One-year sustained cellular and humoral immunities of COVID-19 convalescents

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Summary: SARS-CoV-2-specific humoral and T-cell immune memory are present within ~95% and ~90% convalescents, respectively, until 1-year, with durable NAb, $CD8^+$ and $CD4^+$ T cells, but declined IgG and IgM from 6 to 12 months.

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Abstract

Background: The longitudinal antigen-specific immunity in COVID-19 convalescents is crucial for long-term protection upon individual re-exposure to SARS-CoV-2, and even more pivotal for ultimately achieving population-level immunity. To better understand the features of immune memory in individuals with different disease severities at one year post-disease onset we conducted this cohort study.

Methods: We conducted a systematic antigen-specific immune evaluation in 101 COVID-19 convalescents, who had asymptomatic, mild, moderate, or severe disease, through two visits at months 6 and 12 post-disease onset. The SARS-CoV-2-specific antibodies, comprising NAb, IgG, and IgM, were assessed by mutually corroborated assays, i.e. neutralization, enzyme-linked immunosorbent assay (ELISA), and microparticle chemiluminescence immunoassay (MCLIA). Meanwhile, the T-cell memory against SARS-CoV-2 spike, membrane and nucleocapsid proteins was tested through enzyme-linked immunospot assay (ELISpot), intracellular cytokine staining (ICS), and tetramer staining-based flow cytometry, respectively.

Results: SARS-CoV-2-specific IgG antibodies, and also NAb can persist among over 95% COVID-19 convalescents from 6 months to 12 months after disease onset. At least 19/71 (26%) of COVID-19 convalescents (double positive in ELISA and MCLIA) had detectable circulating IgM antibody against SARS-CoV-2 at 12m post-disease onset. Notably, the percentages of convalescents with positive SARS-CoV-2-specific T-cell responses (at least one of the SARS-CoV-2 antigen S1, S2, M and N protein) were 71/76 (93%) and 67/73 (92%) at 6m and 12m, respectively. Furthermore, both antibody and T-cell memory levels of the convalescents were positively associated with their disease severity.

Conclusions: SARS-CoV-2-specific cellular and humoral immunities are durable at least until one year after disease onset.

Keywords: SARS-CoV-2, COVID-19, neutralizing antibody, T cells, disease severity

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Introduction

The ongoing severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) pandemic has now lasted over one and a half years, resulting in over 229 million coronavirus disease 2019 (COVID-19) cases with 4.7 million deaths (https://covid19.who.int/), and remains a tough challenge for global health [1]. The characteristics of viral pathogeneses and immune responses during acute and convalescent phases of COVID-19 have been widely studied [2-4]. In response to SARS-CoV-2 infection, adaptive immunity, including antibodies, T cells against the virus, is generated [5]. SARS-CoV-2-specific T-cell responses are associated with milder disease in individuals with acute and convalescent COVID-19 [6,7], and neutralizing antibodies (NAbs) contribute to protective immunity against a second infection with SARS-CoV-2 in various animal models [8], indicating protective roles for antigen-specific antibodies and T cells in COVID-19 [9]. This immune memory among the COVID-19 convalescents is crucial for long-term protection upon individual re-exposure to this virus, and even more pivotal for ultimately achieving population-level immunity and interrupting disease transmission, together with the global usage of vaccines.

Here we conducted a systematic antigen-specific immune response evaluation in 101 convalescents of asymptomatic, mild, moderate or severe COVID-19 cases at 6 and 12 months post-disease onset. The SARS-CoV-2-specific antibodies, comprising NAb, IgG, and IgM, were assessed by mutually corroborated in neutralization assay, enzyme-linked immunosorbent assay (ELISA), and microparticle chemiluminescence immunoassay (MCLIA). Moreover, the T-cell memory against SARS-CoV-2 spike (S), membrane (M), and nucleocapside (N) proteins was tested through enzyme-linked immunospot assay (ELISpot), intracellular cytokine staining (ICS), and tetramer staining-based flow cytometry, respectively. This study will expand knowledge of the immune features and their persistence in convalescents recovering from COVID-19 of differing severities.

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Materials and methods

Sample collection

We recruited a total of 101 COVID-19 convalescent patients from Macheng, Hubei Province, China, with two visits in July 2020 and January 2021. A total of 28 healthy controls (HC) who had neither been infected with SARS-CoV-2 nor vaccinated against COVID-19 were recruited at Chinese Center for Disease Control and Prevention (Fig. 1, Supplementary Table 1). Venous blood was collected from each participant, and sera and peripheral blood mononuclear cells (PBMCs) were isolated. Isolated PBMCs were frozen in cell stock solution containing 90% fetal bovine serum (FBS) with 10% dimethylsulfoxide, and stored in liquid nitrogen for later use. Serum samples were preserved at −80°C until use in testing.

Detection of SARS-CoV-2-specific antibodies

SARS-CoV-2-specific IgG and IgM were assessed by ELISA and MCLIA, respectively [10-13]. NAb titer were measured via a live-virus neutralizing assay in Vero E6, as described previously [14]. Sample preparation was performed in a biosafety level-2 (BSL-2) laboratory, and the virus neutralization assay was conducted in a BSL-3 laboratory (Supplementary file 1).

Peptide pools design and culture of PBMCs *in vitro*

Totally, 271 15- to 18-mer SARS-CoV-2 peptides overlapped by 10 amino acids spanning the entire of S, M and N proteins were designed. For *in vitro* PBMC culture, the S1, S2, M and N peptide pools, recombinant IL-7 and IL-2 were added to PBMCs. PBMCs were cultured in a 24-well plate at a density of 3×10^6 cells/well for 9 days, with half of the cultured medium replaced every three days.

Enzyme-linked immunospot (ELISpot) assay

IFN-γ-secreting T cells were detected with human IFN-γ ELISpot assay kits (BD Corp, USA), as described previously [15] (Supplementary file 1). The results are expressed as spot-forming cells (SFCs) per 10^6 PBMCs, counted using an ELISpot Reader System (CTL Corp., USA).

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Tetramers staining

HLA-A*1101 tetramers complexed with SARS-CoV-2-specific peptides M23 (M171-180, ATSRTLSYYK) and N25 (N362-370, KTFPPTEPK) were generated in our laboratory as described previously for the preparation of other HLA class I tetramers [16]. *In vitro* cultured PBMCs were harvested, washed twice with FACS buffer, and then stained with antibodies on ice for 30 min. After the final wash, the cells were re-suspended and immediately analyzed by flow cytometry.

Statistical analysis

Statistical analyses were conducted with GraphPad Prism 8, R, and SAS. The difference between groups was examined by a Wilcoxon matched-pairs signed rank test or Mann-Whitney U-test as appropriate. The comparison of categorical variables was examined by a chi-square test or Fisher' s exact test as appropriate. Correlations were assessed using a Spearman's Rank correlation coefficient (r). Simple linear regression was used to evaluate the impact of disease severity on immune indexes. The statistical significance was set as follows: ns, not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; All tests were two tailed.

Results

Anti-SARS-CoV-2 antibodies persist in COVID-19 convalescents at 6m and 12m

From July 2020 to January 2021, 101 documented COVID-19 convalescent patients responded to the recruitment during their recovery from disease onset for 6 months (denoted as 6m, n=81) to 1-year (denoted as 12m, n=74) with 57 successfully followed up among them (Fig. 1). We measured anti-RBD IgG and IgM levels in the sera of all COVID-19 convalescents visited at 6m and 12m post-disease onset, and in healthy controls, by ELISA and MCLIA (Table 1). There was no significant difference in the percentage of IgG-positive subjects between those followed-up at 12m and 6m. However, the IgG levels were both significantly lower at 12m (P<0.0001 for ELISA and P=0.0011 for MCLIA, Fig. 2A and B, Supplementary Fig. 1). Similarly, the IgM antibody levels at 12m also

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decreased significantly compared to 6m (*P*=0.0004 for ELISA and *P*=0.0067 for MCLIA, Fig. 2C and D, Supplementary Fig. 1). We also calculated the percentage of the convalescents with double positive results from both antibody detection methods (double-positive). IgG and IgM antibodies against SARS-CoV-2 S protein RBD were not detectable in any of the healthy controls with either ELISA or MCLIA.

In addition to quantifying SARS-CoV-2-binding antibodies, we also measured NAbs with live virus neutralization assay in a BSL-3 laboratory. The percentages of convalescents with detectable SARS-CoV-2 NAb were high at both 6m (95%) and 12m (99%), with no significant difference (Table 1). And also no significant difference of the SARS-CoV-2 NAb titers was observed between 6m and 12m (Fig. 2E). Among the 57 participants who provided consecutive samples, 28 (49%) had unchanged NAb titers at 12m compared with 6m (Fig. 2F), (Fig. 2G), 27 (47%) had decreased titers and 2 (4%) had increased titers (Fig. 2H) (Supplementary Fig. 2). No SARS-CoV-2-specific NAb was detected in healthy controls (Fig. 2E).

The relationship assessment between SARS-CoV-2 IgG, IgM levels and the NAb titers showed positive correlations between any two of the three antibody indicators, which confirmed reliability of the methods and the authenticity of the results (Fig. 2I-L, and Supplementary Fig. 3). We also analyzed the maintenance of IgG and IgM levels in COVID-19 convalescents from 6m to 12 based on different disease severities during their acute phase. The level of IgG antibody trended lower at 12m than that at 6m post-disease onset in mild, moderate, or severe cases (Fig. 2M, N). The IgM antibody level significantly decreased at 12m in mild or moderate cases (Fig. 2O, P). However, there was no significant decreasing in the NAb levels between 6m and 12m of convalescents (Fig. 2Q). Furthermore, to assess a possible correlation between anti-SARS-CoV-2 antibodies among convalescents and their disease severity, we converted the severity variable to a rank variable and performed a univariate linear regression. All the relationships between disease severity and IgG, IgM, or NAb levels showed statistically significant fittings; thus, disease severity has an important impact on the humoral immune memory among COVID-19 convalescents (Fig. 2R-V). And this may also indicate that stronger humoral responses were induced at the acute phase in more severe cases.

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Overall T-cell memory is sustained in most COVID-19 convalescents at 12m

The SARS-CoV-2-specific T-cell immunity in COVID-19 convalescent patients were detected by utilizing both freshly isolated PBMCs (*ex vivo*) and 9-days cultured PBMCs (*in vitro*). PBMCs in the IFN-γ ELISpot assay were tested under the stimulation of four pools of overlapping peptides spanning the SARS-CoV-2 S protein (divided into S1 and S2), M protein and N protein. In the *ex vivo* ELISpot detection, only the median of M protein responding T cells at 12m (median: 28 spot-forming cells (SFCs)/10^6 PBMCs; IQR: 0, 103 SFCs/10^6 PBMCs) is above the cutoff (20 SFCs/10^6 PBMCs), which is significantly higher than that at 6m (median: 10 SFCs/10^6 PBMCs; IQR: 0, 28 SFCs/10^6 PBMCs) (Fig. 3A).

We also conducted the *in vitro* expansion of PBMCs for 9 days under the stimulation of the same four antigens. After the expansion, the percentages of convalescents with positive T-cell responses to S1, S2, M protein and N protein at 6m were not differ significantly from their respective percentages at 12m. The percentages of convalescents with positive T-cell responses to at least one of the SARS-CoV-2 antigen peptide pools were 93% and 92% at 6m and 12m, respectively (Table 2). This suggests that robust memory T-cell responses could persist for at least 1 year among most COVID-19 convalescents. We also compared the T-cell memory to peptide pools of different antigens. M and N peptide pool-specific T-cell responses were significantly higher compared with S1 or S2 peptide pool-specific responses (Fig. 3B). Interestingly, we observed T-cell responses to SARS-CoV-2 in healthy controls as well (S1: 7/28(25%), S2: 10/28(36%), M: 8/28(29%) and N: 10/28(36%) which may reflect cross reactivity to common cold coronaviruses in the population.

To evaluate the impact of disease severity on virus-specific T-cell memory, we compared the T-cell response intensities among patients who recovered from COVID-19 cases of differing clinical severity (asymptomatic, mild, moderate, and severe). The response in subjects who had asymptomatic cases was lower than that in subjects who had more severe symptoms, these differences were significant at 6m (mild, P=0.0123; moderate, P=0.0045; and severe, P=0.0115) and the trend continued at 12m (Fig. 3C). We also converted the severity variable to a rank variable and performed

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a univariate linear regression, considering the healthy controls as the lowest rank in this analysis. T-cell memory of the convalescents against different protein peptide pools, both at 6m and 12m, showed a relatively good fit with disease severity, indicating an increasing trend for T-cell memory in convalescent patients with increasing disease severity (Fig. 3D-G).

The T-cell memory against S protein was significantly correlated with antibody responses at 12m. Correlations were also observed among the S1- and S2-specific T-cell responses with antibody levels (Fig. 3H-P). No relationship was observed between the anti-SARS-CoV-2 antibodies and the T-cell responses to other viral antigens, i.e. M and N proteins (Supplementary Fig 4).

Both SARS-CoV-2-specific CD4^{+} and CD8^{+} T cells are durable in convalescents.

We also performed ICS followed by flow cytometry with PBMCs from 12 convalescents at 6m and 12m to further investigate the features of SARS-CoV-2-specific memory T cells, such as the multiple-cytokine-secreting SARS-CoV-2-specific CD4^{+} and CD8^{+} T cells across timepoints (Fig. 4A and Supplementary Fig. 5). The percentages of different CD4^{+} or CD8^{+} T-cell subsets secreting IFN-γ, IL-2, and TNFα with the stimulation of SARS-CoV-2 antigen peptide pools were not significantly different between 6m and 12m in convalescents (Fig. 4B, C). The proportions of single-, double-, and triple-cytokine-secreting T cells tended to be stable between 6m and 12m for both CD4^{+} and CD8^{+} T cells. In detail, single-cytokine-secreting IFN-γ^{+}IL-2^{-}TNFα^{-} and double-cytokine-secreting IFN-γ^{+}IL-2^{-}TNFα^{+} CD4^{+} T cells accounted for most of the SARS-CoV-2-specific CD4^{+} T cells (Fig. 4D, E), while single-cytokine-secreting IFN-γ^{+}IL-2^{-}TNFα^{-} and IFN-γ^{-}IL-2^{+}TNFα^{-} and double-cytokine-secreting IFN-γ^{+}IL-2^{-}TNFα^{+} CD8^{+} T cells accounted for most of the virus-specific CD8^{+} T cells. SARS-CoV-2-specific T cells targeting different virus proteins showed very similar cytokine secretion profiles (Supplementary Fig. 6 and 7).

To investigate the memory phenotypes of SARS-CoV-2-specific CD4^{+} and CD8^{+} T cells, CCR7 and CD45RA expressions on IFN-γ-secreting T cells was investigated and the percentages of naïve (CD45RA^{+}CCR7^{+}), central memory (CD45RA^{-}CCR7^{+}), effector memory (CD45RA^{-}CCR7^{-}), and effector (CD45RA^{+}CCR7^{-}) subsets were determined. The results demonstrate that both virus-specific

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$CD4^+$ and $CD8^+$ T-cell groups were mainly composed of effector memory T cells, and no significant differences were observed across the two timepoints, i.e., at 6m and 12m, for each subset (Fig. 4F, G).

HLA-A*1101/epitope tetramer-based characterization of memory $CD8^+$ T cells among the COVID-19 convalescents

After evaluating T-cell responses to overall antigen peptide pools, we investigated the single epitope-specific T cells within COVID-19 convalescents. Based on results of overlapping peptide-stimulating IFN-γ ELISpot assays performed with PBMCs from COVID-19 convalescent individuals at 6m, two overlapping peptides (nCoV-M23 and nCoV-N25) were identified as the antigenic regions that stimulated T cells to secrete IFN-γ. We predicted potential $CD8^+$ T-cell epitopes within these regions and identified two HLA-A*1101-restricted epitopes M23 (ATSRTLSYYK) and N25 (KTFPPTEPK) derived from the M and N proteins, respectively (Supplementary Fig. 8). Subsequently, we prepared HLA/peptide tetramers comprising these two epitopes bound to the HLA-A*1101 molecules. Using PBMCs from four HLA-A*1101^+ COVID-19 convalescents recovered for 6m, M23 tetramer-positivity was detected in 0.32%–3.63% of the $CD8^+$ T cells, and epitope N25-specificity was detected in 0.83%–2.37% (Fig. 4H and J). Furthermore, we tested the SARS-CoV-2-specific T cells in Participant 16 with HLA-A*1101 restriction at two time points (6m and 12m), using the HLA-A*1101/M23 tetramer. The percentage of M23 tetramer-specific $CD8^+$ T cells at 12m (0.52%) was lower than that at 6m (3.63%) post-disease onset (Fig. 4I). The alignment of the M23 and N25 peptide amino acid sequences with other human coronaviruses and SARS-CoV-2 variants of concern (VOC) showed that the amino acids of these two peptides are conserved in SARS-CoV and the current SARS-CoV-2 VOC, but not in other human coronaviruses (Fig. 4K). Thus, the T-cell responses determined herein are SARS-CoV-2-specific and not influenced by cross-reactivity with common cold coronaviruses.

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Discussion

With the continuous unabated pandemic of SARS-CoV-2, as one of the newly emerging viruses infecting humans [17], the prophylactic interventions, especially the accelerated vaccine inoculation were promoting in various countries with the goal of achieving herd immunity among the population. The attainment of protective population-level immunity requires the induction of long-term immunological memory by SARS-CoV-2 infection or vaccination, as this is crucial for protection upon virus re-exposure and reduction of human-to-human transmission. Thus, the longitudinal assessment of humoral and cellular immune memory against this newly emerging virus among convalescents is critical. Herein, we present a comprehensive longitudinal analysis of SARS-CoV-2-specific humoral and T-cell responses in COVID-19 convalescents who provided follow-up samples at 6m and/or 12m post-symptom onset, conducted using mutually corroborating methods.

The anti-SARS-CoV-2 antibody titers in convalescents were durable. The percentages of NAb-positive COVID-19 convalescents were both above 95% at 6m and 12m post-infection, without a significant decline in NAb titer over time. The IgG against spike RBD, as determined by ELISA and MCLIA, also persisted among nearly 95% patients at 12m post-infection. This finding is in line with previous reports on the relatively stable humoral immunity within the COVID-19 convalescent individuals for up to 6-8 months [18-20]. However, our study found an even higher percentage of convalescents who were positive for anti-SARS-CoV-2 antibodies, supported by the consistency among three different antibody detection methods (NAb, ELISA IgG and MCLIA IgG). Some previous studies have shown clear decay of SARS-CoV-2 NAb and IgG responses in the first several months post-infection [21-23]. Although a significant IgG level decline was also detected among the convalescents in our study, the percentage of IgG-positive individuals was sustained between 6m and 12m. In addition, the SARS-CoV-2 NAb titers of the convalescents did not differ significantly between 6m and 12m. Considering the declining trend in NAb titer among over 40% (27/57) of the convalescents, evaluating the durability of establishing humoral immunity through SARS-CoV-2 infection needs further observation.

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Wheatley et al. found that S-specific IgM fit a two-phase decay (before and after 70 days) in the convalescent time period, through a mixed-effects modelling approach, with a more rapid early decay ($t_{1/2}$=55 days) followed by a slower decay ($t_{1/2}$= 118 days) in late convalescence [23]. In our study, approximately a quarter of the convalescents had anti-SARS-CoV-2 IgM (ELISA and MCLIA double-positive) at 12m. No participants in our study reported reinfection during their convalescent phase. A certain proportion (13%) of individuals who were positive for SARS-CoV IgG had IgM antibodies was also reported among the population in Wuhan, Hubei province, China [20]. Thus, the long-term persistence of anti-S IgM among some of our convalescents may be linked to a certain feature of COVID-19, the mechanism for which needs further investigation.

Post-infection antigen-specific memory T-cell responses are diverse among individuals [24, 25]. Herein, one of our major findings is that the cellular immunity established following acute SARS-CoV infection is maintained for at least 12 months in most convalescents. More than 90% of the convalescents showed T-cell responses to at least one SARS-CoV-2 antigen peptide pool when *in vitro*-cultured PBMCs were used, although the intensities of the T-cell responses were diverse and had a high heterogeneity between individuals.

Disease severity during the acute virus infections plays a pivotal role in the level of antibody and T-cell immune memory among convalescents [25]. One study on COVID-19 convalescents indicated that anti-S IgG titers and memory B cells percentages were higher in hospitalized cases compared with non-hospitalized cases at 120 days post-disease onset [19]. Meanwhile, T-cell responses tended to be lower following asymptomatic SARS-CoV-2 infection than following symptomatic infection [26, 27]. Here, we found a significant linear correlation between patient disease severity during the acute phase and immune memory against SARS-CoV-2, comprising both antibody and T-cell responses. As proposed by Long et al, temperate T-cell responses in asymptomatic patients may clear the virus before they reach higher levels during acute infection, and this may be sufficient to allow reinfection with the virus [28].

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Our data demonstrate that SARS-CoV-2-specific humoral immunity is present within ~95% of convalescents and T-cell memory against at least one viral antigen is measurable among ~90% of subjects at 12m post-infection. From 6m to 12m post-infection, anti-SARS-CoV-2 IgG and IgM levels show a declining trend, but the levels of NAb and $CD8^+$ and $CD4^+$ T cells against SARS-CoV-2 are durable. These findings are encouraging in relation to the longevity of immune memory against this novel virus and indicate that these sustained immune components, which persist, among most SARS-CoV-2-infected individuals, may contribute to protection against reinfection.

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NOTES

Author contributions

W.J.L., G.F.G. and G.W. designed and supervised the study. Jie Z., S.D., M.L., C.Y., Jianbo Z. and Y.J. collected the samples. Jie Z., M.L., B.Y. and M.Z. conducted the experiments. Y.Z., S.L., H.Z., W.X., Y.G., D.Z., M.Y., Jing Z. and P.L. provided technical support and experimental assistance. Jie Z., H.L., M.Z., Y.G., X.L. and W.J.L. analyzed and interpreted data. Jie Z., M.Z., H.L. and W.J.L. wrote the initial draft of the manuscript. All authors contributed intellectually and approved the manuscript.

Ethics

This study was approved by the Ethics Committee of National Institution for Viral Disease Control and Prevention, China CDC (Ethical approval No. IVDC2020-021). Written informed consent was obtained from all participants.

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Declaration of interests

The authors declare no competing interests.

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Tables

Table 1. SARS-CoV-2-specific antibody in COVID-19 convalescents at 6 or 12 months post-disease onset.

Methods[a]	Group[a]	Case Number	Positive Number	Positive Proportions （%）	95% CI[c]	*P* value[d] （6m VS 12m）
Neutralization	HC	28	0	0	NA	
	6m	81	77	95	(88, 99)	
	12m	74	73	99	(93, 100)	0.42
MCLIA-IgG	HC	28	0	0	NA	
	6m	81	79	98	(91, 100)	
	12m	74	70	95	(87, 99)	0.60
MCLIA-IgM	HC	28	0	0	NA	
	6m	81	51	63	(52, 74)	
	12m	74	38	51	(39, 63)	0.19
ELISA-IgG	HC	28	0	0	NA	
	6m	81	78	96	(90, 99)	
	12m	74	71	96	(85, 99)	0.98
ELISA-IgM	HC	28	0	0	NA	
	6m	81	42	52	(40, 63)	
	12m	74	26	35	(24, 47)	0.05
IgG[e]	HC	28	0	0	NA	
	6m	81	78	96	(90, 99)	
	12m	74	70	95	(87, 99)	0.90
IgM[e]	HC	28	0	0	NA	
	6m	81	32	40	(29, 51)	
	12m	71	19	26	(16, 37)	0.09

[a]Neutralization: cutoff: neutralizing antibody titer >3; MCLIA: Microparticle chemiluminescence immunoassay, cutoff: S/CO>1; ELISA: Enzyme-linked immunosorbent assay, cutoff: IgG >0.19, IgM>0.105.

[b]HC: Healthy control; 6m: 6 months post disease onset; 12m: 12 months post disease onset.

[c]95% CI: 95% Confidence Interval.

[d]Chi square test was performed and the corresponding *P* value was listed (α=0.05).

[e]Double-positive, i.e., positive results from both an ELISA and MCLIA.

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Table 2. Percentages of COVID-19 convalescents with positive T-cell responses to SARS-CoV-2[a]

Peptide pool[b]	Group[c]	Case Number[d]	Positive Number	Positive Proportions (%)	95% CI[e]	*p* value[f]	
S1	HC	28	7	25	(11, 45)	6m VS HC	<.0001
	6m	76	53	70	(58, 80)	12m VS HC	<.0001
	12m	73	57	78	(67, 87)	6m VS 12m	0.2467
S2	HC	28	10	36	(19, 56)	6m VS HC	0.0124
	6m	76	48	63	(51, 74)	12m VS HC	0.0027
	12m	73	50	68	(57, 79)	6m VS 12m	0.4926
M	HC	28	8	29	(13, 49)	6m VS HC	<.0001
	6m	76	67	88	(79, 94)	12m VS HC	<.0001
	12m	73	60	82	(71, 90)	6m VS 12m	0.3048
N	HC	28	10	36	(19, 56)	6m VS HC	<.0001
	6m	76	66	87	(77, 94)	12m VS HC	<.0001
	12m	73	60	82	(71, 90)	6m VS 12m	0.4322
SARS-CoV-2	HC	28	20	71	(51, 87)	6m VS HC	0.0026
	6m	76	71	93	(85, 98)	12m VS HC	0.0081
	12m	73	67	92	(83, 97)	6m VS 12m	0.7019

[a]T-cell responses to SARS-CoV-2 were tested by enzyme-linked immunospot assay (ELISpot) with *in-vitro*-cultured PBMCs, the evaluation criteria were as follows: if negative-control wells had < 20 SFCs/10^6 PBMCs, positive responses were defined as having ≥ 40 SFCs/10^6 PBMCs; otherwise, positive responses were defined as having results at least twice that of the negative control.

[b]S1&S2: Spike protein (S) were divided into S1 and S2 pools according to the natural split site.

[c]HC: Healthy control; 6m: 6 months post disease onset; 12m: 12 months post disease onset.

[d]Five recovered patients at 6m and one at 12m had insufficient PBMCs for ELISpot.

[e]95% CI: 95% Confidence Interval.

[f]Chi square test was performed and the corresponding P value was listed ($\alpha=0.05$).

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Figure legends

Fig. 1 Participant characteristics and flow chart of immune memory detection.

A total of 101 COVID-19 convalescent patients were enrolled in two visits within Macheng, Hubei, China. The two visits were conducted in month 6 (n=81) and month 12 (n=74) of the convalescent period. Across the two visits, 57 of these subjects were followed up longitudinally. Three individuals clinically diagnosed with SARS-CoV-2 but lacking nucleic acid diagnostic confirmation were later confirmed by our study as being negative for SARS-CoV-2-specific antibody and T-cell responses; they were excluded from our analyses. Sera were used to measure the titer of SARS-CoV-2-specific antibodies via ELISA, MCLIA and neutralization assays. Whereas PBMCs were used to determine the T-cell memory responses through ELISpot, ICS and tetramer staining assays.

Fig. 2 Humoral immune responses in COVID-19 convalescents.

A-**E**, NAb, IgG, and IgM antibodies of COVID-19 convalescent donors at month 6 (6m, red; n=81) and month 12 (12m, blue; n=74) post-disease onset and of healthy controls (HC, gray; n=28) were detected by virus neutralization assay, ELISA, and MCLIA. **F**-**H**, NAb titers changes in the 57 longitudinally followed up convalescents at 6m and 12m with sustaining (**F**), declining (**G**), or increasing (**H**) trends. The thickness of the line represents different number ranges of convalescent donors. **I-L**, Correlation between NAb titers and IgM/IgG levels at 6m and 12m. **M-Q**, Changes of NAb, IgG and IgM antibody titers at 6m or 12m in asymptomatic (Asym), Mild (Mild), Moderate (Mod), or Severe (Sev) convalescents. **R**-**V**, The influence of disease severity on SARS-CoV-2-specific antibodies among the convalescents by a univariate linear regression. The distance between each point on the abscissa (x-axis) was considered to be equal and was used as an independent variable for simple linear regression. R^2 represents the goodness of fit. *P*-values were calculated based on the slope of the curve. A Mann-Whitney U-test was used for (**A**-**E**) and a Wilcoxon matched-pairs signed rank test was used for (**M**-**Q**). Correlations in (**I**-**L**) were assessed using a Spearman's Rank correlation coefficient (*r*). A simple linear regression (**R**-**V**) was used to evaluate the impact of

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disease severity on antibodies. Two-tailed *P* values were calculated. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Fig. 3 Memory T-cell responses against to SARS-CoV-2 as detected by ELISpot.

A, Memory T-cell responses of COVID-19 convalescent donors at month 6 (6m, red; n=78) and month 12 (12m, blue; n=74) post-disease onset and of healthy controls (HC, gray; n=28) were detected by *ex vivo* ELISpot using freshly isolated PBMCs under the stimulation with the corresponding peptide pool. Medians with interquartile ranges data are presented. **B**, After a 9-day *in vitro* expansion, memory T-cell responses from convalescent patients at 6m (n=76) or 12m (n=73), or from HC (n=28), were detected by ELISpot. “&” and “#” symbols indicate a significant difference with the S1 or S2 peptide pool, respectively. **C**, Memory T-cell responses in HCs and convalescents with different COVID-19 disease severity. Asym (6m, n=8; 12m, n=6); Mild (6m, n=36; 12m, n=36); Mod (6m, n=23; 12m, n=25); Sev (6m, n=9; 12m, n=6). **D**-**G**, Univariate linear regression fitting plot of disease severities vs T-cell responses, with HC considered as the lowest rank in the analysis. **H**-**P**, The correlation between T-cell memory against S (sum of S1 and S2), S1, and S2 proteins and antibody responses at 12m post-infection. A Mann-Whitney U-test was used for (**A**) and (**C**), a Wilcoxon matched-pairs signed rank test was used for (**B**). A simple linear regression (**D**-**G**) was used to evaluate the impact of disease severity on T-cell responses. Correlations in (**H**-**P**) were assessed using a Spearman’s Rank correlation coefficient (r). Two-tailed P-values were calculated. *$P<0.05$; **$P<0.01$; ***$P<0.001$.

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Fig. 4 Functional characterization of SARS-CoV-2-specific memory T cells.

A, Gating strategies for multiple cytokine analyses in $CD4^+$ (left) and $CD8^+$ (right) T cells. **B**, **C**, Percentages of SARS-CoV-2-specific T cells secreting IFN-γ, IL-2, and/or TNFα among the total T cells at month 6 (6m, red) and month 12 (12m, blue) post-COVID-19. **D**, **E**, The constitution ratios of

T cells secreting IFN-γ, IL-2, and/or TNFα in virus-specific $CD4^+$ or $CD8^+$ T cells. **F**, **G**, Phenotypic memory analysis of IFN-γ-secreting $CD4^+$ and $CD8^+$ T cells. **H**, Examples of SARS-CoV-2-specific $CD8^+$ T cells stained by HLA-A*1101 tetramers complexed to either the peptide M23 or the peptide N25 with cultured PBMC cells at 6m post-infection. The controls were stained with an irrelevant tetramer. **I**, HLA-A*1101/Peptide tetramer staining with cultured PBMCs cells from the same participant at 6m and 12m post-infection. **J**, Mean percentage of SARS-CoV-2-specific $CD8^+$ T cells positive for HLA-A*1101/M23 (n=4) or HLA-A*1101/N25 (n=4) in COVID-19 convalescent patients at 6m post-infection. **K**, Alignment of the M23 and N25 peptide amino acid sequences with other human coronaviruses and VOCs. Data are presented as mean ± SEM. The Wilcoxon matched-pairs signed rank test was used for comparison. Two-tailed *P* values were calculated. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

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Figure 1

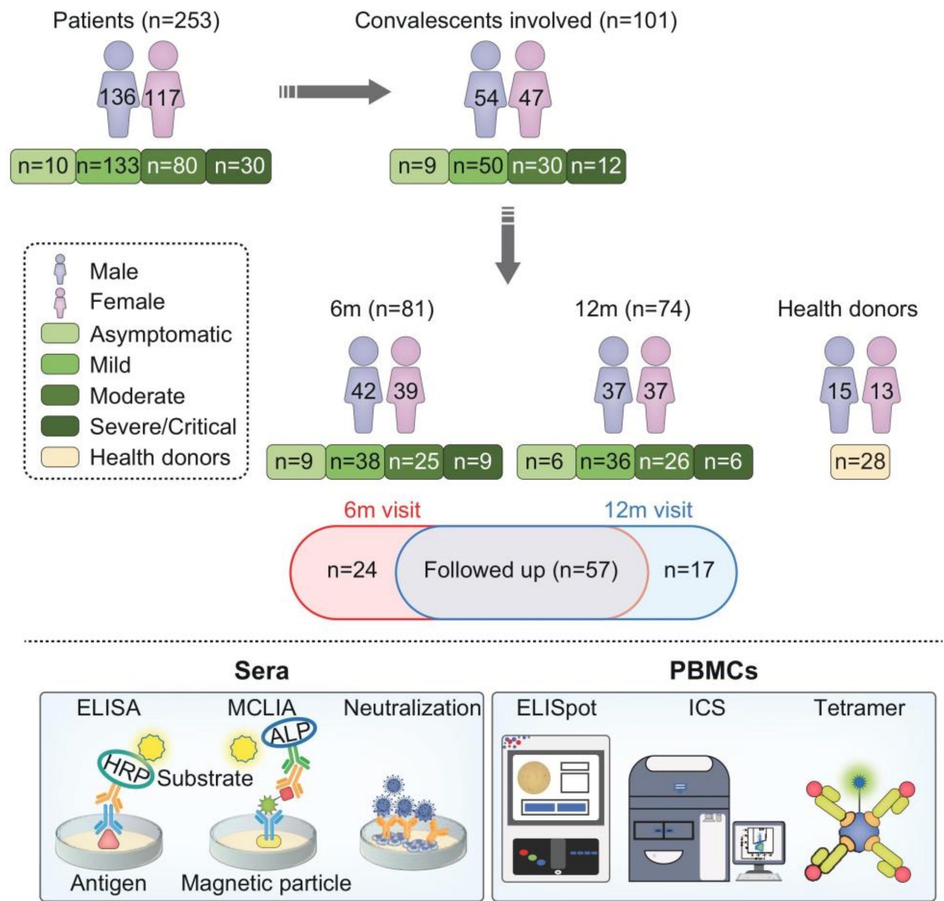

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Figure 2

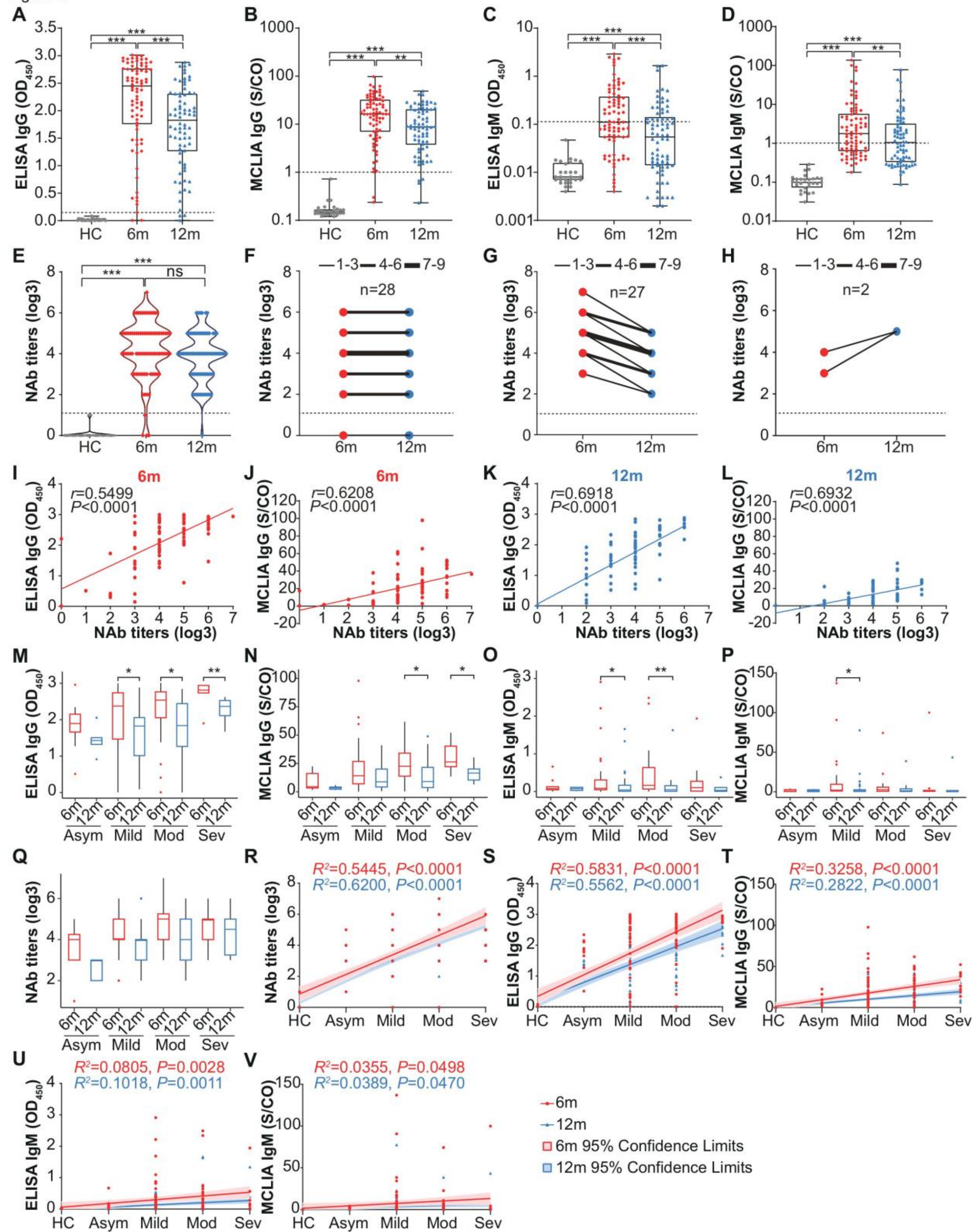

Figure 3

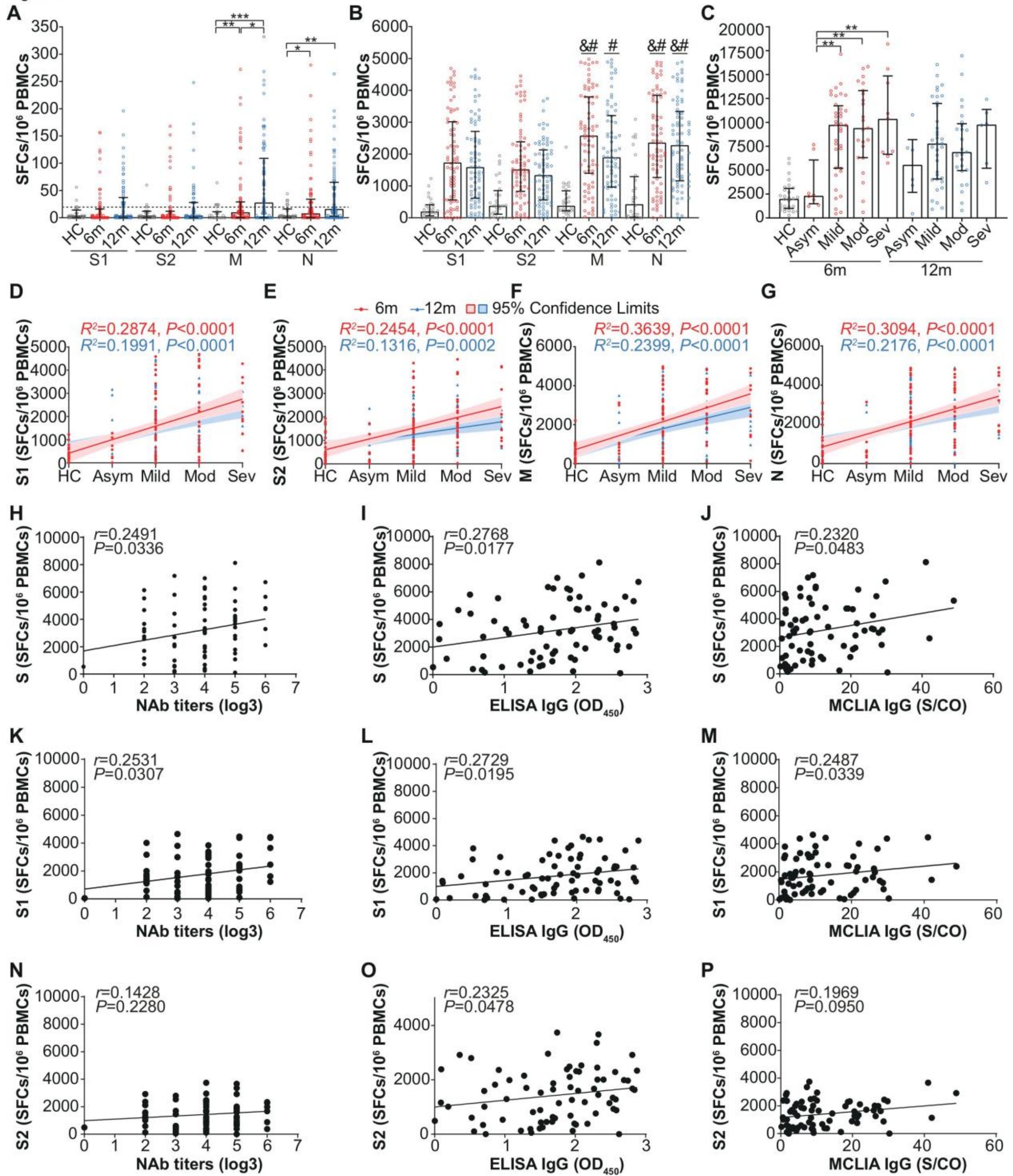

Figure 4