigenic gaps between vaccinated and subsequently circulating strains due to continuous antigenic change, this argument of “critical point” would be an unavoidable and important clinical theme. We believe that serological characterization of humoral immunity in SARS-CoV-2 infected individuals with vaccination through analyzing its quality from the aspects of not only neutralizing activity but also strain-specific antibodies will provide useful information for discussing this theme.

Funding

This study was supported by Shionogi & Co., Ltd.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: [Yong Chong reports equipment, drugs, or supplies was provided by Shionogi and Co Ltd. Natsumi Susai, Tomoyo Yoshinaga reports a relationship with Shionogi and Co Ltd that includes: employment.].

Data availability

Data will be made available on request.

Acknowledgements

First, we would like to thank Dr. Kenichiro Shiraishi and Akiko Yonekawa of the Medicine and Biosystemic Science, Kyushu University Graduate School of Medical Sciences for their cooperation in collecting samples. Next, we sincerely thank Jae Man Lee and Akitsu Masuda of the Laboratory of Creative Science for Insect Industries Laboratory of Insect Genome Science, Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, and Takeru Ebihara of the Laboratory of Insect Genome Science, Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University for their technical support.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2023.10.035.

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