Virologica Sinica 38 (2023) 233–243

Contents lists available at ScienceDirect

Virologica Sinica

journal homepage: www.keaipublishing.com/en/journals/virologica-sinica
www.virosin.org

Research Article

Omicron variants breakthrough infection elicited higher specific memory immunity than third dose booster in healthy vaccinees

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ARTICLE INFO

Keywords:
Inactivated vaccine booster
Ad5-nCoV booster
Omicron variants breakthrough infection
Memory immunity
Binding antibody

ABSTRACT

Homologous booster, heterologous booster, and Omicron variants breakthrough infection (OBI) could improve the humoral immunity against Omicron variants. Questions concerning about memory B cells (MBCs) and T cells immunity against Omicron variants, features of long-term immunity, after booster and OBI, needs to be explored. Here, comparative analysis demonstrate antibody and T cell immunity against ancestral strain, Delta and Omicron variants in Omicron breakthrough infected patients (OBIPs) are comparable to that in Ad5-nCoV boosted healthy volunteers (HVs), higher than that in inactivated vaccine (InV) boosted HVs. However, memory B cells (MBCs) immunity against Omicron variants was highest in OBIPs, followed by Ad5-nCoV boosted and InV boosted HVs. OBIPs and Ad5-nCoV boosted HVs have higher classical MBCs and activated MBCs, and lower naïve MBCs and atypical MBCs relative to both vaccine boosted HVs. Collectively, these data indicate Omicron breakthrough infection elicit higher MBCs and T cells against SARS-CoV-2 especially Omicron variants relative to homologous InV booster and heterologous Ad5-nCoV booster.

1. Introduction

Accumulating studies demonstrated that vaccination might effectively decreases clinical severity of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) breakthrough infected patients (Tregoning et al., 2021). However, the SARS-CoV-2 spreading are not effectively limited due to the gradual diminishing of protective humoral immunity induced by previous vaccination of ancestral strain based vaccine or infection, and the continuous emergence of variants of concerns (VOCs) such as Omicron variants with 32 mutative site in spike protein relative

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https://doi.org/10.1016/j.virs.2022.12.008
Received 16 September 2022; Accepted 30 December 2022
Available online 2 January 2023
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to ancestral isolates (Tseng et al., 2022). To improve the immunity against Omicron variants, homologous third inactivated vaccine (InV) and heterologous boosters were implemented in many countries, such as China (Wang K. et al., 2022; Xue et al., 2022). Our previous data along with other published studies consistently demonstrated the neutralizing antibody against Omicron variants elicited by homogenous third InV was significantly lower than heterologous third vaccination with Ad5-nCoV (Kong et al., 2022), and a recombinant protein subunit vaccine (Zhang et al., 2022), as well as recombinant adenoviral vectored vaccine, and mRNA vaccine (Costa Clemens et al., 2022). Apart from antibody, an early surge of durable SARS-CoV-2 specific memory B cells (MBCs) play key roles in viral clearance in Omicron variant breakthrough infected patients (Kared et al., 2022; Muecksch et al., 2022). However, the comparative analysis of durable MBCs response against SARS-CoV-2 (especially Omicron variants) induced by InV booster, Ad5-nCoV booster and Omicron variants breakthrough infection (OBI) in previous recipients with two doses of InV was not studied.

Durable and conserved T cells immunity response has pivotal protective roles in recovery of coronavirus disease 2019 (COVID-19) patients as well, especially when protective humoral response was not effectively elicited (Sette and Crotty, 2021; GeurtsvanKessel et al., 2022; Woldemeskel et al., 2022). As previous study supported T cell immunity against SARS-CoV-2 might be induced by administration of InV (Melo-Gonzalez et al., 2021; Mok et al., 2022). These cellular immunity against SARS-CoV-2 might be further increased by third booster, with peak cellular immunity response by heterologous Ad5-nCoV booster, followed by recombinant protein subunit vaccine and homogenous InV (Zhang et al., 2022). Another study has demonstrated that T cell response elicited by mRNA-1273 booster was highest in healthy peoples after two doses of InV, and followed by the BNT162b2, AZD1222 and BBIBP booster (Assawakosri et al., 2022). Additionally, robust T cell immunity against spike and non-spike protein was observed in Omicron breakthrough infected patients (OBIPs) with previous administration of two dose of mRNA vaccine (Kared et al., 2022). As a whole, there is no direct comparison of protective memory B and T cell responses induced by OBI and third booster following two doses of InV.

2. Material and methods

2.1. Study cohorts

This study was approved by the Ethics Committee of the Second Affiliated Hospital of Guangzhou Medical University (Approval No. 2021-hs-43) and Dongguan Ninth People's Hospital (Approval No. 2022-8). The OBIPs were from Dongguan Ninth People's Hospital from February 25, 2022 to March 21, 2022, when the COVID-19 epidemic was caused by Omicron subvariant BA.2. The vaccinated healthy volunteers (HVs) were recruited to participating homologous InV (Sinopharm BIBP or CoronaVac) booster or heterologous Ad5-nCoV (CanSinoBio) booster as per their wish in the Second Affiliated Hospital of Guangzhou Medical University. The informed consent was obtained from all participants before participating in this clinical trial. Other clinical parameters including sex, age, and vaccination history were obtained from medical record.

2.2. Whole blood samples collection and processing

The whole blood was drawn from HVs before third dose booster, at median days of 33 [(interquartile range (IQR): 29.5–34.5)] post homologous InV booster, 30.5 (IQR: 28–33) days post Ad5-nCoV booster, and OBIPs at median days of 32 (IQR: 26–36) post first RT-PCR positive. The plasma was obtained by centrifugation at 3000 rpm for 15 min. Then, equal modified RPMI 1640 culture medium (Cat$^{\#}$: 11875093, Gibco, USA) was added in the remaining blood, followed by thorough mixing. Finally, peripheral blood mononuclear cells (PBMCs) were isolated from thorough mixed blood samples by Lymphoprep™ density gradient medium (Cat$^{\#}$: 1114546, Alere Tech, USA).

2.3. SARS-CoV-2 specific antibody detection

SARS-CoV-2 specific antibody titer including anti-spike-trimer antibody (IgG, IgG1, IgG2, IgG3, and IgG4), anti-ancestral-strain-, anti-Delta-, anti-Omicron-receptor binding domain (RBD) IgG was measured using enzyme linked immunosorbent assay (ELISA). In brief, ELISA plate was coated with SARS-CoV-2 specific antigen at proper concentration overnight. The SARS-CoV-2 specific antigen included SARS-CoV-2 ancestral-spike trimer (Cat$^{\#}$: VISC2-S101, Dongkang Biotech, China), ancestral-nucleocapsid protein (NP) (Cat$^{\#}$: VISC2-NP01, Dongkang Biotech, China), ancestral-RBD (Cat$^{\#}$: VISC2-RB04, Dongkang Biotech, China), Delta-RBD (Cat$^{\#}$: ncov-ps-Ag29, Fapon Biotech, China), and Omicron-RBD (Cat$^{\#}$: ncov-ps-Ag41, Fapon Biotech, China). After blocking, 100 μL 3-fold diluted plasma (initially dilute for IgG, IgG1, IgG2 and IgG3:1:20, IgG4:1:10) was added in each well, and incubated at 37 °C for 1 h. After thorough washing, 100 μL HRP labeled secondary antibodies against human IgG (Cat$^{\#}$: 2010-05, 1:10,000, Southern Biotech, USA), IgG1 (Cat$^{\#}$: 9054-05, 1:4000, Southern Biotech, USA), IgG2 (Cat$^{\#}$: 9060-05, 1:2000, Southern Biotech, USA), IgG3 (Cat$^{\#}$: 9210-05, 1:4000, Southern biotech, USA), and IgG4 (Cat$^{\#}$: 9200-05, 1:4000, Southern Biotech, USA) was added in each well. The endpoint titer was determined by the highest dilution, which gives an optical density (OD) value higher than mean + 3 standard deviation (SD) OD values of 3 serum pools from 30 archived serum collected from HVs in the year 2019 at the same dilution.

2.4. Detection of SARS-CoV-2 RBD specific MBCs

Ancestral strain, Delta variants and Omicron variants RBD specific MBCs were determined using enzyme-linked immunospot (ELISPOT) assay, as previously described (Jahnmatz et al., 2013). Briefly, 1.5×10^6 PBMCs were stimulated with 1 μg/mL R848 (Cat$^{\#}$: tlrl-r848, InvivoGen, USA) and 100 IU/mL recombinant human IL-2 (Cat$^{\#}$: 200-02-250, Peprotech, USA) in RPMI-1640 (Cat$^{\#}$: 11875093, Gibco, USA) supplemented with 10% fetal bovine serum (FBS) for 3 days. The ELISPOT plates (Cat$^{\#}$: MBT-3654-WP-10, Mabtech, USA) were pre-coated with 10 μg/mL SARS-CoV-2 ancestral-S1 (Cat$^{\#}$: VISC2–S101, Dongkang Biotech, China), ancestral-RBD (Cat$^{\#}$: VISC2-RB04, Dongkang Biotech, China), Delta variants-RBD (Cat$^{\#}$: ncov-ps-Ag29, Fapon biotech, China), and Omicron variants-RBD (Cat$^{\#}$: ncov-ps-Ag41, Fapon biotech, China) along with 10 μg/mL anti-human IgG (Cat$^{\#}$: 109-035-088, Jackson ImmunoResearch, USA). Following blocking with RPMI-1640 containing 10% FBS (Cat$^{\#}$: CM1002L, Guangzhou Cellcook Biotech, China), 100,000 activated cells were added into ELISPOT plates and incubated at 37 °C in 5% CO_2 for 18 h. To determine the nonspecific binding, control wells without coating target protein and anti-human IgG were also incubated with 100,000 pre-stimulated cells. Then, the cells were discarded and the plates were washed with PBST 6 times. 100 μL HRP-labeled Goat Anti-Human IgG (H + L) (Cat$^{\#}$: A0201, 1/1000, Beyotime, China) in PBS supplemented with 5% FBS were added to the plate wells and incubated for 2 h at room temperature. Afterward, the plates were washed with PBS 6 times. Then, spots were developed with 3-amino-9-ethylcarbazole (AEC) substrate (Cat$^{\#}$: 551951, BD Biosciences, USA) according to the manufacturer's instructions. The ELISPOT plates were fully washed with tap water. Finally, the spots were counted using the ImmunoSpot S6 UV Analyzer (Cellular Technology Limited, USA). The spot-forming units (SFU) of each well were determined by minus spots of the same sample in the control wells. The SFU of each sample was calculated using the means of duplicate wells and expressed as SFU/10^6 PBMCs.

2.5. Detection of SARS-CoV-2 spike specific T-cell frequencies

SARS-CoV-2 (including Ancestral strain, Delta variants, and Omicron variants) spike and specific T lymphocytes were detected using interferon-γ (IFNγ) ELISPOT assay. In brief, 2×10^5 fresh PBMCs were added into each well of anti-IFNγ-antibody pre-coated ELISPOT plate (Cat$^{\#}$:

1110002, Dakewe Biotech, China), and co-cultured with overlapping peptide pools of ancestral spike (Cat#: RP30027, GenScript, China), Delta variants spike (Cat#: RP30033, GenScript, China), Omicron variants spike (Cat#: RP30121, GenScript, China) or ancestral NP (Cat#: RP30013, GenScript, China) for 24 h, with dimethyl sulfoxide (Cat#: 41639-100 ML, Sigma, USA) as a negative control (NC). For positive control, 2×10^4 PBMCs were stimulated with 1 μg/mL staphylococcal enterotoxin B (Cat#: 11100-45-1, Merck, Germany). 100 μL biotinylated antibody working solution was added to each plate well and incubated at 37 °C for 1 h. After that, 100 μL streptavidin-horseradish peroxidase working solution was added into each well and incubated at 37 °C for 1 h. Then, 100 μL AEC solution (Cat#: 551951, BD Biosciences, USA) was added and incubated at room temperature for 30 min. Finally, the spots were counted using the ImmunoSpot®S6 UV Analyzer (Cellular Technology Limited, USA). The SFUs of each well were determined by subtracting spots of the unstimulated wells from the peptide stimulated wells. The SFU of each sample was calculated by using the means of duplicate wells and expressed as SFU/10^6 PBMCs.

2.6. Flow cytometry analysis

Human PBMCs (1×10^7/mL) were resuspended in 100 μL FACS buffer (DPBS contained 1% heat inactivated FBS) with 1×10^6 cells. PBMCs were stained with the following fluorescein-labeled antibodies: anti-human CD19 PE-Cy7 (Cat#: IM3628, Beckman, USA), anti-human CD21 FITC (Cat#: 561372, BD Biosciences, USA), anti-human CD27 BB700 (Cat#: 566449, BD Biosciences, USA), anti-human CD71 APC (Cat#: 334108, Biolegend, USA), anti-human IgD PE (Cat#: 12-9868-42, Invitrogen, USA), anti-human IgG APC-H7 (Cat#: 561297, Biolegend USA) for 30 min. Then, immunophenotype of MBCs was detected using BD FACS LSR Fortessa flow cytometer (BD Biosciences, USA). Data were analyzed using FlowJo software (Tree Star, USA).

2.7. Statistical analysis

Neutralizing antibody titers were expressed as geometric mean titers (GMTs) and 95% confidence interval (CI). The continuous variables were represent as mean (SD) or median (IQR). Categorical variables were described as the count and percentage. All the statistical analysis were performed using GraphPad prism7.0. The Pearson chi-square was used to determine the differences in proportions between two groups. The independent group *t*-test (normal distribution) and Mann-Whitney U (non-normal distribution) were used to compare continuous variables between groups. Paired data were analyzed using Wilcoxon test. A two-side *P*-value < 0.05 was considered statistically significant.

3. Results

3.1. Baseline characteristics of enrolled OBIPs and vaccinated HVs

In total, 33 pre-vaccinated HVs with homologous InV booster, 21 with Ad5-nCoV booster and 10 OBIPs were included in this study. The median age of InV boosted HVs was significantly lower than Ad5-nCoV boosted HVs and OBIPs (Table 1). The sex proportion of InV boosted HVs, Ad5-nCoV boosted HVs and OBIPs was comparable (Table 1). There was no significant difference for the inoculated inactivated vaccine brand among InV boosted HVs, Ad5-nCoV boosted HVs and OBIPs (Table 1). The median time interval between third booster and second dose inactivated vaccine administration of InV boosted HVs was 7 months, which was significantly lower than that of Ad5-nCoV boosted HVs (Table 1). The majority of homologous InV boosted HVs had not apparent adverse effects (AEs) (Table 1). However, all Ad5-nCoV boosted HVs reported apparent AEs, with most reported AEs of myalgia (12/21, 57.1%), followed by fever (8/21, 38.1%), fatigue (4/21, 19.1%), and oropharyngeal pain (2/21, 9.5%) (Table 1). 80% (8/10) enrolled OBIPs had mild manifestations, and 20% (2/10) were asymptomatic (Table 1). In addition, all the enrolled OBIPs were infected by Omicron variants BA.2.2 at median of 9 month after second dose of inactivated vaccine, without third booster (Table 1).

3.2. SARS-CoV-2 specific antibody in pre-vaccinated HVs after InV booster, Ad5-nCoV booster and OBI

As expected, InV and Ad5-nCoV boosted HVs and OBIPs obtained higher anti-spike-trimer IgG titer compared to pre-boosted HVs (Fig. 1A). In addition, Ad5-nCoV booster elicited higher anti-spike-

Table 1
Baseline characteristics of enrolled Omicron variants breakthrough infected patients and vaccinated healthy volunteers.

Variables [n (%) or median (IQR)]	3 doses InV Vaccinated HVs (n = 33)	2 doses InV + 3rd Ad5-nCoV Vaccinated HVs (n = 21)	OBIP (n = 10)	*P*-value
Age (years)	37 (28.5–45.5)	28 (25–38.5)	39 (33.8–56.3)	0.004
Sex				
Female (%)	10 (30.3)	11 (52.4)	1 (10.0)	0.053
Male (%)	23 (69.7)	10 (47.6)	9 (90.0)	
Inactivated Vaccine brand of two dose scheme				0.146
Two doses Sinopharm BIBP n (%)	14	5	5	
Two doses CoronaVac n (%)	16	10	5	
Sinopharm BIBP plus Corona Vac n (%)	3	6	0	
The time interval between third booster or breakthrough infection and second dose inactivated vaccine administration (months)	7 (6–8)	9 (9–10)	9 (8.5–9.5)	0.007
Adverse effects				
No abnormalities n (%)	23 (69.7)	0 (0)		<0.0001
Pain at injection site n (%)	8 (24.2)	0 (0)		
Fatigue n (%)	2 (6.1)	4 (19.1)		
Fever n (%)	0 (0)	8 (38.1)		
Myalgia n (%)	0 (0)	12 (57.1)		
Oropharyngeal Pain n (%)	0 (0)	2 (9.5)		
Clinical manifestation				
Asymptomatic n (%)			2 (20)	
Mild n (%)			8 (80)	
Underlying diseases				
Hypertension n (%)			1 (10)	
Pulmonary inflammation n (%)			1 (10)	
Pulmonary bulla n (%)			1 (10)	

OBIPs: Omicron variants breakthrough infected patients, HVs: Healthy volunteers, InV: inactivated vaccine, IQR: interquartile range.

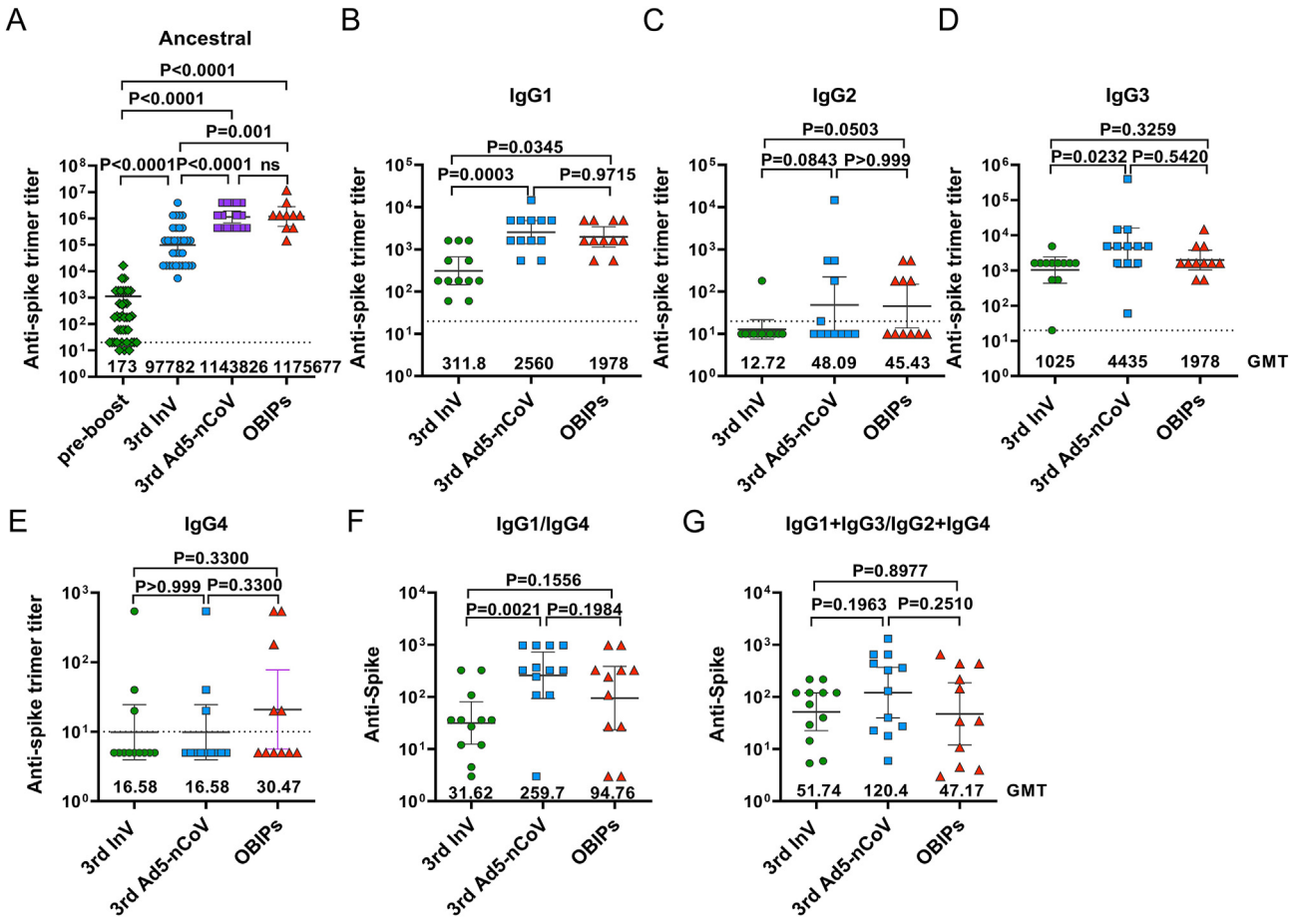


Fig. 1. Comparative analysis of anti-ancestral-spike-trimer-IgG. Serum were collected from vaccinated healthy volunteers (HVs) before third booster, at median of 33 days post inactivated vaccine (InV) booster and 30.5 days post Ad5-nCoV booster, and Omicron variants breakthrough infected patients (OBIPs) at median of 32 days post infection. **A** Anti-spike-trimer-IgG titer in InV boosted HVs, Ad5-nCoV boosted HVs, and OBIPs were determined by ELISA. **B–G** Comparative analysis of SARS-CoV-2 ancestral strain spike-trimer specific IgG subclass. Anti-spike-trimer-IgG1 (**B**), anti-Spike-trimer-IgG2 (**C**), anti-Spike-trimer-IgG3 (**D**), and anti-spike-trimer-IgG4 (**E**) titer in InV boosted HVs, Ad5-nCoV boosted HVs, and OBIPs were determined by ELISA. **F** The ratio of spike-trimer specific IgG1/IgG4 in InV boosted HVs, Ad5-nCoV boosted HVs, and OBIPs. **G** The ratio of spike-trimer specific IgG1+IgG3/IgG4+IgG2 in InV boosted HVs, Ad5-nCoV boosted HVs, and OBIPs. Data presented as geometric mean titers (GMT) and 95% confidence interval. Dotted line: the detection limits. Statistical analyses were performed by Mann-Whitney *U* test.

trimer IgG titer than InV booster (Fig. 1A). Anti-spike-trimer IgG titer in OBIPs was higher than that in HVs with homologous booster, and equal to that in HVs with Ad5-nCoV booster (Fig. 1A). The spike-trimer specific IgG subclass analysis demonstrated anti-spike-trimer-IgG1 titer in InV boosted HVs was significantly lower than that in Ad5-nCoV boosted HVs and OBIPs (Fig. 1B). There was no difference for anti-spike-trimer-IgG1 titer between Ad5-nCoV boosted HVs and OBIPs (Fig. 1B). Regarding anti-spike-trimer-IgG3 titer, there was no significant difference between Ad5-nCoV boosted HVs and OBIPs, InV boosted HVs and OBIPs (Fig. 1D). But, anti-spike-trimer-IgG3 titer in InV boosted HVs was significantly lower than that in Ad5-nCoV boosted HVs (Fig. 1D). The anti-spike-trimer-IgG2 and -IgG4 titer was markedly lower in both vaccine boosted HVs and OBIPs compared to anti-spike-trimer-IgG1 and -IgG3 titer (Fig. 1B–E). Furthermore, there was no significant difference for anti-spike-trimer-IgG2 and -IgG4 titer among both vaccine boosted HVs and OBIPs (Fig. 1C, E). Interestingly, the median of IgG1 and IgG4 titer ratio in individuals after homologous InV booster, Ad5-nCoV booster, and OBI was higher than 1, with highest of Ad5-nCoV booster (259.7), followed by OBI (94.76), and homologous InV booster (31.62) (Fig. 1F), indicating a predominant IgG1 (Th1) response (Perez-Perez et al., 2010). In addition, the ratio of IgG1+IgG3 and IgG2+IgG4 was comparable among homologous InV booster, Ad5-nCoV booster and breakthrough infection (Fig. 1G), indicating both vaccine booster and OBI elicit similar IgG subclass with pro-inflammatory Fc response (Farkash et al., 2021).

The comprehensive circulation of Delta and Omicron variants raise extensive concern of the vaccine efficiency against these two variants induced by third booster and OBI. Our results indicated that anti-ancestral-RBD-IgG titers were significantly increased in InV boosted HVs, Ad5-nCoV boosted HVs and OBIPs relative to pre-boosted HVs, these three groups were comparable (Fig. 2A). Accordantly, the anti-Delta-RBD-IgG and anti-Omicron-RBD-IgG titer were significantly increased in both vaccines boosted HVs and OBIPs compared to pre-boost (Fig. 2B and C). However, anti-Delta-RBD-IgG, anti-Omicron-RBD-IgG titer in InV boosted HVs was consistently lower than Ad5-nCoV boosted HVs and OBIPs, these two groups were comparable (Fig. 2B and C). It was noteworthy that anti-Delta-RBD-IgG and anti-Omicron-RBD-IgG titer elicited by both boosters and OBI was significantly decreased compared to anti-ancestral-strain-RBD-IgG, with 6.9 fold and 55.9 fold decrease for pre-boosted HVs, 3 fold and 55.9 fold decline for InV boosted HVs, 1.1 fold and 3.33 fold decline for Ad5-nCoV boosted HVs, 2.2 fold and 4.2 fold decline for OBIPs (Fig. 2D). This indicates that Ad5-nCoV booster are better alternatives for third booster campaign in countries with these two vaccine approval.

3.3. Ancestral strain, Delta and Omicron variant specific MBCs in pre-vaccinated individuals after InV booster, Ad5-nCoV booster and OBI

Principally, MBCs might rapidly re-activate to differentiate the Ab-secreting cells (ASCs), including plasmablasts and plasma cells

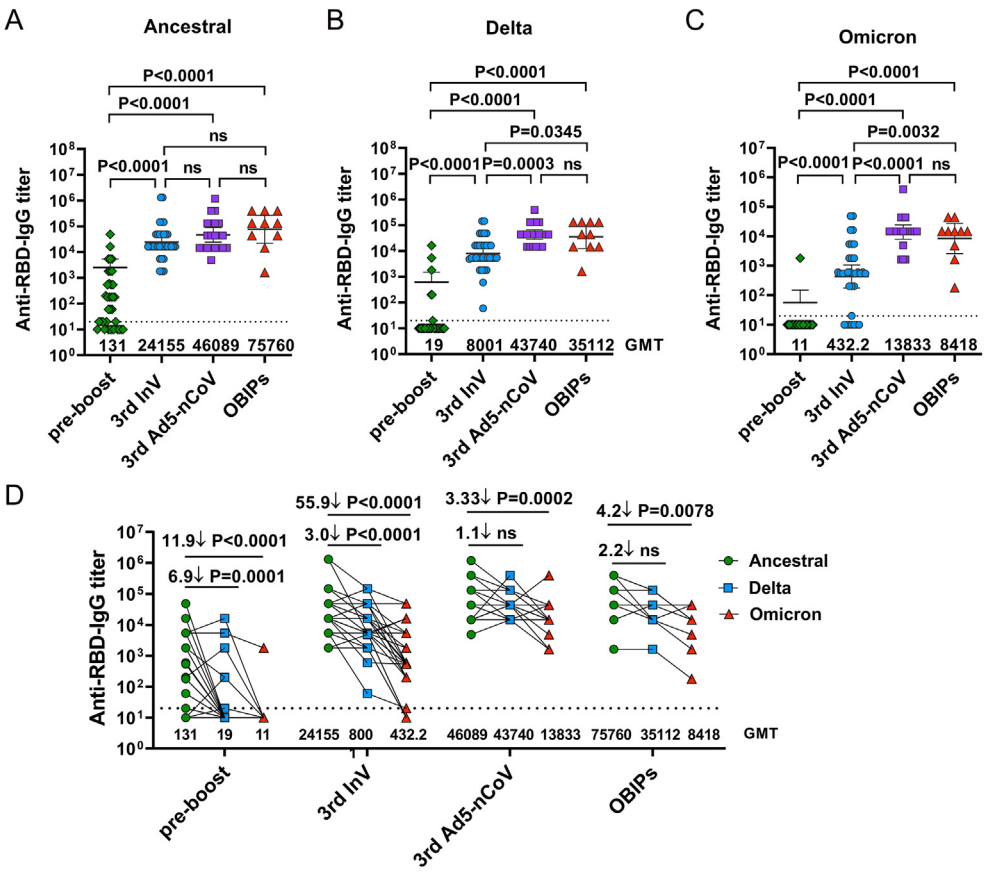


Fig. 2. Comparative analysis of anti-RBD antibody against SARS-CoV-2. Serum were collected vaccinated healthy volunteers (HVs) before third booster, at median of 33 days post InV booster and 30.5 days post Ad5-nCoV booster, and OBIPs at median of 32 days post infection. Anti-ancestral-receptor binding domain (RBD)-IgG (**A**), anti-Delta-RBD-IgG (**B**), and anti-Omicron-RBD-IgG (**C**) titer in pre-boosted HVs, InV boosted HVs, Ad5-nCoV boosted HVs, and OBIPs were determined by ELISA. **D** Paired analysis of anti-ancestral-RBD-, anti-Delta-RBD-, and anti-Omicron-RBD-IgG titer in pre-boosted HVs, InV boosted HVs, Ad5-nCoV boosted HVs, and OBIPs. Data presented as geometric mean titers (GMT) and 95% confidence interval. Dotted line: the detection limits. Statistical analyses were performed by Mann-Whitney *U* test.

following a secondary infection that contributes to protection against severe disease or death in VOC breakthrough infected patients (Sakharkar et al., 2021; Quandt et al., 2022). Therefore, previously developed MBCs ELISPOT assay, which detects antigen specific MBCs after agonist R848 plus Interleukin (IL)-2 stimulation (Pinna et al., 2009), was applied to determine SARS-CoV-2 specific MBCs in our study. By these method, we found that ancestral-spike and ancestral-RBD specific MBCs were significantly improved in both boosters and OBIPs compared to that in pre-boosted HVs (Fig. 3A–C). Interestingly, ancestral-spike and ancestral-RBD specific MBCs induced by Ad5-nCoV booster was significantly higher than that by homologous InV booster (Fig. 3A–C). Markedly, OBI significantly augment higher ancestral-spike and ancestral-RBD specific MBCs than homologous InV booster and Ad5-nCoV booster (Fig. 3A–C). Additionally, Delta-RBD and Omicron-RBD specific MBCs were significantly elevated in Ad5-nCoV boosted HVs and OBIPs compared to that in pre-boosted HVs (Fig. 3A, D, and E). Comparable Delta-RBD specific MBCs were observed in Ad5-nCoV boosted individuals and OBIPs (Fig. 3A, D). However, OBIPs acquired higher Omicron-RBD specific MBCs than Ad5-nCoV boosted individuals (Fig. 3A, E). Compared with ancestral-RBD specific MBCs, Omicron-RBD, but not Delta-RBD specific MBCs, declined significantly in Ad5-nCoV boosted individuals and OBIPs, with 11.8 fold and 5.0 fold decrease for Omicron RBD specific MBCs respectively (Fig. 3F).

3.4. Phenotypic analysis of MBCs in pre-vaccinated individuals after InV booster, Ad5-nCoV booster and OBI

The change of frequency, absolute numbers and phenotype of MBCs subsets in HVs ~32 days after booster vaccination or breakthrough infection by compared to pre-boosted individuals was determined. According to the expression of CD27 and CD21 molecules, B cells ($CD19^+$) were sub-divided into classical MBCs (cMBCs, $CD27^+CD21^+$), activated MBCs (aMBCs, $CD27^+CD21^{-/low}$), and atypical MBCs (atMBCs, $CD27^-CD21^{-/low}$) (Supplementary Fig. S1). The frequency and number of total $CD19^+$ B cell in InV boosted, Ad5-nCoV boosted individuals and OBIPs were markedly increased compared to pre-boosted individuals (Fig. 4A). There were no significant differences in total $CD19^+$ B cells frequency among lymphocyte between InV boosted individuals and Ad5-nCoV boosted individuals, Ad5-nCoV boosted individuals and OBIPs (Fig. 4A), but the number of total $CD19^+$ B cells in Ad5-nCoV boosted individuals higher than in InV boosted individuals and OBIPs (Fig. 4A) in that the lymphocyte counts were significantly higher in Ad5-nCoV boosted individuals compared to InV boosted individuals and OBIPs (Fig. 4B). Subsequent phenotypic analysis revealed that cMBCs and aMBCs frequency and number in InV boosted individuals was not significantly changed compared to pre-boosted individuals, but that in Ad5-nCoV boosted individuals and OBIPs was markedly increased

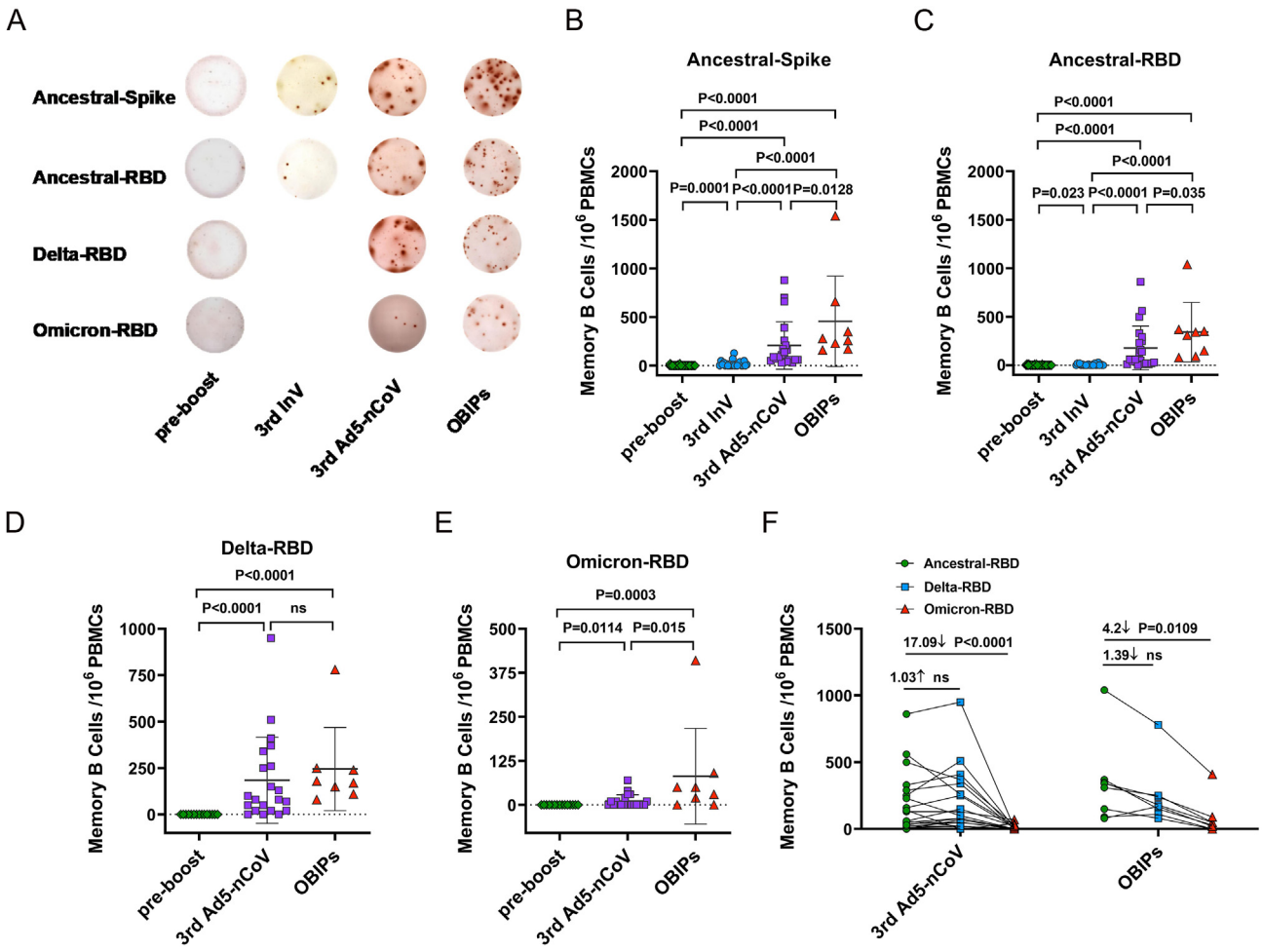
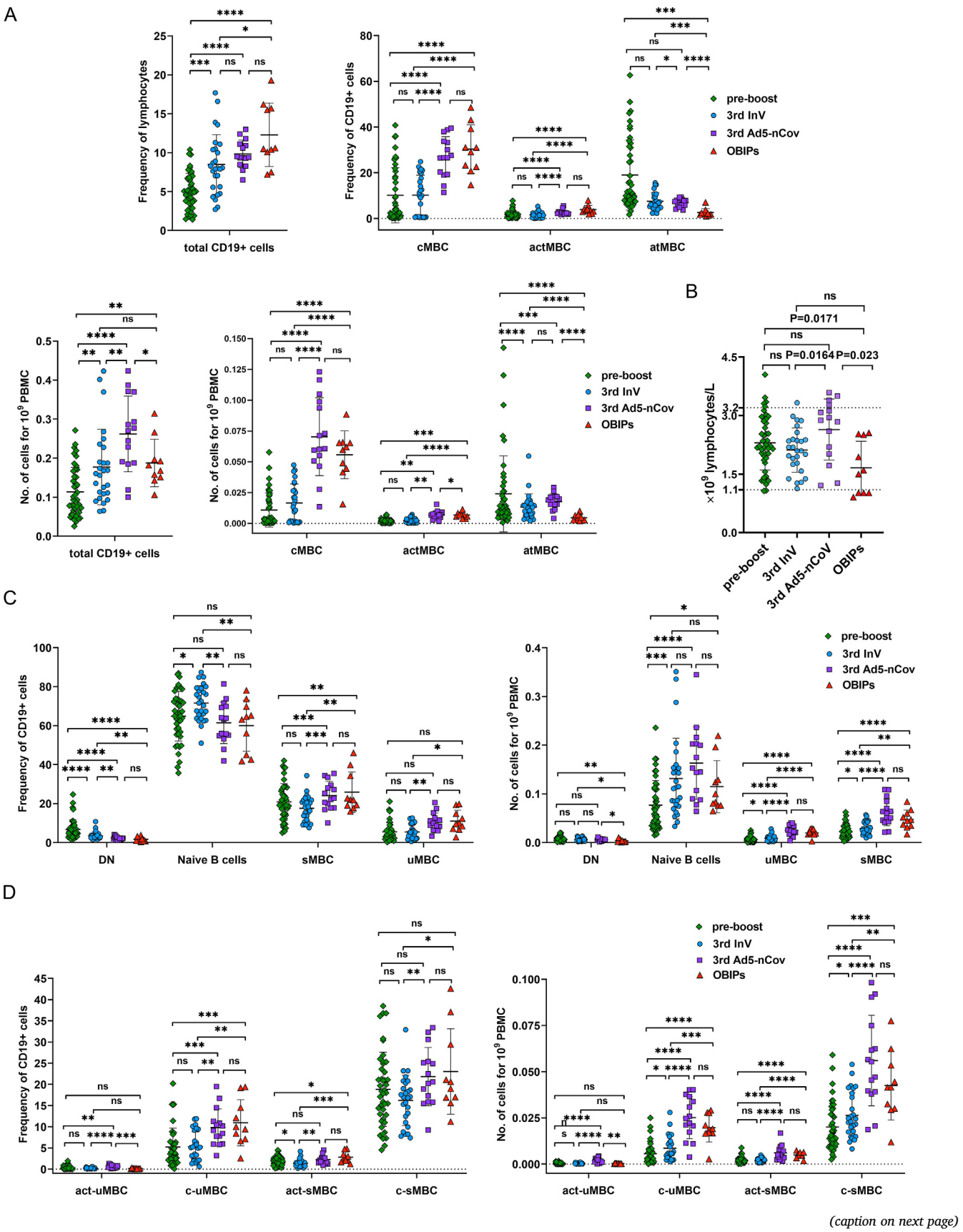


Fig. 3. Comparative analysis of memory B cells against SARS-CoV-2 in vaccinated healthy volunteers (HVs) before third booster, at median of 33 days post InV booster and 30.5 days post Ad5-nCoV booster, and OBIPs at median of 32 days post infection. **A** Representative images of ELISpot wells of ancestral-Spike-specific, ancestral-RBD-specific, Delta-RBD-specific, and Omicron-RBD-specific memory B cells in pre-boosted HVs, InV boosted HVs, Ad5-nCoV boosted HVs, and OBIPs. **B**–**E** ancestral-Spike-specific (**B**), ancestral-RBD-specific (**C**), Delta-RBD-specific (**D**), and Omicron-RBD-specific (**E**) memory B cells frequency were determined by ELISPOT assay in pre-boosted HVs, InV boosted HVs, Ad5-nCoV boosted HVs, and OBIPs. **F** Paired analysis of frequency-RBD-specific, Delta-RBD-specific, and Omicron-RBD-specific memory B cells frequency in Ad5-nCoV boosted HVs and OBIPs. Data presented as spots forming units per 10^6 peripheral blood mononuclear cells. Statistical analyses were performed by Mann-Whitney *U* test.

(Fig. 4A). The frequency of at MBCs was significantly declined in both vaccine boosted individuals and OBIPs compared to pre-boosted individuals (Fig. 4A). The declined at MBC number was only observed in OBIPs (Fig. 4A).

Then, MBCs subsets were assessed based on CD27 and IgD expression level, i.e. B cells were subdivided into naïve B cells ($CD27^-IgD^+$), switched MBCs (sMBC, $CD27^+IgD^-$), un-switched MBCs (uMBCs, $CD27^+IgD^+$), and double negative B cells (DN, $CD27^-IgD^-$) (Supplementary Fig. S1). This study revealed that DN MBCs frequency was significantly decreased in both vaccine boosted individuals and OBIPs compared to pre-boosted individuals, their number was only declined markedly in OBIPs (Fig. 4C). The frequency of naïve B cells were increased in InV boosted individuals, but not in Ad5-nCoV boosted individuals and OBIPs (Fig. 4C). However, naïve B cells number was elevated in both vaccine boosted individuals and OBIPs (Fig. 4C). The frequency of uMBCs increased in Ad5-nCoV boosted individuals and OBIPs, but its number was significantly enhanced in both vaccine boosted individuals and OBIPs, with higher uMBCs frequency and number in Ad5-nCoV boosted individuals and OBIPs compared to InV boosted individuals (Fig. 4C). The number of sMBCs, but not frequency, was increased in both vaccine boosted individuals and OBIPs, along with higher sMBCs frequency and number in Ad5-nCoV boosted individuals and OBIPs compared to InV boosted individuals (Fig. 4C).

Moreover, sMBCs were further sub-divided into activated switched MBCs (act-sMBCs, $CD27^+IgD^-CD71^+$) and classical switched MBCs (c-sMBCs, $CD27^+IgD^-CD71^-$), and uMBCs were further sub-divided into activated un-switched MBCs (act-uMBC, $CD27^+IgD^+CD71^+$) and classical un-switched MBCs (c-uMBCs, $CD27^+IgD^+CD71^-$) according to the expression of activation marker CD71 (Supplementary Fig. S1). Interestingly, the act-uMBCs frequency and number was only enhanced in Ad5-nCoV boosted individuals (Fig. 4D). The frequency of c-uMBCs was increased in Ad5-nCoV boosted individuals and OBIPs, their numbers were increased in both vaccine boosted individuals and OBIPs (Fig. 4D). The frequency of act-sMBCs was decreased in InV boosted individuals, increased in OBIPs, their number was significantly increased in Ad5-nCoV boosted individuals and OBIPs (Fig. 4D). Although there was no significant difference of c-sMBCs frequency in both vaccine boosted individuals and OBIPs compared to pre-boosted individuals (Fig. 4D). The number of c-sMBCs were significantly increased in both boosted individuals and OBIPs, with higher number in Ad5-nCoV boosted individuals and OBIPs relative to InV boosted individuals (Fig. 4D).



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3.5. Ancestral, Delta and Omicron variant specific T cells in pre-vaccinated individuals after InV booster, Ad5-nCoV booster and OBI

Durable and conservative SARS-CoV-2 specific memory T cell immunity could rapidly re-activate after re-infection of VOC, have critical roles in the protection against severe COVID-19 (Sette and Crotty, 2021; Choi et al., 2022; Jung et al., 2022; Kedzierska and Thomas, 2022). Here in this study, we performed an IFN-γ ELISpot following stimulation with pooled overlapping 15-mer peptides spanning the full length of ancestral-, Delta-, Omicron-spike, or ancestral-NP. The number of effector T cells against ancestral-, Delta- and Omicron-spike in both boosters and OBIPs were significantly raised relative to pre-boosted HVs (Fig. 5A–D). The number of effector T cells against ancestral-, Delta- and Omicron-spike in HVs with homologous InV booster were lower than that in individuals with Ad5-nCoV booster and OBIPs, these two groups had comparable effector T cells against ancestral-, Delta- and Omicron-spike (Fig. 5A–D). Of note, the effector T cells counts against Delta-spike and Omicron-spike significantly declined compared to effector T cells against ancestral-spike in HVs with InV booster and Ad5-nCoV booster (Fig. 5E). However, comparable effector T cells counts against ancestral-, Delta- and Omicron-spike were observed in OBIPs (Fig. 5E). Additionally, effector T cells against ancestral-NP were increased in individuals after both booster and OBIPs (Fig. 5F). Among them, OBIPs obtained higher effector T cells response towards ancestral-NP, which were conserved between SARS-CoV-2 VOCs, than HVs following InV booster and Ad5-nCoV booster (Fig. 5F). These data indicate that Ad5-nCoV booster and OBI might lead to higher effector T cells against ancestral-, Delta- and Omicron-spike compared to InV booster. Principally, OBI could also induce higher effector T cells against other key protein of SARS-CoV-2 NP, which are conserved between different VOCs (Kared et al., 2022).

4. Discussion

During the study, comparative analysis demonstrated that Ancestral strain, Delta and Omicron specific binding antibody, MBCs and effector T cells were significantly increased in InV and Ad5-nCoV boosted individuals and OBIPs relative to pre-boosted HVs. Moreover, antibody IgG against ancestral-spike and ancestral-RBD, Delta-RBD, and Omicron-RBD in OBIPs and Ad5-nCoV boosted individuals was comparable and is higher than that in InV boosted individuals. However, the degree of reduction of anti-Delta-RBD- and anti-Omicron-RBD-IgG titer relative to anti-ancestral-RBD-IgG titer in InV boosted individuals was higher than that in Ad5-nCoV boosted individuals and OBIPs, indicating lower antibody affinity maturation potential induced by InV booster relative to Ad5-nCoV booster and OBI (Pape et al., 2021). In agreement with previous study which indicates that SARS-CoV-2 infection is a stronger stimulus for affinity maturation than a single mRNA vaccination (Pape et al., 2021; Sokal et al., 2021), our study showed that OBI might induce stronger MBCs directed to Omicron-RBD than that Ad5-nCoV and InV booster. These stronger Omicron-RBD specific MBCs will benefit the rapid generation of ASCs against the evolving Omicron variants, which finally will contribute to controlling the Omicron infection in the future (Goel et al., 2022).

OBI induced higher binding antibody titers against ancestral-RBD and Delta-RBD compared to that against Omicron-RBD. Consistently, previous studies demonstrated Omicron breakthrough infection induced higher neutralizing antibody titer against Delta than that against Omicron variants as well (Khan et al., 2022). This phenomenon is designated as original antigenic sin, which were reported in some study on the SARS-CoV-2 vaccination or infection (Kong et al., 2022). In addition, recent study demonstrated SARS-CoV-2 infection are more likely to elicit antigenic sin-like antibodies relative to mRNA vaccine administration (Anderson et al., 2022). The underlying mechanism might be related to previous vaccination or infection induced pre-activated memory B cells, which outcompete mutated variants specific naïve B cell after new mutated variants breakthrough infection, thereby boosting high antibody against conserved epitopes (van Zelm, 2022). Compared to Omicron variants spike, Delta variants spike is more similar to ancestral spike. Therefore, the antibody titers against ancestral spike and Delta variants spike was higher than that directed to omicron variants after omicron variant breakthrough infection.

In line with previous study on COVID-19 patients and mRNA vaccine inoculated healthy individuals (Farkash et al., 2021; Luo et al., 2021), SARS-CoV-2 spike trimer specific IgG subclass mainly consist of IgG1 and IgG3 in both vaccine boosted individuals and OBIPs, while IgG2 and IgG4 were barely detected. These increased IgG subclass (IgG1 and IgG3) confer increased capability to engage FcγR pathways, therefore contributes to great antiviral inflammatory reaction in our OBIPs with mild symptoms (Farkash et al., 2021). Besides, the post-translational modifications of IgG Fc region have critical roles in the immunity, such as a trend of higher levels of IgG1 with a fucosylated Fc glycans was observed in severe COVID-19 patients (Chakraborty et al., 2021). Interestingly, elevation in IgG1 sialylation, fucosylation, and galactosylation was found in mRNA vaccinated healthy individuals, which was positively related to the higher anti-SARS-CoV-2 IgG titers (Farkash et al., 2021). Therefore, modification of IgG sub-class in InV boosted and Ad5-nCoV boosted individual still need to be explored further.

Phenotypic analysis indicated that uMBC, and sMBC number was increased in InV boosted individuals, of them c-uMBC, and c-sMBC number, but not act-uMBC and act-sMBC, was enhanced compared to pre-boosted individuals. Correspondingly, recent study has shown that $CD71^+$ RBD specific MBCs percentage at 1 month, after third dose of mRNA vaccine was lower than that after second dose of mRNA vaccine (Muecksch et al., 2022). This phenomenon might be linked to rapid maturation of actMBCs as resting MBCs or lower level of actMBCs at ~32 days post InV booster (Sokal et al., 2021). By contrast, Ad5-nCoV booster and OBI induced higher frequency and total number of uMBCs and sMBCs, among them act-sMBCs number was markedly higher in Ad5-nCoV boosted individuals and OBIPs compared to pre-boosted individuals and InV boosted individuals. Given uMBCs are a transition stage from naïve MBCs to sMBCs (Moroney et al., 2020), the significant enhancement of uMBCs and sMBCs in Ad5-nCoV boosted individuals and OBIPs suggests higher B cell immunity might be induced. While comparison with pre-boosted individuals and InV boosted individuals, Ad5-nCoV boosted individuals and OBIPs obtain higher cMBCs and aMBCs, which are pre-disposed to differentiate into antigen specific antibody-secreting cells upon re-exposure of antigen (Sutton et al.,

Fig. 4. Analysis of the frequency and numbers of B cells and related phenotypes in vaccinated healthy volunteers (HVs) at median of 33 days post inactivated vaccine (InV) booster and 30.5 days post Ad5-nCoV booster, and OBIPs at median of 32 days post infection by comparison with pre-boosted HVs. **A** The frequency of total $CD19^+$ B cells among lymphocyte, classical memory B cells (cMBC, $CD27^+CD21^+$), activated memory B cells (aMBC, $CD27^+CD21^{-/low}$), atypical memory B cells (atMBC, $CD27^-CD21^{-/low}$) among $CD19^+$ B cells and the number of each subsets were determined by flow cytometry analysis in pre-boosted HVs, InV boosted HVs, Ad5-nCoV boosted HVs, and OBIPs. **B** The lymphocyte numbers in vaccinated healthy volunteers (HVs) before third booster, at median of 33 days post InV booster and 30.5 days post Ad5-nCoV booster, and OBIPs at median of 32 days post infection. **C** The frequency of naïve B cells ($CD27^-IgD^+$), double negative B cells (DN, $CD27^-IgD^-$), switched memory B cells (sMBC, $CD27^+IgD^-$), un-switched memory B cells (uMBC, $CD27^+IgD^+$) and the number of each subsets in pre-boosted HVs, InV boosted HVs, Ad5-nCoV boosted HVs, and OBIPs. **D** The frequency of activated switched memory B cells (act-sMBC, $CD27^+IgD^-CD71^+$) and classical switched memory B cells (c-sMBC, $CD27^+IgD^-CD71^-$), activated un-switched memory B cells (act-uMBC, $CD27^+IgD^+CD71^+$) and classical un-switched memory B cells (c-uMBC, $CD27^+IgD^+CD71^-$) among $CD19^+$ B cells and the number of each subsets in pre-boosted HVs, InV boosted HVs, Ad5-nCoV boosted HVs, and OBIPs. Data presented as mean (standard deviation, SD) number per 10^6 peripheral blood mononuclear cells or frequency. Statistical analyses were performed by Mann-Whitney *U* test.

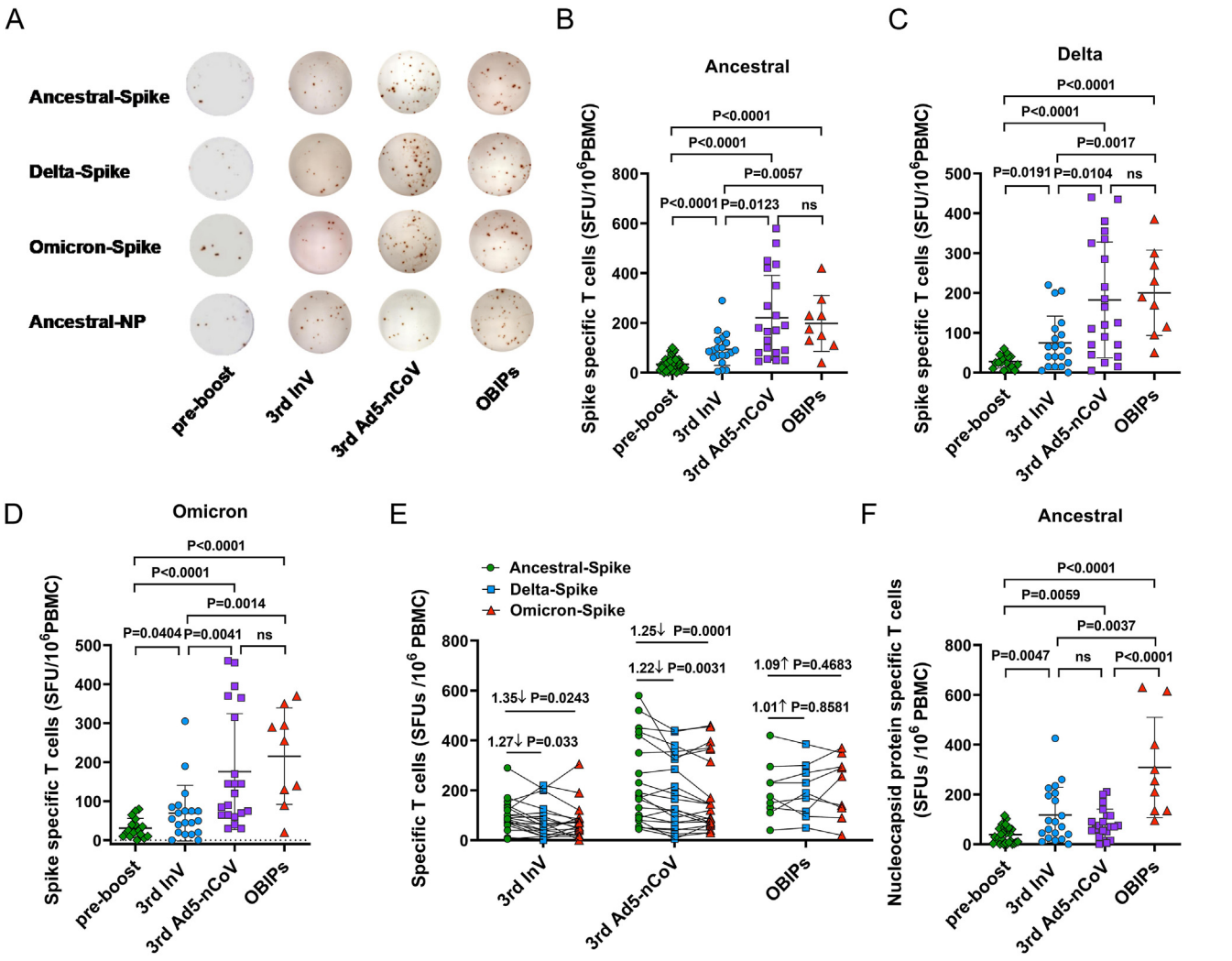

Fig. 5. Comparative analysis of SARS-CoV-2 specific effector T cells in vaccinated healthy volunteers (HVs) before third dose booster, at median of 33 days post inactivated vaccine (InV) booster and 30.5 days post Ad5-nCoV booster, and OBIPs at median of 32 days post infection using interferon-γ (IFNγ) enzyme-linked immunospot (ELISPOT) assay. **A** Representative images of ELISPOT wells of ancestral-Spike-specific, Delta-Spike-specific, Omicron-Spike-specific and ancestral nucleocapsid protein (NP) effector T cells in pre-boosted HVs, InV boosted HVs, Ad5-nCoV boosted HVs, and OBIPs. (**B–D**) ancestral-Spike-specific (**B**), Delta-Spike-specific (**C**), and Omicron-Spike-specific (**D**) effector T cells frequency in pre-boosted HVs, InV boosted HVs, Ad5-nCoV boosted HVs, and OBIPs. **E** Paired analysis of ancestral-Spike-specific, Delta-Spike-specific, and Omicron-Spike-specific effector T cells frequency in InV boosted HVs, Ad5-nCoV boosted HVs and OBIPs. **F** Ancestral NP-specific effector T cells frequency in pre-boosted HVs, InV boosted HVs, Ad5-nCoV boosted HVs, and OBIPs. Data presented as spots forming units per 10^6 peripheral blood mononuclear cells. Statistical analyses were performed by Mann-Whitney *U* test.

2021), indicating robust MBCs response of Ad5-nCoV booster and OBI. Previous study has shown that SARS-CoV-2 specific cMBCs and atMBCs markedly expanded after two doses of mRNA vaccine in SARS-CoV-2 infected subjects compared with SARS-CoV-2 naïve subjects (Pape et al., 2021). However, our study demonstrated total atMBCs frequency was declined in both vaccine boosted subjects and OBIPs. Additionally, atMBCs number in OBIPs, but not in both vaccine boosted individuals, decreased significantly compared to pre-boosted individuals. Accumulating data demonstrated ASCs from atMBC secrete lower level of IgM, IgG1 to IgG3 than ASCs from cMBC (Hopp et al., 2022). Moreover, atMBC are supposed to be exhausted due to overexpression of a large range of inhibitory receptors that reduce their effector functions and proliferative potential (Dauby et al., 2014). Additionally, atMBC are expanded in COVID-19 patients, with significantly higher proportion of atMBCs in patients who died than in those who survived (Oliviero et al., 2020). Herein, decrease of atMBCs induced by both vaccine booster and OBI, especially significantly decline of atMBCs number in OBIPs, along with enhance of cMBCs and aMBCs in Ad5-nCoV boosted individuals and OBIPs indicate Ad5-nCoV and OBI induce robust effector MBCs.

Here, our study demonstrated that Ad5-nCoV booster elicited higher effector T cells against ancestral-, Delta- and Omicron-spike compared to InV booster, along with previous study indicating Ad5-nCoV is a potential alternative for generating robust T cell immunity against SARS-CoV-2 (Zhang et al., 2022). It should be mentioned that InV could induce cellular immunity targeted to spike, NP and other non-structural proteins (Mok et al., 2022). Interestingly, OBI leads to comparable effect on T cells against ancestral-, Delta- and Omicron-spike with Ad5-nCoV booster, and stronger than InV booster. Moreover, effector T cells directed to NP was also robustly elicited in OBIPs relative to Ad5-nCoV and InV booster. It is plausible that other conserved non-structural proteins specific effect T cells might been effectively stimulated by OBI, compared to vaccine booster (Choi et al., 2022; Kared et al., 2022). Therefore, these enhanced T cell immunity against multiple conserved peptide from multiple target protein of SARS-CoV-2 will be of great importance for restricting novel mutated SARS-CoV-2 infection in the future (Koutsakos et al., 2022; Wang C. Y. et al., 2022).

Limitations to this study include low sample size and the age of Ad5-nCoV booster group was significantly lower than InV booster group and

OBIPs. Previous studies showed that age had some degree of influence on the immunogenicity after SARS-CoV-2 vaccination (Toapanta-Yanchapaxi et al., 2022; Wang F. et al., 2022). The negative effects were apparent for age above 80 years when the immunity response was not greatly induced by first dose of mRNA vaccine (Toapanta-Yanchapaxi et al., 2022). However, the heterogeneous immunogenicity related to age was diminished after second dose of mRNA booster (Faro-Viana et al., 2022) and homologous inactivated vaccine booster (Xin et al., 2022), which stimulate higher immunitiy response. In addition, we and others consistantly proved Ad5-nCoV booster on top of two doses inactivated vaccine scheme had higher antibody titers against AS and Omicron variants compared to homolougous inactivated vaccine booster in age matched population (Kong et al., 2022; Li et al., 2022), indicating age difference have little influence on the conclusion on the antibody in our study. But, the younger HVs in Ad5-nCoV booster group might bias to higher SARS-CoV-2 specific effect T cell counts after two doses of incativated vacinne than that in InV booster group (Channappanavar and Perlman, 2020), as shown in our study for ancestral strain-NP specific effector T cells.

5. Conclusions

Conclusively, the Ad5-nCoV booster might leads to stronger SARS-CoV-2 ancestral strain spike specific memory B and T cells immunity response than InV booster. Moreover, Ad5-nCoV booster induce comparable Omicron variants specific T cells immunity to that in OBIPs. Compared to both vaccine boosted healthy individuals, OBIPs could acquire higher memory B cells immunity against Omicron variant.

Data availability

All the data generated during the current study are included in the manuscript.

Ethics statement

This study was approved by the Ethics Committee of the Second Affiliated Hospital of Guangzhou Medical University (Approval No. 2021-hs-43) and Dongguan Ninth People's Hospital (Approval No. 2022-8). The informed consent was obtained from all participants before participating in this clinical trial.

Authors' contributions

Pei Yu: investigation, data curation, and clinical sample collection, Zijian Liu: investigation, Zhuoqi Zhu: data curation, and clinical sample collection, Jiaqing Yang: investigation, Min Deng: investigation, Mingxiao Chen: investigation, Changchun Lai: investigation, Weiya Kong: investigation, Shilong Xiong: investigation, Li Wan: investigation, Weikang Mai: investigation, Lu Chen: investigation, Yu Lei: investigation, Shahzad Akbar Khan: writing - review&editing, Jianfeng Ruan investigation and clinical sample collection, An Kang: clinical sample collection, Xuguang Guo: writing - review&editing Qiang Zhou: methodology, Wenrui Li: clinical sample collection, Zheng Chen: conceptualization and supervision, Yuemei Liang: clinical sample collection and supervision, Pingchao Li: methodology and supervision, Lei Zhang: clinical sample collection and supervision, Tianxing Ji: methodology, conceptualization, writing-review&editing and supervision.

Conflict of interest

All authors declare that there is no competing interests.

Acknowledgment

We thank all participants in this study. This study received the following funding: Guangzhou Health Science and Technology Project (20201A011078), Guangzhou Science and Technology Project (202102010094), Guangdong Basic and Applied Basic Research Foundation (2021A1515012550), Clinical research project of Guangzhou Medical University Second Affiliated Hospital (2021-LCYJ-05), Guangdong Medical Research Fund (A2022255), Key Clinical Specialty of Guangzhou Medical University (0F03031), Guangzhou Laboratory (EKPG21-30-3), and Guangzhou key discipline of urology. The funding sources had no role in the study design, data collection, analysis, interpretation, or writing of the report.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.virs.2022.12.008.

References

Anderson, E.M., Li, S.H., Awofolaju, M., Eilola, T., Goodwin, E., Bolton, M.J., Gouma, S., Manzoni, T.B., Hicks, P., Goel, R.R., Painter, M.M., Apostolidis, S.A., Mathew, D., Dunbar, D., Fiore, D., Brock, A., Weaver, J., Millar, J.S., DerOhannessian, S., Unit, U.P.C.P., Greenplate, A.R., Frank, I., Rader, D.J., Wherry, E.J., Bates, P., Hensley, S.E., 2022. SARS-CoV-2 infections elicit higher levels of original antigenic sin antibodies compared with SARS-CoV-2 mRNA vaccinations. Cell Rep. 41, 111496.

Assawakosri, S., Kanokudom, S., Suntronwong, N., Auphimai, C., Nilyanimit, P., Vichaiwattana, P., Thongmee, T., Duangchinda, T., Chantima, W., Pakchotanon, P., Srimuan, D., Thatsanatorn, T., Klinfueng, S., Yorsang, R., Sudhinaraset, N., Wanlapakorn, N., Mongkolsapaya, J., Honsawek, S., Poovorawan, Y., 2022. Neutralizing activities against the omicron variant after a heterologous booster in healthy adults receiving two doses of coronavac vaccination. J. Infect. Dis. 17, 1372–1381.

Chakraborty, S., Gonzalez, J., Edwards, K., Mallajosyula, V., Buzzanco, A.S., Sherwood, R., Buffone, C., Kathale, N., Providenza, S., Xie, M.M., Andrews, J.R., Blish, C.A., Singh, U., Dugan, H., Wilson, P.C., Pham, T.D., Boyd, S.D., Nadeau, K.C., Pinsky, B.A., Zhang, S., Memoli, M.J., Taubenberger, J.K., Morales, T., Schapiro, J.M., Tan, G.S., Jagannathan, P., Wang, T.T., 2021. Proinflammatory IgG Fc structures in patients with severe COVID-19. Nat. Immunol. 22, 67–73.

Channappanavar, R., Perlman, S., 2020. Age-related susceptibility to coronavirus infections: role of impaired and dysregulated host immunity. J. Clin. Invest. 130, 6204–6213.

Choi, S.J., Kim, D.U., Noh, J.Y., Kim, S., Park, S.H., Jeong, H.W., Shin, E.C., 2022. T cell epitopes in SARS-CoV-2 proteins are substantially conserved in the Omicron variant. Cell. Mol. Immunol. 19, 447–448.

Costa Clemens, S.A., Weckx, L., Clemens, R., Almeida Mendes, A.V., Ramos Souza, A., Silveira, M.B.V., da Guarda, S.N.F., de Nobrega, M.M., de Moraes Pinto, M.I., Gonzalez, I.G.S., Salvador, N., Franco, M.M., de Avila Mendonca, R.N., Queiroz Oliveira, I.S., de Freitas Souza, B.S., Fraga, M., Aley, P., Bibi, S., Cantrell, L., Dejnirattisai, W., Liu, X., Mongkolsapaya, J., Supasa, P., Screaton, G.R., Lambe, T., Voysey, M., Pollard, A.J., Rhhs, team, 2022. Heterologous versus homologous COVID-19 booster vaccination in previous recipients of two doses of coronavac COVID-19 vaccine in Brazil (RHH-001): a phase 4, non-inferiority, single blind, randomised study. Lancet 399, 521–529.

Dauby, N., Kummert, C., Lecomte, S., Liesnard, C., Delforge, M.L., Donner, C., Marchant, A., 2014. Primary human cytomegalovirus infection induces the expansion of virus-specific activated and atypical memory B cells. J. Infect. Dis. 210, 1275–1285.

Farkash, I., Feferman, T., Cohen-Saban, N., Avraham, Y., Morgenstern, D., Mayuni, G., Barth, N., Lustig, Y., Miller, L., Shouval, D.S., Biber, A., Kirgner, I., Levin, Y., Dahan, R., 2021. Anti-SARS-CoV-2 antibodies elicited by COVID-19 mRNA vaccine exhibit a unique glycosylation pattern. Cell Rep. 37, 110114.

Faro-Viana, J., Bergman, M.L., Goncalves, L.A., Duarte, N., Coutinho, T.P., Borges, P.C., Diwo, C., Castro, R., Matoso, P., Malheiro, V., Brennand, A., Kosack, L., Akpogheneta, O., Figueira, J.M., Cardoso, C., Casaca, A.M., Alves, P.M., Nunes, T., Penha-Goncalves, C., Demengeot, J., 2022. Population homogeneity for the antibody response to COVID-19 BNT162b2/comirnaty vaccine is only reached after the second dose across all adult age ranges. Nat. Commun. 13, 140.

GeurtsvanKessel, C.H., Geers, D., Schmitz, K.S., Mykytyn, A.Z., Lamers, M.M., Bogers, S., Scherbeijn, S., Gommers, L., Sablerolles, R.S.G., Nieuwkoop, N.N., Rijsbergen, L.C., van Dijk, L.L.A., de Wilde, J., Alblas, K., Breugem, T.I., Rijnders, B.J.A., de Jager, H., Weiskopf, D., van der Kuy, P.H.M., Sette, A., Koopmans, M.P.G., Grifoni, A., Haagmans, B.L., de Vries, R.D., 2022. Divergent SARS-CoV-2 Omicron-reactive T and B cell responses in COVID-19 vaccine recipients. Sci Immunol 7, eabo2202.

Goel, R.R., Painter, M.M., Lundgreen, K.A., Apostolidis, S.A., Baxter, A.E., Giles, J.R., Mathew, D., Pattekar, A., Reynaldi, A., Khoury, D.S., Gouma, S., Hicks, P., Dysinger, S., Hicks, A., Sharma, H., Herring, S., Korte, S., Kc, W., Oldridge, D.A., Erickson, R.I., Weirick, M.E., McAllister, C.M., Awofolaju, M., Tanenbaum, N., Dougherty, J., Long, S., D'Andrea, K., Hamilton, J.T., McLaughlin, M., Williams, J.C., Adamski, S., Kuthuru, O., Drapeau, E.M., Davenport, M.P., Hensley, S.E., Bates, P., Greenplate, A.R., Wherry, E.J., 2022. Efficient recall of Omicron-reactive B cell memory after a third dose of SARS-CoV-2 mRNA vaccine. Cell 185, 1875–1887. e8.

Hopp, C.S., Skinner, J., Anzick, S.L., Tipton, C.M., Peterson, M.E., Li, S., Doumbo, S., Kayentao, K., Ongoiba, A., Martens, C., Traore, B., Crompton, P.D., 2022. Atypical B

cells up-regulate costimulatory molecules during malaria and secrete antibodies with T follicular helper cell support. Sci Immunol 7, eabn1250.
Jahnmatz, M., Kesa, G., Netterlid, E., Buisman, A.M., Thorstensson, R., Ahlborg, N., 2013. Optimization of a human IgG B-cell elispot assay for the analysis of vaccine-induced B-cell responses. J. Immunol. Methods 391, 50–59.
Jung, M.K., Jeong, S.D., Noh, J.Y., Kim, D.U., Jung, S., Song, J.Y., Jeong, H.W., Park, S.H., Shin, E.C., 2022. BNT162b2-induced memory T cells respond to the Omicron variant with preserved polyfunctionality. Nat Microbiol 7, 909–917.
Kared, H., Wolf, A.S., Alirezaylavasani, A., Ravussin, A., Solum, G., Tran, T.T., Lund-Johansen, F., Vaage, J.T., Nissen-Meyer, L.S., Nygaard, U.C., Hungnes, O., Robertson, A.H., Naess, L.M., Trogstad, L., Magnus, P., Munthe, L.A., Mjaaland, S., 2022. Immune responses in Omicron SARS-CoV-2 breakthrough infection in vaccinated adults. Nat. Commun. 13, 4165.
Kedzierska, K., Thomas, P.G., 2022. Count on us: T cells in SARS-CoV-2 infection and vaccination. Cell Rep Med 3, 100562.
Khan, K., Karim, F., Cele, S., Reedoy, K., San, J.E., Lustig, G., Tegally, H., Rosenberg, Y., Bernstein, M., Jule, Z., Ganga, Y., Ngcobo, N., Mazibuko, M., Mthabela, N., Mhlane, Z., Mbatha, N., Miya, Y., Giandhari, J., Ramphal, Y., Naidoo, T., Sivro, A., Samsunder, N., Kharsany, A.B.M., Amoako, D., Bhiman, J.N., Manickchund, N., Abdool Karim, Q., Magula, N., Abdool Karim, S.S., Gray, G., Hanekom, W., von Gottberg, A., Team, C.-K., Milo, R., Gosnell, B.I., Lessells, R.J., Moore, P.L., de Oliveira, T., Moosa, M.S., Sigal, A., 2022. Omicron infection enhances Delta antibody immunity in vaccinated persons. Nature 607, 356–359.
Kong, W., Zhong, Q., Chen, M., Yu, P., Xu, R., Zhang, L., Lai, C., Deng, M., Zhou, Q., Xiong, S., Liang, Y., Wan, L., Lin, M., Wang, M., Mai, W., Chen, L., Lei, Y., Qin, N., Zhu, J., Ruan, J., Huang, Q., Kang, A., Wang, J., Li, W., Ji, T., 2022. Ad5-nCoV booster and Omicron variant breakthrough infection following two doses of inactivated vaccine elicit comparable antibody levels against Omicron variants. J. Med. 95, e28163.
Koutsakos, M., Lee, W.S., Reynaldi, A., Tan, H.X., Gare, G., Kinsella, P., Liew, K.C., Taiaroa, G., Williamson, D.A., Kent, H.E., Stadler, E., Cromer, D., Khoury, D.S., Wheatley, A.K., Juno, J.A., Davenport, M.P., Kent, S.J., 2022. The magnitude and timing of recalled immunity after breakthrough infection is shaped by SARS-CoV-2 variants. Immunity 55, 1316–1326.e4.
Li, J., Hou, L., Guo, X., Jin, P., Wu, S., Zhu, J., Pan, H., Wang, X., Song, Z., Wan, J., Cui, L., Li, J., Chen, Y., Wang, X., Jin, L., Liu, J., Shi, F., Xu, X., Zhu, T., Chen, W., Zhu, F., 2022. Heterologous Ad5-nCoV plus coronavac versus homologous coronavac vaccination: a randomized phase 4 trial. Nat. Med. 28, 401–409.
Luo, H., Jia, T., Chen, J., Zeng, S., Qiu, Z., Wu, S., Li, X., Lei, Y., Wang, X., Wu, W., Zhang, R., Zou, X., Feng, T., Ding, R., Zhang, Y., Chen, Y.Q., Sun, C., Wang, T., Fang, S., Shu, Y., 2021. The characterization of disease severity associated IgG subclasses response in COVID-19 patients. Front. Immunol. 12, 632814.
Melo-Gonzalez, F., Soto, J.A., Gonzalez, L.A., Fernandez, J., Duarte, L.F., Schultz, B.M., Galvez, N.M.S., Pacheco, G.A., Rios, M., Vazquez, Y., Rivera-Perez, D., Moreno-Tapia, D., Iturriaga, C., Vallejos, O.P., Berrios-Rojas, R.V., Hoppe-Elsholz, G., Urzua, M., Bruneau, N., Fasce, R.A., Mora, J., Grifoni, A., Sette, A., Weiskopf, D., Zeng, G., Meng, W., Gonzalez-Aramundiz, J.V., Gonzalez, P.A., Abarca, K., Ramirez, E., Kalergis, A.M., Bueno, S.M., 2021. Recognition of variants of concern by antibodies and T cells induced by a SARS-CoV-2 inactivated vaccine. Front. Immunol. 12, 747830.
Mok, C.K.P., Cohen, C.A., Cheng, S.M.S., Chen, C., Kwok, K.O., Yiu, K., Chan, T.O., Bull, M., Ling, K.C., Dai, Z., Ng, S.S., Lui, G.C., Wu, C., Amarasinghe, G.K., Leung, D.W., Wong, S.Y.S., Valkenburg, S.A., Peiris, M., Hui, D.S., 2022. Comparison of the immunogenicity of BNT162b2 and coronavac COVID-19 vaccines in Hong Kong. Respirology 27, 301–310.
Moroney, J.B., Vasudev, A., Pertsemlidis, A., Zan, H., Casali, P., 2020. Integrative transcriptome and chromatin landscape analysis reveals distinct epigenetic regulations in human memory B cells. Nat. Commun. 11, 5435.
Muecksch, F., Wang, Z., Cho, A., Gaebler, C., Ben Tanfous, T., DaSilva, J., Bednarski, E., Ramos, V., Zong, S., Johnson, B., Raspe, R., Schaefer-Babajew, D., Shimeliovich, I., Daga, M., Yao, K.H., Schmidt, F., Millard, K.G., Turroja, M., Jankovic, M., Oliveira, T.Y., Gazumyan, A., Caskey, M., Hatziioannou, T., Bieniasz, P.D., Nussenzweig, M.C., 2022. Increased memory B cell potency and breadth after a SARS-CoV-2 mRNA boost. Nature 607, 128–134.
Oliviero, B., Varchetta, S., Mele, D., Mantovani, S., Cerino, A., Perotti, C.G., Ludovisi, S., Mondelli, M.U., 2020. Expansion of atypical memory B cells is a prominent feature of COVID-19. Cell. Mol. Immunol. 17, 1101–1103.
Pape, K.A., Dileepan, T., Kabage, A.J., Kozysa, D., Batres, R., Evert, C., Matson, M., Lopez, S., Krueger, P.D., Graiziger, C., Vaughn, B.P., Shmidt, E., Rhein, J., Schacker, T.W., Khoruts, A., Jenkins, M.K., 2021. High-affinity memory B cells induced by SARS-CoV-2 infection produce more plasmablasts and atypical memory B cells than those primed by mRNA vaccines. Cell Rep. 37, 109823.
Perez-Perez, G.I., Maw, A.M., Feingold-Link, L., Gunn, J., Bowers, A.L., Minano, C., Rautelin, H., Kosunen, T.U., Blaser, M.J., 2010. Longitudinal analysis of serological responses of adults to helicobacter pylori antigens. J. Infect. Dis. 202, 916–923.
Pinna, D., Corti, D., Jarrossay, D., Sallusto, F., Lanzavecchia, A., 2009. Clonal dissection of the human memory B-cell repertoire following infection and vaccination. Eur. J. Immunol. 39, 1260–1270.
Quandt, J., Muik, A., Salisch, N., Lui, B.G., Lutz, S., Kruger, K., Wallisch, A.K., Adams-Quack, P., Bacher, M., Finlayson, A., Ozhelvaci, O., Vogler, I., Grikscheit, K., Hoehl, S., Goetsch, U., Ciesek, S., Tureci, O., Sahin, U., 2022. Omicron BA.1 breakthrough infection drives cross-variant neutralization and memory B cell formation against conserved epitopes. Sci Immunol, eabq2427.
Sakharkar, M., Rappazzo, C.G., Wieland-Alter, W.F., Hsieh, C.L., Wrapp, D., Esterman, E.S., Kaku, C.I., Wec, A.Z., Geoghegan, J.C., McLellan, J.S., Connor, R.I., Wright, P.F., Walker, L.M., 2021. Prolonged evolution of the human B cell response to SARS-CoV-2 infection. Sci Immunol 6, eabg6916.
Sette, A., Crotty, S., 2021. Adaptive immunity to SARS-CoV-2 and COVID-19. Cell 184, 861–880.
Sokal, A., Barba-Spaeth, G., Fernandez, I., Broketa, M., Azzaoui, I., de La Selle, A., Vandenberghe, A., Fourati, S., Roeser, A., Meola, A., Bouvier-Alias, M., Crickx, E., Languille, L., Michel, M., Godeau, B., Gallien, S., Melica, G., Nguyen, Y., Zarrouk, V., Canoui-Poitrine, F., Pirenne, F., Megret, J., Pawlotsky, J.M., Fillatreau, S., Bruhns, P., Rey, F.A., Weill, J.C., Reynaud, C.A., Chappert, P., Mahevas, M., 2021. mRNA vaccination of naive and COVID-19-recovered individuals elicits potent memory B cells that recognize SARS-CoV-2 variants. Immunity 54, 2893–2907 e2895.
Sutton, H.J., Aye, R., Idris, A.H., Vistein, R., Nduati, E., Kai, O., Mwacharo, J., Li, X., Gao, X., Andrews, T.D., Koutsakos, M., Nguyen, T.H.O., Nekrasov, M., Milburn, P., Eltahla, A., Berry, A.A., Kc, N., Chakravarty, S., Sim, B.K.L., Wheatley, A.K., Kent, S.J., Hoffman, S.L., Lyke, K.E., Bejon, P., Luciani, F., Kedzierska, K., Seder, R.A., Ndungu, F.M., Cockburn, I.A., 2021. Atypical B cells are part of an alternative lineage of B cells that participates in responses to vaccination and infection in humans. Cell Rep. 34, 108684.
Toapanta-Yanchapaxi, L., Chiquete, E., Avila-Rojo, E., Lopez-Yanez, S., Luna Del Villar Velasco, S., Rivera Monroy, S., Lopez Gomez, T., Andres Aguilar, J.B., Balcazar Antonio, D.F., Alcaraz-Fuerte, C., Garcia Baysa, M., Lopez Jimenez, J.L., Marquez-Guillen, E., Vilatoba, M., Cruz-Martinez, R., Carpinteyro-Espin, P., Chavez-Villa, M., Romero Morelos, R.D., Torres-Del Real, D., Uscanga-Dominguez, L.F., Garcia-Alanis, M., Tapia Sosa, R., Servin-Rojas, M., Valdez-Echeverria, R.D., Garcia-Juarez, I., 2022. Humoral response to different SARS-CoV-2 vaccines in orthotopic liver transplant recipients. Vaccine 40, 5621–5630.
Tregoning, J.S., Flight, K.E., Higham, S.L., Wang, Z., Pierce, B.F., 2021. Progress of the COVID-19 vaccine effort: viruses, vaccines and variants versus efficacy, effectiveness and escape. Nat. Rev. Immunol. 21, 626–636.
Tseng, H.F., Ackerson, B.K., Luo, Y., Sy, L.S., Talarico, C.A., Tian, Y., Bruxvoort, K.J., Tubert, J.E., Florea, A., Ku, J.H., Lee, G.S., Choi, S.K., Takhar, H.S., Aragones, M., Qian, L., 2022. Effectiveness of mRNA-1273 against SARS-CoV-2 Omicron and Delta variants. Nat. Med. 28, 1063–1071.
van Zelm, M.C., 2022. Immune memory to SARS-CoV-2 Omicron BA.1 breakthrough infections: to change the vaccine or not? Sci Immunol 7, eabq5901.
Wang, C.Y., Hwang, K.P., Kuo, H.K., Peng, W.J., Shen, Y.H., Kuo, B.S., Huang, J.H., Liu, H., Ho, Y.H., Lin, F., Ding, S., Liu, Z., Wu, H.T., Huang, C.T., Lee, Y.J., Liu, M.C., Yang, Y.C., Lu, P.L., Tsai, H.C., Lee, C.H., Shi, Z.Y., Liu, C.E., Liao, C.H., Chang, F.Y., Chen, H.C., Wang, F.D., Hou, K.L., Cheng, J., Wang, M.S., Yang, Y.T., Chiu, H.C., Jiang, M.H., Shih, H.Y., Shen, H.Y., Chang, P.Y., Lan, Y.R., Chen, C.T., Lin, Y.L., Liang, J.J., Liao, C.C., Chou, Y.C., Morris, M.K., Hanson, C.V., Guirakhoo, F., Hellerstein, M., Yu, H.J., King, C.C., Kemp, T., Heppner, D.G., Monath, T.P., 2022. A multitope SARS-CoV-2 vaccine provides long-lasting B cell and T cell immunity against Delta and Omicron variants. J. Clin. Invest. 132, e157707.
Wang, F., Huang, B., Lv, H., Feng, L., Ren, W., Wang, X., Tang, L., Liu, Q., Wu, D., Zheng, H., An, Z., Deng, Y., Zhao, L., Ye, F., Wang, W., Zhang, H., Chang, S., Liao, Y., Chen, F., Rodewald, L.E., Gao, G.F., Yin, Z., Tan, W., 2022. Factors associated with neutralizing antibody levels induced by two inactivated COVID-19 vaccines for 12 months after primary series vaccination. Front. Immunol. 13, 967051.
Wang, K., Jia, Z., Bao, L., Wang, L., Cao, L., Chi, H., Hu, Y., Li, Q., Zhou, Y., Jiang, Y., Zhu, Q., Deng, Y., Liu, P., Wang, N., Wang, L., Liu, M., Li, Y., Zhu, B., Fan, K., Fu, W., Yang, P., Pei, X., Cui, Z., Qin, L., Ge, P., Wu, J., Liu, S., Chen, Y., Huang, W., Wang, Q., Qin, C.F., Wang, Y., Qin, C., Wang, X., 2022. Memory B cell repertoire from triple vaccinees against diverse SARS-CoV-2 variants. Nature 603, 919–925.
Woldemeskel, B.A., Garliss, C.C., Aytenfisu, T.Y., Johnston, T.S., Cox, A.L., Karaba, A.H., Blankson, J.N., 2022. Discordant antibody and T-cell responses to the severe acute respiratory syndrome coronavirus 2 Omicron variant in coronavirus disease 2019 messenger RNA vaccine recipients. Clin. Infect. Dis. 75, 1652–1654.
Xin, Q., Wu, Q., Chen, X., Han, B., Chu, K., Song, Y., Jin, H., Chen, P., Lu, W., Yang, T., Li, M., Zhao, Y., Pan, H., Yu, H., Wang, L., 2022. Six-month follow-up of a booster dose of coronavac in two single-centre phase 2 clinical trials. Nat. Commun. 13, 3100.
Xue, J.B., Lai, D.Y., Jiang, H.W., Qi, H., Guo, S.J., Zhu, Y.S., Xu, H., Zhou, J., Tao, S.C., 2022. Landscape of the RBD-specific IgG, IgM, and IgA responses triggered by the inactivated virus vaccine against the Omicron variant. Cell Discov 8, 15.
Zhang, Z., Wu, S., Liu, Y., Kailiang Li, K., Pengfei Fan, P., Song, X., Wang, Y., Zhao, Z., Zhang, X., Shang, J., Zhang, J., Xu, J., Li, Y., Li, Y., Zhang, J., Fu, K., Wang, B., Hao, M., Zhang, G., Long, P., Qiu, Z., Zhu, T., Liu, S., Zhang, Y., Shao, F., Lv, P., Yang, Y., Zhao, X., Sun, Y., Hou, L., Chen, W., 2022. Aerosolized Ad5-nCoV Booster Vaccination Elicited Potent Immune Response against the SARS-CoV-2 Omicron Variant after Inactivated COVID-19 Vaccine Priming. https://doi.org/10.1101/2022.03.08.22271816 medRxiv.