Persistence of Antibody and Cellular Immune Responses in COVID-19 patients over Nine Months after Infection

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One sentence summary

SARS-CoV-2 specific antibody and memory T and B cell responses were detectable in most patients nearly one year after infection, indicating that durable immunity against secondary COVID-19 disease is possible in most individuals.

Abstract-

Background. The duration of humoral and T and cell response after the infection of SARS-CoV-2 remains unclear.

Methods. We performed a cross-sectional study to assess the virus-specific antibody and memory T and B cell responses in COVID-19 patients up to 343 days after infection. Neutralizing antibodies and antibodies against the receptor-binding domain, spike, and nucleoprotein of SARS-CoV-2 were measured. Virus-specific memory T and B cell responses were analyzed.

Results. We enrolled 59 COVID-19 patients, including 38 moderate, 16 mild, and five asymptomatic patients; 31 (52.5%) were men, and 28 (47.5%) were women. The median age was 41 (interquartile range [IQR]: 30–55). The median day from symptom onset to enrollment was 317 days (range 257 to 343 days). We found that approximately 90% of patients still have detectable IgG antibodies against spike and nucleocapsid proteins and neutralizing antibodies against pseudovirus, whereas ~60% of patients had detectable IgG antibodies against receptor binding domain and surrogate virus-neutralizing antibodies. SARS-CoV-2-specific IgG^+ memory B cell and IFN-γ secreting T cell responses were detectable in over 70% of patients.

Conclusions. SARS-CoV-2-specific immune memory response persists in most patients nearly one year after infection, which provides a promising sign for prevention from reinfection and vaccination strategy.

Keywords: SARS-CoV-2; neutralizing antibody; memory T cell; memory B cell; persistence

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the coronavirus disease (COVID-19) agent, has caused the pandemic worldwide [1-3]. As of February 2, 2021, more than 100 million confirmed cases of SARS-CoV-2 had been reported, with more than 2 million deaths [4]. Effective vaccines are vital for controlling the pandemic. Therefore, understanding the long-term immunological memory response to SARS-CoV-2 after nature infection is critical for developing and implementing a SARS-CoV-2 vaccine. Recent studies showed most patients persist virus-specific antibody response 6-8 months after infection but displayed a waning trend of humoral immunity in patients over time [5-12]. SARS-CoV-2-specific memory $CD8^+$ and $CD4^+$ T cells were detected in most patients but declined with a half-life of 3–5 months [13], whereas virus-specific memory B cells (MBCs) continued to rise or keep unchanged 5–6 months after the infection [7, 8, 13, 14]. However, the antibody and memory T and B cell response beyond eight months or nearly one year after the infection is unclear. Therefore, in this study, we conducted a cross-sectional study of 59 patients up to 343 days after infection and assessed the virus-specific antibody and memory T cells and B cells.

MATERIALS AND METHODS

Study design and participants

Between December 7 and 30, 2020, patients who recovered from the COVID-19 with SARS-CoV-2 infection in Jiangsu, Shandong, and Zhejiang Provinces, China, were invited to participate in this study. All of the patients were laboratory-confirmed positive for SARS-CoV-2 by real-time reverse transcription-polymerase chain reaction (rRT-PCR) results. Each enrolled patient provided a 3 ml blood sample for serum isolation and an additional 5 ml blood sample for peripheral blood mononuclear cells (PBMCs) isolation. Demographics and clinical characteristics of patients were collected upon enrollment. Thirty age- and sex-matched healthy controls (HCs) enrolled before the pandemic were used as control. Each patient signed informed consent. The study protocol was approved by the Institutional Review Board of the Academy of Military Medical Sciences (IRB number: AF/SC-08/02.60).

According to the diagnostic and treatment guidelines for SARS-CoV-2 issued by the Chinese National Health Committee (Trail Version 8), the disease severity was defined as asymptomatic, mild, and moderate. Asymptomatic infection was defined as an individual who had a positive SARS-CoV-2 by RT-PCR without any associated clinical symptoms in the preceding 14 days and during hospitalization for observation as part of the control measures. Mild infection was defined as an individual who had mild clinical symptoms without radiological signs of pneumonia. Moderate was defined according to the following criteria: (i) fever and respiratory symptoms; (ii) radiological signs of pneumonia.

Serum and PMBC isolation

Sera were separated by centrifugation at 2000 rpm for 10 min, aliquoted into three cryovials, and preserved at -80°C until testing. PBMCs were isolated by density gradient centrifugation with Lymphoprep in SepMate tubes (Stemcell Technologies) according to the manufacturer's instruction. Briefly, the blood was placed on top of Lymphoprep in SepMate tubes and centrifuged at 1200 × *g* for 10 min. PBMCs from the top layer were harvested and washed twice with PBS at 400 × *g* for 10 min. Isolated PBMCs were frozen in cell recovery Media containing 10% DMSO (GIBCO), supplemented with 90% heat-inactivated fetal bovine serum, and stored liquid nitrogen before assays analyses.

ELISA analysis of IgG antibody to RBD and spike trimer of SARS-CoV-2

The recombinant receptor-binding domain (RBD) and spike (S) trimer derived from SARS-CoV-2 (Sino Biological, Beijing) were coated onto flat-bottom 96-well enzyme-linked immunosorbent assay (ELISA) plates overnight at 4°C with 0.1 μg/well. Plates were washed with PBS-T (PBS with 0.05% Tween 20) and blocked with blocking buffer (5% skim milk and 2% BSA in PBS) for 1h at room temperature. Duplicate 3-fold 8-point serial dilutions (starting at 1:100) of heat-inactivated serum samples diluted in 1% milk in PBS-T were added to the wells and incubated at 37°C for 1h. Wells were then incubated with the HRP labeled anti-human IgG antibody (1:5000, Promega, W4031) and TMB substrate (Kinghawk, Beijing). The optical density (OD) was measured by a spectrophotometer

at 450nm and 630nm. Endpoint antibody titers were calculated by a fitted curve (4 parameter log regression), and the three times the average value of HCs was used as the detection threshold.

ELISA analysis of IgG antibody to the nucleocapsid (N) protein of SARS-CoV-2

Serum IgG to nucleocapsid (N) protein of SARS-CoV-2 was semiquantitatively measured by ELISA using a well-validated commercial diagnostic ELISA kit (Beijing Wantai Biological Pharmacy Enterprise Co., Ltd) [15] according to manufacturer's instruction. The anti-N IgG antibody was detected using an indirect ELISA kit based on a recombinant N protein of SARS-CoV-2. The cut-off value for IgG is the mean OD value of three negative controls (if the mean absorbance value for three negative calibrators is < 0.03, take it as 0.03) + 0.16. A serum sample with an OD value ≥cut-off OD value was considered anti-N IgG antibody positive.

Surrogate Virus Neutralization Test (sVNT)

sVNT assays were performed by using a Surrogate Virus Neutralization Test Kit (GenScript, L00847) according to the manufacturer's instruction. Briefly, mix the positive control, negative control, or diluted samples (1:10) with diluted HRP-RBD with a volume ratio of 1:1 in tubes, incubate at 37°C for 30 min. Then 100μl mixture of the sample was added to the 96 well microplates pre-coated with recombinant angiotensin-converting enzyme 2 (ACE2) protein and incubate at 37°C for 15 min. After washing the plate four times, 100μl TMB solution was added to each well and incubate at room temperature for 15 min. Then add 50μl stop solution to the plate. OD was measured by a spectrophotometer at 450nm and 570nm. HRP-RBD alone and plasma with no HRP-RBD incubation were used as controls. The percent inhibition was calculated as (1–Sample OD value/Average Negative Control OD value) x 100. The inhibition rate above 30% was considered positive for the SARS-CoV-2 neutralizing antibody.

Pseudovirus Neutralization Test (pVNT)

The SARS-CoV-2 pVNT was performed as described previously [4]. In brief, Huh7 cells were seeded in 96-well plates (200,000 cells/well) and incubated for approximately 24 hours until 90%–100% confluent. Serial 3-fold diluted serum, starting at 1:30, were incubated with 650 $TCID_{50}$ of the pseudovirus for 1h at 37°C. DMEM was used as the negative control. The supernatant was then removed, and luciferase substrate was added to each well, followed by incubation for 2 minutes in darkness at room temperature. Luciferase activity was then measured using GloMax 96 Microplate Luminometer (Promega). Half-maximal inhibitory concentrations (IC50) of the serum samples were determined by luciferase activity 48 hrs after exposure to the virus-serum mixture with a three-parameter non-linear regression inhibitor curve in GraphPad Prism 8.4.1 (GraphPad Software). Titers were determined as the serum dilution that inhibited 50% virus infection (ID50).

Enzyme-linked immunospot (ELISpot) assays

To assess B cells secreting IgG antibodies specific for SARS-CoV-2 RBD and cells secreting IgG (total IgG), we performed an ELISpot assay using the Human IgG SARS-CoV-2 RBD ELISpot^{PLUS} (HRP) kit (3850-4HPW-R1-1, Mabtech AB) according to the manufacturer's protocol. Briefly, PBMCs were incubated for four days in RPMI-1640 medium with 10% fetal calf serum (FCS), supplemented with R848 (1 µg/ml; Mabtech AB) and recombinant human IL-2 (10 ng/ml) for stimulation of memory B cells. The ELISpot plates pre-coated with capturing monoclonal anti-human IgG antibodies were incubated with a total of 200,000 or 40,000 pre-stimulated cells per well for detection of RBD-specific IgG and total IgG secreting cells, respectively.

T cell responses were measured using Human IFN-γ SARS-CoV-2 ELISpot^{PLUS} kit (ALP, 3420-4AST-P1-1, Mabtech AB) according to the manufacture's protocol. Briefly, plates were washed with filtered PBS (Sigma Aldrich, Missouri, USA) and blocked with RPMI-1640 culture media containing 10% batch tested fetal bovine serum (FBS) (Gibco, Thermo Fisher Scientific, Massachusetts, US). The plates pre-coated with capturing monoclonal anti-IFN-γ were incubated 18hrs in RPMI-1640 medium containing 10% FCS supplemented with a mixture containing the SARS-CoV-2 defined

peptide pool. The plates pre-coated with capturing monoclonal anti-IFN-γ were incubated 18hrs in RPMI-1640 medium containing 10% FCS supplemented with a mixture containing the SARS-CoV-2 defined peptide pool. The peptide pool contains 47 synthetic peptides binding to human HLA, derived from the S, N, membrane (M), and the open reading frame (ORF)-3a and ORF-7a proteins (3622-1, Mabtech AB) at a concentration of 2 µg/ml of each peptide, anti-CD28 (0.1 µg/ml) and 250,000 cells per well in a humidified incubator (5% CO_2, 37°C). Negative controls comprising DMSO and positive controls containing anti-CD3 were also included.

Spot numbers were analyzed by the CTL ImmunoSpot S6 universal analyzer (Cellular Technology Ltd., USA). SARS-CoV-2-specific spots were determined as mean spots of the control wells were subtracted from the positive wells, and the results were expressed as spot-forming cells (SFC) per10^6 PBMCs. We defined threefold higher SARS-CoV-2-specific spots versus background together with at least three spots above background as a positive response. This cut-off was set based on negative control values as described previously. If negative control wells had >30 SFC per 10^6 PBMCs or positive control wells (anti-CD3 and CD28 stimulation) were negative, the results were excluded from further analysis.

Statistical analysis

One-way ANOVA with LSD post-hoc testing (normal distribution) or Kruskal-Wallis test with false discovery rate method (non-normal distribution) was used for multiple group comparisons. Mann-Whitney *U* test was used to compare the difference between the two groups. Spearman correlations analyses were used to determine associations between analyzed parameters. All statistical analyses were performed using GraphPad Prism (version 8.4.2, La Jolla, California USA), and all statistical tests were 2-sided with a significance level of 0.05.

RESULTS

Study subjects

We enrolled 59 recovered COVID-19 patients with a time interval of 317 days (range 257 to 343 days, defined 9-11 months after infection) from symptom onset to sampling. Of the 59 patients, 38 were moderate, 16 were mild, and five were asymptomatic (Table 1). The median age was 41 (interquartile range [IQR]: 30–55), and 31 (52.5%) were male. Fever (66.7%), cough (44.4%), and expectoration (16.7%) are the most reported common symptoms (Table 1). No significant differences in sex and age were observed between HCs and patients.

Persistent of SARS-CoV-2 specific IgG and neutralizing antibodies

We first assessed the SARS-CoV-2 RBD binding IgG antibody using ELISA and found that 55.9% (31/59) of patients had detectable anti-RBD IgG antibodies nearly one year after infection. Regarding the disease severity, 66.7% (14/21) of asymptomatic/mild and 50.0% (19/38) of moderate patients were tested positive for anti-IgG antibody, with geometric mean endpoint titer of 53.2, (95% confidence interval [CI], 19.0-148.9) and 17.3 (95%CI, 7.9-33.7) (Figure 1A). We observed that 91.5% of patients had low binding anti-RBD antibody titer (<500) (Figure 1A). In contrast, 93.2% (55/59) patients (100% for asymptomatic/mild and 89.5% for moderate patients) showed a positive detection of anti-S IgG (Figure 1B), with geometric mean endpoint titer of 879 (95%CI, 545.8-1415.8) and 543.3 (95%CI, 319.9-922.6) (Figure 1B). Most (67.8%) patients showed a moderate and strong binding anti-IgG titer (>500). Although our focus was on the S protein, we also semiquantitatively measured the antibody response to the N protein of SARS-CoV-2 since this is the antigen targeted by multiple commercial assays. We observed anti-N IgG antibodies are persistently high in recovered patients, and 98.3% (58/59) of patients (100% for asymptomatic/mild and 97.4% for moderate patients were still positive (Figure 1C). As expected, in the serum of HCs, we observed a minimal reactivity of anti-S or anti-RBD IgG antibodies (Figure 1A and 1B), whereas one serum was detected positive for anti-IgG antibodies (Figure 1C).

To determine whether patients maintain neutralizing antibodies, we detected SARS-CoV-2 neutralization indirectly using a cell-free assay of RBD-ACE2 binding inhibition by sVNT and directly pVNT. sVNT assays showed that 66.1% (39/59) of patients (71.4% for asymptomatic/mild and 63.2% for moderate patients) had antibody to inhibit RBD binding to ACE2 (Figure 1D), and 44% (26/59) patients displayed $\geq$ 50% inhibit rate to ACE2 (Figure 1D). Further pVNT assay revealed that 89.8% (53/59) patients (21/21 asymptomatic/mild and 32/38 moderate patients) had detectable NAb (Figure 1E). The geometric mean titer (GMT) of NAb was significantly higher in asymptomatic/mild (88.0; 95%CI, 66.0-117.3) and moderate patients (56.4; 95%CI, 42.7-74.6) than HC (Figure 1E). We found that IgG antibodies were strongly correlated with each other and neutralizing inhibition rate, whereas they were moderately correlated with pesudovirus-based NAb titer (Supplementary Figure 1).

Although no significant differences were observed for antibody response between asymptomatic/mild and moderate patients, moderate patients tended to a slightly low positive antibody response rate. The fact that most moderate patients (median day of 320, IQR 313-331) had a longer time interval from symptom onset to sampling than those asymptomatic/mild patients (median day of 312, IQR 306-318, $p = 0.0025$). Further analysis showed that the age, sex, and underlying medical conditions were not associated with antibody responses except for a higher anti-S IgG titer (Supplementary Figure 2).

Maintenance of SARS-CoV-2-Specific B and T Cells

We further assessed virus-specific memory B and T cell responses among these patients using ELISpot assay (Figure 2A and 2D). We found that 74.6% (44/59) patients (76.2% and 73.2% for asymptomatic/mild and moderate patients, respectively) had detectable RBD-specific IgG^+ memory B cells, with a mean number of 132 (95%CI, 71-193) (Figure 2C). Similar to memory B cell response, 73.7% (28/38) of moderate patients and 66.7% (14/21) of asymptomatic/mild patients had detectable IFN-γ-secreting T cells, with a mean number of 368 (95%CI, 269-467) IFN-γ-secreting T cells and

significantly higher than in HCs (Figure 2D and 2E). In addition, 2 of 20 (6.7%) (2/30) HCs had detectable IFN-γ-secreting T cells (Figure 2E). There was correlation between SARS-CoV-2 specific MBCs response and anti-RBD IgG (r = 0.350, p = 0.007), anti-S IgG (r = 0.330, p = 0.010), and NAb-inhibition rate (r=0.456, p < 0.001) but not anti-N IgG (Figure 2F). However, no significant correlations were observed between the SFC of SARS-CoV-2 specific T cells secreting IFN-γ and antibody response (Supplementary Figure 3). Further analysis showed that patients older than 60 years old had fewer memory B cells secreting total IgG and RBD-specific IgG than patients under 60 years old (Supplementary Figure 4).

DISCUSSION

Recent studies have shown that most patients had detectable SARS-CoV-2 antibody responses 6–8 months after infection [6, 12, 16-19]. This study evaluated SARS-CoV-2 specific antibody and cellular immune responses in patients up to 11 months after infection. Our data reveal that approximately 70% of COVID-19 patients maintain anti-RBD and -S IgG, ACE2-RBD inhibition rate, and NAb at least 9–11 months after infection. Moreover, we observed that pseudovirus neutralizing antibodies and antibodies against the N and S proteins are longer-lived than those against RBD. Although antibodies to RBD of SARS-CoV-2 S protein accounted for the majority of IgG responses, antibodies to other epitopes such as the N-terminal domain and S2 subunit of S protein also contribute to the IgG response [20]. A previous study also showed that the combination of antibodies to other epitopes of S protein and antibodies against RBD could more effectively block the virus from invading host cells [21]. Therefore, the antibody titer and positivity rates of anti-RBD IgG and ACE2-RBD inhibition might be lower than the NAb and anti-S IgG response. We also observed relatively high (~98%) positive detection of anti-N IgG among patients compared to other antibodies. In human SARS-CoV-1 infection, antibodies against the SARS-CoV-1 N protein are abundant and longer-lived than other viral components such as the S, M, and envelope proteins [22]. In another study, antibodies against S1 or RBD persisted longer than antibodies against N protein in the sera of SARS survivors 17 years after infection [23]. Although this phenomenon's biological significance and mechanistic characterization are beyond this study's scope, further investigation is warranted.

Whether primary infection with SARS-CoV-2 protects individuals from reinfection and how long has yet to be established. Patients with SARS-CoV-2 reinfection are still rare but on the rise [24]. Reinfections may imply that immunity against SARS-CoV-2 may be weak and decay relatively quickly, with implications not just for the risks facing recovered patients but also for how long future vaccines might protect people [25]. Notably, several reported cases with SARS-CoV-2 reinfection displayed a low level or without producing the antibody response after prior infection [26-30], which may hamper a more effective response to the second time around. In contrast, recent studies have shown that the presence of antibodies to SARS-CoV-2 was associated with a significantly reduced risk of SARS-CoV-2 reinfection for six to seven months after prior infection [31, 32]. Moreover, prior infection also reduces the risk of asymptomatic infection and likely to be protective against severe disease or symptomatic infection [31, 32]. These data provide clues on the protection of humoral immune response against reinfection. Collectively, sustained humoral immunity after infection might help apply vaccines for reliable prevention of SARS-CoV-2 transmission, patients who became seronegative for antibody response may be at higher risk of reinfection when they meet the next exposure compared to seropositive individuals.

While most patients had detectable virus-specific antibodies 9–11 months after infection, the longevity of virus-specific memory T cells and MBCs is still unresolved. Previous studies have shown that memory $CD4^+$ and $CD8^+$ T cells can be detected in 70%-100% early convalescent COVID-19 patients [33-35]. In addition, broad and strong memory $CD4^+$ and $CD8^+$ T cells can be found in >90% convalescent COVID-19 patients [36, 37]. Recent studies also showed that memory $CD4^+$ and $CD8^+$ T cell response could persist in 50%-100% of patients at least 3–6 months post symptom onset [13, 14, 38, 39]. Regarding the virus-specific MBCs, previous studies have shown that virus-specific MBCs were detected in >90% of patients and increased over time even five months post symptom onset [8, 14]. Moreover, more abundant S-specific MBCs were detected in approximately 80% of patients at six months compared to one month after infection [13], and S-specific and RBD-specific MBCs can even be respectively detected in 78% and 100% patients for up to eight months post-symptom onset [40, 41]. Consistent with the above evidence, we found that over 70% of patients had detectable both virus-specific T cells producing IFN-γ and RBD-specific IgG^+ MBCs response after 9–11 months after infection. Our data indicate that infection of SARS-CoV-2

could produce long-lasting T and B cell memory in most patients up to nearly one year after infection, which is potentially beneficial for protecting against systemic disease upon reinfection.

There are several limitations of this study. One of the major limitations of this study is a cross-sectional study that limited the observation of the dynamic changes of antibody or immune cells over time that limits generalization. Another limitation is the relatively small number of patients and the paucity of severe patients in this study. Because the most severe patients in the field of the study conducted were elderly, and they and their family members had a very low willingness to participate in the study. However, previous studies have shown that severe patients had a higher antibody titer than other disease conditions [42, 43].

In summary, detectable humoral and cellular immunity can be found in most of the patients 9–11 months after infection with SARS-CoV-2 offers insights into the long-term immune response to SARS-CoV-2 infection. Our findings will provide direct implications for COVID-19 vaccine development and implementation and other public health responses to the COVID-19 pandemic.

Author Contributors

MJM conceived the study. YS, ZYW, BDZ, BL, CS, HHP, YMG, GQW, DMW, MDJ, and GPC collected samples and clinical data of patients. GLW, LY, LJD, and HHP performed PBMC isolation. LY and LJD performed IgG experiment; GLW, LY, and LJD performed ELISpot assay. GLW, LY, and LJD performed sVNT and pVNT test. MJM, GLW, LY, and LJD analyzed the data. MJM, LY, and GLW drafted the manuscript. All authors reviewed and approved the final manuscript.

Acknowledgments

We thank all patients for their participation in this study.

Financial support

This work was supported by grants from the Beijing Natural Science Foundation (L202038 to Mai-Juan Ma), Natural Science Foundation of China (81773494 to Mai-Juan Ma), and the National Key Research and Development Program of China (2019YFC1200502 to Guo-Lin Wang).

Potential conflicts of interest

We declare no competing interests.

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Table 1. Demographic and clinical characteristics of the COVID-19 patients enrolled in this study.

Characteristics	Healthy controls	Asymptomatic	Mild	Moderate	*p* value
No. of participants	30	5	16	38	
Onset to inclusion (days; median, IQR)	NA	303.0 (264.0, 308.0)	315.0 (309.8, 320.5)	319.5 (313.0, 330.8)	
Age (median, IQR)	44.5 (38.5, 52.3)	43.0 (20.0, 49.5)	34.0 (18.5, 47.8)	43.0 (34.8, 59.8)	0.135
Age group (years)					
≤60	25 (83.3)	5 (100.0)	13 (81.3)	29 (76.3)	0.764
＞60	5 (16.7)	0	3 (18.8)	9 (23.7)	
Sex					
Male	15 (50.0)	0	8 (50.0)	23 (60.5)	0.085
Female	15 (50.0)	5 (100.0)	8 (50.0)	15 (39.5)	
Underlying medical condition					

Yes	0	1 (20.0)	4 (25.0)	15 (39.5)	0.566[a]
No	30 (100.0)	4 (80.0)	12 (75.0)	23 (60.5)	
Signs and symptoms					
Fever	NA	No	10 (62.5)	26 (68.4)	
Dry cough	NA	No	10 (62.5)	14 (36.8)	
Expectoration	NA	No	3 (18.75)	6 (15.8)	
Pharyngalgia	NA	No	2 (12.5)	3 (7.9)	
Itchy throat	NA	No	0	2 (5.3)	
Sneeze	NA	No	1 (6.25)	1 (2.6)	
Stuffy nose	NA	No	1 (6.25)	2 (5.3)	
Rhinorrhea	NA	No	0	4 (10.5)	
Dyspnea	NA	No	0	1 (2.6)	
Chest stuffiness	NA	No	0	1 (2.6)	

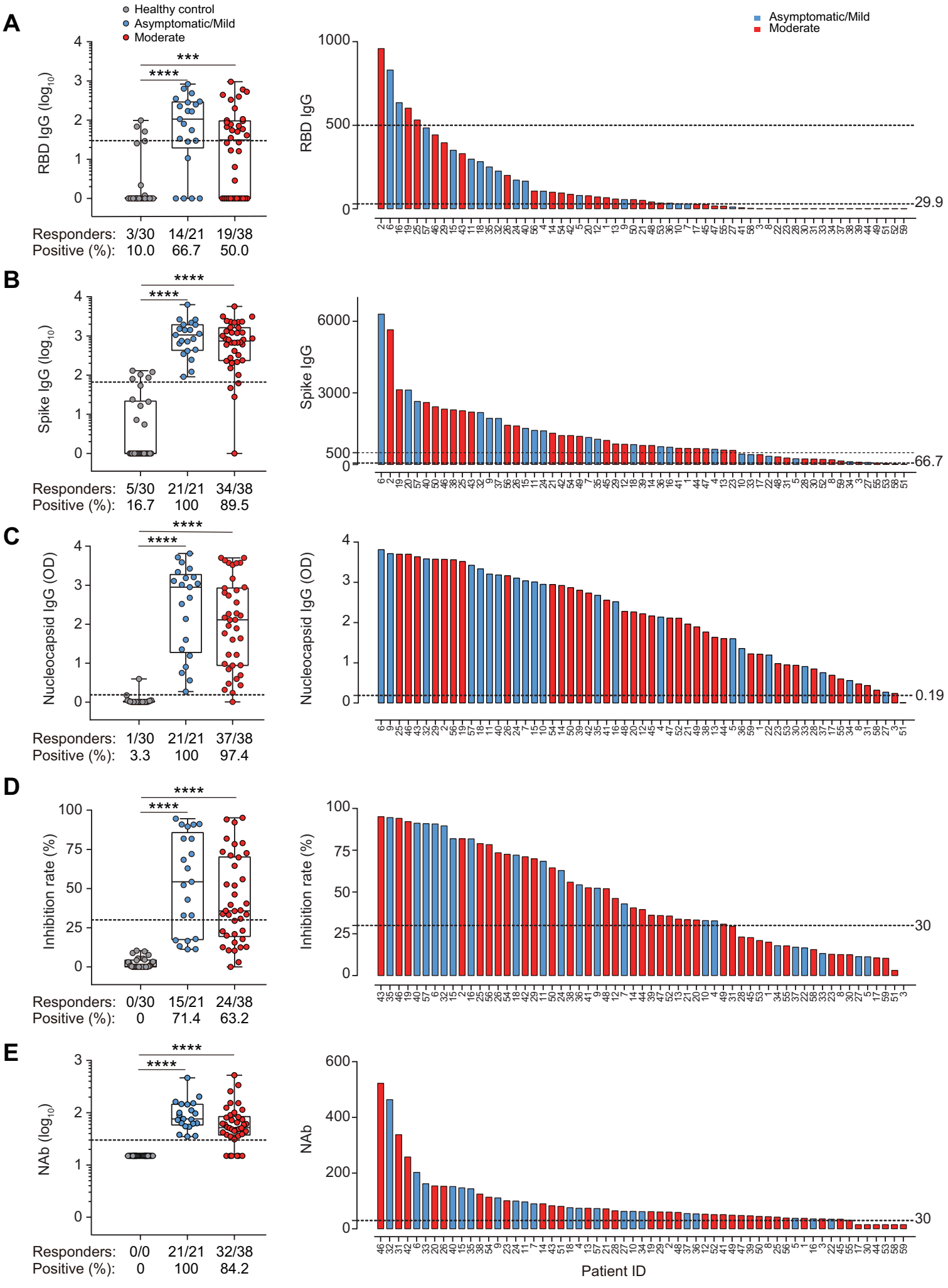
Fatigue	NA	No	0	4 (10.5)
Headache	NA	No	1 (6.25)	4 (10.5)
Inappetence	NA	No	0	1 (2.6)
Nausea	NA	No	0	1 (2.6)
Diarrhea	NA	No	0	4 (10.5)
Chills	NA	No	0	3 (7.9)
Myalgia	NA	No	0	3 (7.9)

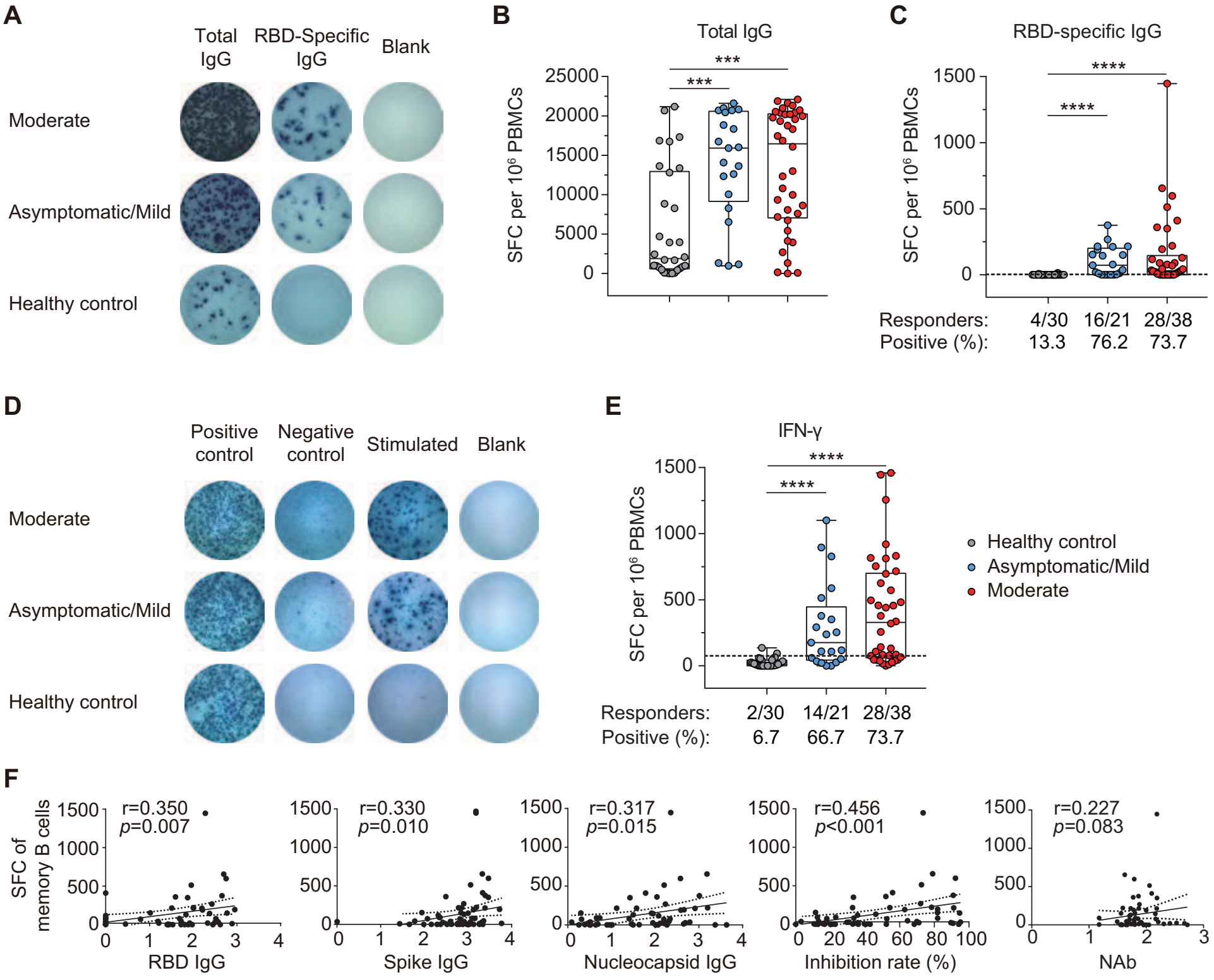
NA, not applicable. [a] *p*-value was calculated between patient groups.

Figure 1. SARS-CoV-2-specific antibody response in recovered patients 9-11 months after infection. (A-E) Left, IgG antibodies against the receptor-binding domain (RBD), spike, and nucleocapsid of SARS-CoV-2 and neutralizing antibodies (NAb) of RBD-ACE2 binding inhibition and pseudovirus in serum samples collected from patients and healthy controls. Right, ranked anti-RBD, anti-spike, and anti- nucleocapsid IgG titers as well as RBD-ACE2 binding inhibition rate and pseudovirus NAb titer of each COVID-19 patients. Each dot represents a titer, OD value, or inhibition rate for each serum sample. The black dashed line indicates the threshold for positivity (anti-RBD IgG=29.9, anti-S IgG=66.7, anti-N IgG=0.19, inhibition rate=30%, and NAb=30). Boxplots indicate median and interquartile range (IQR); and the whiskers represent 1.5 times the IQR. Kruskal-Wallis test and Dunn's multiple comparison test using false discovery rate was used for the comparisons. ***$p < 0.001$, and ****$p < 0.0001$.

Figure 2. B and T cells responses to SARS-CoV-2 in COVID-19 convalescent patients. (A) A representative ELISpot of total IgG^+ and SARS-CoV RBD-specific IgG^+ memory B cells from each type of subjects. (B) Total IgG^+ memory B cell counting in patients and healthy controls. (C) RBD-specific IgG^+ memory B cell counting in patients and healthy controls. (D) A representative ELISpot of IFN-γ producing T cells against a defined peptide pool of S/N/M and ORF3a/7a from each type of subjects, with blank as negative control and anti-CD3 as positive controls. (E) Summary data of patients studied according to S/N/M and ORF3a/7a in patients and healthy controls. (F) Correlations between spot-forming cells (SFC) of memory B cells and antibody responses. The dotted line indicates the cut-off for positive responses (RBD-specific IgG secreting memory B cells=3, and IFN-γ-secreting T cells=75). Boxplots indicate median and interquartile range (IQR); and the whiskers

represent 1.5 times the IQR. Data in the graph (B, C, and E) represented as SFC per $1x10^6$ PBMC, and each point on the dot plot represents an individual subject. Kruskal-Wallis test and Dunn's multiple comparison test using false discovery rate was used for the comparisons in panel B, C, and E. ***$p < 0.001$, ****$p < 0.0001$. Spearman correlations were used in panel F. S, spike; N, nucleoprotein; M, membrane protein; ORF, open reading frame.


A
Healthy control
Asymptomatic/Mild
Moderate
RBD IgG (log10)
***
****
Responders: 3/30 14/21 19/38
Positive (%): 10.0 66.7 50.0
RBD IgG
29.9
B
Spike IgG (log10)
****
****
Responders: 5/30 21/21 34/38
Positive (%): 16.7 100 89.5
Spike IgG
66.7
C
Nucleocapsid IgG (OD)
****
****
Responders: 1/30 21/21 37/38
Positive (%): 3.3 100 97.4
Nucleocapsid IgG (OD)
0.19
D
Inhibition rate (%)
****
****
Responders: 0/30 15/21 24/38
Positive (%): 0 71.4 63.2
Inhibition rate (%)
30
E
NAb (log10)
****
****
Responders: 0/0 21/21 32/38
Positive (%): 0 100 84.2
NAb
30
Asymptomatic/Mild
Moderate
Patient ID


A
Total IgG
RBD-Specific IgG
Blank
Moderate
Asymptomatic/Mild
Healthy control
B
Total IgG
SFC per 10⁶ PBMCs
25000
20000
15000
10000
5000
0
***
***
C
RBD-specific IgG
SFC per 10⁶ PBMCs
1500
1000
500
0
****
****
Responders: 4/30 16/21 28/38
Positive (%): 13.3 76.2 73.7
D
Positive control
Negative control
Stimulated
Blank
Moderate
Asymptomatic/Mild
Healthy control
E
IFN-γ
SFC per 10⁶ PBMCs
1500
1000
500
0
****
****
Responders: 2/30 14/21 28/38
Positive (%): 6.7 66.7 73.7
Healthy control
Asymptomatic/Mild
Moderate
F
SFC of memory B cells
r=0.350
p=0.007
RBD IgG
r=0.330
p=0.010
Spike IgG
r=0.317
p=0.015
Nucleocapsid IgG
r=0.456
p<0.001
Inhibition rate (%)
r=0.227
p=0.083
NAb