Durability and cross-reactive immune memory to SARS-CoV-2 in individuals 2 years after recovery from COVID-19: a longitudinal cohort study

Li Guo, Qiao Zhang*, Xiaoying Gu*, Lili Ren*, Tingxuan Huang, *Yanan Li*, Hui Zhang*, Ying Liu*, Jingchuan Zhong, Xinming Wang, Lan Chen, Yin Zhang, Danyang Li, Meiyu Fang, Liuhui Xu, Haibo Li, Zai Wang, Hui Li, Tao Bai, Wen Liu, Yanchun Peng, Tao Dong, Bin Cao†, Jianwei Wang†*

Summary

Background SARS-CoV-2-specific adaptive immunity more than 1 year after initial infection has not been well characterised. The aim of this study was to investigate the durability and cross-reactivity of immunological memory acquired from natural infection against SARS-CoV-2 in individuals recovered from COVID-19 2 years after infection.

Methods In this longitudinal cohort study, we recruited patients who had recovered from laboratory-confirmed COVID-19 and were discharged from Jinyintan Hospital (Wuhan, China) between Jan 7 and May 29, 2020. We carried out three successive follow-ups between June 16 and Sept 3, 2020 (6 months), Dec 16, 2020, and Feb 7, 2021 (1 year), and Nov 16, 2021, and Jan 10, 2022 (2 years), in which blood samples were taken. We included participants who did not have re-infection or receive a SARS-CoV-2 vaccination (infected–unvaccinated), and participants who received one to three doses of inactivated vaccine 1–2 years after infection (infected–vaccinated). We evaluated the presence of IgG antibodies, neutralising antibodies, and memory B-cell and memory T-cell responses against the prototype strain and delta and omicron variants.

Findings In infected–unvaccinated participants, neutralising antibody titres continually declined from 6-month to 2-year follow-up visits, with a half-life of about 141·2 days. Neutralising antibody responses to omicron sublineages (BA.1, BA.1.1, BA.2, BA.4/5, BF.7, BQ.1, and XBB) were poor. Memory B-cell responses to the prototype strain were retained at 2 years and presented cross-reactivity to the delta and omicron BA.1 variants. The magnitude of interferon γ and T-cell responses to SARS-CoV-2 were not significantly different between 1 year and 2 years after infection. Multifunctional T-cell responses against SARS-CoV-2 spike protein and nucleoprotein were detected in most participants. Recognition of the BA.1 variant by memory T cells was not affected in most individuals. The antibody titres and the frequencies of memory B cells, but not memory T cells, increased in infected–vaccinated participants after they received the inactivated vaccine.

Interpretation This study improves the understanding of the duration of SARS-CoV-2-specific immunity without boosting, which has implications for the design of vaccination regimens and programmes. Our data suggest that memory T-cell responses primed by initial viral infection remain highly cross-reactive after 2 years. With the increasing emergence of variants, effective vaccines should be introduced to boost neutralising antibody and overall T-cell responses to newly emerged SARS-CoV-2 variants.

Funding Chinese Academy of Medical Sciences, National Natural Science Foundation of China, Fundamental Research Funds for the Central Universities for Peking Union Medical College, Beijing Natural Science Foundation, UK Medical Research Council.

Lancet Microbe 2023

Published Online December 1, 2023 https://doi.org/10.1016/S2666-5247(23)00255-0

*Contributed equally as joint first authors

†Contributed equally as joint senior authors

National Health Commission Key Laboratory of Systems Biology of Pathogens and Christophe Mérieux Laboratory, National Institute of Pathogen Biology, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China (Prof L Guo PhD, Q Zhang PhD, Prof L Ren PhD, T Huang MS, Y Li MS, J Zhong MS, X Wang MS, L Chen BS, Y Zhang MS, D Li BS, M Fang BS, L Xu BS, Prof J Wang PhD)**; Key Laboratory of Respiratory Disease Pathogenomics** (Prof L Guo, Prof L Ren, Prof J Wang)**, Department of Clinical Research and Data Management** (X Gu PhD) **and Institute of Respiratory Medicine** (Prof B Cao MD)**, Chinese Academy of Medical Sciences, Beijing, China; Department of Pulmonary and Critical Care Medicine, National Center for Respiratory Medicine** (H Zhang MD, H Li MD, Z Wang MD, H Li MD, Prof B Cao) **and National Clinical Research Center for Respiratory Diseases** (X Gu)**, China–Japan Friendship Hospital, Beijing, China; Department of Respiratory and Critical Care Medicine, West China Hospital, Sichuan University, Chengdu, Sichuan, China** (T Huang, Y Zhang)**; Jinyintan Hospital, Wuhan, Hubei Province, China** (Y Liu MS, T Bai MD, W Liu MS)**; Medical Research Council Human Immunology Unit, Medical Research Council Weatherall Institute of Molecular Medicine, Radcliffe Department of Medicine,**

Introduction

Immunological memory, including circulating antibodies and memory B cells and T cells, is the basis of protective immunity against viral infection. SARS-CoV-2-specific antibodies develop rapidly after infection.[1] However, antibody titres against SARS-CoV-2 decline over time after clearance of SARS-CoV-2 infection.[2] SARS-CoV-2-specific T-cell responses are crucial for protecting against re-infection, providing durable immunological memory, and mediating the recognition of variants.[3] Since adaptive immunity is integral for protecting against viral infection, humoral and cellular immune responses to SARS-CoV-2 have been studied in great detail in convalescent patients and vaccinated individuals.[4–6] Several studies have shown that SARS-CoV-2-specific antibody and T-cell responses could persist up to 1 year after infection.[2,7,8]

With the rapid evolution of the SARS-CoV-2 viral genome, a succession of variants of concern (VOCs) have emerged. After the initial wave of infection by the omicron BA.1 strain,[9] more than 800 omicron sublineages have been identified as of Feb 10, 2023.[10,11] VOCs show increased transmissibility and antibody evasion from the

University of Oxford, Oxford, UK (Y Peng PhD, Prof T Dong PhD)**; Chinese Academy of Medical Science Oxford Institute, University of Oxford, Oxford, UK** (Y Peng, Prof T Dong, Prof B Cao, Prof J Wang)

Correspondence to: Prof Jianwei Wang, National Health Commission Key Laboratory of Systems Biology of Pathogens and Christophe Mérieux Laboratory, National Institute of Pathogen Biology, Chinese Academy of Medical Sciences & Peking Union Medical College, Dongcheng District, Beijing 100730, China
wangjw28@163.com

or

Prof Bin Cao, Department of Pulmonary and Critical Care Medicine, China–Japan Friendship Hospital, Chaoyang District, Beijing 100013, China
caobin_ben@163.com

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Research in context

Evidence before this study

We searched PubMed for longitudinal studies on the adaptive immunity against SARS-CoV-2, published between Jan 1, 2020, and Feb 10, 2023, without language restrictions, using the search terms [(SARS-CoV-2 OR COVID-19 OR Coronavirus Disease 2019 Virus OR 2019 nCoV) AND (Adaptive Immunity OR Adoptive Immunity OR Immunity, Cellular OR Cellular Immunity OR Humoral Immunity OR Immunity, Humoral)] AND (survivor OR recover OR persistent OR follow up OR discharge OR long term). We identified 68 studies reporting that in patients recovered from COVID-19, cellular immunity is maintained but antibodies and neutralising capacity declines several months after infection. Moreover, in recovered patients, SARS-CoV-2 variants of concern (VOCs) escape antibody-mediated neutralisation. By contrast, T cells from most recovered patients cross-react with these VOCs. However, longitudinal studies on humoral and cellular responses to SARS-CoV-2 are about 1 year; therefore, the durability of the adaptive immune response in recovered COVID-19 patients after natural infection over a longer period is poorly understood.

Added value of this study

To our knowledge, this is the longest cohort study on the durability of adaptive immunity against the prototype strain and cross-reactive immune memory to SARS-CoV-2 variants in patients recovered from natural infection without repeated infection. Our findings show that antibody and memory B-cell and T-cell immunities against the SARS-CoV-2 prototype are present in recovered patients 2 years after natural infection. Total SARS-CoV-2 T-cell responses remain able to recognise SARS-CoV-2 variants. Vaccination with an inactivated vaccine improved antibody titres and frequencies of memory B cells, but not memory T cells, in recovered patients.

Implications of all the available evidence

Our data were collected over 2 years from a unique cohort of individuals who had recovered from SARS-CoV-2 prototype strain infection and had not been repeatedly exposed after initial infection. Immunity against the prototype strain in recovered patients is retained 2 years after initial infection. Cross-reactive responses to newly emerged viral variants were observed with T cells but neutralising antibody responses were markedly impaired. Such a dataset is useful for understanding the duration of protection without boosting, and such data are now almost impossible to acquire as SARS-CoV-2 re-infection or vaccination (or both) is ubiquitous in most countries. Given the rapid evolution of SARS-CoV-2, boosting immunity to SARS-CoV-2 variants using effective vaccines is needed to control future SARS-CoV-2 variants. Continuous surveillance is required to assess the duration of infection-induced adaptive immunity and adaptive immunity to VOCs.

serum of vaccinated or naturally infected individuals.[12,13] These properties of rapid evolution emphasise why cross-reactive responses of memory B cells and T cells might be important in controlling severe illness in subsequent infections. Moreover, most individuals naturally infected by the prototype strain have received one to three doses of an inactivated vaccine in China. The distinctive histories of infection and vaccination result in heterogeneous immune-imprinted repertoires.[14] However, the durability of immunity and the ability to cross-protect against VOCs in individuals infected with the prototype strain, as well as in individuals infected with the prototype strain and subsequently vaccinated with an inactivated vaccine, remain poorly characterised.

In this study, we applied our ongoing analysis of individuals recovered from COVID-19 in Wuhan[2] to address the issues of adaptive immunity durability, inactivated vaccine boosting, and immune responses against the prototype strain and VOCs 2 years after SARS-CoV-2 natural infection.

Methods

Study design and participants

In this longitudinal cohort study, we included participants from a previously reported cohort study[15] who recovered from COVID-19 at Jinyintan Hospital (Wuhan, China) between Jan 7 and May 29, 2020. Participants were eligible if they were older than 18 years, had recovered from laboratory-confirmed COVID-19, and were discharged from the hospital between Jan 7 and May 29, 2020. Participants were excluded if they died after discharge; were living in a nursing or welfare home; had psychotic disorder, had dementia, or were re-admitted to hospital due to underlying diseases; or were immobile. All inclusion and exclusion criteria are provided in the appendix (p 2).

See **Online** for appendix

To control the COVID-19 epidemic, stringent action of the dynamic zero COVID policy was taken in China before December, 2022, including periodically carrying out nucleic acid tests, proactive case finding and isolation, tracing and quarantining of close contacts, physical distancing, and home-based quarantining. In such conditions, no participants were re-infected after they had recovered from the initial infection. Recovered patients who did not receive a COVID-19 vaccination (infected–unvaccinated participants), and those who had received one to three doses of inactivated vaccine 1–2 years after infection (infected–vaccinated participants) were asked to attend follow-up visits at Jinyintan Hospital between June 16 and Sept 3, 2020 (6-month follow-up), Dec 16, 2020, and Feb 7, 2021 (1-year follow-up), and Nov 16, 2021, and Jan 10, 2022 (2-year follow-up). Infected–vaccinated participants at the 2-year follow-up were matched (1:1) by age, sex, and

disease severity to infected–unvaccinated participants. Participants gave written informed consent. The study was approved by the institutional review boards of Jinyintan Hospital (KY-2020–80·01).

Procedures

Data on participants' sex were retrieved from electronic medical records in the acute phase and from the hospital's outpatient clinic by clinicians at follow-up visits. Data on participants' race were not collected. Disease severity was characterised by clinicians using the highest seven-category scale during hospital stay (appendix p 2). Clinicians collected 10 mL of venous blood from participants at 1-year and 2-year follow-up visits and processed samples within 12 h to isolate plasma and peripheral blood mononuclear cells (appendix p 2). Not all immunological tests could be done due to a shortage of adequate blood samples. Some samples with sufficient residual blood volume were randomly selected to evaluate the titres of neutralising antibodies and memory B-cell and T-cell responses. Titres of IgG antibodies against the spike protein, receptor-binding domain (RBD), and nucleoprotein of the prototype strain, as well as titres of IgG antibodies against the spike protein of B.1.617.2 (delta) and BA.1 variants, were measured using ELISA on the multifunctional microplate reader SpectraMax M5 (Molecular Devices, Sunnyvale, CA, USA; appendix pp 2–3). Neutralising antibodies against the prototype strain were analysed using an authentic virus microneutralisation assay (MNA; appendix p 3). Neutralising antibodies against the prototype strain and omicron variants (BA.1, BA.1·1, BA.2, BA.4/5, BF.7, BQ.1, and XBB) were measured using a pseudovirus MNA (appendix p 3). SARS-CoV-2-specific memory B-cell responses to the spike protein and RBD were assessed using the flow cytometric assay (appendix p 4).

To detect SARS-CoV-2-specific memory T-cell responses using the enzyme-linked immune absorbent spot (ELISpot) assay and intracellular cytokine staining (ICS; appendix pp 4–5), we designed 236 15–18-mer peptides that overlapped by ten amino acids and spanned the nucleoprotein and spike proteins, which targeted the prototype strain, 21 BA.1 spike protein-mutated region peptides, and 21 prototype spike protein-containing homologous peptides (purity >90% peptides were synthesised by Sangon Biotech, Shanghai, China). The peptide pool contained specific epitopes for cytomegalovirus, Epstein-Barr virus, and influenza viruses as positive controls. The original SARS-CoV-2 spike-specific B cells and RBD-specific B cells, and delta and omicron variant RBD-specific B cells were detected using biotinylated proteins in combination with different streptavidin-fluorophore conjugates on the flow cytometer (appendix p 4). Neutralising antibodies and memory B-cell and T-cell responses were analysed and stratified by the vaccine dose in infected–vaccinated participants. Too few participants had received three doses of vaccine for meaningful comparisons.

Outcomes

The primary outcomes were titres of neutralising antibodies at the 6-month, 1-year, and 2-year follow-ups, memory T-cell responses at the 1-year and 2-year follow-ups, and B-cell responses at the 2-year follow-up. The cutoff for the titre of neutralising antibodies against the authentic virus in MNA was 1:10 or was calculated as the 50% inhibitory dose (ID_{50}); the cutoff was 1:40 in the pseudovirus MNA. T-cell responses were expressed as the magnitude of interferon γ (IFNγ) production and the proportion of IL-2, IFNγ, and tumour necrosis factor-α (TNF-α) produced by SARS-CoV-2-specific CD4+ and CD8+ T cells. Memory B-cell responses were expressed as the proportion of SARS-CoV-2-specific memory B-cells in total B-cells and the proportion of variant RBD memory B-cells in prototype RBD memory B cells. Secondary outcomes included IgG titres against SARS-CoV-2, which were expressed as the area under the curve (AUC). Additional secondary outcomes were immune responses accounting for age (grouped by 18–44 years, 45–59 years, and 60–86 years), disease severity (moderate, severe, or critical), and vaccine doses (one or two). The outcomes reported here were not prespecified in the original cohort study.[15]

Statistical analysis

Demographic characteristics of participants were presented as median (IQR) for continuous variables and absolute values with percentages for categorical variables. Single comparisons between two independent groups were performed using the Mann-Whitney U test. Multiple comparisons of antibody titres at the 2-year follow-up were performed using the Kruskal-Wallis test followed by a post-hoc Dunn's test. Paired antibody and memory B-cell and T-cell responses at the 1-year and 2-year follow-ups were compared using a two-tailed Wilcoxon matched-pairs signed-rank test. Comparisons of paired neutralising antibody titres at the 6-month, 1-year, and 2-year follow-ups were performed using Friedman tests. We set neutralising antibody titres at 1:5 when measurements were below the limit of detection. Spearman correlation analysis was performed for single continuous univariate correlation analyses. A two-sided p value less than 0·05 was considered significant. The half-life of neutralising antibodies was estimated using one-phase decay exponential regression using all the paired neutralising antibody titres of infected–unvaccinated individuals at the 6-month, 1-month, and 2-year follow-ups (appendix p 5). Subgroups of participants were selected using simple randomisation from the total samples. Randomisation schedules were generated using PROC SURVEYSELECT in SAS (version 9.4). All statistical analyses were conducted using GraphPad Prism 9.5.

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

Results

1192 individuals attended a 2-year follow-up visit, of whom 195 were infected–unvaccinated participants with plasma samples available and matched with 195 infected–vaccinated participants (figure 1). We retrospectively found that 171 (88%) of 195 infected–unvaccinated participants and 195 (100%) of 195 infected–vaccinated participants had available plasma samples also at the 1-year follow-up. Among those with samples available at the 1-year follow-up, 156 (91%) of 171 infected–unvaccinated participants and 195 (100%) of 195 infected–vaccinated participants also had available plasma samples from the 6-month follow-up. None of the infected–vaccinated participants were vaccinated at the 6-month or 1-year follow-up. 96 plasma samples in infected–unvaccinated participants and 94 plasma samples in infected–vaccinated participants at 6 months, 1 year, and 2 years were selected using a simple randomisation method to evaluate the decay of neutralising antibodies. Baseline characteristics of the participants are shown in the table.

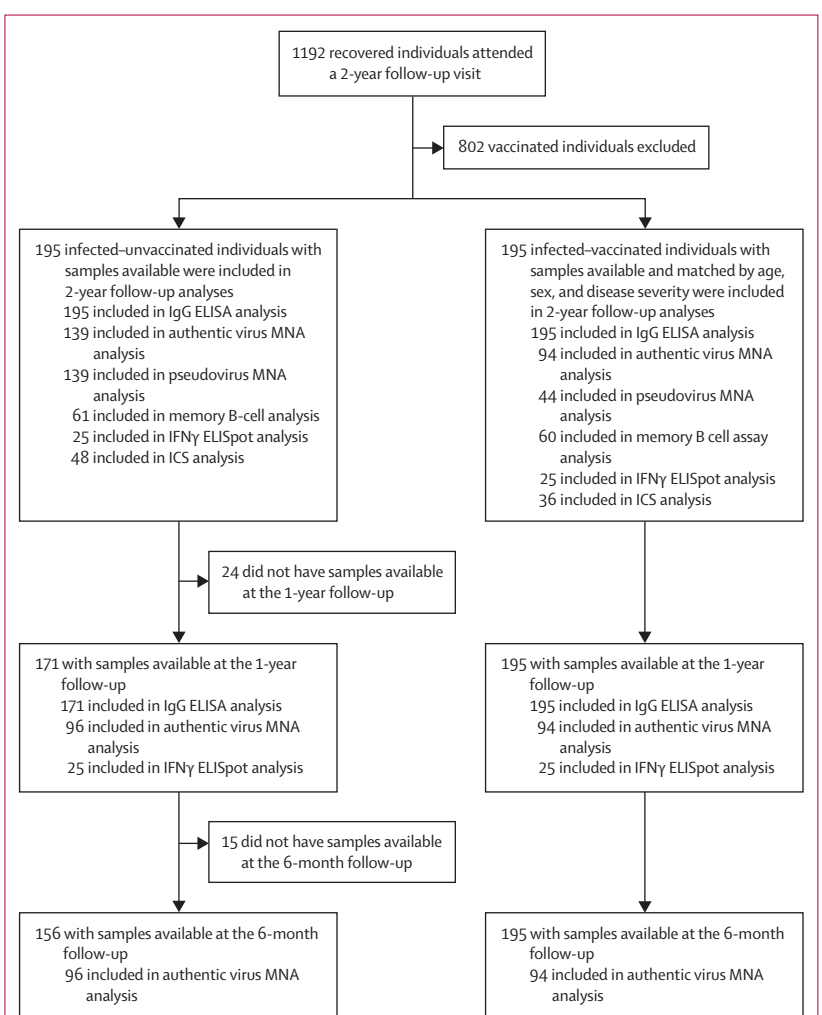

Figure 1: **Study flow chart**
ELISA=enzyme-linked immunosorbent assay. MNA=microneutralisation assay. ELISpot=enzyme-linked immunospot. ICS=intracellular cytokine staining.

Among the prototype spike-specific, RBD-specific, and nucleoprotein-specific IgG responses in infected–unvaccinated participants, spike IgG titres were the most stable over time, with 173 (89%) of 195 individuals being seropositive 2 years after infection (appendix p 7). In infected–unvaccinated participants, titres decreased between the 1-year and 2-year follow-ups for prototype spike-specific IgG (AUC geometric mean titre [GMT] 5579 *vs* 5153; p=0·017), RBD-specific IgG (4222 *vs* 3940; p=0·0032), and nucleoprotein-specific IgG (5073 *vs* 4436; p<0·0001; appendix p 10). At 2 years, binding capacity to the BA.1 spike protein was significantly lower than to the prototype spike protein (4471 *vs* 5153; p<0·0001; appendix p 10). Longitudinal data before and after vaccination in infected–vaccinated participants suggest that vaccination increased the titres of prototype spike-specific IgG (5430 *vs* 6388; p<0·0001), RBD-specific IgG (4266 *vs* 4500; p=0·0009), and nucleoprotein-specific IgG (5122 *vs* 7315; p<0·0001; appendix p 11). We observed a similar pattern in infected–vaccinated participants before and after vaccination for delta spike-specific IgG (5672 *vs* 6672; p<0·0001) and BA.1 spike-specific IgG (5082 *vs* 5565; p<0·0001; appendix p 11).

Of the 195 plasma samples from infected–unvaccinated participants at 2 years, 139 were used for MNA analysis. 96 (69%) of 139 samples were positive for neutralising antibodies against the prototype strain (GMT 1:14·2), independent of age (18–44 years *vs* 45–59 years, p=0·64; 18–44 years *vs* 60–86 years, p=0·28; 49–59 years *vs* 60–86 years, p=0·55; appendix p 12). Cross-sectional analysis of 2-year antibody responses showed that spike-specific IgG titres (r=0·60, p<0·0001) and RBD-specific IgG titres (r=0·66, p<0·0001) remained correlated with neutralising antibody titres (appendix p 12). Neutralisation in 96 infected–unvaccinated participants and 94 infected–vaccinated participants were measured using an authentic virus MNA at 6 months, 1 year, and 2 years after infection. In infected–unvaccinated participants, neutralising antibody titres decreased at the 1-year follow-up (GMT 1:16·6, p<0·0001) and further declined at the 2-year follow-up (1:12·4, p<0·0001) compared with the 6-month follow-up (1:26·5; figure 2A), with a half-life of about 141·2 days (95% CI 79·8–257·9; appendix p 13). Data from longitudinal plasma samples from infected–vaccinated participants at 6 months, 1 year, and 2 years after infection suggest that inactivated vaccines increased neutralising antibody titres (1:14·3 before *vs* 1:41·0 after vaccination, p<0·0001; appendix p 14). Neutralising antibody titres did not differ between

	Infected–unvaccinated participants				Infected–vaccinated participants							p value*
	Total (n=195)	Moderate illness (n=44)	Severe illness (n=133)	Critical illness (n=18)	Total (n=195)	Moderate illness (n=44)	Severe illness (n=134)	Critical illness (n=17)	One vaccine dose (n=61)	Two vaccine doses (n=122)	Three vaccine doses (n=12)	
Age, years	59 (49–68)	62 (51–70)	58 (48–68)	59 (52–69)	59 (48–65)	62 (49–67)	57 (48–64)	62 (55–66)	54 (46–64)	61 (52–65)	60 (52–65)	0·23
Sex												
Male	108 (55·4%)	24 (54·5%)	76 (57·1%)	10 (55·6%)	103 (52·8%)	23 (52·3%)	70 (52·2%)	10 (58·9%)	37 (60·7%)	59 (48·4%)	7 (58·3%)	0·68
Female	87 (44·6%)	20 (45·5%)	57 (42·9%)	8 (44·4%)	92 (47·2%)	21 (47·7%)	64 (47·8%)	7 (41·1%)	24 (39·3%)	63 (51·6%)	5 (41·7%)	0·68
Days after infection	687 (675–697)	676 (667–687)	689 (677–698)	686 (676–700)	673 (669–682)	670 (667–675)	674 (670–684)	689 (673–701)	671 (668–675)	674 (670–684)	693 (676–680)	NA
Days after last vaccine dose	NA	NA	NA	NA	143 (85–164)	145 (87–161)	142 (87–168)	163 (73–172)	131 (86–174)	149 (93–161)	30 (23–41)	NA

Data are n (%) or median (IQR). NA=not applicable. *p values are comparing the totals from both groups.

Table: **Baseline characteristics**

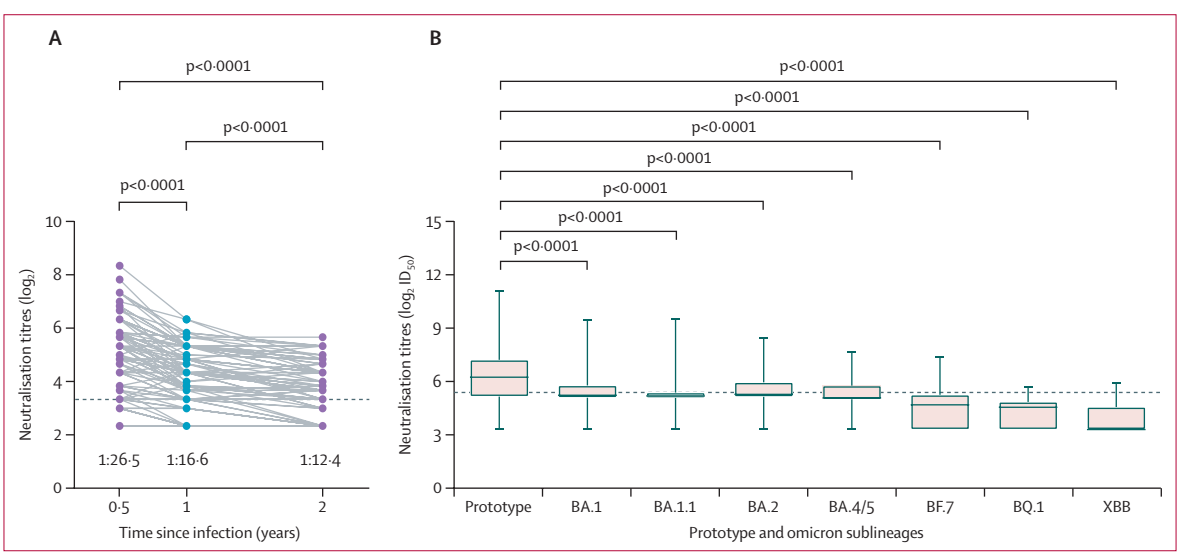

Figure 2: **Neutralising antibody responses in infected–unvaccinated participants**
(A) Dynamic changes of neutralising antibody titres against the SARS-CoV-2 prototype strain. Median titres of neutralising antibody are shown for each timepoint. Lines connect longitudinal samples. (B) Neutralising antibody titres against the prototype strain and omicron sublineages using a pseudovirus microneutralisation assay 2 years after infection. Antibody titres are shown as median (IQR). The dotted lines indicate detection limit of the assays.

participants who received one dose or two doses of the vaccine (p=0·78; appendix p 14). To examine whether plasma from recovered participants can neutralise SARS-CoV-2 variants 2 years after infection, we tested plasma samples from 139 participants at the 2-year follow-up using a pseudovirus MNA. Neutralising antibody ID_{50} titres were significantly lower for BA.1 (GMT 1:45·3; p<0·0001), BA.1.1 (1:42·5; p<0·0001), BA.2 (1:48·5; p<0·0001), BA.4/5 (1:41·7; p<0·0001), BF.7 (1:31·0; p<0·0001), BQ.1 (1:24·3; p<0·0001), XBB (1:22·3; p<0·0001) than the prototype strain (1:85·1; figure 2B).

We next evaluated the magnitude and cross-reactivity of SARS-CoV-2-specific memory B-cell responses in peripheral blood (appendix p 15). In infected–unvaccinated participants, memory B-cell responses to the spike protein and RBD induced by SARS-CoV-2 infection were detected in recovered participants 2 years after infection (figure 3A). The prototype spike-specific and RBD-specific memory B-cell responses as a percentage of total B cells did not differ between recovered participants who had moderate illness and those who had severe or critical illness (p=0·81 for spike-specific responses and p=0·31 for RBD-specific responses; figure 3A). In infected–unvaccinated participants, the frequency of memory B cells cross-reactive to the prototype and delta RBD among prototype RBD-specific memory B cells did not differ from the frequency of memory B cells cross-reactive to the prototype

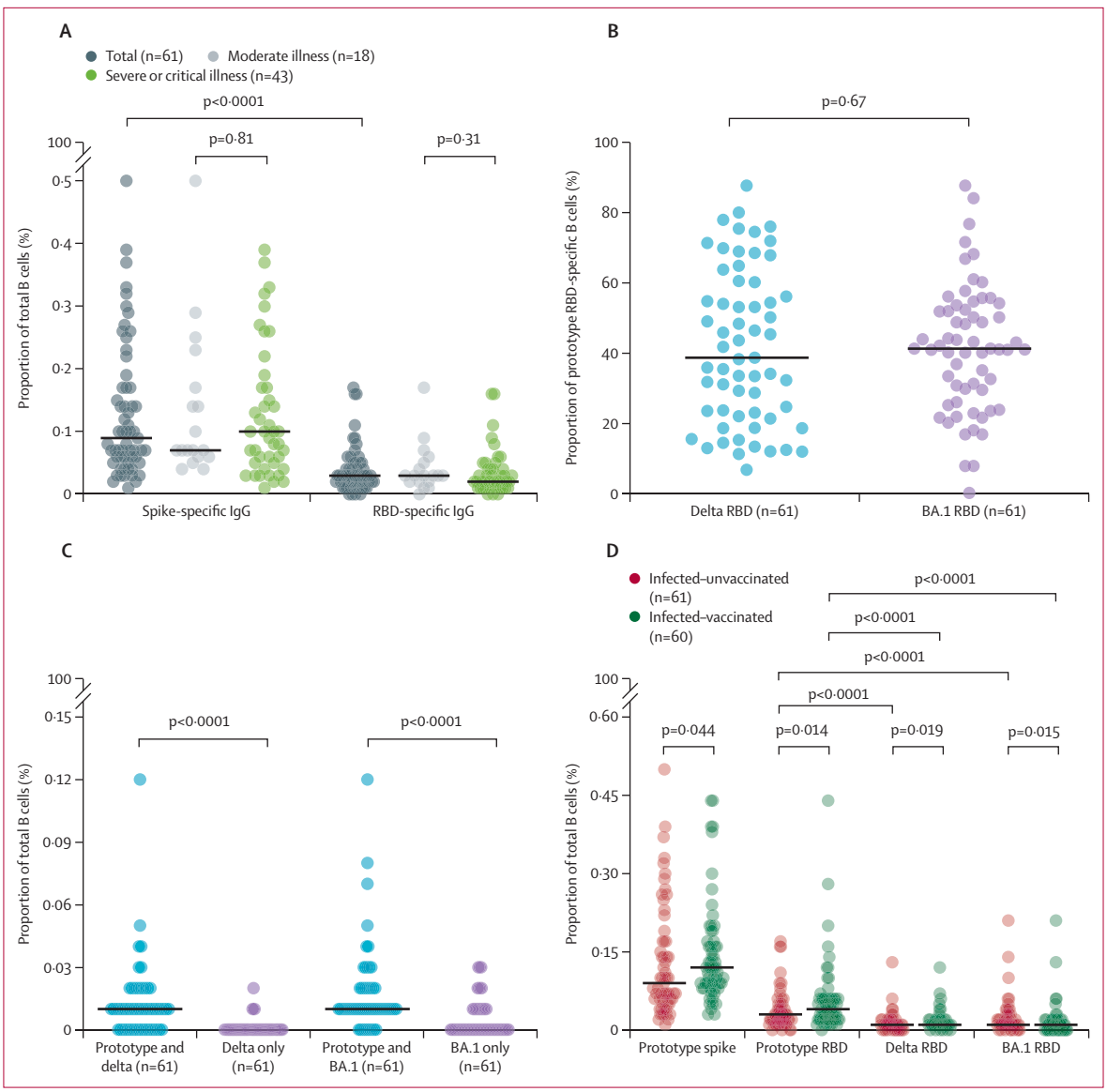

Figure 3: **IgG memory B-cell responses to SARS-CoV-2 infection**
Percentage of prototype strain spike protein-specific and RBD-specific memory B cells in samples of peripheral blood mononuclear cells from infected–unvaccinated participants who recovered from moderate and severe or critical COVID-19 (A), cross-sectional analysis of delta and omicron (BA.1) RBD binding as a percentage of prototype RBD-specific memory B cells 2 years after infection in infected–unvaccinated participants (B), percentage of memory B cells binding prototype and delta, delta only, prototype and omicron (BA.1), and omicron (BA.1) only in infected–unvaccinated participants (C), percentage of memory B cells against prototype strain spike, prototype strain RBD, delta RBD, and BA.1 RBD in samples of peripheral blood mononuclear cells in infected–unvaccinated participants and infected–vaccinated participants (D). Horizontal black lines show median values. RBD=receptor binding domain.

and BA.1 RBD (p=0·67; figure 3B). The proportion of memory B cells reactive against the delta RBD that was reactive against both the prototype and delta RBD was significantly higher than the proportion of memory B cells only recognising the delta RBD (p<0·0001; figure 3C). A similar pattern was observed in memory B cells against BA.1 (p<0·0001; figure 3C). These data indicate that SARS-CoV-2 variants do not completely escape memory B-cell responses induced by prototype infection. The frequency of prototype spike protein (p=0·044), prototype RBD (p=0·014), delta RBD (p=0·019), and BA.1 RBD (p=0·015) memory B cells in the total B-cell population was higher in infected–vaccinated participants than in infected–unvaccinated participants (figure 3D). In infected–vaccinated participants, the proportions of memory B cells cross-reactive to prototype and delta RBD versus prototype and BA.1 RBD among the prototype memory B-cell population did not differ (p=0·13; appendix p 16). Moreover, memory B-cell responses to the prototype spike protein (p=0·24), prototype RBD (p=0·070), delta

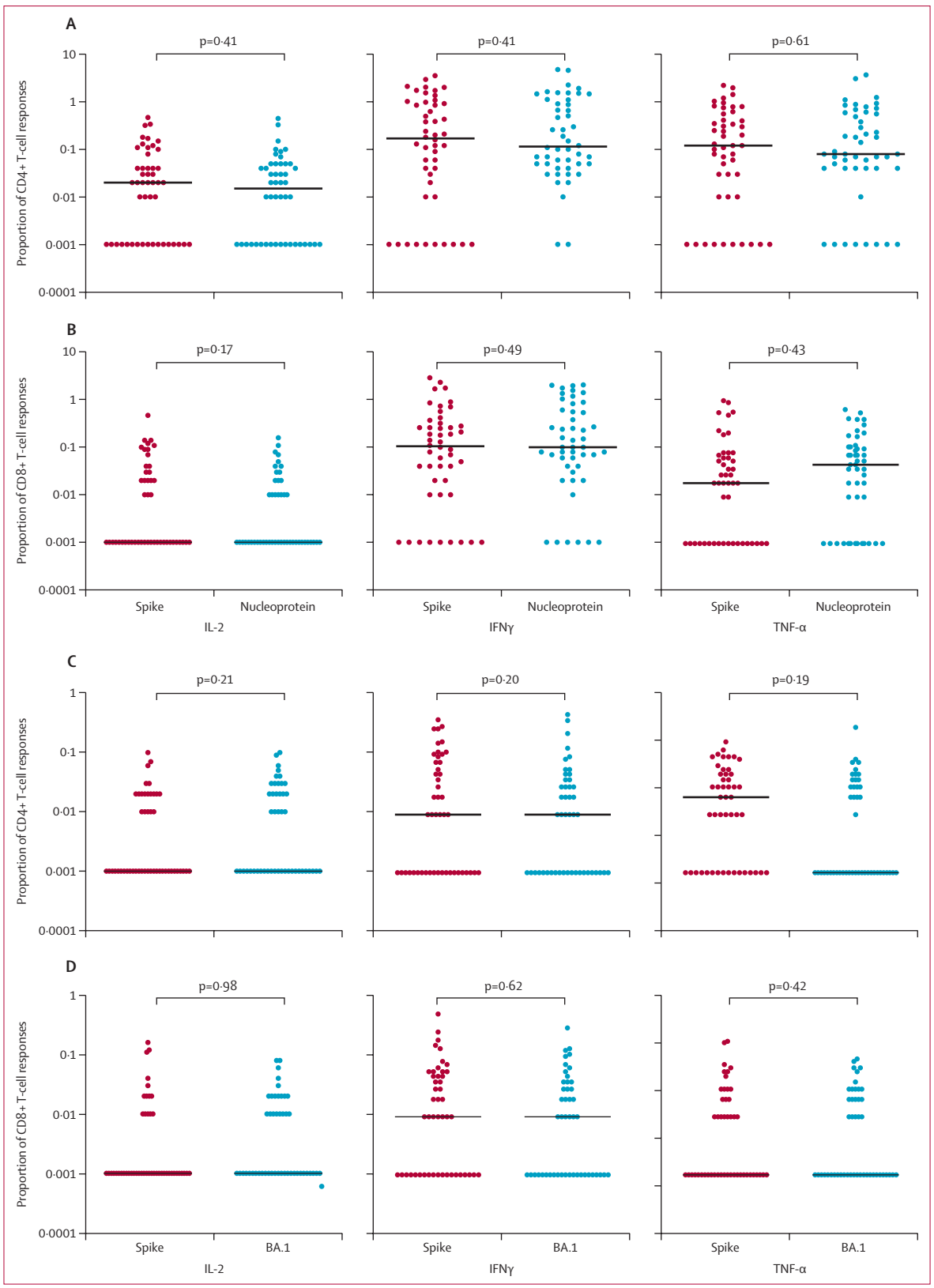

Figure 4: **Memory T-cell responses 2 years after SARS-CoV-2 infection in infected–unvaccinated participants**
Distribution of IL-2, IFNγ, and TNF-α against prototype spike protein and nucleoprotein peptide pools among SARS-CoV-2-specific CD4+ T cells (A) and CD8+ T cells (B) in infected–unvaccinated participants, and comparison of the relative proportion of multifunctional cytokines between SARS-CoV-2 prototype strain and BA.1 spike protein peptide-pool-reactive CD4+ T cells (C) and CD8+ T cells (D) in infected–unvaccinated participants. Horizontal black lines are median. IFNγ=interferon γ. IL-2=interleukin 2. TNF-α=tumour necrosis factor-α.

RBD (p=0·24), and BA.1 RBD (p=0·37) did not differ between participants who received one or two doses of inactivated vaccine (appendix p 16).

Ex-vivo ELISpot assays using spike protein and nucleoprotein peptide pools were done for samples from infected–unvaccinated and infected–vaccinated participants at the 1-year and 2-year follow-ups. The magnitude of IFNγ T-cell responses to the spike protein versus nucleoprotein peptide pools did not significantly differ 1–2 years after infection in infected–unvaccinated participants (p=0·87 *vs* p=0·30; appendix p 17) and 5 months after receipt of inactivated vaccine in infected–vaccinated participants (p=0·76 *vs* p=0·11; appendix p 17). Additionally, no difference was observed in response magnitude (p=0·31 *vs* p=0·47; appendix p 18) between participants who received one or two doses of the inactivated vaccine.

The functional responses of SARS-CoV-2-specific memory T cells in individuals recovered from COVID-19 were then assessed. The production of IL-2, IFNγ, and TNF-α by SARS-CoV-2-reactive T cells were measured. The gating strategy for memory T-cell analysis is shown in the appendix (pp 19–20). No significant differences were observed in the proportions of CD4+ T cells producing IL-2 (p=0·41), IFNγ (p=0·41), and TNF-α (p=0·61) in response to spike protein and nucleoprotein peptide pools (figure 4A). CD8+ T-cell responses showed similar results for IL-2 (p=0·17), IFNγ (p=0·49), and TNF-α (p=0·43; figure 4B). There were no significant differences in the proportion of SARS-CoV-2-specific cytokine responses in CD4+ T cells (IL-2 p=0·21, IFNγ p=0·20, TNF-α p=0·19; figure 4C) and CD8+ T cells (p=0·98, p=0·62, p=0·42; figure 4D) between prototype and BA.1 strains. In infected–vaccinated participants, there were no significant differences in the frequencies of cytokine responses against the spike protein and nucleoprotein in CD4+ T cells (IL-2 p=0·051, IFNγ p=0·70, TNF-α p=0·44), and CD8+ T cells (p=0·53, p=0·068, p=0·18; appendix 21).

Discussion

We describe immunological results from a unique cohort of participants who had recovered from infection with the prototype SARS-CoV-2 strain, had not been re-infected, and had or had not been vaccinated after the initial infection, with plasma samples collected over 2 years. In infected–unvaccinated individuals, neutralising antibody titres continually decreased from 6 months to 1 year and to 2 years after natural infection, with a half-life of approximately 141·2 days. Memory B-cell responses were retained at 2 years and showed cross-reactivity to SARS-CoV-2 delta and omicron BA.1 variants. The magnitude of IFNγ T-cell responses to SARS-CoV-2 were not significantly different 1–2 years after infection. Recognition of the BA.1 variant by memory T cells from most participants was not disrupted. These data—which currently would be difficult to collect since SARS-CoV-2 re-infection or vaccination (or both) is ubiquitous in most countries—are useful for understanding the duration of immune protection without boosting and could have implications for the design of vaccination regimens and programmes.

An effective immune response requires the generation of long-lived immune memory, including circulating antibodies, memory B cells, and memory T cells.[16] Memory B cells and T cells have low activation thresholds and can respond rapidly and efficiently to re-exposure. We show that SARS-CoV-2-specific memory B-cell and T-cell responses are maintained for at least 2 years in individuals recovered from SARS-CoV-2 natural infection, which might contribute to rapid recall immune responses that efficiently limit viral replication and reduce disease severity after re-infection.

The durability of serum antibodies, a key element of protection against viral infection, is a major topic of interest for SARS-CoV-2 adaptive immunity after natural infection. Our study shows that spike-specific, RBD-specific, and nucleoprotein-specific IgG titres decreased from 1 year to 2 years after initial infection. Neutralising antibodies are the markers of immunity most strongly correlated with protection from severe outcomes.[17] We found that neutralising antibodies continually declined from 6 months to 1 year, and to 2 years after infection. Neutralisation titres at the 2-year follow-up in infected–unvaccinated participants were significantly lower than in infected–vaccinated participants. After the appearance of the first omicron variant, omicron sublineages rapidly increased with extensive spike protein mutations. Our results show that neutralising antibody titres against BA.1, BA.1.1, BA.2, BA4/5, BF.7, BQ.1, and XBB were markedly impaired, which might increase the risk of re-infection in vivo. Nevertheless, even low neutralising antibody titres could still slow viral replication and reduce disease severity.[18] By contrast, we found that memory T-cell responses are retained 1–2 years after initial infection regardless of vaccination status, which has important implications for vaccine development.

Immunological memory of B cells develops after infection and has two main components: (1) long-lived plasma cells that produce antibodies to protect against homologous challenges, and (2) memory B cells that activate after re-exposure and rapidly generate antibody responses against homologous or heterologous challenges.[19,20] The fact that SARS-CoV-2-specific neutralising antibody titres are low, especially against omicron sublineages, in recovered individuals 2 years after natural infection raises the concern that neutralising antibody titres would be insufficient to protect against re-infection. However, spike protein-specific and RBD-specific memory B cells were detected in recovered patients 2 years after infection. Studies using mouse models of infections with wild-type strains and variants of West Nile virus or influenza virus show that pre-existing antibodies secreted by long-lived plasma cells

protect against homologous challenges, whereas memory B-cell activation is mainly required to protect against heterologous challenges.[19,21]

The emergence of SARS-CoV-2 VOCs, which harbour mutations in the spike protein, has raised concerns about these variants escaping the immunity induced by vaccination or natural infection by the prototype strain. Our cohort data suggest that omicron sublineages escape infection-elicited antibody and memory B-cell responses, but spike protein-specific T-cell activation is not affected by the mutations in the omicron variants 2 years after infection. In COVID-19-convalescent patients and vaccinated individuals, the SARS-CoV-2 B.1.351 (beta) variant has been shown to partly escape neutralising capacity and Fc-mediated functionality, but there is no difference in CD4+ T-cell activation in response to beta variant antigens.[22] These data suggest that, like other variants, mutations in omicron do not disrupt T-cell responses elicited by natural infection and vaccination, probably due to largely conserved T-cell epitopes.[23]

Analysing the differential adaptive immune responses to VOCs derived from infection and vaccination can help to guide further vaccine design and optimisation. It has been observed that the mRNA vaccine increases all components of the humoral response[24] and leads to the expansion of spike protein-specific T-cells[25] in recovered patients. We show that humoral immunity was boosted against the prototype and omicron sublineages in individuals who were infected by the prototype and who subsequently received the inactivated vaccine. The higher neutralising antibody titres observed in infected–vaccinated participants could also be due to a more recent exposure to the viral antigen. However, memory T-cell responses did not significantly increase after inactivated vaccination. A recent study showed that viral-specific T-cell responses were induced in health-care workers who had received two doses of inactivated vaccine and had no documented history of SARS-CoV-2 infection.[26] The heterogeneous immune repertoires should be further studied. With the rapid evolution of SARS-CoV-2, new variants that are capable of greater immune escape than BQ.1 and XBB might emerge. To control the potentially high incidence of re-infections with omicron sublineages and infection with future variants, improved SARS-CoV-2 vaccines should be developed by optimising the conserved neutralising epitopes and T-cell response epitopes from the prototype and variant strains.

Our study has several limitations. First, the participants were all inpatients moderately to critically ill and were inpatients for the duration of the infection. Asymptomatic individuals and those with mild illness were not included. We believe a more detailed study of the longitudinal effect of immune responses primed by infection with different disease outcomes merits further investigation. Second, the protective efficacy against infection was not evaluated in this study. The protective immune responses should be assessed in a prospective study in this cohort. Finally, since most individuals who had recovered from COVID-19 and were considered for inclusion[15] had been vaccinated, only 195 unvaccinated individuals could be enrolled in the 2-year follow-up and some had insufficient samples to do all the analyses at all three timepoints.

We have evaluated adaptive immune responses in COVID-19-recovered individuals 2 years after infection. Our findings show that immunity against the prototype strain in recovered patients is retained at 2 years. However, cross-reactive neutralising antibody responses to newly emerged viral strains were impaired, whereas cross-reactive T-cell responses were retained regardless of vaccination status. Our data suggest that with the increasing emergence of variants, there is an urgent requirement to introduce an effective vaccine to boost the neutralising antibody and overall T-cell responses to newly emerged SARS-CoV-2 variants.

Contributors

JW and BC conceived and designed the study and take responsibility for data integrity and accuracy of the data analysis. LG, TH, QZ, and LR did the literature review. QZ, LG, TH, JZ, YZ, LC, DL, MF, and LX performed experiments. LG, XG, and HL did the analysis. LG, TD, and JW drafted the manuscript. HZ, YaL, YiL, JZ, XW, TB, and WL completed the follow-up. ZW and HL collected the data. LR and YP verified the underlying data in the study. All authors read and edited the manuscript, approved the final version, had full access to all the data, and had final responsibility for the decision to submit for publication.

Declaration of interests

We declare no competing interests.

Data sharing

Data sharing restrictions apply to the availability of the data; therefore, they are not publicly available. However, data are available from the authors on reasonable request and with permission from the authors' institutions.

Acknowledgments

This study was funded by the Chinese Academy of Medical Sciences Innovation Fund for Medical Sciences (2021-I2M-1–038, 2021-I2M-1–047, 2018-I2M-2–002), the National Natural Science Foundation (81930063), the Fundamental Research Funds for the Central Universities for Peking Union Medical College (3332021092), Science Fund for Creative Research Groups of the National Natural Science Foundation of China (82221004), Beijing Natural Science Foundation (L222055), and the UK Medical Research Council. We thank all the study participants and their families. We also thank all staff of the follow-up study team at Jinyintan Hospital. This manuscript was edited by Life Science Editors.

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