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RESEARCH ARTICLE

Neutralization against SARS-CoV-2 Delta/Omicron variants and B cell response after inactivated vaccination among COVID-19 convalescents

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Abstract **Emerging SARS-CoV-2 variants have made COVID-19 convalescents susceptible to re-infection and have raised concern about the efficacy of inactivated vaccination in neutralization against emerging variants and antigen-specific B cell response. To this end, a study on a long-term cohort of 208 participants who have recovered from COVID-19 was conducted, and the participants were followed up at 3.3 (Visit 1), 9.2 (Visit 2), and 18.5 (Visit 3) months after SARS-CoV-2 infection. They were classified into three groups (no-vaccination (*n* = 54), one-dose (*n* = 62), and two-dose (*n* = 92) groups) on the basis of the administration of inactivated vaccination. The neutralizing antibody (NAb) titers against the wild-type virus continued to decrease in the no-vaccination group, but they rose significantly in the one-dose and two-dose groups, with the highest NAb titers being observed in the two-dose group at Visit 3. The NAb titers against the Delta variant for the no-vaccination, one-dose, and two-dose groups decreased by 3.3, 1.9, and 2.3 folds relative to the wild-type virus, respectively, and those against the Omicron variant decreased by 7.0, 4.0, and 3.8 folds, respectively. Similarly, the responses of SARS-CoV-2 RBD-specific B cells and memory B cells were boosted by the second vaccine dose. Results showed that the convalescents benefited from the administration of the inactivated vaccine (one or two doses), which enhanced neutralization against highly mutated SARS-CoV-2 variants and memory B cell responses. Two doses of inactivated vaccine among COVID-19 convalescents are therefore recommended for the prevention of the COVID-19 pandemic, and vaccination guidelines and policies need to be updated.**

Keywords COVID-19 convalescent; SARS-CoV-2; inactivated vaccination; neutralizing antibody; B cell response

Introduction

Vaccination against SARS-CoV-2 is considered the key strategy to combat against the ongoing COVID-19 pandemic. With re-infection cases being reported frequently [1], the necessity of vaccination among convalescents has become a heated topic. Inactivated vaccines (e.g., CoronaVac and Sinopharm vaccines) have been widely used and account for almost half of COVID-19 vaccine doses delivered globally [2]. Despite the extensive application of vaccines, the humoral immune response of convalescents who have received inactivated vaccines remains largely unknown. In addition, some new SARS-CoV-2 variants (including Delta (B.1.617.2) and Omicron (BA.1/B.1.1.529)) have emerged in recent months and have rapidly become the dominant SARS-CoV-2 viruses worldwide. These SARS-CoV-2 variants of concern (VOC)

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exhibit high transmissibility and raise public concerns about how they reduce the efficacy of existing inactivated vaccines, leading to re-infections among convalescents. Therefore, determining if VOC is resistant to the neutralizing antibody (NAb) induced in COVID-19 convalescents who have received inactivated vaccines requires immediate investigation.

Some studies have reported on the neutralizing activity against SARS-CoV-2 among COVID-19 convalescents who have received mRNA/adenovirus vaccines [3–8] or the heterogenous use of mRNA and inactivated vaccines [9–11]. Meanwhile, a few studies [12–14] have discussed the sole use of inactivated vaccines among these convalescents. However, these prior studies were subject to the limitations of single antibody measurements [4,7,9], relatively short follow-up duration (< 7 months post-infection) [3,9,14], lack of time information after the previous infection [4,7,11,12], or relatively limited sample size [4,7,10,12,13]. In addition, current evidence [3,4,7] on the neutralization activity against SARS-CoV-2 Delta and Omicron variants among vaccinated convalescents remains inconclusive. The fold-decrease in NAb and the specific neutralizing activity varied in existing studies [4,7,9,11,13] despite the fact that compared with neutralization against wild types, neutralization against Omicron is generally acknowledged to be reduced. Moreover, virus-specific B cells and memory B cells provide the basis of protective immunity and contribute to the generation of plasma cells that can produce antigen-specific antibodies [15]. However, previous studies [3,4,7,9,10,12–14,16] have mainly focused on the measurement of antibody responses, and information about SARS-CoV-2-specific B cells and memory B cells among vaccinated convalescents has rarely been reported. Therefore, the present study, which is based on a longitudinal cohort in Wuhan, China, aims to explore NAb (against wild-type, Delta, and Omicron variants) and SARS-CoV-2-specific B cells and memory B cells after natural SARS-CoV-2 infection and vaccination with inactivated vaccines.

Materials and methods

Study design and participants

A longitudinal prospective cohort study was performed to investigate the dynamics of the long-term antibody levels and health status of COVID-19 convalescents. On February 17, 2020, discharged COVID-19 patients without immune system diseases were invited from multiple districts in Wuhan, and a total of 289 participants were enrolled. The participants were scheduled to receive three follow-up visits after SARS-CoV-2 infection. By September 2021, 208 of them had completed the three follow-up visits and were included in the present analysis (Fig. 1). The median durations of the three follow-up visits were 3.3 (IQR: 1.3–4.4), 9.2 (IQR: 9.0–9.6), and 18.5 (IQR: 18.2–19.1) months after infection. During the follow-up period, we collected serum from the 208 participants at three visits to detect NAb. Peripheral blood mononuclear cells (PBMCs) were also collected on Visit 3 to detect SARS-CoV-2 RBD-specific B cells and SARS-CoV-2 RBD-specific memory B cells. The collected serum was stored at −80 °C, and PBMC was stored in liquid nitrogen. Between Visits 2 and 3, 154 participants were voluntarily vaccinated, among whom 92 were vaccinated with two doses and 62 with one dose. The median duration between the last vaccination and Visit 3 was similar for the one-dose (1.7, 1.1–2.9 months) and two-dose (1.6, 1.1–2.3 months) groups. Additionally, 54 participants were unvaccinated during the follow-up.

Vaccination information, including vaccination brand and date of vaccination, was obtained from the Hubei CDC Database of COVID-19 Vaccination, China. The inactivated COVID-19 vaccines, including those from Sinopharm and CoronaVac, were made using conventional whole inactivated SARS-Cov-2 virus technology and adjuvanted with alum. The Sinopharm vaccine was developed from the HB02 strain (Sinopharm's Beijing Institute of Biological Products, Beijing, China) [17] or the WIV04 strain (Sinopharm's Wuhan Institute of Biological Products, Wuhan, China) of SARS-CoV-2 [18]. CoronaVac (Sinovac Life Sciences, Beijing, China) was developed based on the CN02 strain of SARS-CoV-2 [19]. Specifically, 33 (53.2%) and 29 (46.8%) participants from the one-dose group reported receiving Sinopharm and CoronaVac vaccines, and 26 (28.3%), 21 (22.8%), and 45 (48.9%) participants from the two-dose group reported receiving Sinopharm, CoronaVac, and mix-match vaccines, respectively (Fig. S1).

Neutralization assay

We tested NAb against the wild-type virus at the three visits and additionally tested NAb against the Delta and Omicron variants at Visit 3. Plasmid-encoding proteins were transiently expressed in the HEK293T cells. The cDNAs encoding the wild-type S protein from SARS-CoV-2 (GenBank ID: QHD43416.1) and Omicron (B.1.1.529) variant S protein (GenBank ID: 7QO7_A) were human codon-optimized, commercially synthesized, and cloned into the mammalian expression vector pCAGGS with C-terminal 18 amino acid (aa) truncation to improve VSV pseudotyping efficiency. Delta (B.1.617.2) variant S protein containing multiple aa changes (T19R-G142D-del156/157-R158G-L452R-T478K-D614G-P681R-D950N) was generated by overlapping PCR-based mutagenesis using the wild-type S gene as a

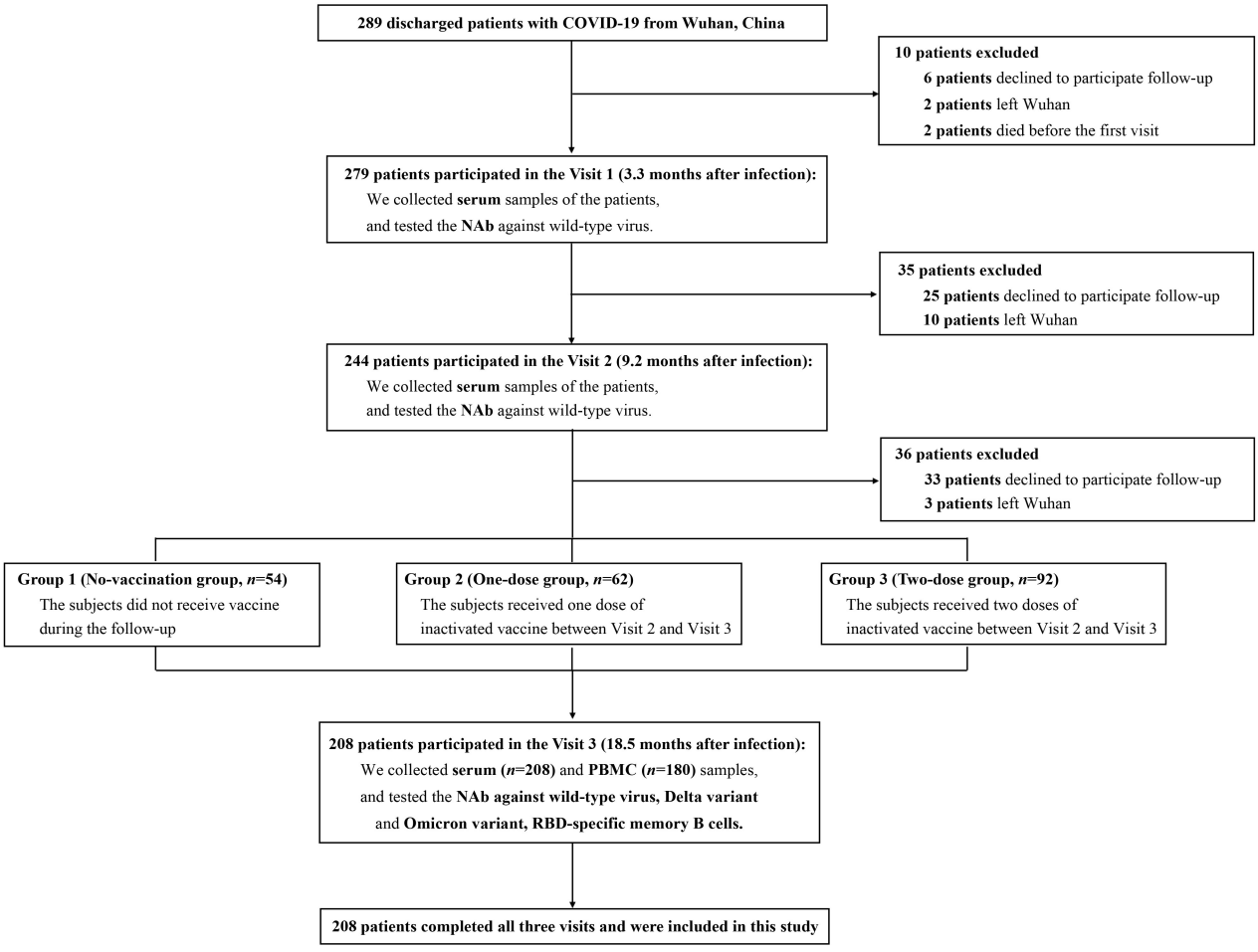

Fig. 1 Study profile. Abbreviation: NAb, neutralizing antibody; PBMC, peripheral blood mononuclear cell; RBD, receptor-binding domain.

template, cloned into pCAGGS with the same 18 aa truncation, and confirmed by sequencing. For the pseudovirus preparation, HEK293T cells were transiently transfected with pCAGGS-SARS-CoV-2-spike$_{\triangle}$18 and cultured at 37 °C with 5% CO_2 for 24 h. The medium was removed, and VSV-$_{\triangle}$G-EGFP was added to infect the cells for 2 h. Then, the acquired cells were washed three times with PBS. Dulbecco's modified eagle medium (Gibco) supplemented with 2% FBS (Gibco) was added. The supernatants were collected on the following day and filtered with a 0.45 μm filter. Then, fourfold of the diluted serum of the participants was incubated with the SARS-CoV-2 pseudovirus at 37 °C for 1 h. The mixture was added to a monolayer Vero-E6 cell on a 96-well plate and incubated for 24 h. The luminescence was measured using the Operatta high-content imaging system combined with Harmony imaging and analysis software (PerkinElmer).

The inhibition rate of each dilution was evaluated, and the half-maximal inhibitory concentration for serum (NT50) was calculated using nonlinear regression (least squares regression method without weighting; constraints: top = 1, bottom = 0). The limit of detection (LOD) of NAb was established as a titer of 1:20. A tested NT50 value below the lower LOD (< 20) was imputed as the value of half of the lower limit (10) of quantification.

To validate the NAb results of the pseudo-typed virus neutralization assay, six sequential samples were selected, and NAb against the wild-type virus was measured using the plaque reduction neutralization test (PRNT) method, which is the gold standard for tests on the blockade of live virus attachment by NAb, in a biosafety level 3 facility. The NT50 of NAb was measured using PRNT and pVNT methods, which are generally similar. The correlation coefficient of the two approaches was determined to be 0.957 (P = 0.003).

SARS-CoV-2 receptor-binding domain (RBD)-specific B cells and memory B cells

The frozen PBMCs were removed from liquid nitrogen and quickly resuscitated in a 37 °C water bath. SARS-CoV-2 RBD-specific B cells and memory B cells were detected using B cell surface markers and fluorescent

probes [20,21]. The wild-type SARS-CoV-2 RBD protein (GenBank: QHR63250.2, residues R319–F541) was biotinylated through biotin (BirA biotin–protein ligase), and the biotinylated protein was desalted through a chromatographic column (Thermo Scientific) when making the probes so that the excess biotin not bound to the protein could be removed. After the quantification of the protein concentration, the biotinylated protein was cross-linked with streptavidin (Thermo Scientific) with different fluorescence to obtain the antigen probe with PE and PE-cy7. The negative of fixable viability stain 510 (BD Biosciences) was defined as living cells when the cells were stained, and cell-surface Fc receptors were blocked using a purified Hu FC block. Then, the cells were mixed and incubated with the probes and antibodies, including PE-RBD, pecy7 RBD, CD19, CD10, CD 38, CD27, and IgD. The samples were fixed with 2% PFA for detection after sample staining. The details of all antibodies and probes are presented in Table S1. All samples were treated through an experiment to eliminate potential batch effects. A CytoFLEX S flow cytometer (Beckman) was used to detect the cells, and the same antigen-specific gates were adopted. Up to 1×10^6 cells were collected per sample by using FlowJo 10.4 for data analysis.

Statistical analysis

The descriptive data were displayed with a number (percentage) for categorical variables and a median (interquartile range, IQR) for continuous variables. The Mann–Whitney U-test or chi-square test was adopted to compare the difference in baseline characteristics among the different subgroups (no vaccination, one-dose, and two-dose groups) in accordance with the data distribution. The Mann–Whitney U-test was also used to test the heterogeneity of NAb titers in the different subgroups, and the paired-sample *t*-test was adopted to test the heterogeneity of different visits (Visits 1, 2, and 3) in the same vaccination group after the NAb titers were log-transformed for normal distribution. A two-sided *P* value < 0.05 was considered the level to reject the null hypothesis. All statistical analyses were performed using SAS (version 9.4) and R (version 3.6.3).

Results

A total of 208 COVID-19 convalescents were included and attended three visits at 3.3 (Visit 1, IQR, 1.3–4.4), 9.2 (Visit 2, 9.0–9.6), and 18.5 (Visit 3, 18.2–19.1) months after infection (Table 1). During the follow-up, 54, 62, and 92 subjects received no vaccination, one dose, and two doses of inactivated vaccines (CoronaVac or Sinopharm), respectively. The median age was 58 years (IQR, 50–65 years), and 100 participants (48.1%) were males. The vaccinated participants received their first dose of vaccine at 16.7 (15.9–17.3 (one-dose)) or 15.9 (15.1–16.6 (two-dose)) months after natural infection. The interval between the first and second vaccination was 1.0 (0.8–1.3) months for the two-dose group, and the median duration between the last vaccination and Visit 3 was similar for both one-dose (1.7, 1.1–2.9 months) and two-dose groups (1.6, 1.1–2.3 months).

Figure 2A and 2B show that the NAb titers (NT50) significantly decreased between Visit 1 and Visit 2 in the no-vaccination, one-dose, and two-dose groups (all $P < 0.001$). The median (IQR) NAb titers for the three groups were 346 (156–767), 298 (144–535), and 274 (138–593) at Visit 2 after infection compared with 1153 (469–2194), 939 (456–2395), and 698 (256–1388) at Visit 1 after infection. The NAb titers dropped continuously to 281 from Visit 2 to Visit 3 in the no-vaccination group and increased to 630 and 969 in the one-dose and two-dose groups, respectively (all $P < 0.001$ for NAb between Visits 2 and 3). No significant difference was observed between the different vaccination groups at Visit 2, but a significant difference was noted among the three groups at Visit 3 (no-vaccination vs. one-dose groups, $P < 0.001$; no-vaccination vs. two-dose groups, $P < 0.001$; one-dose vs. two-dose groups, $P = 0.018$).

The NAb titers against the different variants at Visit 3 are shown in Fig. 3A and 3B. The median titers of NAb against the Delta variant for the no-vaccination, one-dose, and two-dose groups decreased by 3.3, 1.9, and 2.3 folds relative to the wild-type virus, respectively. Neutralization against the Omicron variant was much lower than that against the Delta variant, but the subjects with two doses presented the highest NAb against the Omicron variant. The median titers of NAb against the Omicron variant at Visit 3 after infection were 40, 157, and 255 in the no-vaccination, one-dose, and two-dose groups, respectively, which corresponded to 7.0-, 4.0-, and 3.8-fold decrements relative to the wild-type virus. As shown in Fig. 3C, a significant difference was observed for the fold decrease of NAb titers against the Omicron variant relative to the wild-type virus in the no-vaccination and two-dose groups at Visit 3 ($P = 0.008$). We divided the participants according to the fold decrease of NAb titers against the Delta variant relative to the wild-type virus (Fig. 3D) and the Omicron variant relative to the wild-type virus (Fig. 3E). Then, we categorized the fold decrease into " < 2 folds, 2–4 folds, 4–6 folds, > 6 folds" for the Delta variant and " < 4 folds, 4–8 folds, 8–12 folds, > 12 folds" for the Omicron variant. For the Delta variant, the proportions with a decrease of < 2 folds were 42.6%, 45.2%, and 41.3% in the no-vaccination, one-dose, and two-dose groups, respectively. For the Omicron variant, the proportions with a decrease of < 4 folds were 25.9%, 41.9%, and 47.8% in the no-vaccination, one-

Table 1 Characteristics of COVID-19 patients by vaccination status

	Total (n = 208)	No-vaccination group (n = 54)	One-dose group (n = 62)	Two-dose group (n = 92)	P
Age (year)	58 (50–65)	58 (45–66)	57 (49–65)	59 (52–64)	0.705
Sex					0.341
Men	100 (48.1%)	30 (55.6%)	26 (41.9%)	44 (47.8%)	
Women	108 (51.9%)	24 (44.4%)	36 (58.1%)	48 (52.2%)	
Education					0.296
Middle school or lower	140 (67.3%)	33 (61.1%)	40 (64.5%)	67 (72.8%)	
College or higher	68 (22.7%)	21 (38.9%)	22 (35.5%)	25 (27.2%)	
Cigarette smoking					0.097
Never smoker	183 (88.0%)	47 (87.0%)	59 (95.2%)	77 (83.7%)	
Ever smoker	25 (12.0%)	7 (13.0%)	3 (4.8%)	15 (16.3%)	
Alcohol consumption					0.038
Never drinker	183 (88.0%)	45 (83.3%)	60 (96.8%)	78 (84.8%)	
Ever drinker	25 (12.0%)	9 (16.7%)	2 (3.2%)	14 (15.2%)	
Body mass index	24.3 (22.6– 26.5)	24.1 (22.7–26.0)	24.5 (22.1–26.8)	24.3 (22.8–26.5)	0.793
Waist circumference (cm)	90.0 (83.0–97.0)	92.0 (82.0–96.5)	88.0 (79.3–95.0)	91.0 (85.3–98.0)	0.163
Comorbidity					
Hypertension	75 (36.1%)	19 (35.2%)	22 (35.5%)	34 (37.0%)	0.971
Diabetes	23 (11.1%)	5 (9.3%)	9 (14.5%)	9 (9.8%)	0.582
CVD	14 (6.7%)	4 (7.4%)	5 (8.1%)	5 (2.4%)	0.794
Severity					0.724
Mild	146 (70.2%)	36 (66.7%)	43 (69.4%)	67 (72.8%)	
Severe	62 (29.8%)	18 (33.3%)	19 (30.6%)	25 (27.2%)	
Time from infection to Visit 1 (month)	3.3 (1.3–4.4)	3.0 (1.0–4.0)	3.2 (1.4– 4.2)	3.5 (1.6–4.6)	0.143
Time from infection to Visit 2 (month)	9.2 (9.0–9.6)	9.2 (9.0–9.4)	9.3 (9.0–9.7)	9.2 (8.8–9.9)	0.440
Time from infection to Visit 3 (month)	18.5 (18.2–19.1)	18.4 (18.1–19.0)	18.4 (18.2–18.9)	18.7 (18.2–19.3)	0.148
Time from infection to the first vaccination (month)	–	–	16.7 (15.9– 17.3)	15.9 (15.1– 16.6)	< 0.001
Time from infection to the second vaccination (month)	–	–	–	17.1 (16.4–17.5)	–
Time from infection to the last vaccination (month)[a]	–	–	16.7 (15.9–17.3)	17.1 (16.4–17.5)	0.043
Time from the last vaccination to Visit 3 (month)	–	–	1.7 (1.1–2.9)	1.6 (1.1–2.3)	0.263

Data are median (IQR) or n (%).

P values were calculated by applying a Mann–Whitney U-test or χ^2 test.

[a]Last vaccination: the first vaccination in the one-dose group or the second vaccination in the two-dose group.

dose, and two-dose groups, respectively.

To further examine the SARS-CoV-2 specific B and memory B cells, fluorescence-labeled multimerized probes were used to detect B cells and memory B cells specific to SARS-CoV-2 RBD on the basis of the samples collected at Visit 3. As shown in Fig. 4, all samples were examined using the same antigen-specific gates. The percentage of SARS-CoV-2 RBD-specific B cells was significantly higher in the two-dose group (0.25 (IQR: 0.14–0.92)) than in the no-vaccination group (0.14 (IQR: 0.11–0.22), P = 0.001) and one-dose group (0.18 (IQR: 0.11–0.29), P = 0.011). Meanwhile, the percentage of SARS-CoV-2 RBD-specific memory B cells in the no-vaccination group (0.53 (IQR: 0.30–0.78)) was significantly lower than that in the one-dose and two-dose groups (one-dose group: 0.65 (IQR: 0.44–0.98), P = 0.038; two-dose group: 0.65 (IQR: 0.43–0.98), P = 0.015). Meanwhile, the percentages of SARS-CoV-2 RBD-specific memory B cells were similar between the one-dose and two-dose groups (P > 0.05).

Figures S2 and S3 present the distributions of NAb titers and B cell responses stratified by disease severity and age. The percentage of RBD-specific memory B cells at Visit 3 was significantly higher for severe symptoms compared with that for mild symptoms in the one-dose group (P = 0.025). However, no significant difference in NAb titers was observed between severe and mild symptoms (all P values > 0.05). The NAb titers against

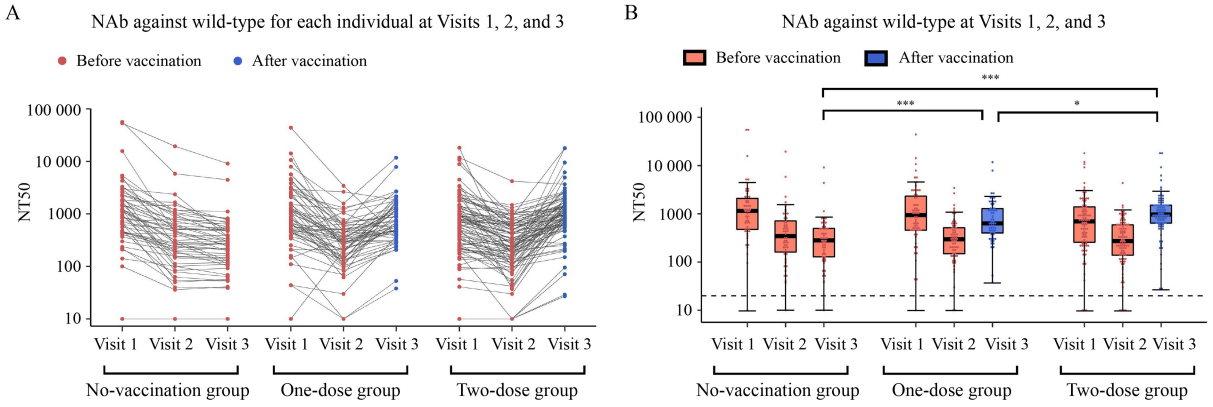

Fig. 2 Longitudinal assessment of neutralizing antibody (NAb) against wild-type SARS-CoV-2. (A) Graph manifesting temporal changes in NAb against the wild-type virus in each individual from the no-vaccination, one-dose, and two-dose groups. (B) Boxplots indicating the median values with the 25th and 75th percentiles of NAb against the wild-type virus at Visits 1, 2, and 3. The whiskers show the 1.5 interquartile range. $*P \leqslant 0.05$, $**P \leqslant 0.01$, $***P \leqslant 0.001$.

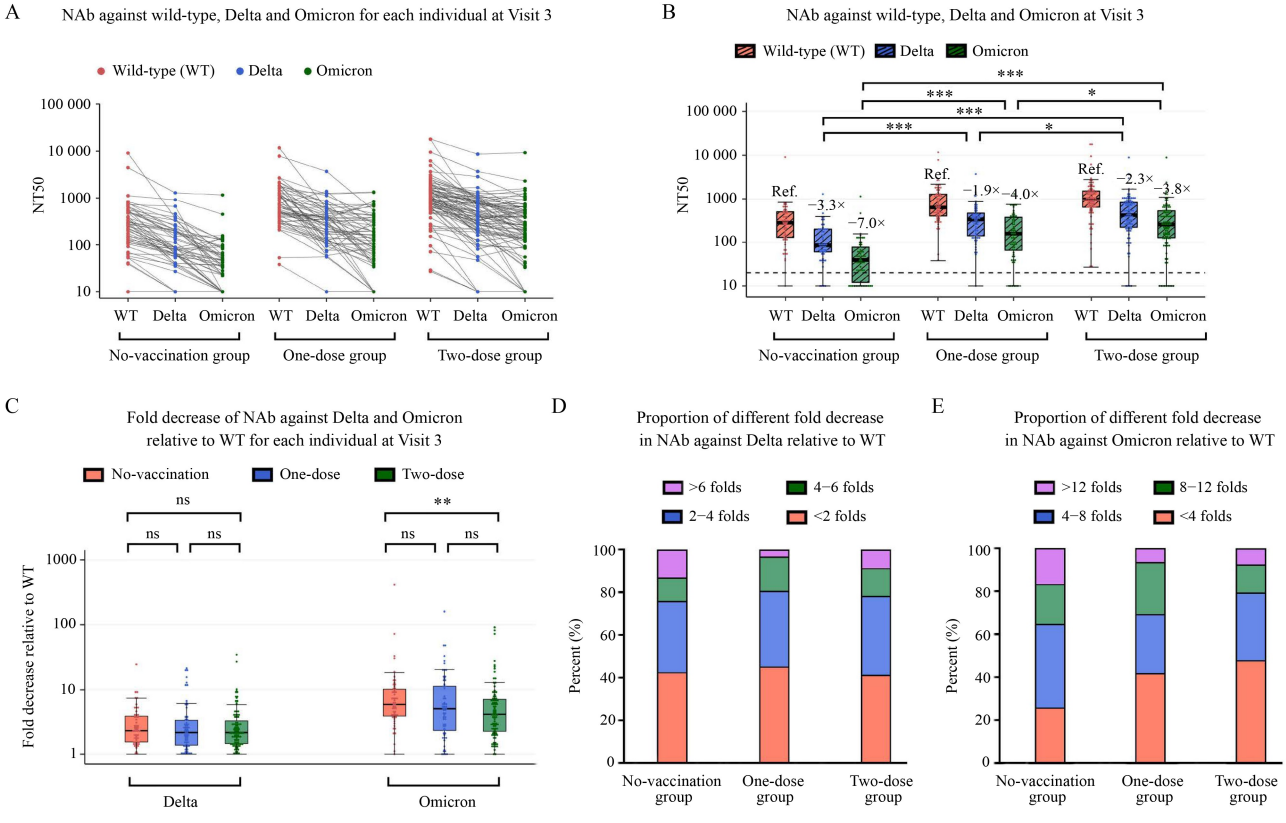

Fig. 3 Neutralizing antibody (NAb) against wild-type, Delta, and Omicron variants of SARS-CoV-2 at Visit 3. (A) NAb against wild-type, Delta, and Omicron variants at Visit 3 in each individual from the no-vaccination, one-dose, and two-dose groups. (B) Boxplots illustrating NAb against the wild-type, Delta, or Omicron variants in the no-vaccination, one-dose, and two-dose vaccination groups at Visit 3. The fold decrease of median NAb titers for the Delta and Omicron variants relative to wild-type variant was calculated and denoted as a number with the × symbol. (C) Calculated fold decrease of NAb titers against the Delta and Omicron variants relative to the wild-type variant in each individual (*Fold decrease =(NAb titers against wild-type / NAb titers against Delta or Omicron variant)*). A significant difference in the fold decrease of NAb titers against the Omicron variant was observed between no-vaccination and two-dose vaccination groups at Visit 3 (calculated by the Mann–Whitney U-test). We divided the participants according to the fold decrease of NAb titers against the Delta variant relative to the wild-type virus (D) and the Omicron variant relative to the wild-type virus (E) and categorized the fold decrease into four groups (Delta variant: < 2 folds, 2–4 folds, 4–6 folds, > 6 folds; Omicron variant: < 4 folds, 4–8 folds, 8–12 folds, > 12 folds). The proportion of participants was expressed as the percentage of each group and presented as stacked bar graphs. $P > 0.05$, not significant (ns); $*P \leqslant 0.05$, $**P \leqslant 0.01$, $***P \leqslant 0.001$.

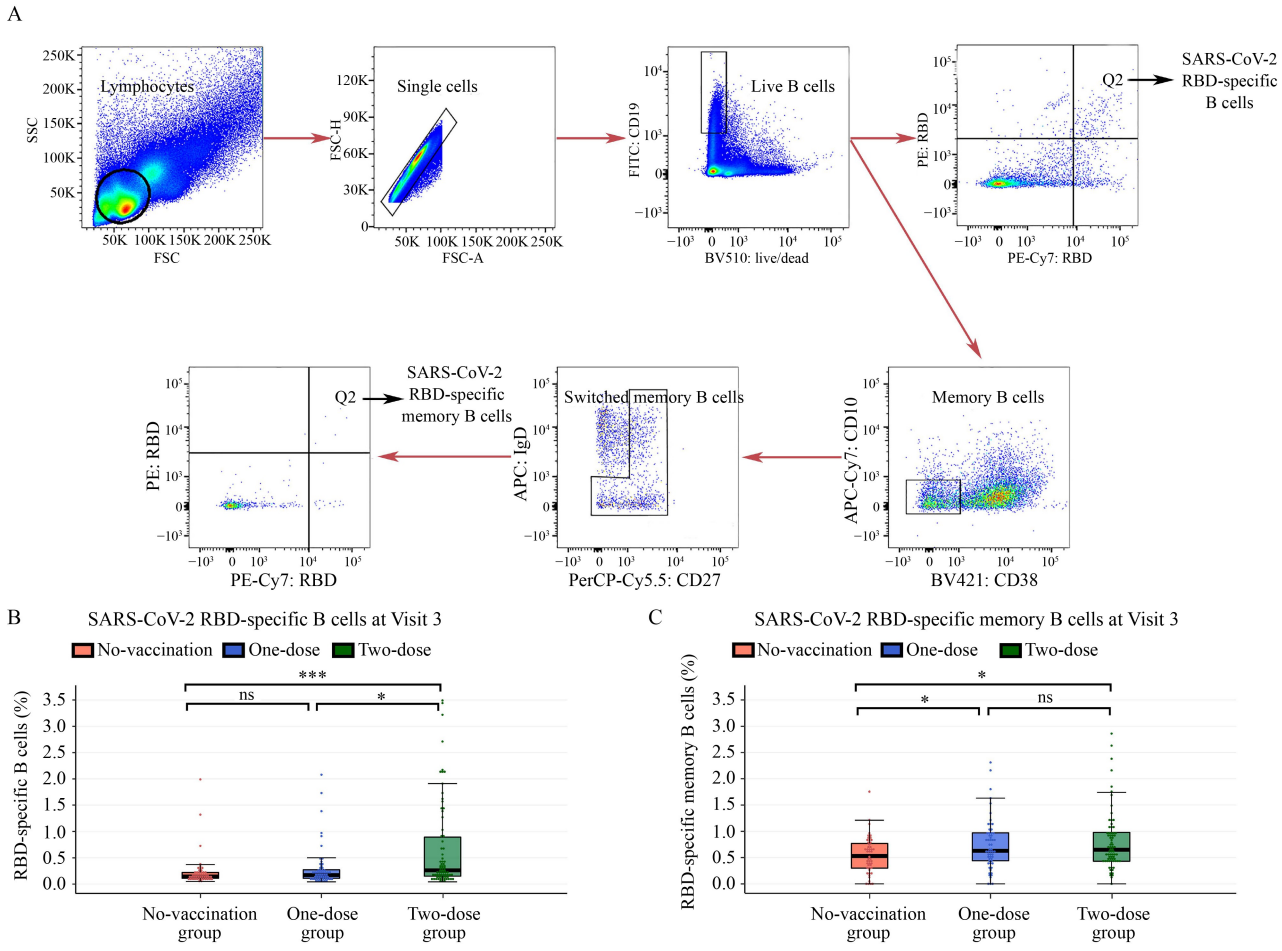

Fig. 4 SARS-CoV-2 RBD-specific B cells and memory B cells among the COVID-19 patients at Visit 3 by vaccination status. (A) Graph summarizing the sorting of SARS-CoV-2 RBD-specific B cells and memory B cells. (B) Percentage of SARS-CoV-2 RBD-specific B cells and (C) percentage of SARS-CoV-2 RBD-specific memory B cells. The boxplots indicate the median values with the 25th and 75th percentiles, and the whiskers show the 1.5 interquartile range. Statistical significance was determined using the Mann–Whitney U-test. $P > 0.05$, not significant (ns); $*P \leqslant 0.05$, $**P \leqslant 0.01$, $***P \leqslant 0.001$.

the wild-type, Delta, and Omicron variants at Visit 3 were all significantly higher among the older convalescents (age ⩾ 60 years) compared with those among the younger convalescents (age < 60 years, all $P < 0.05$) in the two-dose group. We also compared the NAb titers and B cell responses at Visit 3 by vaccine brand (CoronaVac or Sinopharm or mix-match) in the one-dose and two-dose groups (Fig. S4) and found that the NAb titers and RBD-specific memory B cells at Visit 3 tended to be higher for CoronaVac than for Sinopharm, but the difference did not reach statistical significance (Fig. S4, $P > 0.05$).

Discussion

Using a longitudinal cohort of convalescent patients in Wuhan, we monitored the neutralization activity and immune memory after natural SARS-CoV-2 infection and vaccination with inactivated vaccines (CoronaVac or Sinopharm). During the median of 18.5 months of follow-up, the NAb titers against the wild-type virus continued to decrease in the no-vaccination group, but they rose significantly in the one-dose and two-dose groups. The NAb titers against the Delta or Omicron variant were lower than those against the wild-type virus. Notably, receiving two doses of inactivated vaccine boosted the neutralizing activity against the Omicron variant in the convalescent patients. Furthermore, we observed that the B cells and memory B cells against SARS-CoV-2 RBD were robustly boosted by the second vaccination, indicating an enhanced immune memory beyond the antibody response. Our findings prove that convalescent patients benefit from the administration of inactivated vaccines (one or two doses), and two doses of inactivated vaccines are highly recommended and crucial for controlling COVID-19 and informing vaccination guidelines.

The influencing factors of COVID-19 infection may include age [22,23], smoking status [22], working in

high-risk settings [22], living conditions [23,24], level of SARS-CoV-2 specific-antibody [25,26]. Among these factors, the SARS-CoV-2 specific antibody induced by natural infection or vaccination is an important predictor in estimating the risk of COVID-19 infection during the ongoing pandemic [25,26]. Therefore, the persistence of the SARS-CoV-2 specific antibody is currently a popular research issue and has caused a heated debate worldwide [27]. In the best-case scenario, SARS-CoV-2 naturally infected or vaccinated individuals develop sterilizing immunity, and their antibody can be maintained at sufficiently high levels to rapidly block infection upon re-exposure [28,29]. However, previous studies have suggested a gradual decline in the antibody level after SARS-CoV-2 natural infection [30,31] or vaccination [32] in most individuals, demonstrating that COVID-19 vaccine boosting might ultimately be required. Similarly, our study found that the NAb titers continuously decreased within 18.5 months after natural infection in the no-vaccination group. Furthermore, the NAb titers increased significantly after vaccination in the one-dose and two-dose groups, and they were much higher than those among the unvaccinated COVID-19 convalescents (no-vaccination group). These findings indicate that receiving inactivated vaccines can effectively enhance the antibody responses of COVID-19 convalescents.

The NAb elicited by SARS-CoV-2 infection and vaccination is an important factor that affects the estimation of the risks of infection and re-infection during the ongoing pandemic. Meanwhile, the rapid emergence of novel SARS-CoV-2 variants has raised extensive concern about the issue of immune escape and vaccination efficacy [4,6,9,33]. The Delta spike protein fuses membranes efficiently at low levels of the cellular receptor angiotensin-converting enzyme 2 (ACE2) [34]. The Omicron variant is characterized by a high number of mutations on the spike protein, which is mainly on RBD [33]. Although current WHO vaccination guidelines highlight the need for vaccination among COVID-19 convalescents [35,36], a key issue that has not yet been addressed is whether convalescents with inactivated vaccines could maintain antibody-mediated protective immunity against emerging variants. To address this concern, our study examined whether these new variants would be resistant to NAb elicited in convalescent individuals who have received inactivated vaccines. Emerging evidence [3,4,7,9–14] reveals the neutralizing activity against SARS-CoV-2 among vaccinated convalescents, and some studies have discussed neutralization against Delta [7,8,11,13] or Omicron [3,7–9,11,13] variants. In line with our study, prior studies [7,11,13,16,33] have noted that relative to the Delta variant, the neutralizing activity of vaccine-elicited sera against the Omicron variant is substantially reduced. Existing studies [9,11,13,16] have also reported enhanced NAb against the Omicron variant among vaccinated individuals with a booster dose. In addition, the neutralization against Omicron differs for subjects who have received different vaccine plans [7,11]. The majority of studies adopted mRNA/adenovirus vaccines [3–8] or heterogenous use of mRNA and inactivated vaccines [9,11]. Based on a large sample size of convalescents and dynamic data, our study investigated the humoral immune response to inactivated vaccines and provided an in-depth characterization of the humoral immune response to new variants. Our findings suggest that convalescents need to take inactivated vaccines, and two doses of vaccination are recommended.

In addition to the antibody response, the production of B cells (including memory B cells) against SARS-CoV-2 provides another layer of immune protection [37]. Memory B cells offer a diverse repertoire allowing for rapid adaptive responses during re-encounter with the pathogen, especially in the case of variants [38]. Viral variants may evade neutralization by preforming the antibody present in convalescent serum and may lead to a reduced duration of antibody-mediated immunity [37]. In this case, protection against severe disease depends on the re-activation of somatically mutated memory B cells, which recognize antigenically distinct viral variants [37]. However, relevant studies on vaccinated subjects have mainly concentrated on measuring NAb as primary endpoints, and the induction of B cells and memory B cells by inactivated vaccines among convalescents remains poorly understood. Two studies [5,8] have found that RBD-specific memory B cells are significantly higher among convalescents compared with SARS-CoV-2 naïve individuals. After receiving the first dose of mRNA vaccine, convalescent individuals are endowed with substantially boosted RBD-specific memory B cells [5,8]; however, no obvious increase in antigen-specific memory B cells are observed after the second dose. By contrast, an enhanced B cell response was achieved after the second dose of inactivated vaccine in our study. This discrepancy might be due to the difference in vaccines, the different durations after infection or vaccination, and the relatively small sample sizes of convalescents in these studies ($n < 50$).

The main strengths of this study include its prospective design, long-term follow-up, consecutive samples, and in-depth characterizations of the humoral immune response against new variants. However, the present study is still subject to several limitations. First, T cell responses after natural infection or vaccination were not evaluated. Second, although the participants underwent three visits over a median of 18.5 months after natural SARS-CoV-2 infection, we only performed an RBD-specific memory B cell analysis for vaccinated participants at the last visit

(1.5 months after the last vaccination). This time window may be too short to assess the formation of the memory B cell population. Vaccine-induced memory B cell responses play a critical role in long-term immune protection and limiting virus dissemination [5]. Further studies are required to investigate the long-term durability of immunological memory B cells after a longer period of vaccination. Lastly, additional real-world evidence based on large-scale outbreaks is needed in the future to comprehensively evaluate the immune persistence of inactivated vaccines and determine the NAb thresholds associated with preventive clinical outcomes.

In conclusion, our findings provide evidence that convalescent patients benefit from the administration of inactivated vaccines (one or two doses), which enhance neutralization against highly mutated SARS-CoV-2 variants and memory B cell responses. Two doses of inactivated vaccines for COVID-19 convalescents are highly recommended to control the COVID-19 pandemic. Continuous updating of vaccination guidelines and policies is crucial as well.

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Compliance with ethics guidelines

Hao Wang, Yu Yuan, Bihao Wu, Mingzhong Xiao, Zhen Wang, Tingyue Diao, Rui Zeng, Li Chen, Yanshou Lei, Pinpin Long, Yi Guo, Xuefeng Lai, Yuying Wen, Wenhui Li, Hao Cai, Wei Ni, Youyun Zhao, Kani Ouyang, Jingzhi Wang, Qi Wang, Li Liu, Chaolong Wang, An Pan, Xiaodong Li, Rui Gong, and Tangchun Wu declare that they have no conflict of interest. This study was approved by the Ethics Committee of the School of Public Health, Tongji Medical College, Huazhong University of Science and Technology. Written informed consent was obtained from each participant.

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