Waning immune responses against SARS-CoV-2 variants of concern among vaccinees in Hong Kong

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Summary

Background Nearly 4 billion doses of the BNT162b2-mRNA and CoronaVac-inactivated vaccines have been administrated globally, yet different vaccine-induced immunity against SARS-CoV-2 variants of concern (VOCs) remain incompletely investigated.

Methods We compare the immunogenicity and durability of these two vaccines among fully vaccinated Hong Kong people.

Findings Standard BNT162b2 and CoronaVac vaccinations were tolerated and induced neutralizing antibody (NAb) (100% and 85.7%) and spike-specific CD4 T cell responses (96.7% and 82.1%), respectively. The geometric mean NAb IC_{50} and median frequencies of reactive CD4 subsets were consistently lower among CoronaVac-vaccinees than BNT162b2-vaccinees. CoronaVac did not induce measurable levels of nucleocapsid protein-specific IFN-γ^+ $CD4^+$ T or IFN-γ^+ $CD8^+$ T cells compared with unvaccinated. Against VOCs, NAb response rates and geometric mean IC_{50} titers against B.1.617.2 (Delta) and B.1.1.529 (Omicron) were significantly lower for CoronaVac (50%, 23.2 and 7.1%, <20) than BNT162b2 (94.1%, 131 and 58.8%, 35.0), respectively. Three months after vaccinations, NAbs to VOCs dropped near to detection limit, along with waning memory T cell responses, mainly among Corona-Vac-vaccinees.

Interpretation Our results indicate that vaccinees especially CoronaVac-vaccinees with significantly reduced NAbs may probably face higher risk to pandemic VOCs breakthrough infection.

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Research in Context

Evidence before this study

Based on the PubMed search, comparative immunogenicity and durability of BNT162b2 and CoronaVac vaccines in terms of both antibody and T cell responses against major SARS-CoV-2 variants of concern (VOCs) especially Beta (B.1.351), Delta (B.1.617.2) and Omicron (B.1.1.529) have not been studied in a human population with identical genetical background. With the accumulating emergence and spreading of pandemic VOCs by travellers, monitoring vaccine-induced neutralizing antibody (NAb) and T cell memory responses, especially NAbs activity against VOCs, may play a critical role in determining the policy of upcoming boost vaccination.

Added value of this study

By comparing humoral and cellular immune responses over time among Hong Kong vaccinees, we found that initial vaccine-induced NAb and CD4 T cell responses were consistently lower among CoronaVac-vaccinees than BNT162b2-vaccinees. Moreover, NAbs against VOCs dropped to detection limit only three months after vaccinations, along with diminishing memory T cell responses, primarily among CoronaVac-vaccinees. Our results indicate that CoronaVac-vaccinees may face higher risk to pandemic VOCs breakthrough infection.

Implications of all the available evidence

Our findings have significant implications for the third boost vaccination against breakthrough infection among vaccinees, especially among hundreds millions CoronaVac-vaccinees in many countries.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of coronavirus disease 2019 (COVID-19). SARS-CoV-2 has been spreading worldwide since December 2019, leading to the ongoing COVID-19 pandemic with 234 million infections and 4.8 million deaths by 30 September 2021 (https://covid19.who.int/). Due to pressure of the COVID-19 pandemic, the greatest global efforts have been placed for vaccine development. To date, six vaccines have been approved by regulatory agencies for emergency use including (1) two mRNA-based vaccines, namely BNT162b2 (by Pfizer Inc. and BioNTech SE) and mRNA-1273 (by Moderna), expressing full spike (S) glycoprotein with efficacy rates of 94.1-95% against laboratory-confirmed COVID-19,[1,2] (2) the chimpanzee adenovirus-vectored vaccine, named ChAdOx1 nCoV-19 (by the Oxford University and AstraZeneca Inc.), encoding the full S glycoprotein with an efficacy rate of 70.4%,[3] (3) the human adenovirus-vectored vaccine, namely Ad26.COV2.S (by Johnson & Johnson Inc.), encoding the full S glycoprotein with an efficacy rate of 73.1%,[4] (4) two inactivated vaccines CoronaVac and BIBP (by Sinovac Biotech and SinoPharm) with efficacy rates of 83.7%[5] and 78.1%,[6] respectively. In recent reports, however, SARS-CoV-2 variants of concerns (VOCs) have posted great challenges for vaccine-induced protection.[7-10]

Over four million genome sequences of SARS-CoV-2 have been submitted to the hCoV-19 database of the Global initiative on sharing all influenza data (GISAID) since the outbreak of COVID-19. Several VOCs have had significant impacts on the trend of the pandemic. The top five noticeable VOCs include B.1.1.7 variant (Alpha, United Kingdom), B.1.351 (Beta, South Africa), P1 (Gamma, Japan/Brazil), B.1.617.2 (Delta, India), and B.1.427/B.1.429 (Epsilon, United States).[11] The VOC B.1.351 strain was significantly resistant (10.3-12.4-fold) to neutralization by sera derived from vaccinees who received mRNA-1273 or BNT162b2 compared to the VOC D614G strain.[7] Although vaccinations reduced sickness, hospitalization and death rates, vaccine-induced attenuation of peak viral burden has decreased for the VOC B.1.617.2 strain compared to the VOC B.1.1.7 variant in the UK.[12] These results are in line with the increasing number of breakthrough infections among fully vaccinated population.[13] In the recent month, the VOC Omicron (B.1.1.529, South Africa) has caused an alarming global concern due to its stronger immune evasion related to fifteen mutations in the receptor-binding domain (RBD).[14] It is, therefore, critical to study vaccinees to determine their potential risk to the spreading VOCs.

Four waves of SARS-CoV-2 epidemic have hit Hong Kong, resulting in 10950 infections and 198 deaths. To control the epidemic effectively, the BNT162b2 [COMIRNATY™, BioNTech-Fosun Pharma] and CoronaVac [Sinovac Biotech] vaccines have been made available for Hong Kong residents since 26 February 2021 (www.covidvaccine.gov.hk/en/). In mainland China, more than 2.2 billion doses of inactivated vaccines, including mainly CoronaVac, have been inoculated by 30 September 2021. Since both vaccines have been recommended by the World Health Organization for emergency use,[1,5]

66.8% of the 7.5 million Hong Kong people have been fully vaccinated by 30 September 2021. However, the immunogenicity and durability of these two vaccines in terms of antibody and T cell responses against VOCs remain largely unknown. With the accumulating emergence and spreading of pandemic VOCs, monitoring vaccine-induced neutralizing antibody (NAb) and T cell memory responses, especially NAbs activity against VOCs, may play a critical role in determining the policy of boost vaccination. For this reason, we aimed to determine humoral and cellular immune responses in parallel among Hong Kong vaccinees over time with focus on cross-reactive NAbs against VOCs.

Methods

Study subjects

Participants who completed two doses of SARS-CoV-2 vaccination (either BNT162b2 or CoronaVac) before June 2021 were recruited for this study. 95% of the participants were mainly university students and staff members. The exclusion criteria include individuals with: (1) documented SARS-CoV-2 infection, (2) high-risk infection history within 14 days before vaccination, and (3) COVID-19 symptoms such as sore throat, fever, cough and shortness of breath. A total of 62 vaccinated subjects were enrolled in this study including 34 BNT162b2-vaccinees and 28 CoronaVac-vaccinees. Study subjects were immunized at different timepoint with their blood samples collected at the phase with a median of 30 (IQR, 22 to 32) days for BNT162b2 and 28 (IQR, 20 to 39) days for CoronaVac post the second dose. Samples from 27 BNT162b2-vaccinees and 16 CoronaVac-vaccinees were successfully followed up at the memory phase with a median of 113 (IQR, 101 to 115) days and 105 (IQR, 96 to 109) days post the second dose, respectively. Peripheral blood mononuclear cells (PBMCs) and inactivated plasma were freshly isolated for testing. Participants were required to record any adverse reactions related to each dose of vaccination, including local adverse reactions surrounding the injection site and systemic adverse reactions. The demographic characteristics of these two groups were similar in terms of gender, age, nationality, body mass index (BMI) (Table 1). Besides participants with Chinese nationality, there were one CoronaVac recipient from Malaysia and three non-Asian BNT162b2 recipients from France, America and Danish, respectively. Two participants in the BNT162b2 group have mild hypertension and diabetes. The median intervals between two doses and the time of blood collection after second dose were also comparable. 16 gender-matched (8 males, 50%) and age-matched (median 32.5, IQR 24.3-39.8) unvaccinated subjects were included as controls to determine the limit of quantification (LOQ) (Table 1). Their blood samples were obtained from Hong Kong

Red Cross and showed negative results for anti-SARS-CoV-2 nucleocapsid protein (NP) IgG by ELISA.

Ethical statement

This study was approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster (Ref No. UW 21-452). Written informed consent was obtained from all study subjects.

Cell lines

HEK293T (RRID: CVCL_0063) and HEK293T-hACE2 (in-house made) (mycoplasma negative) were maintained in Dulbecco's Modified Eagle Medium (DMEM) (Thermo Fisher Scientific) containing 10% fetal bovine serum, 2 mM L-glutamine and 100 U/mL penicillin and were incubated at 37°C in 5% CO_2 setting.[15,16] High-level and stable expression of human ACE2 on HEK293T-hACE2 cell line surface was validated by flow cytometry analysis.

Enzyme-linked immunosorbent assays (ELISA)

ELISA was used for determining the IgG binding to RBD and full spike as we previously described.[17] The area under the curve (AUC), representing the total peak area based on ELISA OD values as previously described,[18] of each sample was plotted using the GraphPad Prism v8, and the baseline with the defined endpoint was set as the average of negative control wells +10 standard deviation. The LOQ (cut-off value) was established based on the geometric mean of 16 non-vaccinated donors without prior SARS-CoV-2 infection history as previously reported.[19]

Pseudotyped viral neutralization assay

The S-expression plasmids encoding wildtype, D614G, B.1.1.7, B.1.351, P1, B.1.617.2, B.1.429 and B.1.1.529 variants were used to generate pseudoviruses. P1 and B.1.429 were purchased from InvivoGen (pUNO1-SpikeV4, CAT#: p1-spike-v4; pUNO1-SpikeV5, CAT#: p1-spike-v5) while others were made by us or collaborators. Briefly, SARS-CoV-2 pseudoviruses were generated by co-transfection of 293T cells with a pair of plasmids, the S-expression plasmid for wildtype or VOCs and the pNL4-3Luc_Env_Vpr plasmid in a human immunodeficiency virus type 1 backbone.[15,20] At 48 hours post-transfection, virus-containing supernatant was collected, quantified by determining $TCID_{50}$ value in HEK293T-hACE2 cell line and frozen at -150°C. The pseudotyped neutralization assay of vaccinated samples was performed as previously described.[15,20] Serially diluted and heat-inactivated plasma samples were incubated with 200 $TCID_{50}$ of pseudovirus at 37°C for 1 hour. The plasma-virus mixtures were then added into pre-seeded

	BNT162b2(n=34)	CoronaVac(n=28)	Non-vaccinated(n=16)	*P* value
Gender, male (%)	16 (47.1%)	14 (50.0%)	8 (50.0%)	0.967
Age, median years (IQR)	30.5 (26.8-35.3)	29.0 (26.0-31.0)	32.5 (24.3-39.8)	0.248
Nationality				0.376
Chinese	31 (91.2%)	27 (96.4%)	16 (100.0%)	
Non-Chinese	3 (8.8%)	1 (3.6%)	0 (0.0%)	
Place of vaccination				0.345
Hong Kong	33 (97.1%)	24 (85.7%)	-	
Mainland	0 (0.0%)	4 (14.3%)	-	
Others	1 (2.9%)	0 (0.0%)	-	
BMI (Kg/m^2) (IQR)	21.3 (19.2-24.0)	21.0 (18.8-26.6)	22.0 (19.5-26.7)	0.246
Underlying diseases				0.264
Yes	2 (5.9%)	0 (0.0%)	0 (0.0%)	
No	32 (94.1%)	28 (100.0%)	16 (100.0%)	
The median interval days between two vaccinations (IQR)	30 (22-32)	28 (28-30)	-	0.493
The median interval days between the second dose and the first blood collection (IQR)	30 (22-32)	28 (20-39)	-	0.858

Table 1: **Demographic characteristics of study subjects.**

HEK293T-hACE2 cells. After 48 hours, infected cells were lysed, and luciferase activity was measured using the Luciferase Assay System kit (Promega) in a Victor3-1420 Multilabel Counter (PerkinElmer). The 50% inhibitory concentrations (IC$_{50}$) of each specimen were calculated using non-linear regression in GraphPad Prism v8 to reflect anti-SARS-CoV-2 antibody potency. Samples that failed to reach 50% inhibition at the lowest serum dilution of 1:20 were considered to be non-neutralizing, and the IC$_{50}$ values were set to 10.

Peptide pools

We purchased peptide pool of 15 amino acid (aa) overlapping by 11 aa spanning the full length of SARS-CoV2-spike (a total of 316 peptides), receptor binding domain (RBD) (S306-S543, a total of 57 peptides) and nucleocapsid protein (NP) (a total of 102 peptides) from GenScipt. As a control, we utilized a peptide pool spanning the entire region pp65 protein (15-mers overlapping by 11 aa) of human cytomegalovirus (CMV), which was obtained from the NIH HIV reagent program (CAT# ARP-11549). The peptides were dissolved in H$_2$O, then diluted with the RPMI-1640 culture medium. The peptides in the RBD peptide pool were also included in the Spike pool.

Intracellular cytokine staining (ICS)

To measure antigen-specific T cell response, PBMCs were stimulated with 2 μg/mL of indicated COVID-19 antigen peptide pools (RBD or Spike or NP) in the presence of 0.5 μg/mL anti-CD28 (Biolegend Cat#302902) and anti-CD49d mAbs (Biolegend Cat#304302). Cells were incubated at 37°C overnight, and BFA (Sigma Cat#B654225MG) was added at 2 h post incubation, as previously described.[17] CMV (pp65) peptide pool was included as an internal positive control. Stimulation alone with anti-CD28 and anti-CD49d was used as negative control. After overnight incubation, cells were washed with staining buffer (PBS containing 2% FBS) and stained with mAbs against surface markers including Zombie Aqua (Biolegend Cat#423102), Pacific blue anti-CD3 (Biolegend Cat#344824), PerCP/Cyanine 5.5 anti-CD4 (BioLegend Cat# 317428), APC/Fire^{TM} 750 anti-CD8 (Biolegend Cat#344746), BV711^{TM} anti-CD45RA (Biolegend Cat#304138) and APC anti-CCR7 (Biolegend Cat#344746) . For intracellular staining, cells were fixed and permeabilized with BD Cytofix/Cytoperm (BD Biosciences) prior to staining with the mAbs against cytokines including PE anti-IFN-γ (Biolegend Cat#506507), Alexa Fluor® 488 anti-TNF-α (Biolegend Cat#502915) and PE/Cyanine 7 anti-IL-2 (Biolegend Cat#500326) with Perm/Wash buffer (BD Biosciences). After gating on CD4$^+$T and CD8$^+$T cells, intracellular IFN-γ/TNF-α/IL-2 were calculated (Figure S1a to 1c). All percentages of antigen-specific CD4$^+$ and CD8$^+$ T cells were reported as background subtracted data from the same sample stimulated with negative control (anti-CD28/CD49d only). The LOQ for antigen-specific CD4$^+$ (0.01%) and CD8$^+$ T cell responses (0.02%) was calculated using a twofold median value of all negative controls. Responses >LOQ and a stimulation index >2 for CD4$^+$ and CD8$^+$ T cells were considered positive responders. Values higher than the threshold of positive responders after spike peptide pool stimulation were considered for the analysis of multifunctional antigen-specific T cell responses. Phenotype

profiles were further analyzed by gating on IFN-γ^+CD4 or IFN-γ^+CD8 T cells for expression of CCR7 and/or CD45RA in response to spike, respectively (Fig. S1a-c).

Statistical analysis

Flow cytometric data were analysed using FlowJo 10.6.0. Statistical analysis was performed using the GraphPad Prism v8 Software. Mann-Whitney U test or Kruskal-Wallis test were used to compare between-group or multiple-group continuous values. Wilcoxon signed-rank test was used for paired comparisons. For between-group or multiple-group categorical values comparison, two-sided chi-square tests or fisher's exact test were used. The non-parametric Spearman test was used for correlation analysis. The statistical method of aggregation used for the analysis of binding and neutralizing antibody titers (NAbTs) is geometric mean titer (GMT) with the corresponding 95% confidence intervals (95%CI), and median with interquartile (IQR) for antigen-specific T cell frequencies. The statistic details are depicted in the respective legends. $P < 0.05$ was considered statistically significant.

Role of funding source

The funders of this study had no role in study design, data collection, data analysis, interpretation or writing of the report.

Results

Safety profiles of BNT162b2 and CoronaVac in Hong Kong vaccinees

After the first vaccination, the overall incidence rate of any adverse reactions was higher for BNT162b2 than CoronaVac (79.4% [27/34] versus 53.6% [15/28], P=0.03). The most common adverse reaction was the injection-site pain (73.5% [25/34] versus 35.7% [10/28], P=0.003), followed by fatigue (41.2% [14/34] versus 25.0% [7/28], P=0.18) and myalgia (17.6% [6/34] versus 14.3% [4/28], P=0.991), respectively (Fig. S2a). After the second vaccination, the overall incidence of any adverse reactions was consistently higher for BNT162b2 than CoronaVac (88.2% [30/34] versus 57.1% [16/28], P=0.005), including the major injection-site pain (64.7% [22/34] versus 53.6% [15/28], P=0.374) and other systematic symptoms (e.g., headache, fever and fatigue) (Fig. S2b). Moreover, significantly more BNT162b2-vaccinees had more than two adverse reactions (86.7% [26/30] versus 31.3% [5/16], $P < 0.001$). Most of the adverse reactions, however, were considered tolerable with an average recovery time of 48-72 hours. No vaccine-associated severe adverse reactions were reported among our study subjects.

Humoral and cellular immune responses induced by BNT162b2 and CoronaVac

The amounts of anti-Spike and RBD IgG as well as pseudovirus NAbTs were firstly determined at the acute phase after vaccination. Anti-Spike and RBD IgG were induced among 100% vaccinees in the BNT162b2 group. In contrast, only 85.7% (24/28) of CoronaVac vaccinees showed a detectable amount of anti-Spike and RBD IgG. Compared to CoronaVac, BNT162b2 induced a significantly higher GMT of anti-Spike IgG (1400 [95CI% 1035 to 1894] versus 217.8 [95CI% 152.7 to 310.5], $P < 0.0001$) and anti-RBD IgG [683.3 (95CI% 498.4 to 936.9) versus 17.8 (95CI% 4.1 to 76.7), $P < 0.0001$] responses (Fig. 1a). Encouragingly, the majority of BNT162b2 and CoronaVac vaccinees (100% [34/34] versus 85.7% [24/28]) developed NAb responses against the wildtype Wuhan-Hu-1 pseudovirus. Moreover, immunized sera from BNT162b2 vaccinees showed 19 times higher NAbTs against wildtype than that from CoronaVac vaccinees based on the geometric mean IC_{50} (1400.5[95CI% 1076 to1823] versus 73.7 [95CI% 43.4 to 125], $P < 0.0001$) (Fig. 1b). Since the neutralization potency index calculated by the NT_{50}/IgG ratio was suggested as a predictor of survival,[21] we also evaluated this factor by calculating the ratio of IC_{50} to AUC of anti-Spike and RBD IgG (Fig. 1c). The neutralization potency index of BNT162b2 (geometric mean of 1.0 [95% CI 0.73 to 1.37] for IC_{50}/Spike IgG and 0.88 [95% CI 0.66 to 1.17] for IC_{50}/RBD IgG) was significantly higher than that of CoronaVac (0.36 [95% CI 0.24 to 0.55] for IC_{50}/Spike IgG and 0.18 [95% CI 0.09 to 0.35] for IC_{50}/RBD IgG) (both P values <0.0001). These results demonstrated that while anti-Spike IgG, anti-RBD IgG and NAbs were induced by both vaccines, the NAbTs of CoronaVac vaccinees was 19-fold lower than that of BNT162b2 vaccinees, together with the lower neutralization potency index.

Besides humoral immune response, we also measured antigen-specific T cell response because it may play an important role in protection against SARS-CoV-2 infection.[17,22] Vaccine-specific T cell responses were determined by ICS after stimulation by the peptide pools covering RBD, spike and NP antigen (Figure S1a-c). Spike-specific IFN-γ^+CD4$^+$T cells were induced in 96.7% (32/33) of BNT162b2 and 82.1% (23/28) of CoronaVac subjects. The frequencies of spike-specific IFN-γ^+CD4$^+$T cells in both vaccinated groups were significantly higher than those of the non-vaccinated controls. The median frequency was 0.11 (IQR 0.08 to 0.22) for BNT162b2 and 0.03 (IQR 0.01 to 0.08) for CoronaVac as compared with controls' 0.00 (IQR 0.00 to 0.02) ($P < 0.0001$ and P=0.0057), respectively (Fig. 1d, top). Meantime, both BNT162b2 (81.8% [27/33]) and CoronaVac (71.4% [20/28]) vaccinees displayed higher frequencies of spike-specific IFN-γ^+CD8$^+$T cells than the non-vaccinated group (0.07 [IQR 0.02 to 0.17] for BNT162b2 and 0.04 (IQR 0.02 to 0.15) for CoronaVac
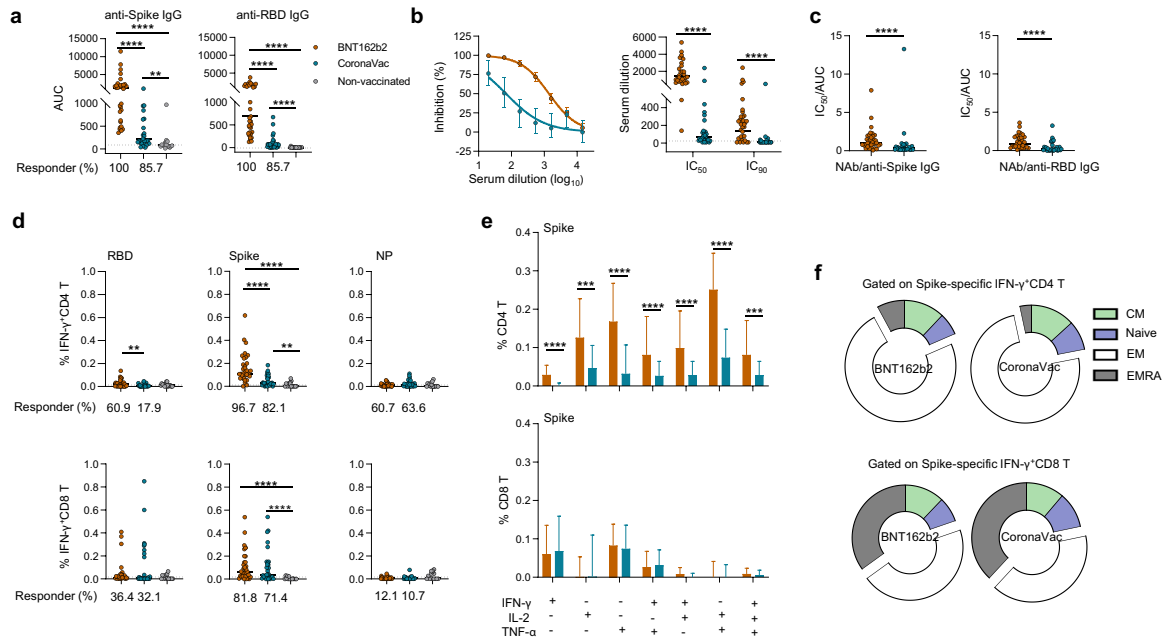
Figure 1. Immune responses measured at the acute phase after the second dose among BNT162b2 and CoronaVac vaccinees. (**a**) The area under curve (AUC) of anti-Spike and anti-RBD IgG in BNT162b2 (orange) (n=34), CoronaVac (green) (n=28) and non-vaccinated volunteers (grey) (n=16). The AUC represents the total peak area calculated from ELISA OD values by the GraphPad Prism v8. (**b**) Percentage inhibition and IC_{50}/IC_{90} values against wild type SARS-CoV-2 pseudoviruses in BNT162b2 and CoronaVac vaccinees. (**c**) The neutralization antibody potency index defined by the ratio of IC_{50}/AUC of anti-Spike IgG and anti-RBD IgG in BNT162b2 and CoronaVac group. Data showed geometric mean values in each group in **a-c**. (**d**) Quantified results depict the percentage of RBD, spike and NP-specific $IFN-\gamma^+$ $CD4^+$ T (top) and $IFN-\gamma^+$ $CD8^+$ T (bottom) cells in BNT162b2 (n=33), CoronaVac (n=28) and non-vaccinated volunteers (n=15), respectively. Fresh PBMC were subjected to T cell response measurement by ICS after RBD-, spike- and NP-specific *ex vivo* peptide pool stimulation, respectively. Numbers under the x-axis in **a** and **d** indicate the rate of positive responders. (**e**) The proportions of spike-specific polyfunctional $CD4^+$ T (top) and $CD8^+$ T (bottom) cells were compared in BNT162b2 and CoronaVac-vaccinated responders. After gating on $IFN-\gamma^+CD4^+/CD8^+$ T cells and $IFN-\gamma^-CD4^+/CD8^+$ T cells, single cytokine ($IFN-\gamma^+$ or $TNF-\alpha^+$ or $IL-2^+$only), double cytokines ($IFN-\gamma^+$ $TNF-\alpha^+$ or $IFN-\gamma^+$ $IL-2^+$ or $TNF-\alpha^+$ $IL-2^+$), and triple cytokines ($IFN-\gamma^+$ $TNF-\alpha^+$ $IL-2^+$) producing cells were analyzed in response to spike-specific *ex vivo* peptide pool stimulation, respectively. Background-subtracted data was analyzed in all cases in **d** and **e**. The bars in D and E indicated median value. (**f**) Phenotypic analysis depicted antigen-specific T cell subsets of BNT162b2 and CoronaVac vaccinees. After gating on $IFN-\gamma^+$ $CD4^+$ or $IFN-\gamma^+$ $CD8^+$ T cells, T cell subsets expressing CCR7 and/or CD45RA were analyzed in response to spike-specific *ex vivo* peptide pool stimulation. Data were analyzed for statistical significance using Mann-Whitney U test. Dotted black lines indicate the limit of quantification (LOQ). **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.

versus 0.00 ([IQR 0.00 to 0.01], both $P < 0.0001$) (Fig. 1d, bottom). BNT162b2 elicited significantly higher proportions of spike-specific $IFN-\gamma^+CD4^+$T ($P < 0.0001$) but not $IFN-\gamma^+CD8^+$T cells ($P=0.6376$) including all polyfunctional subsets (all $P < 0.001$) as compared with the CoronaVac group (Fig. 1d and e). Spike-specific $CD8^+$T cells were consistently detected in 81.8% of our BNT162b2-vaccinees similar to recent reports by others.[19,23,24] BNT162b2, however, did not elicited significantly higher frequencies of spike-specific $IFN-\gamma^+CD8^+$T cells ($P=0.6376$) as compared with the CoronaVac group (Fig. 1d and e). Surprisingly, CoronaVac did not induce measurable levels of NP-specific $IFN-\gamma^+CD4^+$T or $IFN-\gamma^+CD8^+$T cells compared with the unvaccinated group (Fig. 2d, right). As expected, CMV-

specific $IFN-\gamma^+CD4^+$ and $CD8^+$ T cells were comparable between these two vaccinated groups (Figure S3a-b). The slightly lower levels of CMV-specific $IFN-\gamma^+CD4^+$ and $CD8^+$ T cells in the BNT162b2 group than the non-vaccinated control ($P=0.0457$ and 0.0464, respectively) might indicate that there was unlikely vaccine-elicited bystander spike-specific T cell responses (Fig. S3a and 1d). In addition, spike-specific $IFN-\gamma^+CD4^+$ T cells induced by both vaccines showed similar phenotypic profiles of dominated effector memory subsets (Fig. 1f). These results demonstrated that while spike-specific $CD4^+$T and $CD8^+$T cells were generated by both vaccines, CoronaVac induced significantly weaker spike-specific $CD4^+$T cell responses including polyfunctional subsets as compared with BNT162b2.
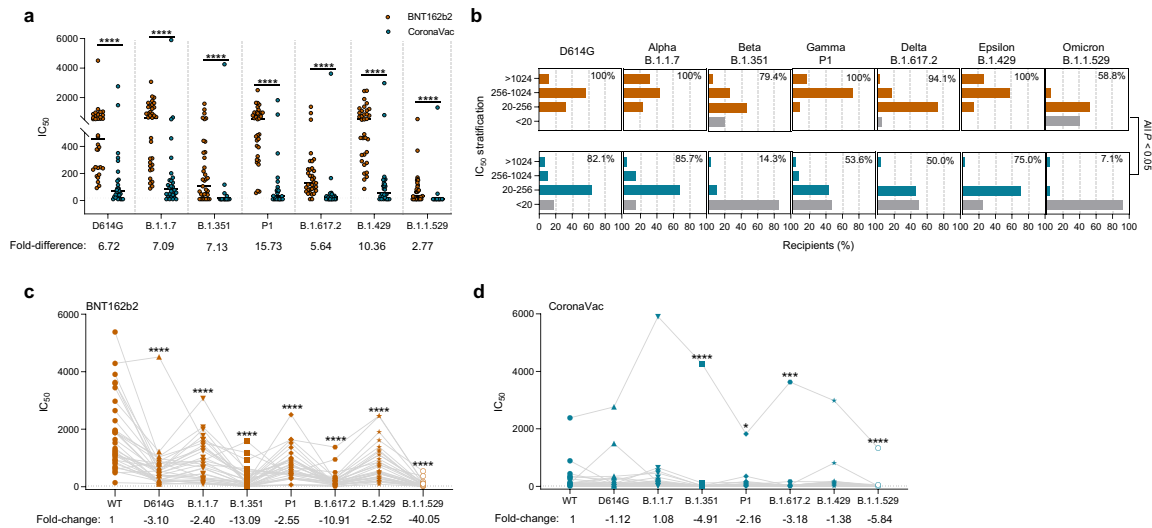
Figure 2. Neutralizing antibody activity against SARS-CoV-2 variants of concerns elicited by BNT162b2 and CoronaVac. (**a**) Neutralizing antibody titers (NAbTs) against seven SARS-CoV-2 strains from BNT162b2 (orange) (*n*=34) and CoronaVac (green) (n=28) participants at acute phase after the second vaccination. NAbTs represent serum dilution required to achieve 50% virus neutralization (IC_{50}). Numbers under the x-axis indicate the fold difference of BNT162b2 to CoronaVac. (**b**) shows neutralizing IC_{50} of four response levels from BNT162b2 (orange) and CoronaVac (green) recipients. Grey bars indicate the percentage of non-responders. Numbers in the top right corner represent the percentage of responders for each VOC. Neutralizing IC_{50} against wild type compared to D614G, B.1.1.7, B.1.351, P1, B.1.617.2, B.1.429 and B.1.1529 in BNT162b2 (**c**) and CoronaVac (**d**) vaccinees. Numbers under the x-axis indicate the fold change of different VOC relative to Wuhan-Hu-1wild type. Mann-Whitney U test was used for between-group comparison in **a, c** and **d**. Two-sided chi-square test was used in **b**. The bars represent geometric mean in **a, c, d**. Dotted black lines indicate the limit of quantification (LOQ). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.

NAbs against SARS-CoV-2 VOCs among BNT162b2 and CoronaVac vaccinees

Considering the rising issues of SARS-CoV-2 variants of concern (VOCs) on the ongoing pandemic,[7−9,11,25] we tested plasma neutralization against multiple VOCs including B.1.1.7, B.1.351, P1, B.1.617.2, B.1.429 and B.1.1.529. In general, the amounts of cross-NAbs against VOCs elicited by BNT162b2 were significantly stronger than those by CoronaVac with 2.7-16-fold differences of the NAbTs including 6.72-fold against D614G (451.8 [95%CI 341.1 to 598.6] versus 67.15 [95%CI 38.15 to 118.2]), 7.09-fold against B.1.1.7 (593.5 [95%CI 422.7 to 833.2] versus 83.72 [95%CI 47.68 to 147.0]), 7.13-fold against B.1.351 (107.0 [95%CI 62.1 to 184.4] versus 15.0 [95%CI 9.24 to 24.37]), 15.73-fold against P1 (548.9 [95%CI 403.5 to 746.6] versus 34.9 [95%CI 20.01 to 60.9]), 5.64-fold against B.1.617.2 (131.0 [95%CI 91.56 to 187.3] versus 23.19 [95%CI 14.49 to 37.11]), 10.36-fold against B.1.429 (565.8 [95%CI 425.7 to 752.0] versus 54.59 [95%CI 31.67 to 94.11]) and 2.77-fold against B.1.1.529 (35.0 [95%CI 22.59 to 54.18] versus 12.6 [95%CI 8.69 to 18.32]) (all *P* values <0.0001) (Fig. 2a). Based on NAbTs, study subjects were stratified into four groups: no response (<20: lower than LOQ), low (20-256), medium (256-1024), and high (>1024). We found that significantly fewer CoronaVac vaccinees than BNT162b2 recipients had

measurable NAbTs against VOCs, including 82.1% (23/28) against D614G (*P*=0.015), 85.7% (24/28) against B.1.1.7 (*P*=0.039), 14.3% (4/28) against B.1.351 (*P* < 0.0001), 53.6% (15/28) against P1 (*P* < 0.0001), 50.0% (14/28) against B.1.617.2 (*P* < 0.0001), 75.0% (21/28) against B.1.429 (*P*=0.002) and 7.1% (2/28) against B.1.1.529 (*P* < 0.0001). Moreover, most CoronaVac responders showed low NAbTs. In contrast, 7 (20.6%), 2 (5.9%) and 14 (41.2%) out of 34 BNT162b2 vaccinees lacked NAbTs against B.1.351, B.1.617.2 and B.1.1.529, respectively (Fig. 2b). Notably, NAbTs elicited by BNT162b2 were significantly reduced against VOCs relative to the wildtype virus including -3.1-fold against D614G, -2.4-fold against B.1.1.7, -13.09-fold against B.1.351, -2.55-fold against P1, -10.91-fold against B.1.617.2, -2.52-fold against B.1.429 and -40.05-fold against B.1.1529 (all *P* <0.0001) (Fig. 2c). Similar NAbTs reduction against VOCs was also observed among CoronaVac responders including -1.12-fold against D614G, -4.91-fold against B.1.351 (*P* < 0.0001), -2.16-fold against P1 (*P*=0.0111), -3.18-fold against B.1.617.2 (*P*=0.0001), -1.38-fold against B.1.429 and -5.84-fold against B.1.1.529 (*P* < 0.0001) (Fig. 2d). Unexpectedly, however, the highest amount of cross-NAbs against VOCs, especially B.1.351, B.1.617.2 and B.1.1.529, was observed in a single CoronaVac-vaccinee (Fig. 2d). These results demonstrated that NAb positive

rates and titers against VOCs, especially against B1.351, B.1.617.2 and B.1.1.529 strains, were lower among CoronaVac-vaccinees than BNT162b2-vaccinees.

In addition, correlation analysis between age (Fig. S4a-l) and BMI (Fig. S5a-l) with immune response parameters, as well as stratified comparison by gender (Fig. S6a-c), were performed in each vaccine group. These results showed that age, BMI and gender did not appear to have any consistent or strong correlation with host immune responses among our study subjects.

Vaccine-induced NAbs against VOCs and T cell responses in the memory phase

Since the epidemic was well controlled in Hong Kong, no SARS-CoV-2 infection was found among our study subjects at the follow-up time of 3 months after the second vaccination. We were able to follow-up 43 longitudinal subjects to measure their immune responses at the memory phase. There is a -1.92-fold reduction of anti-Spike IgG ($P=0.0131$) in the CoronaVac group (Fig. 3a) while a -1.31-fold reduction of anti-RBD IgG ($P=0.0229$) was found in the BNT162b2 group (Fig. 3b). Surprisingly, there were significant reductions of NAbTs

against the wildtype in both BNT162b2-vaccinees (-2.72-fold, $P < 0.0001$) and CoronaVac-vaccinees (-4.03-fold, $P=0.0001$) (Fig. 3c). Similar reductions were found against the panel of VOCs among BNT162b2- and CoronaVac-vaccinees including D614G (-4.68-fold, $P < 0.0001$ and -5.32-fold, $P=0.0084$) (Fig. 3d), B.1.1.7 (-4.38-fold, $P < 0.0001$ and -5.14-fold, $P=0.0002$) (Fig. 3e), B.1.351 (-2.22-fold, $P=0.0003$ and no difference) (Fig. 3f), P1 (-6.33-fold, $P < 0.0001$ and -3.06-fold, $P=0.0017$) (Fig. 3g), B.1.617.2 (-3.38-fold, $P < 0.0001$ and -1.53-fold, $P=0.0078$) (Figure 3h), B.1.429 (-2.52-fold, $P < 0.0001$ and no difference) (Fig. 3i) and B.1.1.529 (-2.66-fold, $P=0.0004$ and no difference) (Fig. 3j), respectively. In terms of spike-specific T cell responses, a significant decrease of spike-specific IFN-γ^+CD4$^+$T cells at the memory phase was observed for both vaccine groups (-2.69-fold, $P < 0.0001$ and -2.4-fold, $P=0.029$), respectively (Fig. 3k). A similar decrease of spike-specific IFN-γ^+CD8$^+$T cells at the memory phase was mainly observed for BNT162b2-vaccinees (-2.94-fold, $P < 0.0001$). A similar trend was found with CoronaVac-vaccinees but without statistical significance and one individual showed a big increase of IFN-γ^+CD8$^+$T cells in the memory phase (Fig. 3l). These
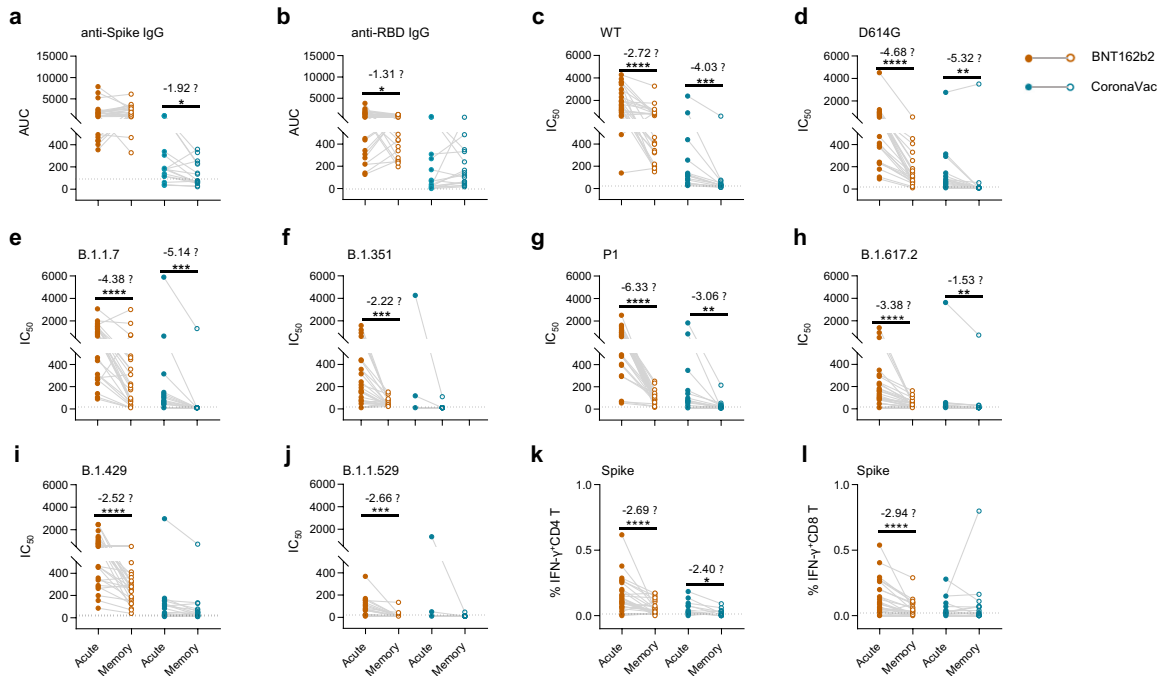

Figure 3. Changes of humoral and cellular responses from the acute phase to the memory phase among BNT162b2- and Corona-Vac-vaccinees. Longitudinal samples from 27 BNT162b2 (orange) and 16 CoronaVac (green) vaccinees were available to track immune response from acute phase to memory phase. Longitudinal binding antibodies to anti-Spike (**a**) and RBD IgG (**b**), neutralizing IC$_{50}$ to wild type (**c**) and different VOCs including D614G (**d**), B.1.1.7 (**e**), B.1.351 (**f**), P1 (**g**), B.1.617.2 (**h**), B.1.429 (**i**), B.1.1.529 (**j**) and spike-specific IFN-γ^+ CD4$^+$ (**k**) or CD8$^+$ (**l**) T cells were measured and compared. Significant differences between acute phase and memory phase of both vaccine groups were determined by Wilcoxon signed-rank test. Dotted black lines indicate the limit of quantification (LOQ) . *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.
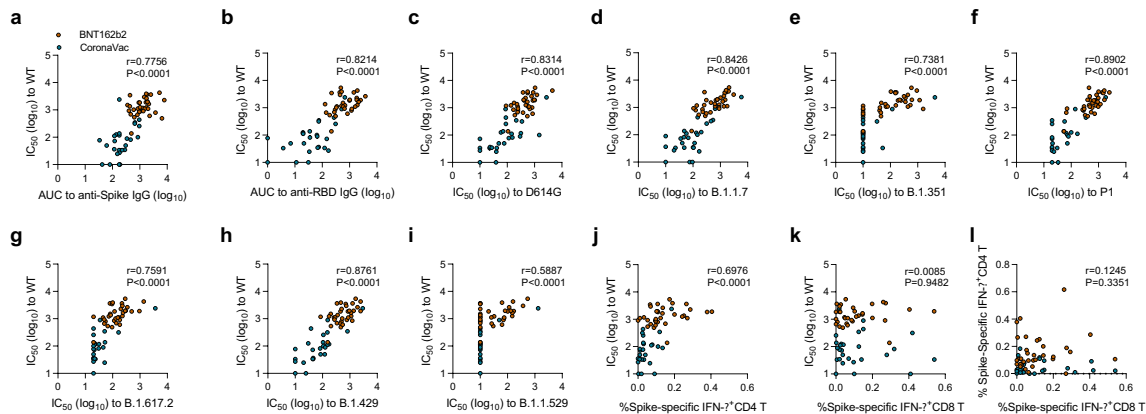
Figure 4. Correlation analysis of vaccine-induced humoral and cellular immune responses. Correlation analysis of anti-Spike (**a**) and RBD IgG (**b**) ($\log_{10}$), IC_{50} against different VOCs including D614G (**c**), B.1.1.7 (**d**), B.1.351 (**e**), P1 (**f**), B.1.617.2 (**g**), B.1.429 (**h**), B.1.1.529 (**i**) and spike-specific IFN-γ^+ CD4$^+$ (**j**) or CD8$^+$ T cells (**k**) to IC_{50} against wild type (WT) as well as spike-specific IFN-γ^+ CD4$^+$ and CD8$^+$ T cells (**l**). The non-parametric Spearman test was used for correlation analysis. P and r values were indicated.

Correlation analysis of vaccine-induced humoral and cellular immune responses

Considering that NAbTs correlate with viral infectivity,[26] we conducted similar correlation analysis at acute phase. Similar to the findings described previously by us[15] and others,[19,27] strong positive correlations were found between NAbTs against wildtype pseudovirus and anti-spike IgG (Fig. 4a, r=0.7756 and $P < 0.0001$) or anti-RBD IgG (Fig. 4b, r=0.8241 and $P < 0.0001$). Furthermore, strong positive correlations were found between NAbTs against wildtype and NAbTs against D614G (Fig. 4c, r=0.8314 and $P < 0.0001$) or NAbTs against B.1.1.7 (Fig. 4d, r=0.8426 and $P < 0.0001$) or NAbTs against B.1.351 (Fig. 4e, r=0.7381 and $P < 0.0001$) or NAbTs against P1 (Fig. 4f, r=0.8902 and $P < 0.0001$), or NAbTs against B.1.617.2 (Fig. 4g, r=0.7591 and $P < 0.0001$) or NAbTs against B.1.429 (Fig. 4h, r=0.8761 and $P < 0.0001$) or NAbTs against B.1.1.529 (Fig. 4i, r=0.5887 and $P < 0.0001$). These results indicated that NAbTs against wildtype at peak immunity predicted NAbs cross-reactivity despite the titer drops against these VOCs tested. In addition, NAbTs against wildtype was positively correlated with the frequency of spike-specific IFN-γ^+CD4$^+$T cell response (Fig. 4j, r=0.6976 and $P < 0.0001$) but not with the frequency of spike-specific IFN-γ^+CD8$^+$T cell frequencies (Fig. 4k, P=0.9482). However, no correlation was found between spike-specific IFN-γ^+CD4$^+$T

and CD8$^+$T cell responses (Fig. 4l, P=0.3351). Similar correlations were also observed in terms of the aforementioned parameters in each separate vaccine group (Fig. S7a−l). Collectively, these results indicated that CD4 helper T cells had likely contributed to the induction of NAbs.

Discussion

Here we report a prospective longitudinal study of antibody and T cell immune responses among BNT162b2- and CoronaVac-vaccinees in Hong Kong. To the best of our knowledge, our results present the most comprehensive clinical study on NAb responses against the global panel of VOCs and T cell responses to wildtype induced by the standard 2-dose BNT162b2 and Corona-Vac vaccinations in parallel. Both vaccines were safe and well-tolerated among our study subjects although BNT162b2 induced more frequent but transient side effects. While both vaccines induced NAb and spike-specific T cell responses to the wildtype virus similar to previous findings,[2,6,28] the geometric NAbTs of CoronaVac-vaccinees was 19-fold lower than that of BNT162b2-vaccinees, which is consistent to recent publications.[29,30] The NAb response correlated positively with CD4 but not CD8 responses, suggesting that vaccine-induced CD4 helper probably contributes to B cell activation. Moreover, our findings on waning NAb responses to wildtype virus among BNT162b2-vaccinees are consistent to several studies published recently.[19,27,31,32] Importantly, against the global panel of VOCs, NAb response rates and titers among Corona-Vac-vaccinees were not only significantly lower but also disappeared more dramatically just three months after the vaccinations as compared with BNT162b2-vaccinees. CoronaVac-vaccinees also exhibited lower neutralization potency index and waning spike-specific IFN-

results demonstrated that both vaccine-induced NAb and T cell responses have waned significantly at the memory phase just three months after the second immunization. In particular, since most CoronaVac-vaccinees have low or unmeasurable NAbTs at this stage, they may face higher risk to infection by the spreading VOCs.

γ^+CD4^+T cells while they showed no obvious advantage for inducing spike-specific IFN-γ^+CD8^+T cells. The neutralization potency index is predictive of immune protection against symptomatic SARS-CoV-2 infection.[21] Our findings are in line with better clinical efficacy against infection of BNT162b2 than that of CoronaVac during phase 3 trials against COVID-19 (95.0% versus 50.65% - 83.5% based on CoronaVac trials from Brazil, Turkey and Indonesia).[1,5,33] Since CoronaVac is one of the most extensively used vaccine with approaching 1.9 billion doses administrated in many countries, our findings have significant implication to CoronaVac-vaccinees who may probably face higher risk than BNT162b2-vaccinees to the spreading VOCs breakthrough infection and should be considered as a priority for the third vaccination.

SARS-CoV-2 VOCs continue to emerge globally, which have brought new challenges to the efficacy of COVID-19 vaccines in emergency use.[7−9,11,25] The B.1.351 variant escaped not only RBD-specific monoclonal NAbs but also vaccine-induced NAbs and convalescent sera.[7] Similarly, we found that our subjects contained the lowest NAbTs against B.1.351 in sera elicited by both BNT162b2 and CoronaVac. In particular, the NAbTs induced by BNT162b2 against B.1.351 decreased by 13-fold, which is worse than the 6.5-8.6-fold decrease among American vaccinees.[7] In this study, while 79.4% BNT162b2-vaccinees developed NAbs to B.1.351, only 14.3% CoronaVac-vaccinees had similar NAbs. Fortunately, B.1.351 and its variants have not becoming a major circulating VOC globally. Instead, the B.1.617.2 variant has become the major VOC after its first detection at the end of March 2021 in India.[34] Due to its extremely high transmissibility and infectivity, cases of breakthrough B.1.617.2 infections have been increasing dramatically even in regions with high vaccination coverage. We found that 94.1% BNT162b2-vaccinees and 50% CoronaVac-vaccinees have developed cross-NAbs to B.1.617.2 mainly at low NAbTs (20-256). Compared with NAbTs to the wildtype, there were 10.91-fold and 3.18-fold reduced NAbTs observed for BNT162b2- and CoronaVac-vaccinees, respectively, in line with the 5.8-fold decrease against B.1.617.2 induced by mRNA vaccine in the UK and other studies.[25,35,36] Since the geometric mean NAbTs further dropped by 3.38- and 1.53-fold just three months after the vaccination, especially with most CoronaVac-vaccinees to the detection limit (<20), the efficacy of preventing breakthrough B.1.617.2 infections is indeed worrisome. Nevertheless, the efficacy of 2-dose vaccinations against B.1.617.2 was 88% for BNT162b2 and 67% for ChAdOx1 nCoV-19,[37] while the efficacy of 2-dose inactivated vaccinations was 59% in a test-negative case-controlled study.[38] In past months, an exploratory trial of boosting with the third dose of inactivated SARS-CoV-2 showed induction of 7.2-fold higher NAbTs, together with 5.9- and 2.7-fold higher spike-specific CD4$^+$ and CD8$^+$ T cells one week after the third dose.[39] This finding is consistent with our results of spike-specific CD4$^+$ and CD8$^+$ T cells among our CoronaVac-vaccinees. The third timely boost vaccination is likely helpful especially for Corona-Vac-vaccinees against breakthrough infections against the pandemic VOCs. In the recent month, breakthrough infections with the recently emerged VOC B.1.1.529 (Omicron) have already been reported in individuals who received booster mRNA vaccination in South Africa.[10] The astonishing 40-fold reduction of NAbTs among BNT162b2-vaccinees and only 2 out of 28 (7.1%) Corona-Vac-vaccinees had remaining NAbs to neutralize the Omicron variant are consistent with the striking antibody evasion found with convalescent patients and vaccinees as well as most monoclonal NAbs used for clinical therapy.[40,41] Collectively, the results from others and us support the roll-out of the third dose of vaccination.[42,43] Our findings also urge unvaccinated people to receive the complete 2-dose vaccinations with the highest priority to reduce sickness, hospitalization and death.

This study has some limitations. The sample size of this study was relatively small and most of our subjects have not reached 6 months for follow-up testing. Extending the follow-up to 6 months and one year or longer is necessary in future studies. Due to the lack of baseline samples, we cannot exclude the possibility of an undocumented SARS-CoV-2 infection prior to vaccination in the single CoronaVac-vaccinee who had the highest amount of NAbs. Such a prior infection, however, was unlikely because there was no reported infections in his family and living community before and during this study. While NAbs have been indicated as correlates of protection,[26,44] the protective role of vaccine-induced T cell responses remains to be further investigated. During acute SARS-CoV-2 infection, we and others demonstrated that antigen-specific T cell responses have likely been associated with viral control and limited pathogenesis.[17,45] In this study, while we consistently found antigen-specific CD4$^+$T cells after vaccinations in both vaccine groups like previously reported by others,[19,46,47] majority of our subjects did not show measurable RBD-specific CD4$^+$ T cells. Why only spike-specific CD4$^+$ but not CD8 T$^+$ cell responses correlated to NAbTs remain unclear. VOC spike-specific T cell responses were not explored due to limited number of cells received, although some studies indicated that the mutations in VOCs might modify single T cell specificities but could not fully escape the whole repertoire of spike-specific T cells.[48,49] Future studies are needed to address these limitations.

Declaration of interest
The authors declare no competing interests.

Contributors
Z.C. supervised the collaborative team, conceived of and designed the study, and wrote the manuscript. K.M. coordinated donor recruitment and specimen collection.

Q.P., R.Z., Y.W. designed some experiments, analysed the data, and prepared the manuscript. M.Z., N.L., S.L., H.H., K.A. and D.Y. performed immune assays, H.W. and K.Y.Y. provided critical comments, supports and materials. Q.P. and R.Z. have analysed and verified the data reported in the manuscript. Q.P. drafted the manuscript. All authors read and approved the final version of the manuscript.

Data sharing statement

The authors declare that the data supporting the findings of this study are available from the corresponding author upon request.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ebiom.2022.103904.

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