

# Durability of neutralizing antibodies and T-cell response post SARS-CoV-2 infection

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**Abstract** The ongoing pandemic of coronavirus disease 19 (COVID-19) is caused by a newly discovered  $\beta$  coronavirus named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). How long the adaptive immunity triggered by SARS-CoV-2 can last is of critical clinical relevance in assessing the probability of second infection and efficacy of vaccination. Here we examined, using ELISA, the IgG antibodies in serum specimens collected from 17 COVID-19 patients at 6–7 months after diagnosis and the results were compared to those from cases investigated 2 weeks to 2 months post-infection. All samples were positive for IgGs against the S- and N-proteins of SARS-CoV-2. Notably, 14 samples available at 6–7 months post-infection all showed significant neutralizing activities in a pseudovirus assay, with no difference in blocking the cell-entry of the 614D and 614G variants of SARS-CoV-2. Furthermore, in 10 blood samples from cases at 6–7 months post-infection used for memory T-cell tests, we found that interferon  $\gamma$ -producing CD4<sup>+</sup> and CD8<sup>+</sup> cells were increased upon SARS-CoV-2 antigen stimulation. Together, these results indicate that durable anti-SARS-CoV-2 immunity is common in convalescent population, and vaccines developed from 614D variant may offer protection from the currently predominant 614D variant of SARS-CoV-2.

**Keywords** SARS-CoV-2; neutralizing antibodies; T-cell response

## Introduction

Coronavirus disease 2019 (COVID-19) is a newly emerging infectious acute respiratory disease that has caused arguably the most challenging pandemic since the 1918 influenza [1–3]. Several lines of evidence have shown that convalescent COVID-19 patients developed both humoral and T cell responses against SARS-CoV-2 [4–7]. However, there have been seemingly conflicting reports on the durability of serum antibodies post SARS-CoV-2 infection in COVID-19. For example, some studies showed that the levels of antibodies against SARS-CoV-2

measured by ELISA gradually declined after a few weeks post infection [8], while others found that these antibodies increased and then remained at a plateau till four months after diagnosis [9]. Hypothesis on two waves of specific antibody generation by short- or long-lived plasma cells has also been raised [10]. Here, we report the persistence of neutralizing antibodies and T cell response post 6–7 months of SARS-CoV-2 infection.

## Materials and methods

### Patient cohort

This study was performed with approval by the Shanghai Public Health Clinical Center Ethics Committee. Informed consent was obtained from all enrolled cases. Enrolled cases included donors infected with SARS-CoV-2 since February 21, 2020. The plasma at 2 weeks to 1 month, 1–2 months, and 6–7 months post infection were collected for

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antibody testing. Peripheral blood mononucleated cells (PBMC) of samples collected between 6 and 7 months post-infection were used for T cell activation test.

### **ELISA-based anti-SARS-CoV-2 antibody detection**

IgG antibodies against receptor binding domain (RBD) of S-protein and N-protein were detected with anti-SARS-CoV-2 S-RBD protein Human IgG ELISA Kit (KE30003, ProteinTech, Rosemont, IL, USA) and anti-SARS-CoV-2 N protein Human IgG ELISA Kit (KE30001, ProteinTech) according to the manufacturer's instructions. Briefly, serum samples were diluted and added into SARS-CoV-2 S-RBD protein or N-protein-coated plates for 30 min. After 4 rounds of washes, horseradish peroxidase-conjugated anti-human IgG antibodies were added and incubated for 30 min at room temperature. After 4 more rounds of washes, tetramethylbenzidine substrates were added and incubated for 8 min before adding the stop solution. The plates were read at 450 nm and 630 nm using Varioskan® Flash (Thermo Fisher Scientific, Waltham, MA, USA).

### **Pseudovirus-based SARS-CoV-2 neutralizing antibody detection**

Pseudovirus-based neutralizing antibodies were detected as previously described [11,12]. Briefly, the collected plasma was first heated at 56 °C to inactivate the complement proteins. The plasma was diluted with culture media and incubated with pseudovirus for one hour at 37 °C. Then the virus-plasma mix was added into Huh7 cells for 24 h. Luciferase activity was detected using the EnSight® Multimode Microplate Reader (HH34000000, PerkinElmer, Singapore). The 50% neutralization titer (NT<sub>50</sub>) was used to evaluate the neutralizing ability of the antibodies in plasmas.

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

PBMC were separated using Ficoll-Paque PLUS (17144003, GE Healthcare, Pittsburgh, USA) according to the manufacturer's instructions. An aliquot of the PBMCs was used to produce antigen-presenting dendritic cells (DC) as previously described [13,14]. Briefly, the aliquot of PBMCs were cultured in RPMI 1640 (Hyclone, Logan, UT, USA) supplemented with 5% Human AB serum (100-512, GeminiBio, CA, USA), 20 ng/mL GM-CSF (300-03-5, PeproTech, Rocky Hill, NJ, USA), and 20 ng/mL IL-4 (200-04-5, PeproTech) for five days. Lipopolysaccharides (LPS) (L2630, Sigma-Aldrich, St. Louis, MO, USA) was then added to a final concentration of 50 ng/mL for 24 h. After washing the cells twice with PBS, 5 µg each of SARS-CoV-2 S-RBD- and N-proteins were added to the media to stimulate DC cells for five

hours. The stimulated DC cells were then incubated with the autologous PBMC cells for four hours, followed by 1 h of incubation with BD GolgiPlug™ Protein Transport Inhibitor (Containing Brefeldin A) (554724, BD Bioscience, San Jose, CA, USA) before T cell detection using flow cytometry. Cells were harvested, washed once with PBS, and incubated with the PerCP/Cyanine5.5 anti-human CD8 Antibody (clone SK1, Biolegend, San Diego, CA, USA) and the Pacific Blue™ anti-human CD4 Antibody (clone OKT4, Biolegend) for 15 min. After washing with PBS, the cells were permeabilized, washed with the BD Perm/Wash™ buffer (554723, BD Bioscience), and then labeled with the PE anti-human IFN-γ Antibody (clone 4S.B3, Biolegend). Flow cytometry analysis was performed with BD LSRFortessa.

### **Statistical analysis**

Unpaired two-sided Student's *t*-tests were used to compare two unpaired groups of variables. Paired two-sided Student's *t*-tests were used to compare the significance of paired samples. GraphPad Prism 7 was used for plotting.

## **Results**

### **Detection of anti-SARS-CoV-2 S-protein and N-protein antibodies 6–7 months post-infection**

We examined the IgG antibodies against the RBD of S-protein and the N-protein of SARS-CoV-2 by ELISA in blood samples from COVID-19 cases at different time points after diagnosis. The antibody levels from three groups of patients, without significant differences in terms of gender, age, and disease severity, were compared: 15 cases at 2 weeks to 1 month, 20 cases at 1–2 months and 17 cases at 6–7 months post SARS-CoV-2 infection. A group of 12 normal individuals with comparable gender and age distribution was set as control (Table 1). Notably, all plasma samples from 17 cases were positive for both IgG antibodies 6–7 months after diagnosis, but the levels were lower than those in the samples collected at 2 weeks to 2 months after diagnosis (Fig. 1A).

### **Persistence of neutralizing antibody activities against both 614G and 614D SARS-CoV-2 variants**

Since neutralizing antibodies against SARS-CoV-2 are considered as protective antibodies of clinical relevance, we used pseudoviral-based assay for their detection in available samples from 8 cases at 2 weeks to 2 months and 14 ones at 6–7 months after diagnosis in our patient cohort (Table 2). We found that all investigated plasma samples (8/8 at 2 weeks to 2 months and 14/14 at 6–7 months) showed significant neutralizing effect on the pseudovirus and the levels of neutralizing activities, as evaluated by

**Table 1** General information about cases enrolled in the ELISA-based SARS-CoV-2 antibody tests

	2 weeks to 1 month (n = 15)	1–2 month (n = 20)	6–8 months (n = 17)	Healthy donors (n = 12)	P value
Gender					0.89 <sup>b</sup>
Female, n (%)	6 (40.0%)	9 (45.0%)	9 (52.9%)	6 (50.0%)	
Male, n (%)	9 (60.0%)	11 (55.0%)	8 (47.1%)	6 (50.0%)	
Age (year)					0.87 <sup>b</sup>
<60, n (%)	10 (66.7%)	11 (55.0%)	11 (64.7%)	8 (66.7%)	
≥60, n (%)	5 (33.3%)	9 (45.0%)	6 (35.3%)	4 (33.3%)	
Severity of COVID-19					0.76 <sup>b</sup>
Mild/moderate, n (%)	12 (80.0%)	14 (70.0%)	13 (76.5%)		
Severe/critical, n (%)	3 (20.0%)	6 (30.0%)	4 (23.5%)		
Days post infection <sup>a</sup>	20 (17–22)	43.5 (42.25–47)	206 (193.5–212)		<0.0001 <sup>c</sup>
Concentration of S-RBD protein IgG <sup>a</sup> (µg/mL)	31.89 (27.38–35.36)	29.96 (24.74–32.36)	15.38 (8.43–22.01)	0.11 (0.02–0.17)	<0.0001 <sup>c</sup>
Concentration of N protein IgG <sup>a</sup> (µg/mL)	11.81 (10.91–13.47)	10.97 (10.00–11.39)	8.96 (3.71–11.14)	0.03 (0.01–0.03)	<0.0001 <sup>c</sup>

<sup>a</sup>Data are presented as median (IQR); <sup>b</sup>Chi-square test; <sup>c</sup>Kruskal–Wallis test.

NT<sub>50</sub>, remained high at 6–7 months post-infection (Fig. 1B).

Recently, the 614G SARS-CoV-2 variant has been shown to be more infectious than the initial 614D one in *in vitro* and animal studies, and 614G now represents the most frequent allele among infected population [4]. Because antibodies recognizing the S-ACE2 interface have higher neutralization activity [15], one public concern is that the 614D-infected people may not possess antibody protection from the 614G mutant infection. The majority of cases in our study were infected by 614D SARS-CoV-2 variants (Table 2). Of note, the plasma at 6–7 months post-infection of these individuals strongly neutralized 614G SARS-CoV-2 in pseudoviral assay (Fig. 1B).

### Persistence of T cell response post SARS-CoV-2 infection

We also tested the existence of the memory T cells in 10 cases at 6–7 months after diagnosis (9 with available data of neutralizing antibody) by an antigen-presenting DC stimulation assay. Both interferon  $\gamma$ -producing CD4<sup>+</sup> and CD8<sup>+</sup> cells were increased upon SARS-CoV-2 antigen stimulation as compared to non-stimulated samples (Fig. 1C). These observations indicate that memory T cells for SARS-CoV-2 can persist for up to 6–7 months post-infection, in agreement with the status of humoral immunity.

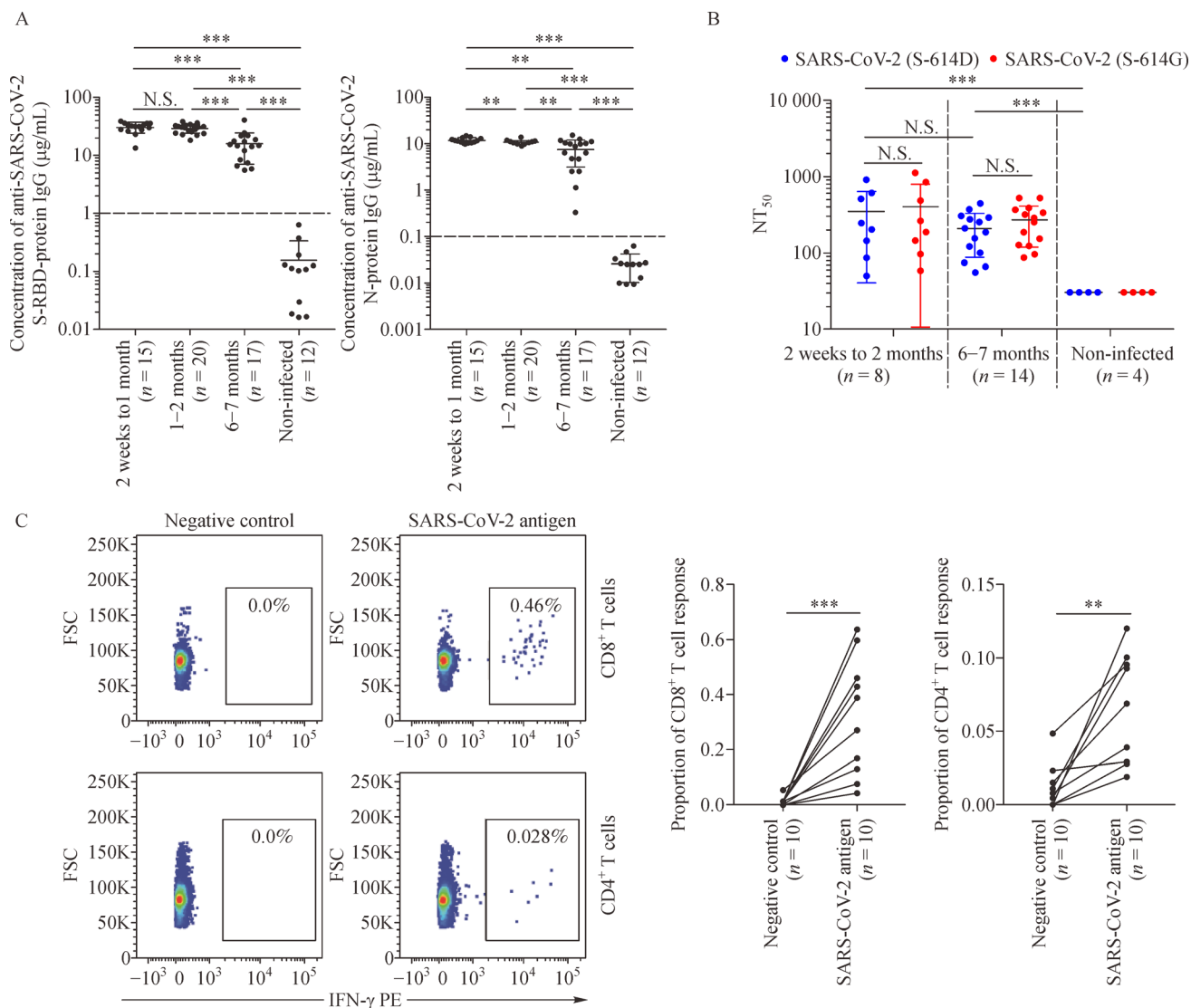
## Discussion

The durability of humoral and cellular response in COVID-19 is a key issue for understanding the risk of second infections. Here, we report the neutralizing antibody activities and memory T cells against the

SARS-CoV-2 can remain stable for up to 6–7 months post-infection.

In our work, both ELISA-based and pseudovirus-based analysis demonstrated the high levels of anti-SARS-CoV-2 antibodies post-infection for 6–7 months. These results are in support of the existence of long-lived plasma cell specifically against SARS-CoV-2 [10]. The persistence of neutralizing antibody activities is essential, since they not only reduce the probability of antibody-dependent enhancement (ADE) effect but may also provide the protection for the infected people from the second infection. We note that, in two cases who developed mild/moderate disease phenotype with low viral loads during hospitalization, the levels of neutralizing antibody activities at 6 months post-infection were also relatively low (Table 2). This might suggest a correlation between SARS-CoV-2 viral load and the strength of long-term humoral immune response. Plasma samples from another two cases with severe/critical symptoms showed relatively high levels of neutralizing activities against SARS-CoV-2 at 6–7 months after diagnosis (Table 2), suggesting that B cell response to SARS-CoV-2 was recovered during the convalescent stage due to an effective immune repair mechanism. More studies are required to confirm these possibilities.

Genomic epidemiology studies by us and others have shown that the SARS-CoV-2 genome has been relatively stable since it appeared last December [16], although a recently emerged D614G mutation in the S-protein appears to increase its binding affinity with the host membrane receptor ACE2 [15]. We found that plasma samples in patients infected with 614D SARS-CoV-2 also had a strong neutralizing efficacy to the 614G variant, probably because the majority of antibody recognition sites were not located near 614D/G. Indeed, other groups reported that the antibodies isolated from 614D variant-infected patients



**Fig. 1** Persistent antibody and T cell response to SARS-CoV-2 after six-month of infections. (A) The levels of antibodies against SARS-CoV-2 S-protein and the N-protein in plasma samples at 2 weeks to 1 month, 1–2 months, and 6–7 months post-infection are plotted. The levels of non-infected donors were included as negative control. (B) The neutralizing antibodies against the SARS-CoV-2 (S-614D) and SARS-CoV-2 (S-614G) pseudovirus in plasma samples of cases collected at 2 weeks to 2 months or 6–7 months post infection are plotted. Plasma samples of non-infected donors were used as negative control. (C) T cell response to the SARS-CoV-2 antigen stimulation. The interferon  $\gamma$ -producing CD4<sup>+</sup> T cells and CD8<sup>+</sup> T cells were detected with or without stimulation by SARS-CoV-2 antigens. The blood samples of cases collected at 6–7 months post infection were used. \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

could neutralize the D614G mutant in distinct experimental settings [15,17]. We therefore deduce that this cross-protection may also significantly reduce the risk of ADE upon vaccination against the D614G mutant using vaccines based on 614D SARS-CoV-2.

In addition, this work shows a durable response of memory T cells to SARS-CoV-2 antigens, in consistence to previous reports that convalescent sera contained high levels of interferon  $\gamma$  [4]. Similar long-term persistence of memory T cells was reported in SARS-CoV infection [18–21]. Though longer observations are needed in future

studies, our finding that all of the patients investigated including those with mild symptoms can develop a cellular immune response to SARS-CoV-2 antigens is promising in terms of protection from second infection.

In summary, our study suggests that the majority of convalescent individuals of COVID-19 have persistent immune protection. It may also be speculated that vaccination against SARS-CoV-2 infection may trigger relatively durable immune response, and that people exposed to the D614G variant may be protected by vaccines designed with the 614D SARS-CoV-2.

**Table 2** Detailed information of cases in the analysis of neutralizing antibodies against SARS-CoV-2 pseudovirus

No.	Gender	Age	Days post infection <sup>c</sup>	Severity of COVID-19	S protein variant	Viral load <sup>d</sup> (CT value)	NT <sub>50</sub> (SARS-CoV-2 pseudovirus)	NT <sub>50</sub> (SARS-CoV-2 D614G pseudovirus)
1	Male	65	20	Severe/critical	WT	21.23	244.5	186.7
2	Female	64	22	Severe/critical	WT	28	85.6	95.8
3	Male	37	43	Mild/moderate	H49Y	29.31	507	481.4
4	Female	65	43	Mild/moderate	WT	18.47	200.5	261.2
5	Female	68	44	Mild/moderate	NA	23.63	606.1	850.2
6	Male	58	45	Mild/moderate	WT	NA	141.7	143.6
7	Female	53	52	Mild/moderate	WT	31.73	50.2	58.8
8	Male	68	59	Severe/critical	WT	26.56	912.2	1109.6
9 <sup>a</sup>	Female	58	186	Severe/critical	WT	19.72	368.9	523.2
10	Male	37	188	Mild/moderate	NA	NA	73.7	125.7
11	Male	40	193	Mild/moderate	NA	NA	289.8	251.6
12	Female	42	194	Mild/moderate	WT	23.52	436.2	519.9
13	Female	62	196	Mild/moderate	NA	NA	118.4	152.1
14	Male	39	197	Mild/moderate	WT	27.71	183.8	331.2
15	Male	40	206	Mild/moderate	WT	NA	303.2	385.1
16	Male	68	207	Mild/moderate	WT	29	251.5	315.9
17	Male	60	208	Mild/moderate	WT	26.96	154.2	281.6
18 <sup>b</sup>	Female	71	208	Mild/moderate	NA	36.5	55.1	95.3
19	Female	33	211	Mild/moderate	WT	29.46	270.7	363.8
20	Female	47	215	Mild/moderate	D614G	32.52	98.4	122
21 <sup>a</sup>	Male	71	219	Severe/critical	WT	NA	207.9	185.5
22 <sup>b</sup>	Female	51	220	Mild/moderate	NA	30.14	64.5	86

<sup>a</sup>Cases underwent severe/critical COVID-19 at 6–7 months before. <sup>b</sup>Cases with relatively low NT<sub>50</sub> post 6–7 months of infection. <sup>c</sup>Viral load represented results from the real-time quantitative PCR analysis of the SARS-CoV-2 RNA using the throat swab samples for the first time of hospitalization. <sup>d</sup>Days post-infection was defined by the number of days between the day post symptom onset and the day of specimen collection for the antibody and T cell detection. NA, not available; WT, wild-type (the NC\_045512.2 was used as reference genome of SARS-CoV-2); CT, cycle threshold; NT<sub>50</sub>, 50% neutralizing titer.

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## Compliance with ethics guidelines

Yun Tan, Feng Liu, Xiaoguang Xu, Yun Ling, Weijin Huang, Zhaoqin Zhu, Mingquan Guo, Yixiao Lin, Ziyu Fu, Dongguo Liang, Tengfei Zhang, Jian Fan, Miao Xu, Hongzhou Lu, and Saijuan Chen declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the *Helsinki Declaration* of 1975, as revised in 2000 (5). Informed consent was obtained from all patients for being included in the study.

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