SARS-CoV-2-specific antibody and T-cell responses 1 year after infection in people recovered from COVID-19: a longitudinal cohort study

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Summary

Background The memory immune response is crucial for preventing reinfection or reducing disease severity. However, the robustness and functionality of the humoral and T-cell response to SARS-CoV-2 remains unknown 12 months after initial infection. The aim of this study is to investigate the durability and functionality of the humoral and T-cell response to the original SARS-CoV-2 strain and variants in recovered patients 12 months after infection.

Methods In this longitudinal cohort study, we recruited participants who had recovered from COVID-19 and who were discharged from the Wuhan Research Center for Communicable Disease Diagnosis and Treatment at the Chinese Academy of Medical Sciences, Wuhan, China, between Jan 7 and May 29, 2020. Patients received a follow-up visit between Dec 16, 2020, and Jan 27, 2021. We evaluated the presence of IgM, IgA, and IgG antibodies against the SARS-CoV-2 nucleoprotein, Spike protein, and the receptor-binding domain 12 months after initial infection, using ELISA. Neutralising antibodies against the original SARS-CoV-2 strain, and the D614G, beta (B.1.351), and delta (B.1.617.2) variants were analysed using a microneutralisation assay in a subset of plasma samples. We analysed the magnitude and breadth of the SARS-CoV-2-specific memory T-cell responses using the interferon γ (IFNγ) enzyme-linked immune absorbent spot (ELISpot) assay and intracellular cytokine staining (ICS) assay. The antibody response and T-cell response (ie, IFN-γ, interleukin-2 [IL-2], and tumour necrosis factor α [TNFα]) were analysed by age and disease severity. Antibody titres were also analysed according to sequelae symptoms.

Findings We enrolled 1096 patients, including 289 (26·4%) patients with moderate initial disease, 734 (67·0%) with severe initial disease, and 73 (6·7%) with critical initial disease. Paired plasma samples were collected from 141 patients during the follow-up visits for the microneutralisation assay. PBMCs were collected from 92 of 141 individuals at the 12-month follow-up visit, of which 80 were analysed by ELISpot and 92 by ICS assay to detect the SARS-CoV-2-specific memory T-cell responses. N-IgG (899 [82·0%]), S-IgG (1043 [95·2%]), RBD-IgG (1032 [94·2%]), and neutralising (115 [81·6%] of 141) antibodies were detectable 12 months after initial infection in most individuals. Neutralising antibodies remained stable 6 and 12 months after initial infection in most individuals younger than 60 years. Multifunctional T-cell responses were detected for all SARS-CoV-2 viral proteins tested. There was no difference in the magnitude of T-cell responses or cytokine profiles in individuals with different symptom severity. Moreover, we evaluated both antibody and T-cell responses to the D614G, beta, and delta viral strains. The degree of reduced in-vitro neutralising antibody responses to the D614G and delta variants, but not to the beta variant, was associated with the neutralising antibody titres after SARS-CoV-2 infection. We also found poor neutralising antibody responses to the beta variant; 83 (72·2%) of 115 patients showed no response at all. Moreover, the neutralising antibody titre reduction of the recovered patient plasma against the delta variant was similar to that of the D614G variant and lower than that of the beta variant. By contrast, T-cell responses were cross-reactive to the beta variant in most individuals. Importantly, T-cell responses could be detected in all individuals who had lost the neutralising antibody response to SARS-CoV-2 12 months after the initial infection.

Interpretation SARS-CoV-2-specific neutralising antibody and T-cell responses were retained 12 months after initial infection. Neutralising antibodies to the D614G, beta, and delta viral strains were reduced compared with those for the original strain, and were diminished in general. Memory T-cell responses to the original strain were not disrupted by new variants. This study suggests that cross-reactive SARS-CoV-2-specific T-cell responses could be particularly important in the protection against severe disease caused by variants of concern whereas neutralising antibody responses seem to reduce over time.

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Research in context

Evidence before this study

We searched PubMed on Nov 2, 2021, using the following search terms [("SARS-CoV-2" OR "COVID-19" OR "Coronavirus Disease 2019 Virus" OR "2019 Novel Coronavirus") AND ("Adaptive Immunity" OR "Adoptive Immunity" OR "Immunity, Cellular" OR "Cellular Immunity" OR "Humoral Immunity" OR "Immunity, Humoral")], with no date or language restrictions, and identified 793 results. Studies identified by the search reported that SARS-CoV-2-specific antibodies gradually decreased over several months. However, analyses of lymphocytes from COVID-19-convalescent individuals indicate that B cells and CD4 or CD8 T cells play an important role in mediating memory responses after natural infection. Therefore, it is important to explore these pathways to improve vaccination efficacy. The longest study spanned 12 months. However, the characteristics of adaptive immunity in patients 12 months after they contracted COVID-19 was not well understood. Given the rapid emergence of variants of concern, the ability of immunological memory to protect previously infected individuals from new variants has been examined, but no firm conclusions were reached.

Added value of this study

To our knowledge, this is the first comprehensive evaluation on the durability and robustness of antibody and T-cell responses

against the SARS-CoV-2 original strain and its variants in recovered patients 1 year after natural infection without repeat exposure or vaccination in Wuhan, China. Our findings show that robust antibody and T-cell immunity against SARS-CoV-2 is present in the majority of recovered patients 12 months after moderate-to-critical infection. Total SARS-CoV-2-specific T-cell responses remain effective against variants, but neutralising antibodies diminish by 12 months.

Implications of all the available evidence

These data have important implications for vaccine efficacy against SARS-CoV-2 variants; the presence of cellular immune responses to SARS-CoV-2 variants and patients who lost their neutralising antibody responses provide additional important information on broad B-cell and T-cell immunity for future vaccine strategies targeting SARS-CoV-2. In particular, when neutralising antibody responses are reduced, cross-reactive SARS-CoV-2-specific T-cell responses could be important in protection against severe disease caused by variants of concern. Continuous surveillance is required to assess the duration of infection-induced immunity and the antibody and T-cell responses to these variants.

Introduction

The SARS-CoV-2 pandemic remains a serious public health threat to the global population.[1] Consistent with other viral infections, there is evidence that humans develop SARS-CoV-2-specific humoral and cellular immunity that mediates viral clearance and inhibits viral dissemination. A study of patients with COVID-19 suggests that CD4 and CD8 T cells play a dominant role in reducing disease severity during initial SARS-CoV-2 infection.[2] In terms of neutralising antibodies, the data are inconclusive; one study suggested that neutralising antibodies against SARS-CoV-2 did not generally correlate with reduced disease severity in initial infections,[3] but in another study, they seemed to have an important role in vaccination against and treatment of COVID-19.[4] To date, few data are available on long-term immunity against SARS-CoV-2. Available reports mainly suggest that the titres of antibodies against SARS-CoV-2 decline over time after clearance of COVID-19 infection.[5,6] Studies have shown that SARS-CoV-2-specific cellular immune responses developed in patients with COVID-19[7,8] and remained detectable 8 months after infection.[9] Studies on patients who have recovered from SARS-CoV indicated that cellular immune responses were maintained for nearly two decades, whereas memory B cells and antibody responses could not be detected in most individuals at that point.[10] However, the durability of antibody and

T-cell memory against SARS-CoV-2 in recovered individuals remains poorly characterised.

Emerging SARS-CoV-2 variants are a major public health concern. SARS-CoV-2 variants of concern (VOCs), including the alpha (B.1.1.7), beta (B.1.351), gamma (P.1), delta (B.1.617.2), and omicron (B.1.1.529) variants, spread more efficiently[11] and lead to a substantial loss of neutralising activity by vaccine-elicited and monoclonal antibodies.[12] However, the impact of VOCs on the durability of SARS-CoV-2 immunity, and whether these VOCs evolved to escape from natural infection-elicited immunity, is not well understood.

In this study, we characterised SARS-CoV-2-specific humoral and cellular immune responses in a follow-up cohort of patients recovered from COVID-19 12 months after infection, without repeat exposure or SARS-CoV-2 vaccination, in Wuhan, China. We also tested in vitro the impact of SARS-CoV-2 variants on B-cell and T-cell responses 12 months after infection in this cohort.

Methods

Study design and participants

In this longitudinal cohort study, we recruited participants who had recovered from laboratory-confirmed COVID-19 and who were discharged from the Wuhan Research Center for Communicable Disease Diagnosis and Treatment at the Chinese Academy of Medical Sciences, Wuhan, China, between Jan 7 and May 29, 2020.[5]

Patients were asked to attend a follow-up visit at the research centre between Dec 16, 2020, and Jan 27, 2021. Inclusion and exclusion criteria are provided in the appendix (p 2). The individuals studied are part of a larger longitudinal cohort study, whose outcomes were described in detail elsewhere.[5,13] Written informed consent was obtained from each individual. The study was approved by the Institutional Review Boards of the Wuhan Research Center for Communicable Disease Diagnosis and Treatment, Chinese Academy of Medical Sciences (KY-2020-80.01).

Procedures

Disease severity was characterised by clinicians using the highest seven-category scale during hospital stay (appendix p 2); for this study, patients in the third category (admitted to hospital but who did not require supplemental oxygen) were categorised as moderate, patients in the fourth category (admitted to hospital requiring supplemental oxygen) were categorised as severe, and patients in the fifth and sixth categories (admitted to hospital requiring high-flow nasal cannula, non-invasive mechanical ventilation, extracorporeal membrane oxygenation, or invasive mechanical ventilation) were categorised as critical.[5] 10 mL of venous blood was collected from participants by clinicians when they attended the 12-month follow-up visit and processed within 12 h to isolate plasma (for the antibody assays) and peripheral blood mononuclear cells (PBMCs; for the T-cell response assays; appendix p 2). Titres of IgM, IgA, and IgG antibodies against the nucleoprotein, Spike protein, and receptor-binding domain of SARS-CoV-2 were evaluated using ELISA (appendix pp 2–3). The neutralising antibodies against the original, beta, and delta SARS-CoV-2 strains were titred on Vero cells using a microneutralisation assay (appendix p 3). SARS-CoV-2-specific memory T-cell responses to overlapping peptides spanning the SARS-CoV-2 Spike protein, nucleoprotein, membrane protein, and envelope protein–open reading frame (E/ORF) were detected using both cryopreserved (ex vivo) and cultured PBMCs (in vitro) and assessed using the interferon enzyme-linked immune absorbent spot (ELISpot) and intracellular cytokine staining (ICS) assays (appendix pp 3–4). The antibody and T-cell responses (ie, interferon γ [IFNγ], interleukin-2 [IL-2], and tumour necrosis factor α [TNFα]) were analysed by age and disease severity. Antibody titres were also analysed according to sequelae symptoms.

Outcomes

The primary outcomes were neutralising antibody titres and T-cell responses. The cutoff for neutralising antibody titre was 1/10. T-cell responses were expressed as the magnitude of IFNγ production and proportion of IL-2, IFNγ, and TNFα produced by SARS-CoV-2-specific CD4 and CD8 T cells. Secondary outcomes included IgM antibodies against the nucleoprotein (N-IgM), Spike protein (S-IgM), and receptor-binding domain (RBD-IgM), and IgA and IgG antibodies, which were expressed as optical density at 450 nm. Further secondary outcomes were the demographic characteristics of recovered patients, such as age, sex, days after infection, and sequelae symptoms.

Statistical analysis

Demographic characteristics and sequelae symptoms of COVID-19 in patients are presented as median (IQR) for continuous variables and n (%) for categorical variables. The comparison of seropositivity of IgM, IgA, IgG, and neutralising antibodies, and the escape percentage of the D614G, beta, and delta variants from neutralising antibodies was done with the χ² test, or Fisher's exact test when appropriate. Single comparisons between other metrics were done using the Mann-Whitney U test. Multiple comparisons of antibody titres and memory T-cell responses were done using the Kruskal-Wallis test followed by a post-hoc Dunn's correction. Paired plasma antibody titres and T-cell responses were compared using a two-tailed Wilcoxon matched-pairs signed-rank test. Spearman correlation analysis was done for single continuous variate correlation analyses. A two-sided p value less than 0·05 was considered to be statistically significant. All statistical analyses were done using

	Total (n=1096)	Moderate (n=289)	Severe (n=734)	Critical (n=73)
Age, years	58 (48–65)	59 (48–66)	58 (48–65)	53 (47–64)
Sex				
Male	587 (53·6%)	146 (50·5%)	393 (53·5%)	48 (65·8%)
Female	509 (46·4%)	143 (49·5%)	341 (46·5%)	25 (34·2%)
Days after infection	347 (336–358)	345 (335–355)	347 (336–358)	360 (351–372)
Sequelae symptoms				
Overall	514 (46·9%)	131 (45·3%)	345 (47·0%)	38 (52·1%)
Fatigue	202 (18·4%)	51 (17·6%)	136 (18·5%)	15 (20·5%)
Sleep difficulties	183 (16·7%)	45 (15·6%)	128 (17·4%)	10 (13·7%)
Muscle weakness	147 (13·4%)	39 (13·5%)	98 (13·4%)	10 (13·7%)
Joint pain	138 (12·6%)	35 (12·1%)	88 (12·0%)	15/58 (25·9%)
Palpitations	117 (10·7%)	24 (8·3%)	86 (11·7%)	7 (9·6%)
Hair loss	114 (10·4%)	26 (9·0%)	83 (11·3%)	5 (6·8%)
Chest pain	91 (8·3%)	23 (8·0%)	63 (8·6%)	5 (6·8%)
Cough	80 (7·3%)	25 (8·7%)	48 (6·5%)	7 (9·6%)
Dizziness	74 (6·8%)	18 (6·2%)	47 (6·4%)	9 (12·3%)
Headache	69 (6·3%)	17 (5·9%)	45 (6·1%)	7 (9·6%)
Skin rash	62 (5·7%)	14 (4·8%)	46 (6·3%)	2 (2·7%)
Myalgia	53 (4·8%)	14 (4·8%)	32 (4·4%)	7 (9·6%)
Smell disorder	45 (4·1%)	13 (4·5%)	28 (3·8%)	4 (5·5%)
Sore throat or difficult to swallow	43 (3·9%)	13 (4·5%)	26 (3·5%)	4 (5·5%)
Decreased appetite	33 (3·0%)	5 (1·7%)	24 (3·3)	4 (5·5%)
Taste disorder	33 (3·0%)	4 (1·4%)	29 (4·0%)	0
Diarrhoea or vomiting	10 (0·9%)	4 (1·4%)	4 (0·5%)	2/71 (2·8%)

Data are n (%), n/N (%), or median (IQR).

Table: **Demographic characteristics of study patients recovered from COVID-19**
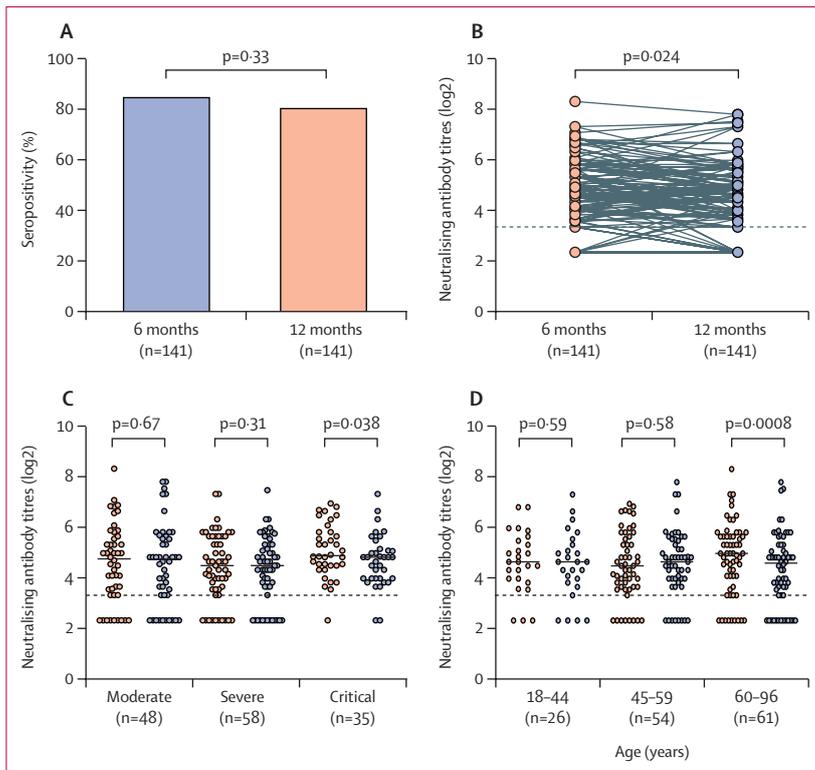
Figure 1: Neutralising antibody titres 6 and 12 months after SARS-CoV-2 infection
(A) Seropositivity of neutralising antibodies against the original SARS-CoV-2 strain from Wuhan, China.
(B) Neutralising antibody titres against the original SARS-CoV-2 strain (IPBCAMS-WH-01/2019, number
EPI_ISL_402123). (C) Neutralising antibody titres against the original SARS-CoV-2 strain in moderate, severe,
and critical patients. (D) Neutralising antibody titres against the original SARS-CoV-2 strain in different
age groups. The dotted line denotes the cutoff value for positive neutralising antibody titre. The solid lines
denote the median value.

GraphPad Prism 9.1 (GraphPad Software, San Diego, CA,
USA) and SAS (version 9.4).

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

We assessed 1107 patients for eligibility, of whom
1096 patients who had recovered from COVID-19 and for
whom 12 months had passed after the initial infection
were enrolled. Demographic characteristics of recovered
COVID-19 patients recruited in the 12-month follow-up
visit are listed in the table; the baseline characteristics of
these individuals are listed in the appendix (p 6).
Participants included 289 (26·4%) patients with moderate
initial disease, 734 (67·0%) with severe disease, and
73 (6·7%) with critical disease. The median duration
from symptom onset to the follow-up visit was 347 days
(IQR 336–358). The median age of the patients was
58 years (range 21–95; IQR 48–65), and 587 (53·6%) were
male. In this cohort, 514 (46·9%) of 1096 patients
reported at least one symptom at the 12-month follow-up

visit, with fatigue, sleep difficulties, and muscle weakness
being the most common self-reported symptoms (table).

Plasma samples were taken from all 1096 patients to
perform the ELISA assay. 141 paired plasmas were
collected from 141 patients during the follow-up visits 6
(between June 16 and Sept 3, 2020) and 12 months after
infection for the microneutralisation assay. PBMCs were
collected from 92 of 141 individuals at the 12-month
follow-up visit, of which 80 were analysed by ELISpot
and 92 by ICS assay to detect the SARS-CoV-2-specific
memory T-cell responses (appendix p 7).

Seropositivity for N-IgM, N-IgA, S-IgM, S-IgA, RBD-IgM,
and RBD-IgA ranged from 11 (1·0%) to 49 (4·5%; appendix
p 8). 899 (82·0%) were seropositive for N-IgG, 1043 (95·2%)
were seropositive for S-IgG, and 1032 (94·2%) were
seropositive for RBD-IgG (appendix p 8). S-IgG antibody
titres were higher in those with severe symptoms
(0·63 [IQR 0·44–0·83]) than in those with moderate
symptoms (0·59 [0·42–0·75]; p=0·017). The IgG titres
against SARS-CoV-2 increased with age at the 12-month
follow-up visit (appendix p 11). We found that SARS-CoV-2-
specific antibody titres for some age groups were higher in
patients with muscle weakness, hair loss, and headache
than in patients with no related symptoms reported at the
12-month follow-up visit (appendix p 12).

Among 141 paired plasmas, 121 (85·8%) were positive
for neutralising antibodies at the 6-month follow-up visit
and 115 (81·6%) were positive for neutralising antibodies
at the 12-month follow-up visit (p=0·33; figure 1A). There
were no significant differences in neutralising antibody
titres between critical, moderate, and severe individuals
at the 6-month (p=0·088) and 12-month (p=0·53) visits,
as well as between age groups of 18–44, 45–59, and
60–95 years at 6 months (p=0·48) and 12 months
(p=0·73). When comparing overall titres, neutralising
antibody titres decreased between the 6-month
(median 1/25·1 [IQR 1/12·6 to 1/40]) and 12-month visits
(1/25·1 [1/13·4 to 1/50·1]; p=0·024; figure 1B).
Neutralising antibody titres did not differ significantly
between the 6-month and 12-month visits in moderate
(p=0·67) and severe (p=0·31) patients, or in the groups
aged 18–44 (p=0·59) and 45–59 (p=0·58) years
(figure 1C–D). However, neutralising antibody titres did
decrease in the critical group (from 1/29·5 [1/22·4 to
1/56·2] at 6 months to 1/28·2 [1/15·8 to 1/35·1] at
12 months; p=0·038) and the group aged 60 years or older
(from 1/31·6 [1/15·9 to 1/50·1] at 6 months to 1/24·3
[1/11·3 to 1/40·0] at 12 months; p=0·0008; figure 1C–D).
Seropositivity for neutralising antibodies did not differ
significantly between the 6-month and 12-month visits in
cohorts with different disease severity and across age
groups (appendix p 13).

ELISpot responses against SARS-CoV-2 were measured
in five (26%) of 19 healthy individuals sampled between
October and December, 2018 (appendix p 14). The overall
magnitude and breadth of SARS-CoV-2-specific IFNγ
responses against viral peptides is shown in figure 2A
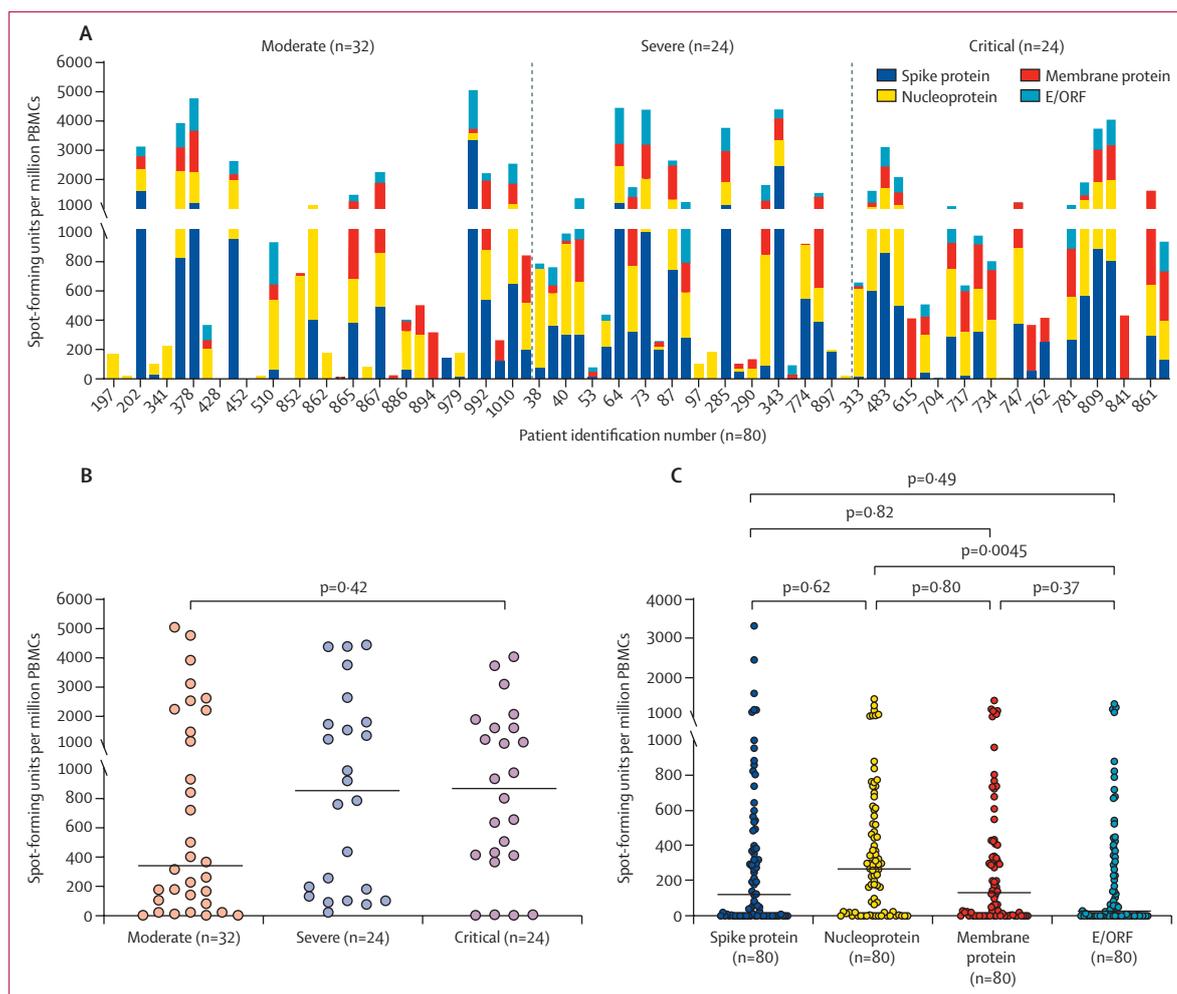
***Figure 2*: Memory T-cell responses to SARS-CoV-2 peptides measured by IFNγ ELISpot**
(A) Magnitude of IFNγ T-cell responses for each individual. Each bar shows the total T-cell response of each individual specific to all the SARS-CoV-2 protein peptide pools tested. Each coloured segment represents the source protein corresponding to peptide pools eliciting the IFNγ T-cell responses. (B) Magnitude of IFNγ T-cell responses in individuals with different disease severity. (C) Magnitude of IFNγ T-cell responses against different peptide pools. The solid lines in panels B and C denote the median of magnitude of IFNγ T-cell responses. ELISpot=enzyme-linked immune absorbent spot assay. IFNγ=interferon γ. PBMC=peripheral blood mononuclear cells.

and the appendix (p 15). Memory T-cell responses were detected in 72 (90%) of 80 recovered patients, showing SARS-CoV-2-specific T-cell responses to at least one of the SARS-CoV-2 peptide pools. However, there was high interindividual heterogeneity in the magnitude of SARS-CoV-2-specific T-cell responses (figure 2A). No significant correlations were observed between the magnitude of SARS-CoV-2-specific memory T-cell responses and disease severity (p=0·42; figure 2B). Differences in the magnitude of IFNγ T-cell responses to the Spike (p=0·62), nucleoprotein (p=0·82), and membrane protein (p=0·80) peptide pools were not significant (figure 2C). However, the IFNγ T-cell responses to the E/ORF peptide pool were lower than those to nucleoprotein peptide pools (p=0·0045; figure 2C).

Ex-vivo ELISpot assays for T-cell responses to the Spike and nucleoprotein peptide pools were performed on

37 individuals (15 moderate and 22 severe patients; appendix p 16). We did not see any significant difference between Spike protein and nucleoprotein T-cell responses overall, or between severe versus moderate patients, in line with data from the expanded (in vitro) patient T-cells (figure 2).

To assess functional SARS-CoV-2-specific memory T-cell responses in COVID-19-convalescent individuals, we isolated PBMCs and exposed them to overlapping Spike, nucleoprotein, and membrane or E/ORF peptide pools. We then measured the production of IFNγ, IL-2, and TNFα by SARS-CoV-2-reactive T cells (appendix pp 17–18). For 92 individuals tested, both CD4 and CD8 antigen-specific T cells produced at least one of these three cytokines. The proportion of CD4 and CD8 T-cell responses to the Spike protein and the membrane and E/ORF protein peptide pools showed no significant
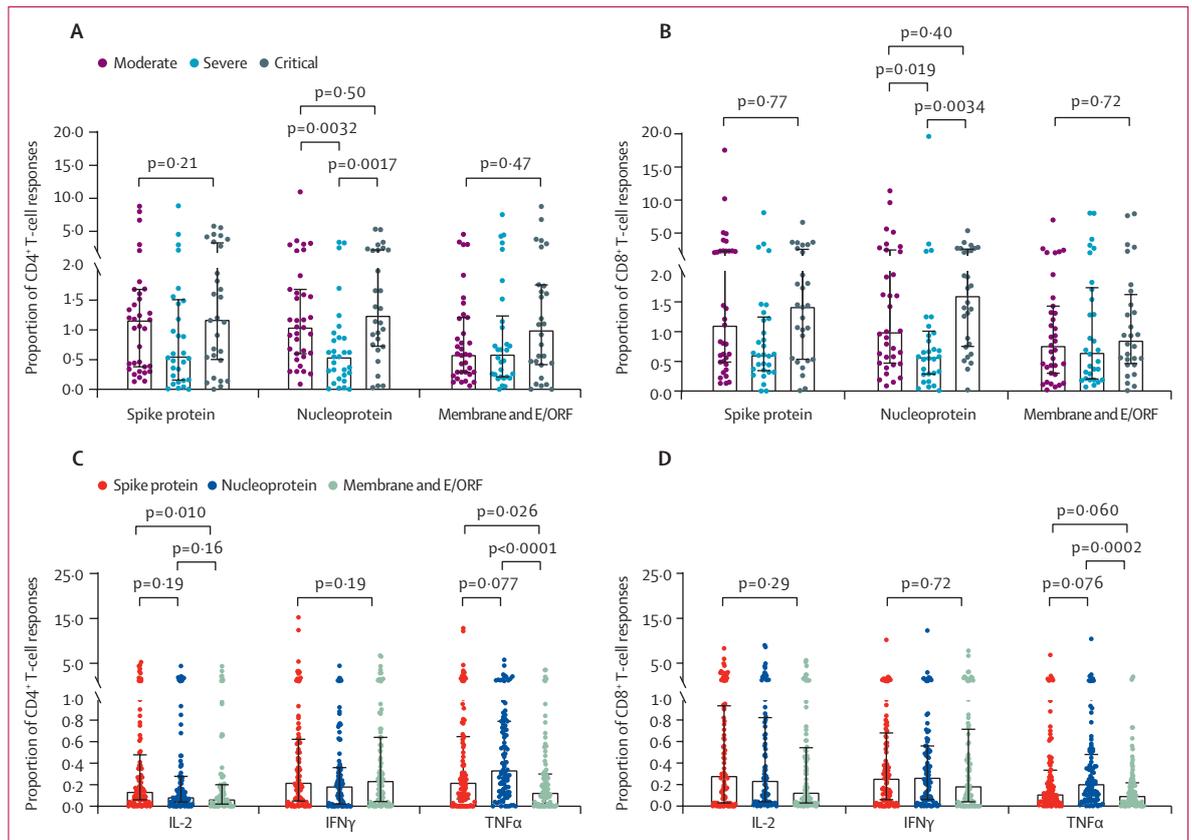
Figure 3: **Functional characteristics of SARS-CoV-2-specific T cells in recovered COVID-19 patients**
Cytokine-producing T cells were detected by ICS after incubation with SARS-CoV-2 peptides in 92 recovered patients. Comparison of the relative proportion of SARS-CoV-2 peptide-pool-reactive CD4 (A) and CD8 (B) T cells among moderate (n=35), severe (n=29), and critical (n=28) patients recovered from COVID-19. The SARS-CoV-2 peptide-pool-reactive CD4 or CD8 T cells were identified with at least one of the three cytokines (IFNγ, TNFα, and IL-2) detected. Bar graphs summarise the distribution of multifunctional cytokines against different peptide pools among SARS-CoV-2-specific CD4 (C) and CD8 (D) T cells in 92 recovered patients. Data are presented as median (IQR). ICS=intracellular cytokine staining. IFNγ=interferon γ. IL-2=interleukin 2. TNFα=tumour necrosis factor α.

differences among moderate, severe, and critical patients (figure 3A–B). However, there was a lower proportion of CD4 (p=0·0032 for severe *vs* moderate and p=0·0017 for severe *vs* critical) and CD8 (p=0·019 for severe *vs* moderate and p=0·0034 for severe *vs* critical) T-cell responses to the nucleoprotein in severe patients than in moderate and critical patients (figure 3A–B). Spike and nucleoprotein peptide pools expressed more IL-2 and TNFα than did membrane and E/ORF peptide pools (figure 3C–D).

To establish whether plasma from recovered patients can neutralise circulating SARS-CoV-2 variants, we tested plasma from 141 recovered patients against authentic viruses of the D614G, beta, and delta variants using microneutralisation assays. 12 months after infection, 115 (82%) of 141 individuals had neutralising antibodies against the original strain from Wuhan, China. By contrast, only 68 (48%) had neutralising antibodies against D614G, 32 (23%) had neutralising antibodies against the beta variant, and 69 (49%) had neutralising antibody responses against the delta variant (all p<0·0001; figure 4A). The neutralising antibody titres were significantly lower for the D614G (median 1/5·0 [IQR 1/5·0–1/14·1];

p<0·0001), beta (1/5·0 [1/5·0–1/5·0]; p<0·0001), and delta (1/5·0 [1/5·0–1/15·8]; p<0·0001) variants than for the original strain (1/25·1 [1/12·6–1/40·0]). Moreover, the neutralising antibody titres against the D614G and delta variants were similar (p=0·42), and both were higher than those against the beta variant (p=0·036 for D614G *vs* beta and p=0·0019 for delta *vs* beta; figure 4A).

Of the 115 samples positive for neutralising antibodies to the original strain, 28 (24%) had a titre of 1/10 to 1/20, 43 (37%) had a titre of 1/20 to 1/32, and 44 (38%) had a titre of 1/32 or more. 21 (75%) of 28 patients with a titre of 1/10 to 1/20 (p=0·047), 21 (49%) of 43 patients with a titre of 1/20 to 1/32 (p<0·0001), and five (11%) of 44 patients with a titre of 1/32 or more (p=0·0001) lost neutralising activity to the D614G variant at 12 months. 19 (68%) of 28 patients with a titre of 1/10 to 1/20 (p=0·32), 23 (53%) of 43 with a titre of 1/20 to 1/32 (p<0·0001), and seven (16%) of 44 with a titre of 1/32 or more (p=0·0003) lost neutralising activity against the delta variant at 12 months (appendix p 19). By contrast, 22 (79%) of 28 patients with a titre of 1/10 to 1/20, 34 (79%) of 43 with a titre of 1/20 to 1/32, and 27 (61%) of 44 with a titre of 1/32 or more lost neutralising activity
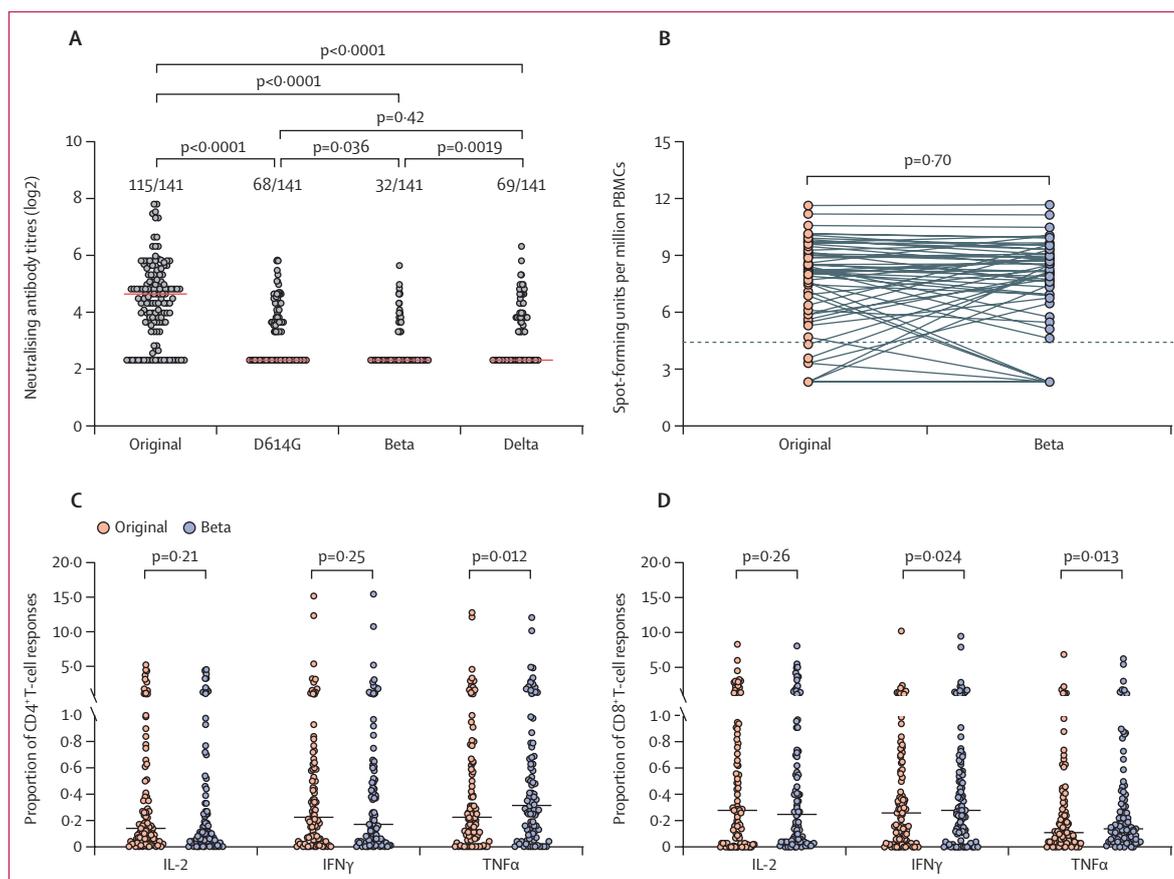
Figure 4: Humoral and cellular immune responses to the original SARS-CoV-2 strain, and the D614G, beta, and delta variants, in recovered patients 12 months after infection
(A) Neutralising antibody titres against the original SARS-CoV-2 strain from Wuhan, China (IPBCAMS-WH-01/2019, number EPI_ISL_402123), and the D614G, beta (B.1.351), and delta (B.1.617.2) variants in 141 patients. The lines denote the median of neutralising antibody titres. (B) Magnitude of IFNγ T-cell responses to the original SARS-CoV-2 strain, and the beta variant Spike protein peptide pool, plotted pairwise in 80 individuals. (C–D) Comparison of the relative proportion of multifunctional cytokines between the original SARS-CoV-2 strain and the beta variant Spike protein peptide-pool-reactive CD4 (C) and CD8 (D) T cells in 92 recovered patients. The lines denote the median proportion of T-cell responses. IFNγ=interferon γ. IL-2=interleukin 2. TNFα=tumour necrosis factor α.

against the beta variant (p=0·13; appendix p 19). The D614G (p=0·36) and beta (p=0·82) variants escaped from neutralising antibodies against the original strain, and there were no differences between age cohorts (appendix p 19). However, the escape percentage of the delta variant was higher in those aged 18–44 years (p=0·020) and 45–59 years (p=0·021) than in those aged 60–95 years (appendix p 19).

To investigate the difference in T-cell recognition between the original strain and the beta variant, we did IFNγ ELISpot and ICS assays using the original strain and the beta variant peptide pools spanning the full length of the Spike protein. The SARS-CoV-2-specific IFNγ responses were heterogeneous. However, the overall SARS-CoV-2-specific IFNγ responses in ELISpot showed no differences between the original strain and the beta variant peptide pool (p=0·70; figure 4B) and showed a strong correlation in the magnitude of IFNγ responses (Spearman r=0·85, p<0·0001; appendix p 19). Furthermore, we examined whether there was T-cell immune deviation

between the original strain and the beta variant using ICS. The beta variant induced a stronger TNFα response in CD4 T-cell responses (p=0·012), and stronger IFNγ (p=0·024) and TNFα (p=0·013) responses in CD8 T-cell responses (figure 4C–D).

No correlation was observed between the magnitude of the T-cell ELISpot responses and the neutralising antibody (Spearman r=0·10, p=0·34), S-IgG (Spearman r=–0·062, p=0·59), N-IgG (Spearman r=–0·052, p=0·65), and RBD-IgG (Spearman r=0·14, p=0·23) titres 12 months after infection (appendix p 20). To assess the persistence of the T-cell response, we measured the SARS-CoV-2-specific cellular immune response in 16 patients who were negative for neutralising antibodies after 12 months, selected from 26 (18%) of 141 recovered patients. We found that 15 (94%) of 16 individuals showed memory IFNγ responses in the ELISpot assay. All 16 samples produced at least one of the three cytokines (IL-2, IFNγ, and TNFα) in combination in CD4 and CD8 specific T-cell responses in the ICS assay (appendix p 21).

Discussion

The data presented here show that 82·0% of recovered COVID-19 patients had N-IgG antibodies, 95·2% had S-IgG antibodies, 94·2% had RBD-IgG antibodies, and 81·6% had neutralising antibodies 12 months after a SARS-CoV-2 infection. Although the decline in neutralising antibody titres between 6 and 12 months after infection mainly occurred in older people and critical patients, seropositivity of neutralising antibodies was stable in this population. Virus-specific T cells were detectable in all patients that had recovered from COVID-19. Both the D614G and the delta variants escape from the neutralising antibodies against the original strain, an effect that depends on neutralising antibody titres. By contrast, an absence of response to the beta variant is not related to neutralising antibody titres against the original strain. Importantly, although neutralising antibody responses are less efficient to this variant, T-cell responses are cross-reactive to the beta variant in patients recovered from infection. Memory T cells retained the ability to mediate cellular immunity in patients who had lost their neutralising antibody responses.

At present, long-term immune responses in recovered patients have been investigated. Cohen and colleagues observed that broad and effective antibody responses, and memory B-cell and T-cell responses, might persist for 8 months following SARS-CoV-2 infection.[14] Li and colleagues found that the positive rate of RBD-IgG exceeded 70% 12 months after diagnosis.[15] However, they did not evaluate the T-cell responses to SARS-CoV-2 and neutralising antibody responses to variants. Rank and colleagues found that about two-thirds of participants maintained IFNγ-specific T-cell responses at the 12-month follow-up. The antiviral T cells were lower in frequency than those in this study.[16] The major reason for this discrepancy might be because we used in-vitro expanded short-term T-cell lines after peptide pool stimulation. Recently, Zhang and colleagues reported that SARS-CoV-2-specific cellular and humoral immunities are durable 1 year after disease onset, and PBMCs were expanded for 9 days in vitro.[17] The results were similar to our findings. However, the neutralising antibody and T-cell responses to SARS-CoV-2 variants were not assessed in the study by Zhang and colleagues.

SARS-CoV-2 variants, especially alpha, beta, gamma, delta, and omicron, have been associated with rapid increases in cases at multiple locations.[18–22] These variants harbour mutations in the Spike protein that might alter virus–host cell interactions and escape neutralising antibody responses. Because beta is the most probable variant to escape the approved vaccines in comparison with the alpha, gamma, and delta variants,[23] we evaluate the neutralising antibody and T-cell responses to the beta variant in this study. Our data showed that neutralising antibodies from individuals who had recovered from natural infection against the original strain are less able to neutralise effectively the D614G, beta, and delta variants.

However, higher neutralising antibody titres against the original strain contributed to the protection from infection of D614G and delta variants in vitro. Neutralising antibodies against the original strain had a lower ability to neutralise the beta variant than the D614G and delta variants. The ability to escape from the neutralising antibodies against the original strain has no association to antibody titres, suggesting that the beta variant affects the binding of neutralising antibodies to the viral Spike protein. Structural information provides a basis for how SARS-CoV-2 variants have evolved to evade the immune system. The three mutations characterising the beta variant (K417N, E484K, and N501Y) are located at the receptor-binding domain, making the variant resistant to some potent neutralising antibodies.[24] However, the D614G variant enhances infectivity mainly through increasing the stability of the trimer, rather than through more exposed receptor-binding domains.[25] Recently, a new SARS-CoV-2 variant (omicron [B.1.1.529]) was reported.[22] How it interacts with immune cells needs to be assessed.

Current SARS-CoV-2 vaccines are mainly focused on neutralising antibodies induced by the viral Spike or receptor-binding domain protein. However, mutations in the Spike protein can cause epitope changes, potentially resulting in the virus escaping from neutralising antibodies. It has been reported that the beta and gamma variants could not be efficiently blocked by plasma from convalescent patients with COVID-19 and serum from individuals vaccinated with the BNT162b2 vaccine (tozinameran, Pfizer–BioNTech),[26] indicating that vaccine efficacy could be compromised by the emergence of viral variants. Although neutralising antibodies were less efficient at mediating in-vitro protection in naturally infected individuals, we found that the beta variant seemed to have no substantial impact on cellular immune responses 12 months after infection. Thus, the lack of sufficient neutralising antibodies against SARS-CoV-2 variants in individuals recovered from previous SARS-CoV-2 infection could be mitigated by T-cell responses. Despite the existence of cellular immune responses, it is urgent to use viral targets that are less likely to mutate for future SARS-CoV-2 vaccine platforms.

Our findings indicate that SARS-CoV-2 cellular immunity decays more slowly over time than neutralising antibody titres. In addition, SARS-CoV memory T cells have been detected 17 years after infection in individuals who recovered from SARS, and displayed robust cross-reactivity to SARS-CoV-2.[27] It has been reported that in the absence of neutralising antibodies, T-cell memory correlates with protection from influenza disease severity in humans.[28] Future studies are needed to evaluate the role of T-cell memory of SARS-CoV-2 in protection against reinfections.

Our study has several limitations. First, we did not obtain consecutive samples. Longitudinal data from cohorts will help to further analyse the SARS-CoV-2 humoral and

cellular immunity in individuals recovered from COVID-19. Second, because moderate-to-critical cases represent most inpatients, asymptomatic and mild cases are not included here. Third, we evaluated neutralising antibody responses to the D614G, beta, and delta variants, and the cellular responses to the beta variant. Further studies should be done to characterise humoral and cellular immunity against other SARS-CoV-2 VOCs. In addition, because of the ethical limitations to sampling, we were not able to obtain enough samples for T-cell analysis. Instead, we cultured PBMCs in vitro before analysing T-cell responses in ELISpot assays, as described previously.[17] This expansion protocol could potentially alter both the magnitude and polyfunctionality of the T cells due to differences in the proliferative capacity of different antigen-specific T cells.

In summary, we evaluated antibody and cellular immunity in COVID-19-convalescent individuals 12 months after infection. Our findings show that humoral and cellular immunity against SARS-CoV-2 is present in most recovered patients 12 months after moderate-to-critical infection. Neutralising antibody responses to the D614G, beta, and delta variants are much poorer than those to the original strain from Wuhan, China. Our results also showed the presence of cellular immune responses to SARS-CoV-2 variants and patients who lost their neutralising antibody responses. These data underline the importance of broad B-cell and T-cell immunity for future vaccine strategies targeting SARS-CoV-2.

References
1   Ren LL, Wang YM, Wu ZQ, et al. Identification of a novel coronavirus causing severe pneumonia in human: a descriptive study. *Chin Med J (Engl)* 2020; **133**: 1015–24.
2   Karlsson AC, Humbert M, Buggert M. The known unknowns of T cell immunity to COVID-19. *Sci Immunol* 2020; **5**: eabe8063.
3   Sette A, Crotty S. Adaptive immunity to SARS-CoV-2 and COVID-19. *Cell* 2021; **184**: 861–80.
4   Du L, Yang Y, Zhang X. Neutralizing antibodies for the prevention and treatment of COVID-19. *Cell Mol Immunol* 2021; **18**: 2293–306.
5   Huang C, Huang L, Wang Y, et al. 6-month consequences of COVID-19 in patients discharged from hospital: a cohort study. *Lancet* 2021; **397**: 220–32.
6   He Z, Ren L, Yang J, et al. Seroprevalence and humoral immune durability of anti-SARS-CoV-2 antibodies in Wuhan, China: a longitudinal, population-level, cross-sectional study. *Lancet* 2021; **397**: 1075–84.
7   Braun J, Loyal L, Frentsch M, et al. SARS-CoV-2-reactive T cells in healthy donors and patients with COVID-19. *Nature* 2020; **587**: 270–74.
8   Sekine T, Perez-Potti A, Rivera-Ballesteros O, et al. Robust T cell immunity in convalescent individuals with asymptomatic or mild COVID-19. *Cell* 2020; **183**: 158–168.
9   Dan JM, Mateus J, Kato Y, et al. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. *Science* 2021; **371**: eabf4063.
10   Jarjour NN, Masopust D, Jameson SC. T cell memory: understanding COVID-19. *Immunity* 2021; **54**: 14–18.
11   Krause PR, Fleming TR, Longini IM, et al. SARS-CoV-2 variants and vaccines. *N Engl J Med* 2021; **385**: 179–86.
12   Chen X, Chen Z, Azman AS, et al. Neutralizing antibodies against SARS-CoV-2 variants induced by natural infection or vaccination: a systematic review and pooled meta-analysis. *Clin Infect Dis* 2021; published online July 24. https://doi.org/10.1093/cid/ciab646.
13   Huang L, Yao Q, Gu X, et al. 1-year outcomes in hospital survivors with COVID-19: a longitudinal cohort study. *Lancet* 2021; **398**: 747–58.
14   Cohen KW, Linderman SL, Moodie Z, et al. Longitudinal analysis shows durable and broad immune memory after SARS-CoV-2 infection with persisting antibody responses and memory B and T cells. *Cell Rep Med* 2021; **2**: 100354.
15   Li C, Yu D, Wu X, et al. Twelve-month specific IgG response to SARS-CoV-2 receptor-binding domain among COVID-19 convalescent plasma donors in Wuhan. *Nat Commun* 2021; **12**: 4144.
16   Rank A, Tzortzini A, Kling E, et al. One year after mild COVID-19: the majority of patients maintain specific immunity, but one in four still suffer from long-term symptoms. *J Clin Med* 2021; **10**: 3305.
17   Zhang J, Lin H, Ye B, et al. One-year sustained cellular and humoral immunities of COVID-19 convalescents. *Clin Infect Dis* 2021; published online Oct 5. https://doi.org/10.1093/cid/ciab884.
18   Galloway SE, Paul P, MacCannell DR, et al. Emergence of SARS-CoV-2 B.1.1.7 lineage—United States, December 29, 2020–January 12, 2021. *MMWR Morb Mortal Wkly Rep* 2021; **70**: 95–99.
19   Tegally H, Wilkinson E, Giovanetti M, et al. Detection of a SARS-CoV-2 variant of concern in South Africa. *Nature* 2021; **592**: 438–43.
20   Faria NR, Mellan TA, Whittaker C, et al. Genomics and epidemiology of the P.1 SARS-CoV-2 lineage in Manaus, Brazil. *Science* 2021; **372**: 815–21.
21   Vaidyanathan G. Coronavirus variants are spreading in India—what scientists know so far. *Nature* 2021; **593**: 321–22.
22   Torjesen I. Covid-19: omicron may be more transmissible than other variants and partly resistant to existing vaccines, scientists fear. *BMJ* 2021; **375**: n2943.
23   Khateeb J, Li Y, Zhang H. Emerging SARS-CoV-2 variants of concern and potential intervention approaches. *Crit Care* 2021; **25**: 244.
24   Cai Y, Zhang J, Xiao T, et al. Structural basis for enhanced infectivity and immune evasion of SARS-CoV-2 variants. *Science* 2021; **373**: 642–48.
25   Zhang J, Cai Y, Xiao T, et al. Structural impact on SARS-CoV-2 spike protein by D614G substitution. *Science* 2021; **372**: 525–30.
26   Hoffmann M, Arora P, Groß R, et al. SARS-CoV-2 variants B.1.351 and P.1 escape from neutralizing antibodies. *Cell* 2021; **184**: 2384–2393.
27   Le Bert N, Tan AT, Kunasegaran K, et al. SARS-CoV-2-specific T cell immunity in cases of COVID-19 and SARS, and uninfected controls. *Nature* 2020; **584**: 457–62.
28   Sridhar S, Begom S, Bermingham A, et al. Cellular immune correlates of protection against symptomatic pandemic influenza. *Nat Med* 2013; **19**: 1305–12.