Clinical & Translational Immunology

Australian and New Zealand Society for Immunology Inc.

Clinical & Translational Immunology 2021; e1319. doi: 10.1002/cti2.1319
www.wileyonlinelibrary.com/journal/cti

ORIGINAL ARTICLE

Decline in neutralising antibody responses, but sustained T-cell immunity, in COVID-19 patients at 7 months post-infection

Jun Chen[1], Xiaomin Liu[2], Xinyu Zhang[2], Yixiao Lin[1], Danping Liu[2], Jingna Xun[2], Zhenyan Wang[1], Ling Gu[2], Qian Li[2], Dan Yin[2], Junyang Yang[1,3] & Hongzhou Lu[1]

[1]Department of Infectious Diseases and Immunology, Shanghai Public Health Clinical Center, Fudan University, Shanghai, China
[2]Scientific Research Center, Shanghai Public Health Clinical Center, Fudan University, Shanghai, China
[3]Wenzhou Medical University, Wenzhou, Zhejiang, China

Correspondence
J Chen and H Lu, Department of Infectious Diseases and Immunology, Shanghai Public Health Clinical Center, Fudan University, 2901 Caolang Road, Shanghai 201508, China.
E-mails: qtchenjun@163.com or luhongzhou@fudan.edu.cn

Received 8 February 2021;
Revised 27 April, 3 June and 6 July 2021;
Accepted 7 July 2021

doi: 10.1002/cti2.1319

Clinical & Translational Immunology
2021; **10**: e1319

Abstract

Objectives. This study aimed to explore the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-specific humoral responses and T-cell responses in patients who have recovered from coronavirus disease 2019 (COVID-19) to understand the natural protective immune responses and to facilitate the development of vaccines. **Methods.** We conducted a combined assessment of the changes in neutralising antibody levels and SARS-CoV-2-specific T-cell responses over time in 27 patients up to 7 months after infection. **Results.** The neutralising antibody remained detectable in 96.3% of the patients at their second visit at about 7 months post-onset of symptoms. However, their humoral responses, including titres of the spike receptor-binding domain IgG and neutralising antibody, decreased significantly compared with those at first clinic visit. By contrast, the proportions of spike-specific $CD4^+$ T cells, but not $CD8^+$ T cells, in COVID-19 patients after recovery were persistently higher than those in healthy controls. No significant change was observed in the proportion of spike-specific $CD4^+$ T cells in patients who had recovered from COVID-19 within 7 months. **Conclusion.** The SARS-CoV-2-specific T-cell immune responses persisted, while the neutralising antibodies decayed. Further studies are needed to extend the longevity of neutralising antibodies and to evaluate whether these T cells are sufficient to protect patients from reinfection.

Keywords: COVID-19, neutralising antibody, SARS-CoV-2, T cells

INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has led to over 190 million cases and more than 4.1 million deaths to date. Therefore, there is an urgent need to develop an effective vaccine that can be used to immunise the global population to halt the transmission of the virus. There is considerable

interest in understanding the nature of the immune response to SARS-CoV-2 in patients who have recovered from COVID-19 to shed light on the requirements and likelihood of achieving durable protection from SARS-CoV-2 infection.

The immune system comprises several components that work together to develop protective immunity. Adaptive immune responses, which comprise both humoral and T-cell responses specific to SARS-CoV-2, are important for protection against viral infections. Neutralising antibodies against SARS-CoV-2, especially the surface spike protein that mediates viral entry, have been identified in acute and convalescent COVID-19 patients.[1–9] These neutralising antibodies are currently under development as promising therapeutic options.[10,11] Most COVID-19 vaccines that induce the production of neutralising antibodies also target the spike protein of SARS-CoV-2.[12] However, a concern has been raised regarding the longevity of the antibody response to the spike protein in convalescent COVID-19 patients. Although recent studies have shown that neutralising antibodies last for at least 3 months, some earlier studies have also shown that the level of SARS-CoV-2 IgG declines over time and may become undetectable in a substantial proportion of patients.[5,13–15] Helping B cells generate neutralising antibody responses and maintain durable antibody responses is a major function of $CD4^+$ T cells. In addition, recent studies have suggested that T-cell response could be induced by SARS-CoV-2 in the absence of humoral immune responses.[16] Therefore, the balance between humoral and cellular immune responses might be important for protection from COVID-19 and avoidance of vaccine-enhanced disease.[8,17] Consequently, several COVID-19 vaccines have been designed to elicit robust $CD4^+$ or $CD8^+$ T-cell responses based on neutralising antibodies.[18–20]

However, there is a lack of longitudinal studies that conduct a combined examination of neutralising antibodies and $CD4^+$ T-cell and $CD8^+$ T-cell responses against SARS-CoV-2 in the same patient population. Addressing these fundamental questions is important in understanding the natural protective immune responses, which may facilitate the development of COVID-19 vaccines. In this study, we aimed to perform a combined assessment of changes in neutralising antibody levels and SARS-CoV-2-specific T-cell responses over time in patients at 7 months after infection.

RESULTS

Declined, but still detectable, humoral response against SARS-CoV-2

The levels of IgG and IgM against spike receptor-binding domain (RBD), as well as surrogate markers of neutralising antibodies, were measured in all collected samples. In the 11 samples from healthy controls, the spike-RBD IgM, IgG and neutralising antibodies were undetectable. Spike-RBD IgM was detected in 48.1% (13/27) of the patients, while high titres of spike-RBD-specific IgG were detected in all patients at their first visit (Figure 1a). By contrast, the titres of neutralising antibodies ranged from low to robust (Figure 1b). Despite the detectable levels of neutralising antibodies in all patients, the 50% inhibitory dilutions were below 1:100 in 29.6% (8/27) of the patients. Disease severity was associated with the titres of neutralising antibodies, but not with those of spike-RBD IgG and IgM (Figure 1c). Patients with previous severe diseases had higher titres of neutralising antibodies than those with mild diseases. Other demographic characteristics, including age and sex, did not affect the titres of neutralising antibodies, spike-RBD-specific IgG or IgM.

IgM titres decayed rapidly as they became undetectable in 96.3% (26/27) of the patients at their second visit. The only patient who had detectable IgM was followed up at 63 days post-onset of symptoms (POS). Spike-RBD IgG titres also decreased sharply in most patients since their first visit (Figure 1a, d). However, spike-RBD IgG remained detected in 92.6% of patients (25/27). Similarly, the titres of neutralising antibodies also decreased significantly at the patient's second visit compared with that at the first visit (Figure 1b). However, the speed of decay of neutralising antibodies was slower than that of IgG (Figure 1d, e). Despite the detectable levels of neutralising antibodies in the majority (92.6%, 25/27) of the patients at this time point, the 50% inhibitory dilutions higher than 1:100 were only observed in 25.9% (7/27) of these patients. The two patients whose samples were obtained at 214 and 222 days POS, respectively, had loss of neutralising antibodies. The 50% inhibitory dose values of samples obtained from these patients at their first visit were 1:34 and 1:112, respectively. The titres of neutralising antibodies were comparable between patients with different

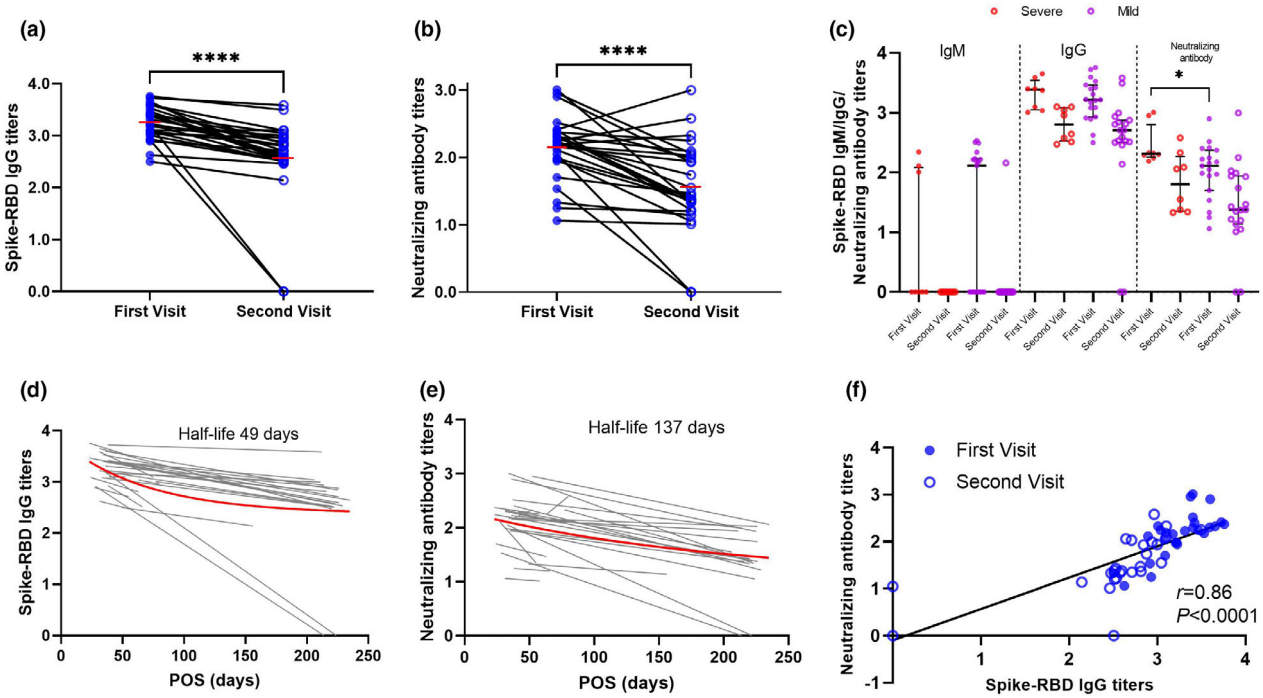

Figure 1. Dynamic changes in the SARS-CoV-2-specific humoral response. **(a)** Changes in the spike-RBD IgG titres and **(b)** neutralising antibody titres at each visit. Each symbol in **a** and **b** represents one patient (n = 27). **(c)** The different humoral responses among patients with various disease severities at their first visit (n = 8 and n = 19 in the severe disease group and mild disease group, respectively). **(d)** Decay of the spike-RBD IgG titres and **(e)** neutralising antibody titres over time. **(f)** Correlation between titres of spike-RBD IgG and neutralising antibodies.

disease severities. The titres of neutralising antibodies were positively correlated with the IgG titres (Figure 1f).

Sustained SARS-CoV-2-specific T-cell responses during follow-up

SARS-CoV-2-specific $CD4^+$ T-cell and $CD8^+$ T-cell responses were measured by quantification of T-cell receptor activation-induced markers (AIM) after *in vitro* stimulation with two SARS-CoV-2 spike peptides. The data were obtained after subtracting the background control from the DMSO-negative control (Figure 2a). The cumulative SARS-CoV-2-specific $CD4^+$ T-cell and $CD8^+$ T-cell measurements were calculated as the sum of the two peptides specific $CD4^+$ T cells and $CD8^+$ T cells, respectively. Adequate living cells were collected from 26 and 25 patients during the first and second visits, respectively.

During their initial visits, SARS-CoV-2-specific $CD4^+$ T cells were detected in 96.2% (25/26) of the patients (Figure 2b). Of them, 24 had robust levels of $CD4^+$ SARS-CoV-2-specific T cells in the circulation. Despite the low proportion of these cells, spike-specific $CD4^+$ T cells were identified in 72.7% (8/11) of healthy controls. The proportion of SARS-CoV-2-specific $CD4^+$ T cells in COVID-19 patients was significantly higher than that of healthy controls (Figure 2b). Spike-specific $CD8^+$ T cells were also observed in 92.3% (24/26) of the patients. However, the proportions of spike-specific $CD8^+$ T cells in COVID-19 patients were statistically comparable to those detected in healthy controls (0.46 [0.13–0.90] % vs 0.14 [0.01–0.38] %, P = 0.052, Figure 2c). In patients without detectable circulating SARS-CoV-2-specific $CD4^+$ T cells, 0.14% of SARS-CoV-2-specific $CD8^+$ T cells could be identified. Therefore, all patients had measurable SARS-CoV-2-specific $CD4^+$ T cells or $CD8^+$ T cells.

Both SARS-CoV-2-specific $CD4^+$ T cells and $CD8^+$ T cells decayed slowly during the follow-up period (Figure 2d, e). SARS-CoV-2-specific $CD4^+$ T cells were detectable in 96% (24/25) of the patients at their second visit, which occurred at 212 days POS. Robust SARS-CoV-2-specific $CD4^+$ T-cell responses could still be identified in 68.0% (17/25) of the patients. When compared to the levels of SARS-CoV-2-specific $CD4^+$ T cells among these patients

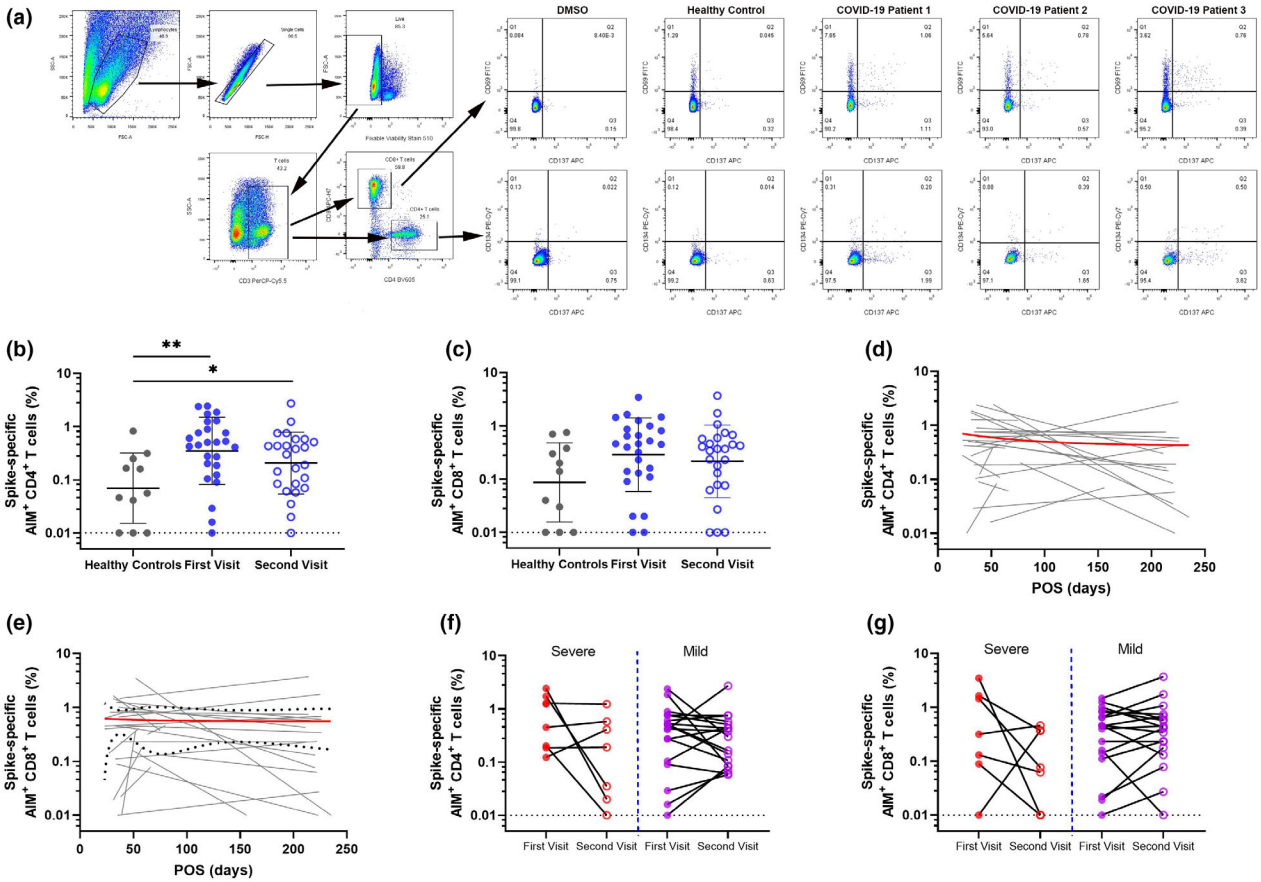

Figure 2. T-cell response to spike peptide pools in patients who have recovered from COVID-19. **(a)** Representative flow cytometry gating of AIM^+ $CD4^+$ T cells and $CD8^+$ T cells. **(b)** Different proportions of spike-specific $CD4^+$ T cells and **(c)** $CD8^+$ T cells in patients who have recovered from COVID-19 (n = 26 at the first visit and n = 25 at the second visit) and healthy controls (n = 11). **(d)** Decay of the proportions of the spike-specific $CD4^+$ T cells and **(e)** $CD8^+$ T cells over time. **(f)** The disease severity does not have an effect on the changes in spike-specific $CD4^+$ T-cell and **(g)** $CD8^+$ T-cell response. The dashed line indicates the limit of detection.

at the first visit, no significant change was observed. The proportion of SARS-CoV-2-specific $CD4^+$ T cells at this time point was persistently higher than that detected in healthy controls (Figure 2b). Similarly, SARS-CoV-2-specific $CD8^+$ T-cell responses were identified in 88% (22/25) of the patients, with relatively constant levels compared with those identified in the first visit (Figure 2c). No significant differences were observed in the proportion of spike-specific $CD8^+$ T cells between patients who had recovered from COVID-19 and healthy controls ($P = 0.17$). In patients whose levels of spike-specific $CD4^+$ T cells were not detectable, the proportion of spike-specific $CD8^+$ T cells was also low, but remained detectable (0.062%). The relative levels of circulating SARS-CoV-2-specific $CD4^+$ T cells were comparable to those of SARS-CoV-2-specific $CD8^+$ T cells, while a significant association was observed between these two cell populations ($r = 0.54$, $P < 0.01$). A similar trend was also observed in the changes in SARS-CoV-2 specific $CD4^+$ and $CD8^+$ T-cell levels during these two visits (Figure 2d, e). In this cohort, the differences in the levels of SARS-CoV-2-specific T cells were not significantly associated with age, sex and disease severity (Figure 2f, g).

Correlation between SARS-CoV-2-specific T-cell responses and antibody titres

We then evaluated the association between SARS-CoV-2-specific T-cell responses and antibody titres. We pooled the data from patients who had recovered from COVID-19. A moderate positive correlation was observed between the levels of

$CD4^+$ T-cell responses and neutralising antibody titres ($r = 0.47$, $P < 0.01$, Figure 3a). The titres of spike-RBD IgG were also positively associated with the proportion of SARS-CoV-2-specific $CD4^+$ T cells ($r = 0.54$, $P < 0.001$, Figure 3b). Moreover, weak correlations were found between the levels of circulating SARS-CoV-2-specific $CD8^+$ T cells and titres of spike-RBD IgG, as well as between the levels of circulating SARS-CoV-2-specific $CD8^+$ T cells and titres of neutralising antibodies (Figure 3c, d).

DISCUSSION

Ascertaining the magnitude and quality of humoral and T-cell immunological memory against SARS-CoV-2 is critical to understanding durable protection. Doing so could also help in the development of effective vaccines. Although our understanding of COVID-19 is expanding, our knowledge on immunity to SARS-CoV-2 after recovery is still limited. Comprehensive evaluations of SARS-CoV-2-specific humoral and T-cell responses in the same patients who have recovered from COVID-19 are essential to expand our understanding. The results of our exploratory study suggest that although the titres of neutralising antibodies against SARS-CoV-2 decay, immunity mediated by T cells, predominantly SARS-CoV-2-specific $CD4^+$ T cells, is sustained at 7 months after primary infection.

Our results show that the spike-RBD-specific IgM and IgG titres, as well as the neutralising antibody titres, declined significantly within the first 7 months after SARS-COV-2 infection. The results of our study are in line with the reports of previous studies, which showed that SARS-CoV-2-specific IgG decayed sharply in convalescent COVID-19 patients.[3,13] Recent studies also revealed that the neutralising antibody waned after the titre peaked, which was detected 3–4 weeks after infection.[6,21] Taken together, these results support the notion that the humoral response against SARS-CoV-2 is a typical response after an acute viral infection. Hence, the primary concern is the longevity of the neutralising antibody after an acute SARS-COV-2 infection. Several longitudinal studies have shown that there was little to no decrease in the neutralising antibody titres at 75 days POS and that only modest declines were observed within 5 months POS.[5,15] In this study, 92.5% of the patients still had detectable neutralising antibodies approximately 7 months after infection, despite a decrease in titres. However, two patients with relatively low neutralising antibody titres at their first visit lost

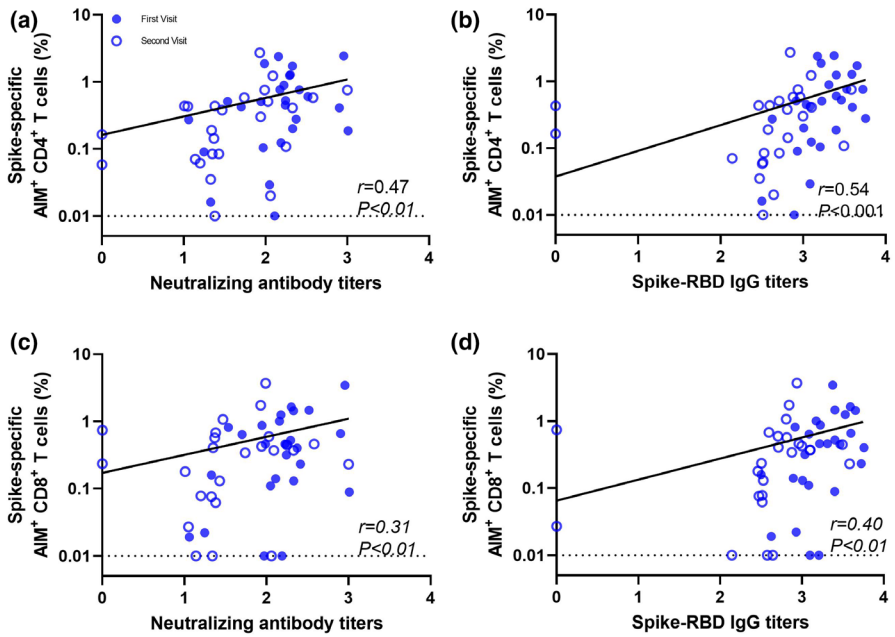

Figure 3. Correlations of spike-specific humoral response and T-cell response. **(a)** Neutralising antibody titres and **(b)** spike-RBD IgG titres are both positively correlated with the proportions of spike-specific $CD4^+$ T cells and **(c, d)** $CD8^+$ T cells. The dashed line indicates the limit of detection.

their neutralising antibodies at 7 months POS. Consistently, a previous study also suggested that some individuals with low peak neutralising antibody titres were unable to persistently produce neutralising antibodies for 7 weeks.[6] Further follow-ups in these longitudinal cohorts are needed to determine whether the levels of neutralising antibodies will continue to decline or will plateau to a steady level. Investigations to extend the longevity of neutralising antibodies are also warranted.

In contrast to the decreased humoral response against SARS-CoV-2, the levels of specific T cells were maintained at 7 months post-infection. In the current study, the spike-specific $CD4^+$ T-cell responses persisted in 96% of the patients at their second visit. In patients with undetectable spike-specific $CD4^+$ T cells, the levels of AIM^+ $CD8^+$ T cells were still measurable. Therefore, all patients had spike-specific T-cell responses at approximately 7 months after infection. In line with our results, several recent studies also reported that the T-cell responses to SARS-CoV-2 were maintained in patients up to 6 months after the onset of COVID-19 symptoms.[22–24] However, another group found that the frequencies of spike-specific $CD4^+$ T cells declined in the first 4 months POS.[25] Indeed, in our study, either $CD4^+$ or $CD8^+$ T-cell response waned in some patients. Therefore, further studies are needed to explain why T-cell responses are maintained in some patients while decaying in others.

Interestingly, only the proportions of spike-specific $CD4^+$ T cells in the patients who had recovered from COVID-19 were higher than those in healthy controls. Moreover, the T-cell responses to SARS-CoV-2 in patients who had recovered from COVID-19 were predominantly $CD4^+$ T-cell responses with strong IL-2 cytokine expression.[22] Therefore, the different immunological roles of memory $CD4^+$ T cells and $CD8^+$ T cells warrant further investigation.

Evidence from previous studies on related viruses, such as SARS-CoV, has shown that SARS-CoV-specific T-cell responses against these viruses are maintained for a long-term period compared with antibody responses. The half-life of the neutralising antibodies against SARS-CoV in patients who had recovered from SARS was approximately 6.4 weeks.[26] In a longitudinal study, the neutralising antibodies disappeared in 16.1% of patients at 36 months POS.[27] However, memory T cells that are reactive to the N protein of SARS-CoV were detectable in patients who had recovered from SARS 17 years after the outbreak this disease in 2003.[28] In the current study, the humoral response to SARS-CoV-2 declined significantly after 7 months, while the levels of specific T-cell responses remained stable, indicating that cellular responses to SARS-CoV-2 may last longer than humoral responses.

Emerging evidence suggests that both humoral and T-cell immune responses are required for effective protection against SARS-CoV-2 infection.[8,29] In a mouse study, the intravenous adoptive transfer of SARS-CoV-immune splenocytes or *in vitro*-generated T cells to mice enhanced their survival and reduced the virus titres in the lungs, suggesting that T cells play a crucial role in SARS-CoV clearance.[30] In another mouse model of SARS, $CD4^+$ T cells alone, without antibodies or $CD8^+$ T cells, could protect the mouse against the lethal effects of SARS-CoV infection.[31] However, it is still unknown whether the persistence of the cellular immune response can prevent SARS-CoV-2 reinfection or reduce the severity of infection when neutralising antibodies are present. In a recent study, higher T-cell responses to SARS-CoV-2 were reported to be associated with a lower risk of developing COVID-19.[32] In addition, a few case reports of laboratory-confirmed SARS-CoV-2 reinfections have also suggested that T-cell responses may prevent reinfection.[33–36]

Our study has some limitations. First, we used the ACE2-RBD binding inhibition assay, which is a surrogate neutralisation test, and may be less helpful compared with a living virus assay. Second, we only used peptide pools from the spike protein. Although the neutralising antibodies are generally considered against the spike protein, humoral and T-cell responses against other proteins, including the M and N proteins, and full epitope mapping in the future could provide comprehensive information on human coronavirus-specific humoral and T-cell responses. Third, the sample size used in our study was limited by expediency, which may be underpowered for detecting subtle differences. Finally, all COVID-19 patients enrolled in the present study were admitted in the hospital. Therefore, it remains unknown whether these asymptomatic patients have a similar pattern of changes in humoral and T-cell responses.

In conclusion, our study suggests that in patients who have recovered from COVID-19,

SARS-CoV-2-specific T-cell immune responses persist, while the neutralising antibodies are waning. Hence, further studies are needed to determine the longevity of neutralising antibodies and to evaluate whether these T cells are sufficient to protect patients from reinfection.

METHODS

Ethical statement and clinical definitions

This study was approved by the ethics committee of the Shanghai Public Health Clinical Center. Written informed consent was obtained from all donors. All donors were COVID-19 patients admitted in the hospital between January and February 2020. They were discharged after showing symptom relief and clearance of SARS-CoV-2. They were routinely followed up at the outpatient clinic. Blood samples for antibody tests and cellular analyses were collected and stored at each visit. The samples were collected at a median of 36 (range: 23–77) and 212 (range: 52–235) days after the onset of self-reported symptoms at the first and second visits, respectively. The disease severity of the patients was identified based on the degree of hypoxemia. Eight patients were classified as having a severe case because they had a PaO_2/FiO_2 ratio lower than 300 and met the criteria for acute respiratory distress syndrome, while 19 were classified as having a mild case. Details regarding the patients' information are provided in Table 1. Eleven healthy donors were enrolled as controls.

Table 1. Demographic and clinical characteristics of the study population

Characteristics	Value
Age, median (IQR)	52 (39–64)
Sex, *n* (%)	
Male	12 (44.4)
Female	15 (55.6)
Days post-onset of symptoms, median (IQR)	
First visit	36 (31–39)
Second visit	212 (70–222)
Disease severity, *n* (%)	
Mild	19 (70.4)
Severe	8 (29.6)
Comorbidities, *n* (%)	
Hypertension	8 (29.6)
Diabetes	4 (14.8)
Coronary heart disease	1 (3.7)
COPD	3 (11.1)
Symptoms, *n* (%)	
Fever	25 (92.6)
Cough	15 (55.6)
White blood cells count ($\times 10^9$ L^{-1})	3.91 (3.54–5.49)
< 3.5, *n* (%)	6 (22.2)
Lymphocytes ($\times 10^9$ L^{-1})	0.85 (0.59–1.16)
< 1.1, *n* (%)	17 (63.0)
Baseline $CD4^+$ T-cell count, median (IQR), cells μL^{-1}	316 (211–595)
Baseline $CD8^+$ T-cell count, median (IQR), cells μL^{-1}	192 (103–281)
Baseline CD4/CD8 ratio	1.78 (1.32–2.54)
Use of glucocorticoid (*n*, %)	4 (14.8)
Use of intravenous immunoglobulin (*n*, %)	7 (25.9)

Peripheral blood mononuclear cells (PBMCs) from healthy donors and patients were isolated from fresh blood samples by Ficoll-Paque density gradient centrifugation on the day of blood collection. The majority of the purified PBMCs were used for immune cell phenotyping, whereas the plasma samples were subjected to antibody tests.

Surrogate virus neutralisation test

The levels of serum neutralising antibodies against the RBD of SARS-COV-2 were measured using a novel surrogate virus neutralisation test with a commercial kit provided by GenScript (L00847; GenScript, Nanjing, China).[37] The serum samples were diluted (at 1:10) with twofold serial gradients and incubated with an equal volume of horseradish peroxidase-conjugated RBD (HRP-RBD) at 37°C for 30 min. Then, the serum/HRP-RBD mix (100 μL) was added to each well and incubated at 37°C for 15 min. Unbound HRP-RBD was removed by four washes, the chromogenic substrate TMB was added, and the mixture was incubated at 25°C for 15 min. The colorimetric reaction was terminated by adding a stop solution. The absorbance at 450 nm was measured using a microplate reader (iMark, Bio-Rad, CA, USA). The percentage inhibition for each sample was calculated using the following formula: % reduction = [1 − OD450 (sample)/average OD450 (negative control)] × 100%. The neutralising antibody titre was calculated with a half-maximal inhibitory concentration.[37]

Spike S1-receptor-binding domain-specific IgG and IgM test

The serum titres of IgG and IgM antibodies against SARS-CoV-2 spike S1-RBD were determined using commercial kits (L00845; GenScriptChina) according to the manufacturer's instructions. The serum samples were diluted to 10-fold serial gradients. Briefly, 100 μL of serum sample was incubated at 37°C for 30 min, followed by four washes. Then, 100 μL of HRP-conjugated mouse anti-human IgG or IgM was added and incubated at 37°C for 15 min, after which the chromogenic substrate (TMB) was added and incubated at 25°C for another 15 min. The colorimetric reaction was terminated by adding a stop solution, and the absorbance of each sample at 450 nm was measured with a microplate reader (iMark). The sample/cut-off ratio (S/CO) was calculated according to the manufacturer's instructions. The serum IgG and IgM antibody titres were calculated as the reciprocal of the dilution factor when S/CO = 1.

Detection of SARS-CoV-2 antigen-responsive T cells

The PBMCs were separated using a Lymphoprep (AS1114546; Axis-Shield, Dundee, UK). The PBMCs (1 million) were cultured in RMPI 1640 medium (Gibco, Gaithersburg, MD, USA) supplemented with 10% foetal

bovine serum (Gibco, Logan, UT, USA) and an antibiotic cocktail containing 100 μg mL^{-1} penicillin and 100 μg mL^{-1} streptomycin. Five nanograms of the SARS-CoV-2 peptide pool at a concentration of 5 ng mL^{-1} was added to the culture media to stimulate the production of T cells for 3 days. The same volume of DMSO and PHA served as the negative control and positive control, respectively, for each sample. The peptide pool, including 316 peptides (delivered in two subpools of 158 and 158 peptides), was derived via a peptide scan (15 mers with 11 aa overlap) through the entire spike glycoprotein (protein ID: P0DTC2) of SARS-CoV-2 (GenScript). Cell culture was performed at 37°C in a 5% CO_2 humidified environment. Then, the SARS-CoV-2-specific $CD4^+$ T-cell and $CD8^+$ T-cell responses were measured by quantification of T-cell receptor AIMs using flow cytometry in living cells. Briefly, the cells were harvested, washed twice with PBS, incubated with fixable viability stain 510 (BDTM Horizon, 564406) to distinguish whether the cells were alive or dead for 30 min and washed once with PBS. Then, the cells were incubated with the APC-H7 mouse anti-human CD8 antibody (clone SK1; BD Bioscience, Franklin Lakes, NJ, USA), BV605 mouse anti-human CD4 antibody (clone RPA-T4, BD Bioscience, Franklin Lakes, NJ, USA), Percp mouse anti-human CD3 antibody (clone SK7; BD Bioscience, Franklin Lakes, NJ, USA), APC anti-human CD137 antibody (clone 4B4-1; BioLegend, San Diego, CA, USA), PE/cyanine7 anti-human CD134 antibody (clone Ber-ACT35; BioLegend) and FITC-CD69 monoclonal antibody (11-0699-42; Thermo Fisher Scientific, Waltham, MA, USA) for 15 min. After washing with PBS, the cells were permeabilised and washed with BD Perm/Wash™ buffer (554723; BD Bioscience, Franklin Lakes, NJ, USA), and flow cytometry analysis was performed using BD LSRFortessa. The AIM^+ $CD4^+$ T cells ($CD134^+CD137^+$) and AIM^+ $CD8^+$ T cells ($CD69^+CD137^+$) were gated. Robust T-cell responses were defined as more than 0.1% of AIM^+ $CD4^+$ or $CD8^+$ T cells detected in the circulation.[8,38] Flow cytometry data were analysed using the FlowJo version 10 software (FlowJo LLC, Ashland, OR, USA).

Statistical analyses

Statistical analyses were performed using the GraphPad Prism 9 software (GraphPad, San Diego, CA, USA). The data were represented as mean values with SDs or medians (interquartile ranges) depending on their distribution. A Mann–Whitney two-tailed *U*-test was used to compare variables between the two groups, and a Wilcoxon matched-pairs signed-rank test was used to compare paired non-parametric data. The correlations were calculated using Spearman's rank correlation coefficient.

ACKNOWLEDGMENTS

The work was supported by the Shanghai Science and Technology Committee (20411950200 and 20Z11900900), the Development Fund for Shanghai Talents (2020089) and the Shanghai 'Rising Stars of Medical Talent' Youth Development Program, Specialist Program (no. 2019-72). We thank Editage (www.editage.com) for English-language editing.

AUTHOR CONTRIBUTIONS

Jun Chen: Conceptualization; Formal analysis; Investigation; Writing-original draft. **Xiaomin Liu:** Investigation; Methodology. **Xinyu Zhang:** Investigation; Methodology. **Yixiao Lin:** Investigation; Methodology. **Danping Liu:** Investigation; Methodology. **Jingna Xun:** Investigation; Methodology. **Zhenyan Wang:** Investigation. **Ling Gu:** Investigation; Methodology. **Qian Li:** Investigation; Methodology. **Dan Yin:** Project administration. **Junyang Yang:** Formal analysis; Project administration. **Hongzhou Lu:** Conceptualization; Funding acquisition; Supervision; Writing-review & editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Wang X, Guo X, Xin Q *et al*. Neutralizing antibody responses to severe acute respiratory syndrome coronavirus 2 in coronavirus disease 2019 inpatients and convalescent patients. *Clin Infect Dis* 2020; **71**: 2688–2694.
2. Ni L, Ye F, Cheng ML *et al*. Detection of SARS-CoV-2-specific humoral and cellular immunity in COVID-19 convalescent individuals. *Immunity* 2020; **52**: 971–977.e973.
3. Long QX, Liu BZ, Deng HJ *et al*. Antibody responses to SARS-CoV-2 in patients with COVID-19. *Nat Med* 2020; **26**: 845–848.
4. Robbiani DF, Gaebler C, Muecksch F *et al*. Convergent antibody responses to SARS-CoV-2 in convalescent individuals. *Nature* 2020; **584**: 437–442.
5. Wajnberg A, Amanat F, Firpo A *et al*. Robust neutralizing antibodies to SARS-CoV-2 infection persist for months. *Science* 2020; **370**: 1227–1230.
6. Seow J, Graham C, Merrick B *et al*. Longitudinal observation and decline of neutralizing antibody responses in the three months following SARS-CoV-2 infection in humans. *Nat Microbiol* 2020; **5**: 1598–1607.
7. Chen Y, Zuiani A, Fischinger S *et al*. Quick COVID-19 healers sustain anti-SARS-CoV-2 antibody production. *Cell* 2020; **183**: 1496–1507.e1416.
8. Rydyznski Moderbacher C, Ramirez SI, Dan JM *et al*. Antigen-specific adaptive immunity to SARS-CoV-2 in acute COVID-19 and associations with age and disease severity. *Cell* 2020; **183**: 996–1012.e1019.
9. Amanat F, Stadlbauer D, Strohmeier S *et al*. A serological assay to detect SARS-CoV-2 seroconversion in humans. *Nat Med* 2020; **26**: 1033–1036.
10. Chen P, Nirula A, Heller B *et al*. SARS-CoV-2 neutralizing antibody LY-CoV555 in outpatients with covid-19. *N Engl J Med* 2021; **384**: 229–237.
11. Bloch EM, Shoham S, Casadevall A *et al*. Deployment of convalescent plasma for the prevention and treatment of COVID-19. *J Clin Invest.* 2020; **130**: 2757–2765.
12. Renn A, Fu Y, Hu X, Hall MD, Simeonov A. Fruitful neutralizing antibody pipeline brings hope to defeat SARS-Cov-2. *Trends Pharmacol Sci* 2020; **41**: 815–829.

13. Ibarrondo FJ, Fulcher JA, Goodman-Meza D *et al*. Rapid decay of anti-SARS-CoV-2 antibodies in persons with mild Covid-19. *N Engl J Med* 2020; **383**: 1085–1087.
14. Long QX, Tang XJ, Shi QL *et al*. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. *Nat Med* 2020; **26**: 1200–1204.
15. Iyer AS, Jones FK, Nodoushani A *et al*. Persistence and decay of human antibody responses to the receptor binding domain of SARS-CoV-2 spike protein in COVID-19 patients. *Sci Immunol* 2020; **5**: eabe0367.
16. Gallais F, Velay A, Nazon C *et al*. Intrafamilial exposure to SARS-CoV-2 associated with cellular immune response without seroconversion, France. *Emerg Infect Dis* 2021; **27**: 113–121.
17. Graham BS. Rapid COVID-19 vaccine development. *Science* 2020; **368**: 945–946.
18. Folegatti PM, Ewer KJ, Aley PK *et al*. Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial. *Lancet* 2020; **396**: 467–478.
19. Corbett KS, Flynn B, Foulds KE *et al*. Evaluation of the mRNA-1273 vaccine against SARS-CoV-2 in nonhuman primates. *N Engl J Med* 2020; **383**: 1544–1555.
20. Keech C, Albert G, Cho I *et al*. Phase 1–2 trial of a SARS-CoV-2 recombinant spike protein nanoparticle vaccine. *N Engl J Med* 2020; **383**: 2320–2332.
21. Prevost J, Gasser R, Beaudoin-Bussieres G *et al*. Cross-sectional evaluation of humoral responses against SARS-CoV-2 spike. *Cell Rep Med* 2020; **1**: 100126.
22. Zuo J, Dowell AC, Pearce H *et al*. Robust SARS-CoV-2-specific T cell immunity is maintained at 6 months following primary infection. *Nat Immunol* 2021; **22**: 620–626.
23. Rodda LB, Netland J, Shehata L *et al*. Functional SARS-CoV-2-specific immune memory persists after mild COVID-19. *Cell* 2021; **184**: 169–183.e117.
24. Bilich T, Nelde A, Heitmann JS *et al*. T cell and antibody kinetics delineate SARS-CoV-2 peptides mediating long-term immune responses in COVID-19 convalescent individuals. *Sci Transl Med* 2021; **13**: eabf7517.
25. Wheatley AK, Juno JA, Wang JJ *et al*. Evolution of immune responses to SARS-CoV-2 in mild-moderate COVID-19. *Nat Commun* 2021; **12**: 1162.
26. Ho MS, Chen WJ, Chen HY *et al*. Neutralizing antibody response and SARS severity. *Emerg Infect Dis* 2005; **11**: 1730–1737.
27. Cao WC, Liu W, Zhang PH, Zhang F, Richardus JH. Disappearance of antibodies to SARS-associated coronavirus after recovery. *N Engl J Med* 2007; **357**: 1162–1163.
28. 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**: 457–462.
29. Tay MZ, Poh CM, Renia L, MacAry PA, Ng LFP. The trinity of COVID-19: immunity, inflammation and intervention. *Nat Rev Immunol* 2020; **20**: 363–374.
30. Zhao J, Zhao J, Perlman S. T cell responses are required for protection from clinical disease and for virus clearance in severe acute respiratory syndrome coronavirus-infected mice. *J Virol* 2010; **84**: 9318–9325.
31. Zhao J, Zhao J, Mangalam AK *et al*. Airway memory $CD4^+$ T cells mediate protective immunity against emerging respiratory coronaviruses. *Immunity* 2016; **44**: 1379–1391.
32. Wyllie D, Mulchandani R, Jones HE *et al*. SARS-CoV-2 responsive T cell numbers are associated with protection from COVID-19: a prospective cohort study in keyworkers. *medRxiv* 2020. https://doi.org/10.1101/2020.11.02.20222778
33. Tillett RL, Sevinsky JR, Hartley PD *et al*. Genomic evidence for reinfection with SARS-CoV-2: a case study. *Lancet Infect Dis* 2021; **21**: 52–58.
34. Van Elslande J, Vermeersch P, Vandervoort K *et al*. Symptomatic SARS-CoV-2 reinfection by a phylogenetically distinct strain. *Clin Infect Dis* 2021; **73**: 354–356.
35. To KK, Hung IF, Ip JD *et al*. COVID-19 re-infection by a phylogenetically distinct SARS-coronavirus-2 strain confirmed by whole genome sequencing. *Clin Infect Dis* 2020. https://doi.org/10.1093/cid/ciaa1275. Online ahead of print.
36. To KK, Hung IF, Chan KH *et al*. Serum antibody profile of a patient with COVID-19 reinfection. *Clin Infect Dis* 2021; **72**: e659–e662.
37. Tan CW, Chia WN, Qin X *et al*. A SARS-CoV-2 surrogate virus neutralization test based on antibody-mediated blockage of ACE2-spike protein-protein interaction. *Nat Biotechnol* 2020; **38**: 1073–1078.
38. Grifoni A, Weiskopf D, Ramirez SI *et al*. Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals. *Cell* 2020; **181**: 1489–1501.