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Anti-viral memory T cell responses in the absence of IgG production in a COVID-19 convalescent individual

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Abstract: Cellular and humoral immunity are both important in host defense against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Although defects in SARS-CoV-2-specific T cell immunity have been found in patients with severe lung pathology, it is still largely unclear whether virus-specific T cells are sufficient for host protection. Here, we found that in a previously characterized cohort of convalescent subjects, one individual, though lacking detectable anti-viral neutralizing IgG antibodies, showed virus-specific T cell responses, both in $CD4^+$ and $CD8^+$ T cells. SARS-CoV-2-specific T cells in this and other individuals are maintained for up to 10 months. This study thus further supports a critical role of T cells in host defense against SARS-CoV-2, offering new insights into the design and evaluation of COVID-19 vaccines.

Keywords: SARS-CoV-2, adaptive immunity, T cells, memory

INTRODUCTION

The current global pandemic of COVID-19, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1], is affecting many countries all over the world. The high infection rate and rapid spread have paralyzed society and in turn dramatically expedited global efforts in vaccine development.

The adaptive immune system plays a key role in controlling viral infection. There has been rapid progress in understanding adaptive immunity to SARS-CoV-2. Less than two months after declaration of a global pandemic on 11 March 2020, we and other groups reported the detection of anti-SARS-CoV-2 antibodies and

T cells in convalescent individuals [2–4]. Grifoni *et al.* [3] found that nearly 70% and 100% of COVID-19 recovered patients mounted SARS-CoV-2-specific $CD8^+$ and $CD4^+$ T cell responses, respectively, while a report by Wu *et al.* [4] showed that ~94% of COVID-19 recovered subjects developed neutralizing antibodies. Moreover, most acute COVID-19 patients with severe lung pathology exhibited anti-SARS-CoV-2 neutralizing antibody responses, but with largely undetectable SARS-CoV-2-specific $CD8^+$ and $CD4^+$ T cell responses [5,6]. Of note, a few reports showed that COVID-19 convalescent subjects still had anti-viral memory T and B cells at 6 or 8 months after infection, though their antibody titers had declined [7,8]. Virus-specific memory $CD4^+$ T cells were more prevalent than memory $CD8^+$ T cells. Interestingly, the frequency of spike receptor binding domain (RBD) memory B cells was reported to increase over time [8,9].

Neutralizing antibodies can prevent viral infection and further viral transmission. The US Food and Drug Administration (FDA) has authorized the emergency use of neutralizing monoclonal antibodies (mAbs) to treat mild to moderate COVID-19 patients in order to reduce the hospitalization and death rates. In addition, many types of vaccine have been developed and tested. In December 2020, the FDA granted emergency use authorization for COVID-19 mRNA vaccines (BTN162 and mRNA-1273) based on a final efficacy of 95% in a Phase III trial [10,11]. In a phase 1 trial, BNT162b2 (full-length spike) induced neutralizing antibody titers better than natural infection over the short term (T cell data was unavailable) [12], while the phase 1 trial of BNT162b1(RBD domain) demonstrated robust $CD4^+$ and $CD8^+$ T cell responses, with $CD4^+$ T cells largely consisting of Th1 cells [13]. Whether the protection by these vaccines (BTN162 and mRNA-1273) is attributable to humoral immunity, cellular immunity or both needs extensive investigation.

In non-human primates (NHPs), SARS-CoV-2 infection has been shown to prevent re-infection [14]. NHP vaccination experiments have shown that neutralizing antibodies, but not T cell responses, are related to protection [15]. Unlike the NHP study, neutralizing antibodies have not been associated with reduced COVID-19 disease severity [6,16,17]. In addition, several reports support the possibility of SARS-CoV-2 clearance in the absence of neutralizing antibodies. Soresina *et al*. showed that two unrelated adults with agammaglobulinemia and no circulating B cells developed COVID-19 and recovered from SARS-CoV-2 infection [18]. Another three studies of COVID-19 patients on B cell depletion therapy reported that all of them recovered with intensive care [19–21]. Unfortunately, no virus-specific T cell data are available in any of those reports. Whether SARS-CoV-2-specific T cells are sufficiently protective warrants investigation.

Here we analyzed memory SARS-CoV-2-specific T cells in a cohort of convalescent individuals and the data reveal that SARS-CoV-2-specific T cells can be maintained for up to 10 months, the latest time point analyzed. Interestingly, we detected them in one subject in the absence of neutralizing antibodies. This study provides new insights into the design and evaluation of candidate COVID-19 vaccines.

MATERIALS AND METHODS

COVID-19 patient blood samples

The blood samples of mild COVID-19 convalescent patients were obtained from Chui Yang Liu Hospital, affiliated with Tsinghua University, in Beijing. All procedures were in accordance with the ethical standards of the responsible committee on human experimentation (The institutional review board at Tsinghua University) and with the Helsinki Declaration of 1975, as revised in 2000. All studies were approved by the

Medical Ethical Committee at Tsinghua University (approval No. 20200009). Informed consent was obtained from all subjects for being included in the study. All patient data were anonymized before study inclusion.

Purification of recombinant proteins

The recombinant His-tagged nucleocapsid protein (NP) of SARS-CoV-2 was expressed in *Escherichia coli* using a T7 expression system, while the recombinant His-tagged receptor-binding domain of the spike protein (S-RBD) (amino acids 319–541) was expressed using a mammalian system in 293F cells. The proteins were purified and then analyzed by SDS-PAGE. The purity of the His-tagged NP and the His-tagged S-RBD was ~90% and the endotoxin levels of NP and S-RBD were 0.01 and 0.02 EU per μg, respectively.

Anti-SARS-CoV-2 IgG/IgM ELISA

For SARS-CoV-2-specific IgM/IgG testing, 96-well ELISA plates were coated overnight with recombinant NP and S-RBD (80 ng/well) at 4°C. After plates were washed, serially diluted sera from COVID-19 subjects were added and incubated for 1 h at 37°C. After extensive washing, anti-human IgG-biotin conjugated monoclonal antibodies (Cat. SSA009, Sino Biological Inc., Wayne, PA) and anti-human IgM-HRP conjugated monoclonal antibodies (Cat. bs-0345G-HRP, Biosynthesis Biotechnology Inc., Beijing, China) were added at a dilution of 1:5000 and 1:1000 as indicated. After extensive washing, the second antibody (streptavidin-HRP) was used at a dilution of 1:250. TMB substrate solution was added and the OD value at 450 nm was determined. The area under the curve (AUC) was calculated by Prism 8 (Graphpad).

Anti-SARS-CoV-2 IgG1 ELISA

For assaying IgG1/IgG3, 96-well ELISA plates were coated (80 ng/well) overnight with recombinant NP and S-RBD at 4°C. Plates were washed and the serially diluted sera from COVID-19 subjects were incubated for 1 h at 37°C. After washing, anti-human IgG1-HRP conjugated monoclonal antibody (Cat. C030248, BaiaoTong Experiment Center, LY) and anti-human IgG3-HRP conjugated monoclonal antibody (Cat. C030246, BaiaoTong Experiment Center, LY), both validated by the company for their specificity, were used at a dilution of 1:4000 for 1 h at room temperature. After extensive washing, TMB substrate solution was added and the OD value at 450 nm was determined. The area under the curve (AUC) was calculated by Prism 8 (Graphpad).

Neutralizing antibody assay

Pseudovirus expressing the SARS-CoV-2 S protein (Wuhan strain) was produced as described [22]. pNL43Luci and GP-pCAGGS were co-transfected into 293T cells. Forty-eight hours later, SARS-CoV-2 pseudovirus-containing supernatants were mixed with at least six serially diluted serum samples from the COVID-19 subjects at 37°C for 1 h. Then the mixtures were transferred to 96-well plates containing monolayers of Huh-7 cells [23]. Three hours later, the medium was replaced. After incubation for 48 h, the

cells were washed, harvested in lysis buffer and analyzed for luciferase activity by the addition of luciferase substrate. Inhibition rate = [1−(the sample group−the cell control group) / (the virus control group−the cell control group)]×100%. The neutralizing antibody titers (NAT50) were calculated by performing S-fit analysis via Graphpad Prism 7 software.

Isolation of PBMCs

Peripheral blood mononuclear cells (PBMCs) were isolated from anti-coagulant blood using Ficoll-Hypaque gradients (GE Healthcare Life Sciences, Philadelphia, PA) as previously described [24] in a biosafety level 2 facility. The whole blood was centrifuged for 5 min to separate plasma and cells. Then blood cells diluted with PBS were gently layered over an equal volume of Ficoll in a tube and centrifuged for 30–40 min at 400–500×*g* without a brake. Four layers formed and the cells in the second layer were PBMCs.

In vitro expansion

PBMCs were plated in 96-well U bottom plates at 1×10^6 PBMCs per well in complete RPMI1640 supplemented with 10% human AB serum in the presence of SARS-CoV-2 megapools (MP)-S (a megapool of 253 15-mer peptides covering the entire S protein with overlapping by 10 residues) or MP-NP (a megapool of 82 15-mer peptides covering the entire NP protein with overlapping by 10 residues) at 1 μg/mL for 7 days. The cells were then rested overnight in serum-free medium. The expanded T cells were restimulated with MP-S or MP-NP, respectively, followed by IFNγ ELISpot assay or FACS staining. Supernatants were harvested for multiplex detection of cytokines.

IFNγ ELISpot

IFNγ-secreting T cells were detected using a Human IFNγ ELISpot^{pro} kit (MABTECH AB, Sweden) according to the manufacturer's protocol. The PBMCs after *in vitro* expansion were plated in duplicate at 150k/well and then incubated for 48 h with 1 μM of MP-S or MP-NP. Anti-CD3 antibody (0.1 μg/mL) was used as a positive control and medium alone as a negative control. Spots were then counted using an AID ELISpot Reader System (iSpot, AID GmbH). The number of spots was converted into the number of spots per million cells. Mean spots in the negative control wells were subtracted from the experimental and positive control wells.

Intracellular cytokine staining

The PBMCs were stimulated with phorbol myristate acetate (PMA)/Ionomycin for 4 h with GolgiPlug (brefeldin A, BD) or MP-S/NP for 7 h with GolgiPlug. For flow cytometry staining, dead cells were first stained with live/dead fixable aqua dye. Next, surface markers were stained. Cells were then washed, fixed with Cytofix/Cytoperm^{TM} (BD Biosciences) and stained with anti-IFNγ and anti-tumor necrosis factor alpha (TNFα). The samples were acquired on BD FACS Fortessa (BD Biosciences, San Jose, CA) and analyzed with FlowJo^{TM} v.10 software for Mac (Version 10.0.8, Tree Star Inc., Ashland, OR).

Cytokine cytometric bead array

The concentration of cytokines secreted from *in vitro* expanded T cells was measured using a Human Th1/Th2/Th9/Th17/Th22/Treg cytokine panel (18 plex, EPX180-12165-901) (Invitrogen, USA) as per the manufacturer's instructions. Then, the plate was run on a Luminex 200. The sensitivities of the cytometric bead array for each cytokine were 17.9 pg/mL (IFNγ), 9.5 pg/mL (IL-12p70), 3.0 pg/mL (IL-13), 2.5 pg/mL (IL-1β), 8.1 pg/mL (IL-2), 15.0 pg/mL (IL-4), 3.6 pg/mL (IL-5), 10.5 pg/mL (IL-6), 5.9 pg/mL (TNFα), 17.3 pg/mL (GM-CSF), 18.6 pg/mL (IL-18), 2.2 pg/mL (IL-10), 3.1 pg/mL (IL-17), 9.3 pg/mL (IL-21), 21.2 pg/mL (IL-22), 20.5 pg/mL (IL-23), 24.7 pg/mL (IL-27) and 9.7 pg/mL (IL-9).

Statistical analysis

Prism 8 software was used for statistical analysis. Student's *t* test was performed for two-group analysis. Two-way ANOVA followed by multiple comparison test was used for three-group analysis. *P* values less than 0.05 were considered to be statistically significant.

RESULTS

Lack of a memory SARS-CoV-2-specific IgG response in a convalescent individual

Our previous work identified one COVID-19 convalescent individual (c#9) without SARS-CoV-2-specific IgG or neutralizing antibody at 2 weeks post discharge (PD) (first follow-up) [2]. To explore if c#9 could generate neutralizing antibodies thereafter, we collected blood samples at 5 months PD (second follow-up) and analyzed the levels of serum SARS-CoV-2-specific IgM/IgG and neutralizing antibodies by a pseudo-virus particle-based neutralization assay as previously described [2]. As shown in Figure 1A, c#9 still did not show neutralizing antibodies. For the other convalescent subjects, the neutralizing antibody titers (NAT50) declined dramatically with only two (40%, 2/5) showing detectable amounts. The clinical features of these convalescent subjects and the sampling days are summarized in Table 1. In addition, compared to the negative controls, their IgM specific to both NP and S-RBD (Figures 1B and 1C) also declined significantly at the second follow-up (4–5 months PD). However, there was no significant difference in terms of virus-specific IgG levels between the first and the second follow-ups (Figure 1C). Of note, we still did not observe S-RBD or NP-specific IgG in the serum of c#9 at the second follow-up, but her levels of total IgG were similar to other convalescent subjects and healthy donors (data not shown). In terms of IgG subclass, our previous study showed that anti-NP and S-RBD IgG was mainly of the IgG1 isotype. As shown in Figure 1D, the levels of IgG1 specific to both NP and S-RBD between the two time-points did not differ significantly. These results indicate that c#9 had consistent defects in serum virus-specific IgG production, even months after infection.

SARS-CoV-2-specific memory T cell responses in c#9

Since we previously detected SARS-CoV-2-specific T cells in c#9, we further explored memory T cell

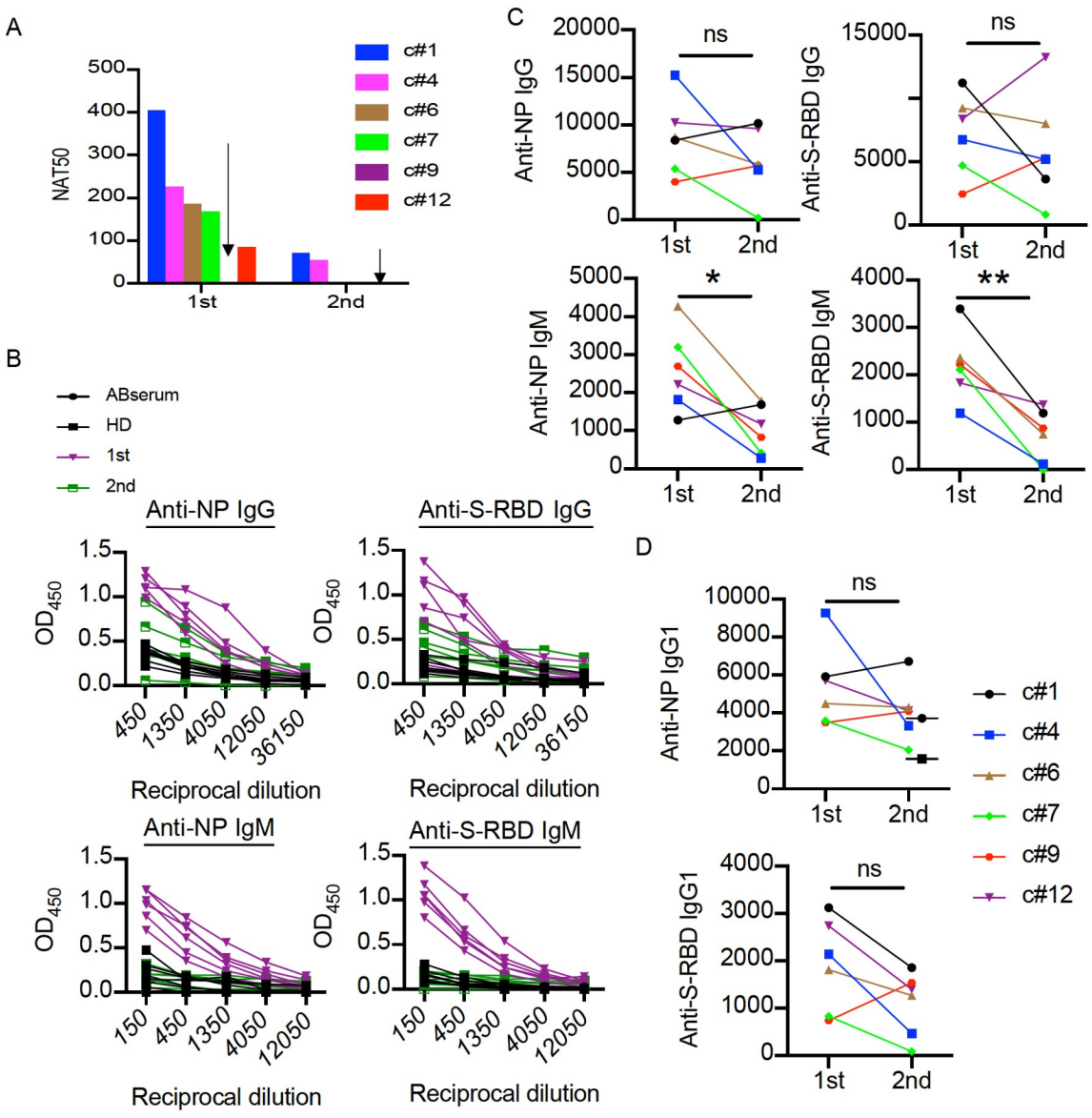

Figure 1 Maintenance of SARS-CoV-2-specific antibodies in convalescent COVID-19 subjects. (A) Measurement of neutralizing antibody titers in six convalescent subjects by a pseudovirus-based assay. c#9 is indicated by an arrow. (B) Titration of individual serum samples for detection of anti-virus Ig. (C) Data from the same experiments as (B) are presented as area under the curve (AUC). (D) Levels of IgG1 antibody reactive with recombinant NP and S-RBD of convalescent subjects at their first (2 weeks) and second (4–5 months) post discharge visits. The experiment was done in duplicate. Data in C are presented as mean±SEM. NP, nucleocapsid protein. S-RBD, receptor binding domain of spike protein. HD, healthy donor. c, convalescent subject; AUC, area under the curve. **P*<0.05, ***P*<0.01, ns., not significant.

Table 1 Clinical characteristics of the convalescent COVID-19 subjects

Subject	Sex	Age	Fever	Fatigue	Severity	Sampling days PD
#1	F	51	Yes	Yes	Mild	2 weeks, 4 months
#4	M	49	Yes	No	Mild	2 weeks, 4 months, 5 months, 9 months
#6	M	32	Yes	Yes	Mild	2 weeks, 4 months, 5 months, 9 months
#7	M	32	Yes	Yes	Mild	2 weeks, 4 months, 5 months, 9 months
#9	F	26	Yes	No	Mild	2 weeks, 5 months, 6 months, 10 months
#12	F	29	Yes	Yes	Mild	2 weeks, 5 months, 6 months

Notes: F, female; M, male; PD, post discharge.

responses in this subject. To that end, blood samples from five convalescent subjects were sampled at their third follow-up (5–6 months PD). Previously, we found very weak T cell responses following exposure to the recombinant proteins (NP and S-RBD) in some convalescent subjects at their first follow-up analysis. Thus, we synthesized two megapools, MP-S and MP-NP. PBMCs from the five subjects were incubated for 7 days with the two megapools separately. The expanded T cells were then restimulated with MP-S or MP-NP, followed by an IFNγ ELISpot assay. Interestingly, c#9 had readily detectable IFNγ-producing SARS-CoV-2-specific memory T cells at 6 months PD (Figures 2A and 2B). Moreover, comparison of stimulation with anti-CD3 and megapools showed that most expanded T cells were specific to spike or NP. The magnitude of the IFNγ responses to the two megapools for all the participants are shown in Figure 2C. Among healthy

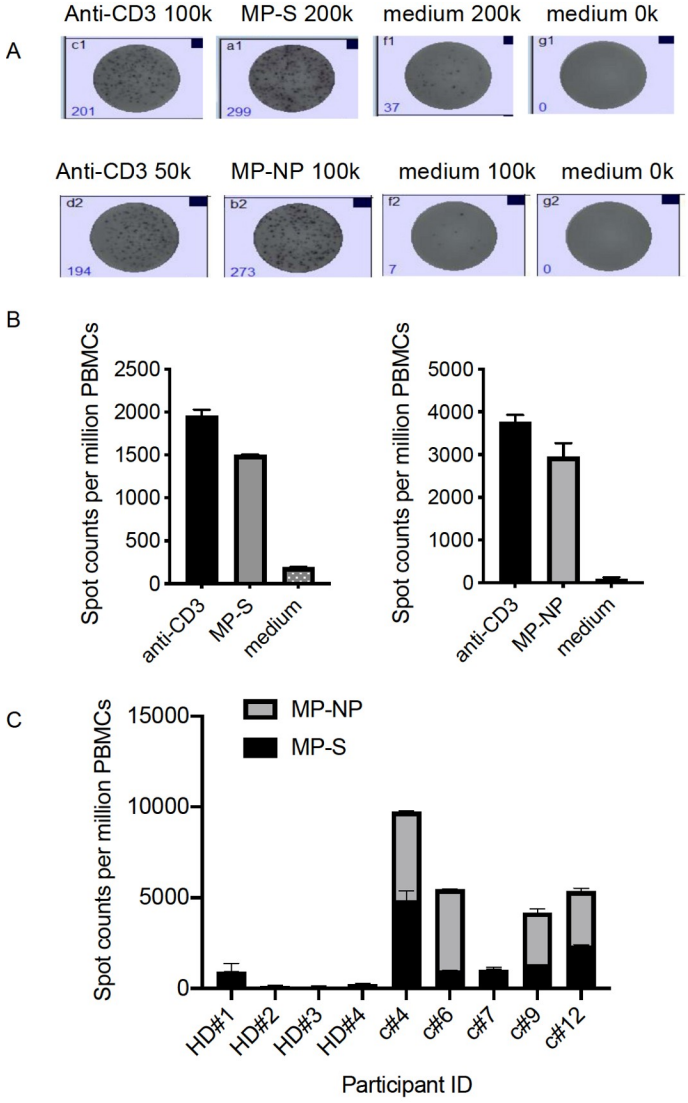

Figure 2 Detection of SARS-CoV-2-specific T cells in the convalescent subjects at 5–6 months post discharge. (A) Representative IFNγ ELISpot image. 0–200k means 0–200k of PBMCs per well. (B) IFNγ ELISpot assay of spike- (left panel) and NP- (right panel) specific T cells from c#9 post-expansion. (C) Summarized IFNγ ELISpot data showing the frequency of S- and NP-specific T cells in the convalescent subjects. The experiment was done in duplicate. Data are presented as mean±SEM. HD, healthy donor; c, convalescent subject. MP-NP, NP peptide pool; MP-S, spike peptide pool.

volunteers, a weak IFNγ-secreting spike-specific T cell response was observed only for HD#1. The magnitude of T cell responses varied widely among the convalescent subjects. c#4 exhibited the strongest IFNγ-producing spike/NP-specific T cell responses, while a weak T cell response only to spike was detected in c#7. c# 6, 9 and 12 showed a similar magnitude of T cell responses (Figure 2C). Interestingly, there was no obvious difference in the frequency of the NP/S-RBD-specific T cells between c#6 and c#7 on discharge, but that in c#7 was much lower than in c#6 at 5 months PD, indicating that this individual did not develop long-lasting NP-specific memory T cells. Taken together, our results indicate that memory SARS-CoV-2-specific T cells could persist for months after infection in a majority of recovered subjects. Moreover, T cells from c#9, who had no neutralizing antibodies, likely played a protective role in the clearance of SARS-CoV-2.

Detection of SARS-CoV-2-specific memory $CD4^+$ and $CD8^+$ T cells

Since spike/NP-specific memory T cells were identified in the convalescent subjects at 5–6 months PD, we further analyzed antigen-specific $CD4^+$ and $CD8^+$ T cells for their production of IFNγ and TNFα by flow cytometry. In c#9, a greater proportion of T cell response to spike was observed among $CD8^+$ T cells than $CD4^+$ T cells (Figure 3A). The patterns of virus-specific TNFα-producing T cell responses to spike were similar to those of the IFNγ-producing T cells (Figure 3A). However, in contrast, we observed a high frequency of NP-specific IFNγ or TNFα-expressing $CD4^+$ T cells, but not NP-specific $CD8^+$ T cells (Figure 3B). In total, two out of five subjects (40.0%) had IFNγ-producing S-specific $CD8^+$ T cells, while four out of five subjects (80%) had IFNγ-producing S-specific $CD4^+$ T cells (Figure 3C). Three out of four (75%) of the subjects had IFNγ-producing NP-specific $CD8^+$ T cells, whereas four out of four (100%) had NP-specific $CD4^+$ T cells (Figure 3D). Moreover, both S-specific or NP-specific IFNγ-producing $CD4^+$ and $CD8^+$ T cells also produced TNFα (Figures 3C and 3D), indicating that those memory T cells are poly-functional. Interestingly, we also detected IFNγ-producing spike-specific $CD4^+$ T cells in HD#1, but not $CD8^+$ T cells, which was in line with the ELISpot assay data (Figure 2C). Taken together, SARS-CoV-2-specific memory $CD4^+$ and $CD8^+$ T cell responses were observed in most convalescent subjects, with $CD8^+$ T cell responses from c#9 mainly targeting the spike protein but not NP.

Cytokine profiling of NP-specific T cells from convalescent subjects

To investigate the function of NP-specific T cells, we measured their cytokine profiles at 9–10 months PD by a Luminex assay using a human Th1/Th2/Th9/Th17/Treg cytokine panel. As shown in Figure 4, we did not detect any type of cytokines from T cells of c#7, which was consistent with the ELISpot data, suggesting that there was no T cell response or memory T cells to NP in this individual. Of note, $CD4^+$ T cells from c#9 did not secrete the Tfh cytokine IL-21 following exposure to MP-NP, which might account for the humoral response defects. However, compared to other convalescent subjects, c#9 produced increased levels of IL-22, which plays a pivotal role in mucosal immunity and tissue repair, suggesting that SARS-CoV-2-specific $CD4^+$ T cells might be involved in lung tissue repair during COVID-19. Moreover, SARS-CoV-2 IL-22-producing memory $CD4^+$ T cells could be sustained for at least 10 months. Th1 (IFNγ, TNFα and IL-18), Th2 (IL-4, IL-5 and IL-13), Th17 (GM-CSF and IL-17A) and Treg (IL-10) cytokine production could also be detected in this subject. The highest amount of NP-specific IL-17A production was observed in c#6, while

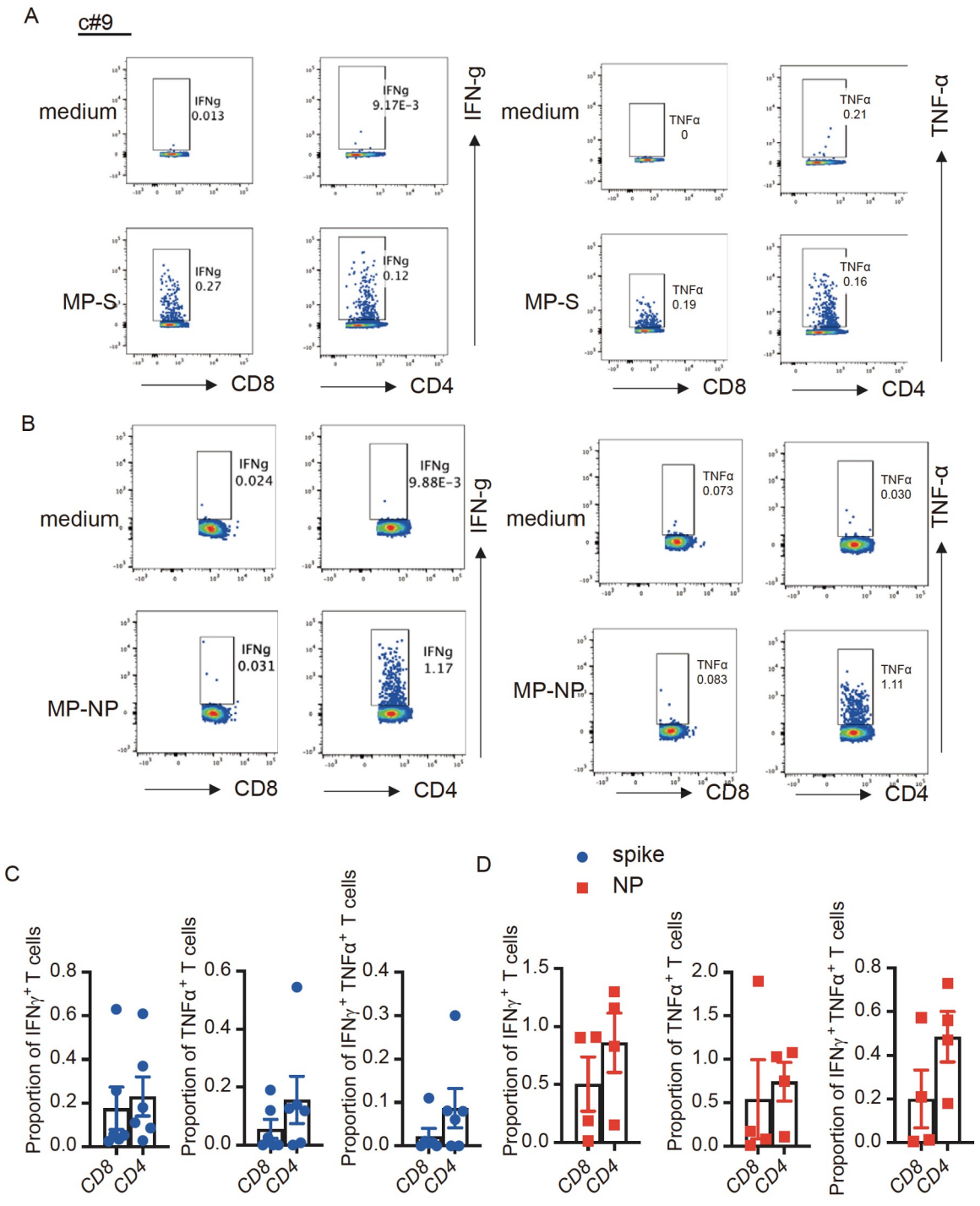

Figure 3 Detection of memory SARS-CoV-2-specific $CD8^+$ and $CD4^+$ effector T cells in convalescent subjects. (A) Flow cytometry plot showing S-specific IFNγ (left panel) and TNFα (right panel)-producing $CD8^+$ and $CD4^+$ T cells from c#9 at 6 months post discharge. (B) Flow cytometry plot showing NP-specific IFNγ (left panel) and TNFα (right panel)-expressing $CD8^+$ and $CD4^+$ T cells from c#9 at 10 months post discharge. (C) Summarized data showing spike-specific IFNγ, TNFα and IFNγ+TNFα-producing T cells in the convalescent subjects at 5–6 months post hospital discharge (n=5). (D) Summarized data showing NP-specific IFNγ, TNFα and IFNγ+TNFα T cells in the convalescent subjects at 9–10 months post hospital discharge (n=4). Data are presented as mean±SEM. MP-NP, NP peptide pool; MP-S, spike peptide pool.

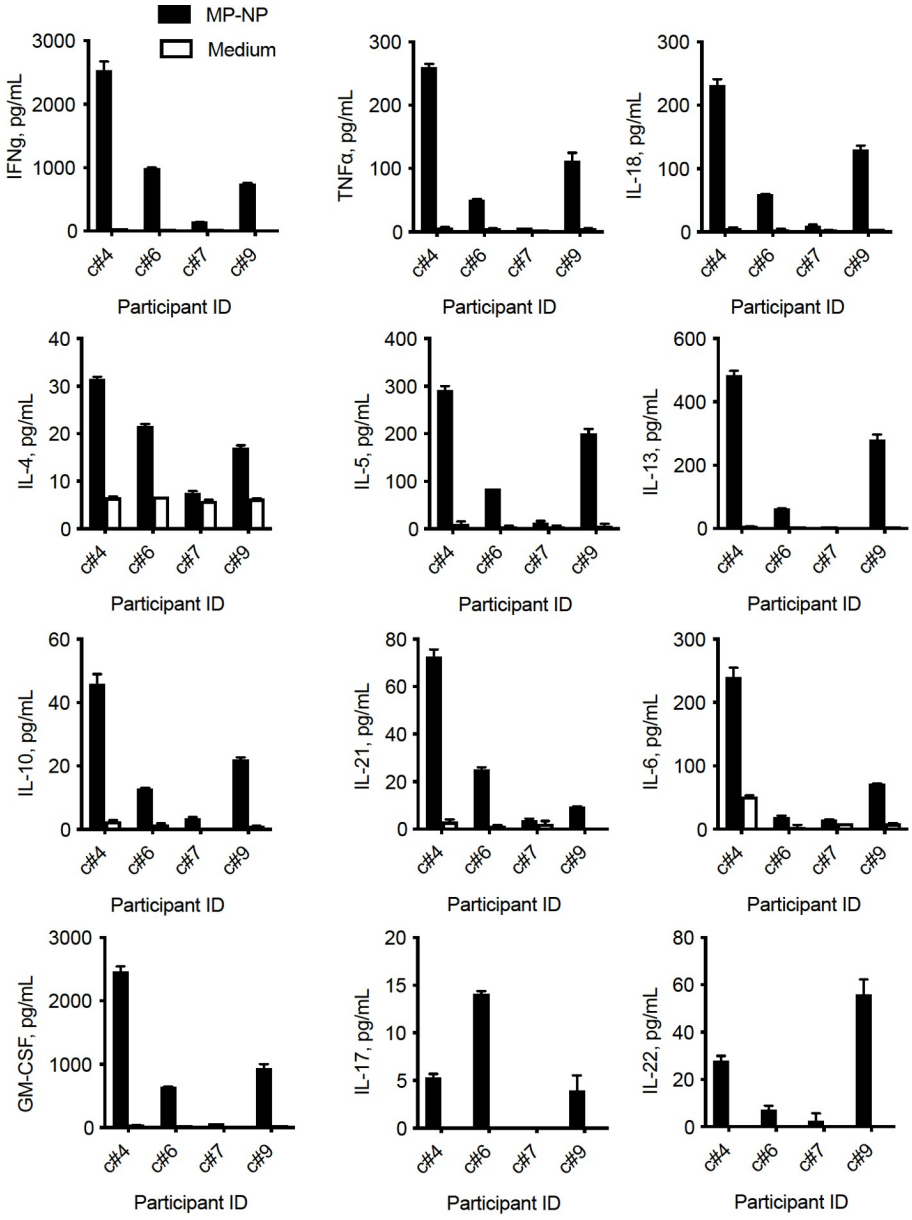

Figure 4 Cytokine profiling of NP-specific T cells from the convalescent subjects. T cells from convalescent subjects at 9–10 months post hospital discharge were expanded in the presence of MP-NP and then restimulated with MP-NP. The culture supernatants were analyzed by a Luminex assay. The experiment was done in duplicate. Data are presented as mean±SEM. MP-NP, NP peptide pool; c, convalescent subjects.

c#4 produced the highest levels of NP-specific Th1 and Th2-type cytokines. IL-9, IL-23 and IL-27 were not detected in any of the samples (data not shown).

Kinetics of SARS-CoV-2 memory T cells

NP-specific T-cell cytokine production in c#9 was still observed up to 10 months post discharge. To explore the kinetics of SARS-CoV-2 memory T cells in this subject and to predict the durability of protective T cells,

we compared the magnitude of NP-specific T cell responses in c#9 between 6 and 10 months after release from the hospital. In general, memory T cell responses declined over time (Figure 5). However, NP-specific memory $CD4^+$ T cells in c#9 expressed higher levels of IL-5 at 10 months than at 6 months post discharge. However, production of another Th2-type cytokine IL-13 was reduced over time. NP-specific T cells in c#9 had a 57% reduction in the production of IFNγ and a 33% reduction in IL-22 at 10 months PD. NP-specific IL-21 production was not detected either at 6 months or 10 months of follow-up, further confirming that this subject failed to generate NP-specific Tfh cells and in turn failed to generate humoral immunity. For c#4, there was no obvious difference in the production of IFNγ and IL-21 between 5 months and 9 months after

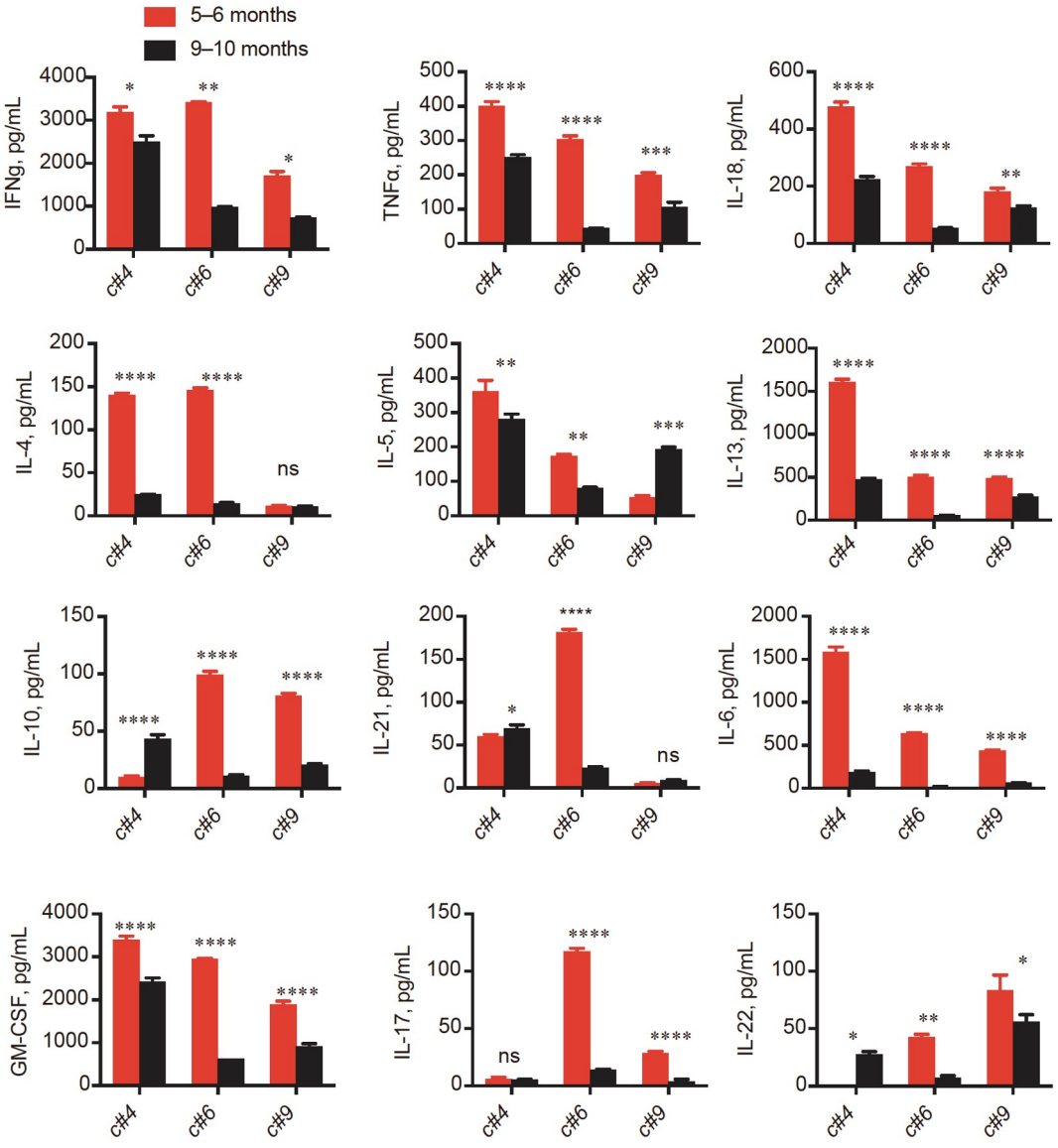

Figure 5 Kinetics of NP-specific T cells from the convalescent subjects. Comparison of cytokine profiles of NP-specific T cells from the convalescent subjects between 5–6 months and 9–10 months post-hospital discharge. T cells from convalescent subjects were expanded in the presence of MP-NP and then restimulated with MP-NP. The culture supernatants were analyzed by a Luminex assay. The experiment was done in duplicate. Data are presented as mean±SEM. MP-NP, NP peptide pool; c, convalescent subject. **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001, ns., not significant.

discharge, but a dramatic reduction in the expression of Th2 cytokines IL-4 and IL-13. Interestingly, compared with cytokine production at 5–6 months PD, c#4 showed increased production of IL-10 and IL-22 at 9 months PD.

In summary, overall SARS-CoV-2 memory T cell responses declined over time. How long these memory T cells can be maintained warrants further investigation.

DISCUSSION

In this study, we characterized antibody and memory T responses in convalescent subjects and found that SARS-CoV-2-specific memory T cells could be detected in most cases, accompanied by a decline in neutralizing antibody titers.

In a previous report [25], 93.3% of asymptomatic and 96.8% of symptomatic COVID-19 patients had a reduction in virus-specific IgG levels at 8 weeks PD. Two other reports also showed a rapid decline in neutralizing antibody levels at 3 months following SARS-CoV-2 infection [26,27]. Our study indicates that three in five convalescent individuals (c#9 with undetectable IgG) had a decline in anti-virus IgG at 4–5 months PD, consistent with the above-mentioned reports. Due to the small sample size, our data failed to reach statistical significance. Despite the decline in antibody titers, several studies showed detectable memory B cells at 6 months post infection [8,9], which could offer durable humoral immunity. The memory B cells detected in all the subjects were specific for spike, RBD and NP, and the isotype of the memory anti-spike B cells was mainly IgG with only ~ 5% IgA [8].

In this study, memory $CD4^+$ and $CD8^+$ T cell responses were observed in most of convalescent subjects at 10 months post hospital release. Among the subjects analyzed (c#4, #6, #7 and #9), only c#4 still had neutralizing antibodies at the second follow-up, and also showed the strongest SARS-CoV-2-specific cytokine responses, except for IL-17 and IL-22. In c#9, the subsets of NP-specific memory $CD4^+$ T cells consisted of Th1 and Th2, but not Tfh, and NP-specific memory $CD8^+$ T cells were undetectable. Mucosal antibody responses have been shown in COVID-19 patients [28], which indicates that mucosal immunity might play a critical role in the clearance of SARS-CoV-2. c#9 had not produced SARS-CoV-2-specific IgG and neutralizing antibodies on hospital discharge and thereafter. However, it is very important to note that there were increased NP-specific IL-22-producing T cells in this subject. At the mucosal surface, IL-22 provides innate immune protection as well as enhances epithelial cell proliferation and repair [29]. It would be very informative to study mucosal IL-22-producing T cells and analyze their roles in maintaining the mucosal barrier. Interestingly, we detected $CD4^+$ T cells recognizing MP-S and producing IFNγ in one healthy donor. This individual was negative for SARS-CoV-2 nucleic acids by PCR, suggesting a possible cross-reactivity due to past infection with ‘common cold’ coronaviruses.

With the clearance of SARS-CoV-2, neutralizing antibody titers dropped and memory T developed. Although c#7 had memory S-specific, but not NP-specific, T cells producing S-specific IFN-γ, his neutralizing antibodies were undetectable at the second follow-up visit. Only c#4 still maintained the neutralizing antibodies at the second follow-up with a similar level of IL-21 expression between 5 and 9 months PD, whereas NP-specific IL-4 and IL-13 at 9 months PD dropped dramatically, which might contribute to the decreased neutralizing antibodies.

As to the defects in the anti-SARS-CoV-2 IgG response in c#9, we envision two possible explanations. One is disease severity. Favresse *et al.* showed that 100% of patients with moderate-severe disease had positive neutralizing antibodies (mainly contributed by IgG) while 80.2% of patients with mild disease were positive [30], indicating that the generation of neutralizing antibodies might be associated with disease severity. In fact, the magnitude of neutralizing antibody titers was dependent on COVID-19 severity [22]. The second is impaired generation of Tfh cells. Kaneko *et al*. reported that absence of germinal centers correlated with a defective Bcl-6$^+$ Tfh cell generation in COVID-19 patients, which may lead to the short durability of antibody responses against SARS-CoV-2 or even the absence of neutralizing antibodies. In the case of c#9, there was a mild COVID-19 disease. We also found that NP-specific CD4$^+$ T cells in this individual did not produce IL-21. All of these factors might lead to the absence of anti-SARS-CoV-2 IgG. Of note, this individual did not exhibit a lack of total IgG, suggesting that the humoral defect was specifically associated with SARS-CoV-2 infection.

In summary, in the absence of anti-SARS-CoV-2 IgG, c#9 developed strong memory anti-viral CD4$^+$ and CD8$^+$ T cell responses, indicating that SARS-CoV-2-specific T cells play a protective role in the clearance of SARS-CoV-2, at least in this individual. These data highlight the importance of T cell immunity in COVID-19 and provide new insights into the design and evaluation of COVID-19 vaccines.

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Author contributions

L.N. and C.D. designed the research and analyzed the data. Y.F. and F.C. collected clinical convalescent specimens with COVID-19; Q.Q. did FACS staining and Y.F. did ELISA experiment. Y.Y. and M.Y. did ELISpot assay. H.Z., M.C. and C.Q. did the experiments with pseudotype viruses. G.Z., X.L. and P.W. performed some experiments or prepared key reagents. L. N. and C.D. wrote the manuscript.

Conflict of interest

The authors declare that there are no conflicts of interest to disclose.

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