



RESEARCH ARTICLE

Alterations in Phenotypes and Responses of T Cells Within 6 Months of Recovery from COVID-19: A Cohort Study

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Received: 11 October 2020 / Accepted: 30 November 2020 / Published online: 9 February 2021
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Abstract

The COVID-19 pandemic, caused by the SARS-CoV-2 infection, is a global health crisis. While many patients have clinically recovered, little is known about long-term alterations in T cell responses of COVID-19 convalescents. In this study, T cell responses in peripheral blood mononuclear cells of a long-time COVID-19 clinically recovered (20–26 weeks) cohort (LCR) were measured via flow cytometry and ELISpot. The T cell responses of LCR were comparatively analyzed against an age and sex matched short-time clinically recovered (4–9 weeks) cohort (SCR) and a healthy donor cohort (HD). All volunteers were recruited from Wuhan Jinyintan Hospital, China. Phenotypic analysis showed that activation marker PD-1 expressing on CD4⁺ T cells of LCR was still significantly lower than that of HD. Functional analysis indicated that frequencies of Tc2, Th2 and Th17 in LCR were comparable to those of HD, but Tc17 was higher than that of HD. In LCR, compared to the HD, there were fewer IFN- γ producing T cells but more IL-2 secreting T cells. In addition, the circulating Tfh cells in LCR were still slightly lower compared to HD, though the subsets composition had recovered. Remarkably, SARS-CoV-2 specific T cell responses in LCR were comparable to that of SCR. Collectively, T cell responses experienced long-term alterations in phenotype and functional potential of LCR cohort. However, after clinical recovery, SARS-CoV-2 specific T cell responses could be sustained at least for six months, which may be helpful in resisting re-infection.

Keywords COVID-19 · Long-time recovery · Peripheral T cells

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s12250-021-00348-0>) contains supplementary material, which is available to authorized users.

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Introduction

The worldwide pandemic COVID-19 caused by SARS-CoV-2, remains a global threat to both humans and society. As of November 30, 2020, there were over 61 million confirmed infections globally, with more than 1.4 million deaths. Seeing as tens of thousands of patients have clinically recovered, whether this population could resist re-infection for a long period of time is a question attracting more and more attention.

Long-lasting B cell memory and the highest affinity pathogen-specific antibodies are critical for resistance against re-infection. However, antibody responses to SARS-CoV-2 appear to have declined in a large percentage of individuals (Long *et al.* 2020). The striking absence of germinal centers, the defective generation of Bcl6⁺ T follicle helper (Tfh) cells, and dysregulated humoral immune induction early in COVID-19 disease, provide a mechanistic explanation for the limited durability of antibody responses (Kaneko *et al.* 2020).

The T cell is another important cell type for eliminating infection and establishing long-term immunity from the coronavirus infection (Liu *et al.* 2017; Callaway 2020; Corey *et al.* 2020; Leslie 2020). For SARS-CoV-2 infection, T cells were activated (Mathew *et al.* 2020; Thevarajan *et al.* 2020), and the specific T cell response could be established (Cox and Brokstad 2020; Grifoni *et al.* 2020; Peng *et al.* 2020; Sekine *et al.* 2020). However, a decreased number of T cells with impaired function is prominent in COVID-19 patients, especially in severe cases (Qin *et al.* 2020; Zhang *et al.* 2020; Zhou *et al.* 2020; Zhu *et al.* 2020). Moreover, broad phenotypic alterations and potential dysfunctions were still prominent in those T cells in individuals clinically recovered from COVID-19 (Yang *et al.* 2020a, b). It is still unknown, after being recovered for a long time, whether the altered non-specific T cell responses could bounce back and whether the specific T cell responses are sustainable.

In August, 2020, individuals who had clinically recovered from COVID-19 were initially enrolled in this study at Wuhan Jinyintan Hospital. A COVID-19 long-time clinically recovered (20–26 weeks) cohort (LCR) was recruited amongst these individuals. To analyze changes in T cell responses of LCR, a healthy donor cohort (HD) who matched the age and sex of the LCR, and a short-time clinically recovered (4–9 weeks) cohort (SCR) were also established. Phenotype and functional potential of the T cells in the freshly isolated peripheral blood mononuclear cells (PBMCs) were analyzed by FACS and ELISpot assay.

Materials and Methods

Clinical Laboratory Measurements

Clinical laboratory measurements including complete blood count, SARS-CoV-2 receptor binding domain (RBD) specific antibody detection, and SARS-CoV-2 specific nucleotide detection were performed at Wuhan Jinyintan Hospital. Nasopharyngeal swab samples were collected on the day of peripheral blood collection and were measured via qRT-PCR for amplification of *E* gene, *RdRp* gene, and *N* gene of SARS-CoV-2 as previously described (Cao *et al.* 2020).

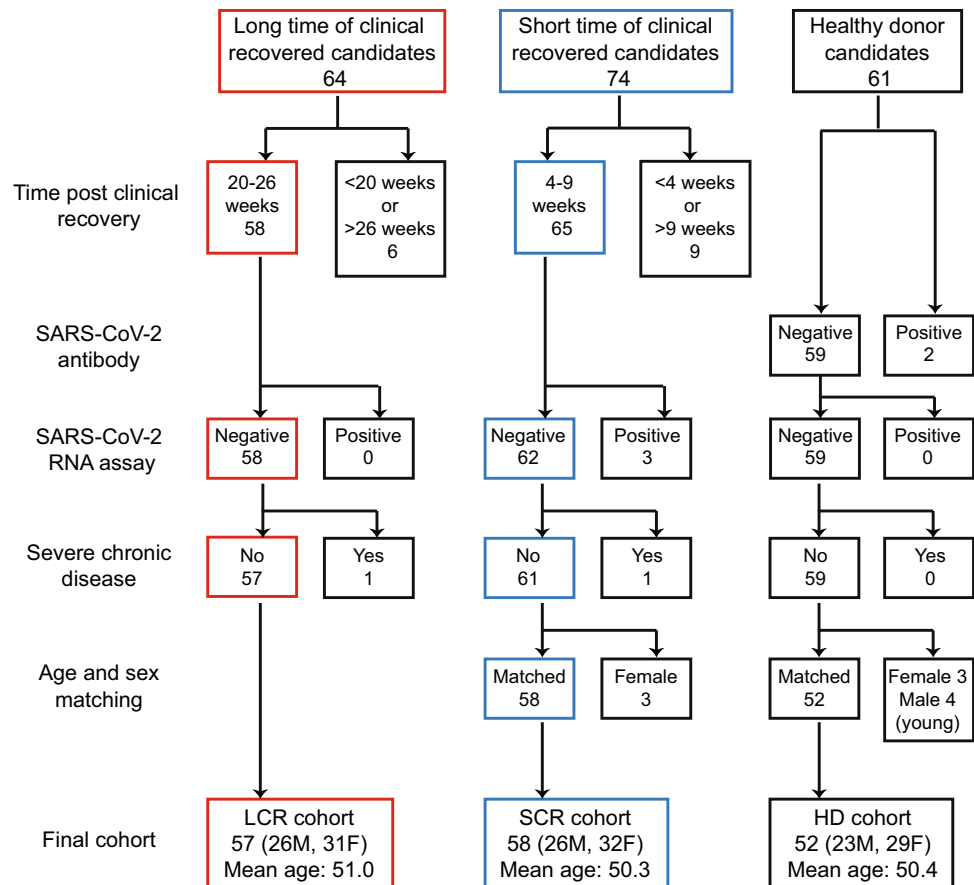
Study Design and Participants

In August, 2020, a total of 64 individuals who had clinically recovered from documented COVID-19 were recruited at Wuhan Jinyintan Hospital. Fifty-eight of them were enlisted following recovery from COVID-19 within 20–26 weeks, and were confirmed negative for SARS-CoV-2 on the day of sampling. Then, one individual with severe chronic disease was excluded. The remaining 57 subjects (26 males and 31 females) with a mean age of 51 years old composed the long-time clinically recovered cohort (LCR) (Fig. 1). Among the individuals in LCR cohort, six subjects experienced severe COVID-19.

Among the 74 enrolled COVID-19 clinically recovered volunteers in April, 2020, 65 of them (recovery within 4–9 weeks) were initially recruited. Three individuals with detectable virus RNA, and one individual with severe chronic disease was not admitted to the study. To match the age and sex of the LCR cohort, three young females were omitted (Supplementary Fig. S1). The remaining 58 subjects (26 males and 32 females) with a mean age of 50 years old, were designated as a short-time clinically recovered cohort (SCR). There were three participants who had a history of severe COVID-19 in SCR cohort.

In addition, 61 healthy individuals without documented SARS-CoV-2 infection were initially recruited when having their regular physical examination at Wuhan Jinyintan Hospital in April of 2020. Two subjects were excluded due to the positive IgM and IgG antibodies response against the SARS-CoV-2 in serum. In order to match the other two cohorts in age and sex, three young females and four young males were excluded (Supplementary Fig. S1). Finally, a total of 52 subjects were included in the healthy donor cohort (HD) containing 23 males and 29 females. The mean age of this cohort was 50 years old.

Fig. 1 The process of participants selection. Participants in the three cohorts were selected based on the parameters listed to the left of the figure.



For ELISpot assay, due to the cost and labor-intensity, a sub-cohort of only 27 individuals in SCR and a sub-cohort of 18 individuals in HD were involved in the test.

Flow Cytometry

Cell pellets from the fresh peripheral blood of participants were separated by centrifugation. After density centrifugation, the white cell layers were aspirated and washed by cold PBS twice. The obtained PBMCs were resuspended in RPMI 1640 medium for further use.

In order to evaluate the activation, proliferation, and differentiation of T cells, single-cell suspensions of PBMCs were surface stained with monoclonal antibodies (mAbs) specific to CD3 (HIT3a), CD4 (OKT4), CD8 (RPA-T8), CD45RO (UCHL1), CD27 (O323), HLA-DR (L243), CD38 (HB-7), PD-1 (EH12.2H7) and Fixable Viability Dye eFluorTM 506 (eBioscience, San Diego, CA, USA) at 4 °C for 30 min. After washing, cells were fixed and permeabilized with Foxp3/Transcription Factor Staining Buffer Set (eBioscience, San Diego, CA, USA) for 12 h, and were then incubated with mAbs specific to T-bet (4B10), ROR γ t (AFKJS-9) and Ki-67 (Ki-67) at 4 °C for 30 min.

To detect the proliferation and activation of Tfh cells, PBMCs were surface stained with mAbs specific to CD3 (HIT3a), CD4 (RPA-T4), CD8 (RPA-T8), CXCR5 (J252D4), CXCR3 (G025H7), CCR6 (G034E3), PD-1 (EH12.2H7), ICOS (C398.4A), and Fixable Viability Dye eFluorTM 506 at 4 °C for 30 min. Cells were then fixed and permeabilized (eBioscience, San Diego, CA, USA) overnight prior to incubation with mAb specific to Ki-67 (Ki-67).

For detection of cytokine production with non-specific stimulation, obtained PBMCs were stimulated for 4.5 h at 37 °C with 200 ng/mL PMA (Beyotime, Shanghai, China) and 2000 ng/mL ionomycin (Beyotime, Shanghai, China) in the presence of 1 μ mol/L monensin (BioLegend, San Diego, CA, USA) and 2.5 μ g/mL Brefeldin A (BioLegend, San Diego, CA, USA). Cells were then labeled with Fixable Viability Dye eFluorTM 506 and mAbs specific to CD3 (HIT3a), CD4 (OKT4) and CD8 (RPA-T8) at 4 °C for 30 min. After being fixed using Fixation buffer for 30 min at 4 °C, those cells were permeabilized overnight and were then intracellularly stained with mAbs specific to IL-2 (MQ1-17H12), IL-21(3A3-N2), IFN- γ (4S.B3), IL-4 (MP4-25D2), IL-17A (BL168) and granzyme B (QA16A02) at 4 °C for 30 min.

All antibodies were bought from BioLegend (San Diego, CA, USA) or eBioscience (San Diego, CA, USA). Stained cells were run on an LSRFortessa (BD, Heidelberg, Germany) and analyzed with FlowJo V 7.0 software.

Enzyme-Linked Immunospot Assay (ELISpot)

IFN- γ producing T cells were assessed by ELISpot under the manufacturer's instructions. Briefly, PVDF-membrane-bottomed plates (Millipore, Bedford, MA, USA) were coated overnight with an anti-human IFN- γ coating antibody at 4 °C. After blocking, single cell suspensions of PBMCs were seeded onto the plates (1×10^5 cells/well), and were stimulated with N, S1, and S2 proteins of SARS-CoV-2, and p24 protein of HIV-1 for 45 h at 37 °C, 5% CO₂, respectively. The final concentration of each protein was 2 μ g/mL. Then cells were removed, and biotin-conjugated mAb was applied prior to adding streptavidin-HRP. After washing, spots were developed with AEC coloring system, and the number of spots were counted by an automatic ELISpot reader (AID, Strassberg, Germany).

IFN- γ ELISpot antibody pair was purchased from U-CyTech biosciences (Utrecht, Netherlands), the SARS-CoV-2 N protein was kindly provided by Professor Ning-shao Xia at Xiamen University, while the SARS-CoV-2 S1 and S2 proteins were bought from Sino Biological Company (Beijing, China).

Statistical Analysis

Results were expressed as mean \pm S.D. unless otherwise indicated. Statistical analyses were performed using GraphPad Prism version 8.3 software (GraphPad Software Inc., San Diego, CA, USA), and data were considered to be statistically significant if $P < 0.05$. One-way analysis of variance (ANOVA) with Tukey's multiple comparison was carried out to compare differences between the three cohorts. Two-way ANOVA was used to analyze ELISpot results. The chi-square test was used to analyze the difference in sex distribution of the three cohorts.

Results

Phenotypic Alteration in T Cells of LCR

In the LCR cohort, despite the fact that the percentage of CD4⁺ T cells was significantly higher than that in SCR (Fig. 2A), percentages of CD8⁺ T and CD4⁺ T cells were comparable to those of the HD cohort (Fig. 2A). After learning this, we focused on the phenotypic changes in the T cells of LCR.

In terms of activation, frequencies of HLA-DR⁺ in CD8⁺ T or CD4⁺ T cells of the LCR cohort were both comparable with those of SCR and HD, respectively (Fig. 2B). However, similar to that of SCR, the frequency of PD-1⁺ in CD4⁺ T cells of LCR was still significantly lower than that of the HD cohort (Fig. 2C). Moreover, in terms of proliferation, also similar to that of SCR, frequency of Ki-67⁺ in CD8⁺ T cells of LCR was still significantly higher than that of the HD cohort (Fig. 2D). Whereas, in terms of differentiation, frequencies of effector T cells (CD45RO⁻CD27⁻, Teff) and effector memory T cells (CD45RO⁺CD27⁻, Tem) in CD8⁺ T cells of LCR both became lower than those of SCR (Fig. 2E). Furthermore, the frequencies of naïve T cells (Tna) in CD8⁺ T cells and central memory T cells (CD45RO⁺CD27⁺, Tcm) in the CD4⁺ T cells of LCR both became higher than those of SCR. For LCR, the frequencies of Teff, Tem, Tcm and Tna in either CD8⁺ T cells or CD4⁺ T cells all became comparable to those of HD (Fig. 2E).

Functional Potential Alteration in T Cells of LCR

Next, we evaluated the functional potential of non-specific T cells. In CD8⁺ T and CD4⁺ T cells, transcription factor T-bet expressing cells were often deemed as Tc1 and Th1 cells respectively (Lucas *et al.* 2020). Without stimulation, the frequencies of transcription factor T-bet⁺ in CD8⁺ T or CD4⁺ T cells of the LCR cohort showed no difference to that of the SCR or HD cohort (Fig. 3A, 3B). Surprisingly, under polyclonal stimulation, frequencies of IFN- γ ⁺ in either CD8⁺ T or CD4⁺ T cells of the LCR cohort were remarkably lower than that of HD (Fig. 3C, 3D), but almost the same as that of SCR.

Intriguingly, frequencies of IL-2⁺ in either CD8⁺ T or CD4⁺ T cells of LCR became significantly higher than that of SCR and HD (Fig. 3E, 3F). Further analysis showed that compared to the HD cohort, frequencies of IFN- γ ⁺IL-2⁻ in CD8⁺ T or CD4⁺ T cells of the LCR cohort were reduced, while frequencies of IFN- γ ⁻IL-2⁺ in CD8⁺ T or CD4⁺ T cells were elevated (Supplementary Fig. S2A). In addition, compared to HD, the frequencies of IFN- γ ⁺GZMB⁻ in CD8⁺ T or CD4⁺ T cells of the LCR cohort were reduced, however, frequencies of IFN- γ ⁻GZMB⁺ in CD8⁺ T cells of LCR were surprisingly elevated (Supplementary Fig. S2B), though the frequencies of GZMB⁺ in CD8⁺ T or CD4⁺ T cells showed no difference between the three cohorts (Fig. 3G, 3H).

For LCR, frequencies of IL-4⁺ in CD8⁺ T and CD4⁺ T cells were comparable to that of SCR and HD (Fig. 3I, 3J). It should be noted that the frequency of IL-17A⁺ in CD8⁺ T cells of LCR were much higher than that of SCR, and even higher than that of HD (Fig. 3K). The frequency of IL-17A⁺ in CD4⁺ T cells of LCR was also much greater

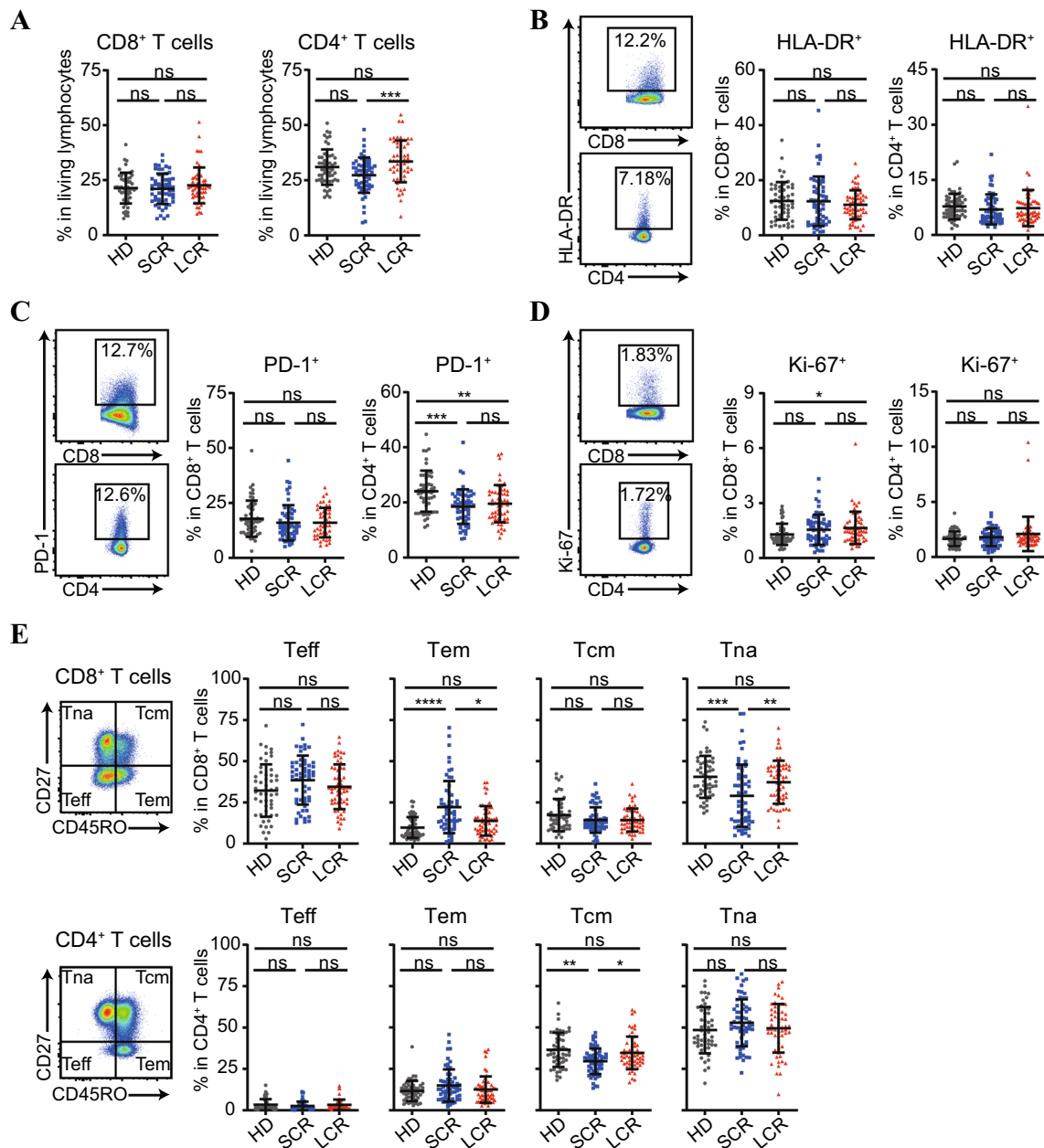


Fig. 2 The percentages, activation, proliferation and differentiation of CD8⁺ T and CD4⁺ T cells in PBMCs of HD, SCR and LCR cohorts. PBMCs were stained and analyzed by flow cytometry. **A** Frequencies of living CD8⁺ T cells and living CD4⁺ T cells in lymphocytes. **B**, **C** Representative plots and cumulative frequencies of activation markers HLA-DR (**B**) and PD-1 (**C**) expressing on CD8⁺ T and CD4⁺ T cells. **D** Representative plots and cumulative frequencies of

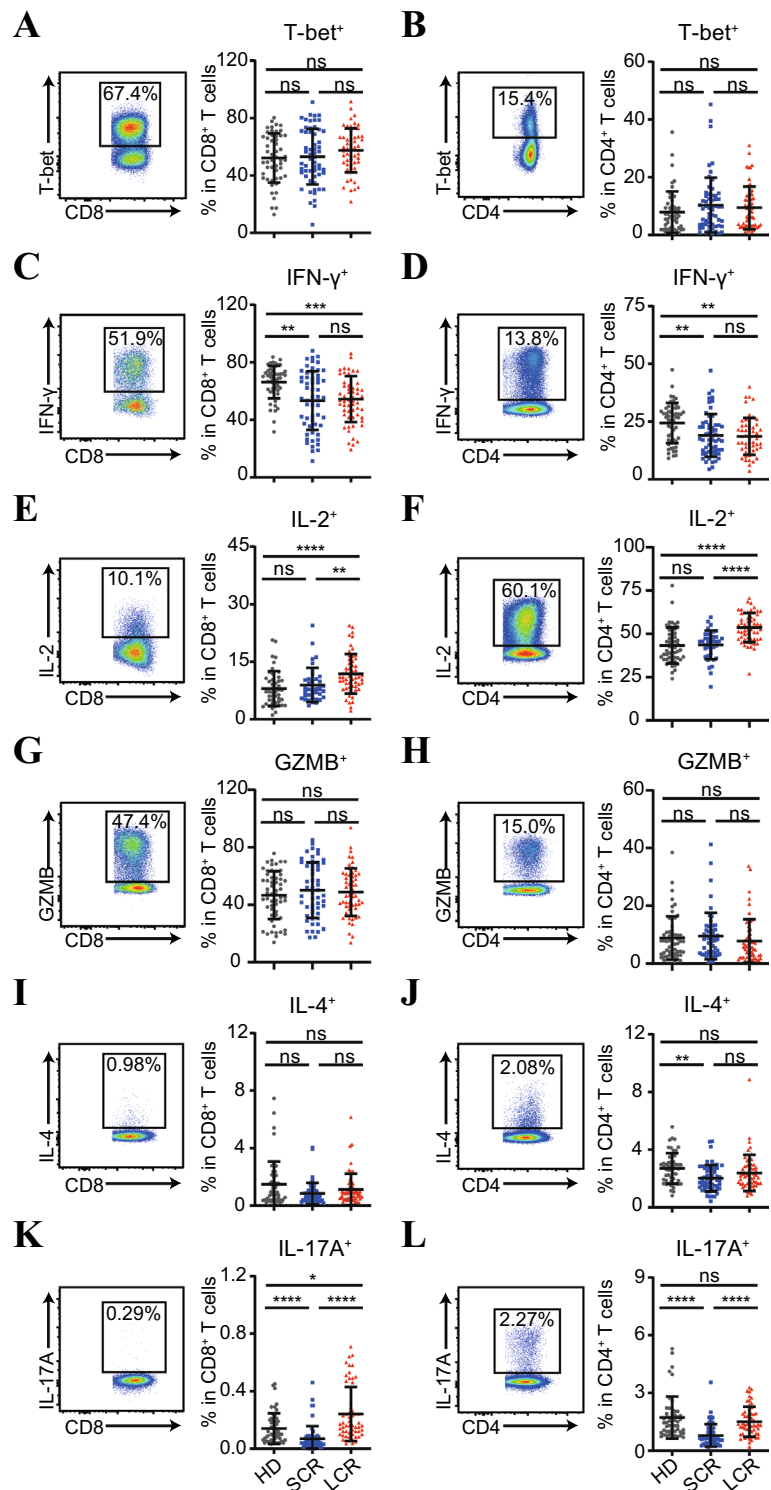
proliferated Ki-67⁺ CD8⁺ T and CD4⁺ T cells. **E** Gating strategies and frequencies of Teff (CD45RO⁻ CD27⁻), Tem (CD45RO⁺ CD27⁻), Tcm (CD45RO⁺ CD27⁺), and Tna (CD45RO⁻ CD27⁺) cells in CD8⁺ T and CD4⁺ T cells. Results are expressed as mean \pm S.D. Data were analyzed with one-way ANOVA. ns, non-significant; **P* < 0.05; ***P* < 0.01; ****P* < 0.001; *****P* < 0.0001.

than that of SCR, but comparable with HD (Fig. 3L). Moreover, in CD4⁺ T cells of LCR, the frequency of IL-21⁺ cells, which are mainly composed of T follicular helper (Tfh) cells and Th17 cells (Long *et al.* 2019), was much higher than that of SCR, but similar to HD (Supplementary Fig. S3).

Eventually Resumed Circulating Tfh Cells

Tfh cells, with CXCR5 as a marker, are the specialized subset of CD4⁺ T cells needed for germinal centers and related B cell responses (Crotty 2019). Both the frequencies of CXCR5⁺ cells and circulating Tfh (cTfh, PD-1⁺CXCR5⁺) cells in CD4⁺ T cells of the LCR cohort

Fig. 3 Potential capability of CD8⁺ T and CD4⁺ T cells in PBMCs of HD, SCR and LCR cohorts to secrete cytokines. **A**, **B** PBMCs were co-stained with phenotypic markers and nuclear transcription factor T-bet, representative plots and cumulative frequencies of T-bet⁺ CD8⁺ T (**A**) and CD4⁺ T (**B**) cells were shown. **C–I** PBMCs were stimulated with PMA and ionomycin for 4.5 h in the presence of BFA and monensin. Production of IFN- γ (**C**, **D**), IL-2 (**E**, **F**), granzyme B (**G**, **H**), IL-4 (**I**, **J**) and IL-17A (**K**, **L**) by CD8⁺ T or by CD4⁺ T cells were intracellularly stained and analyzed by flow cytometry. Results are shown as mean \pm S.D. Data were analyzed with one-way ANOVA. ns, non-significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.



were much higher than that of the SCR cohort, but were still slightly lower than that of the HD cohort (Fig. 4A). The cTfh cells could be further organized into three subsets with different capabilities: cTfh1 (CCR6⁻CXCR3⁺), cTfh2 (CCR6⁻CXCR3⁻), and cTfh17 (CCR6⁺CXCR3⁻) (Morita *et al.* 2011). Different from the SCR cohort, all

three of the cTfh subsets in LCR were almost comparable with the HD cohort (Fig. 4B), from the perspective of the composition of cTfh. The frequency of the three cTfh subsets in CD4⁺ T cells of LCR were also recovered, nearly the same as that of HD (Supplementary Fig. S4). Additionally, the expression level of activation marker

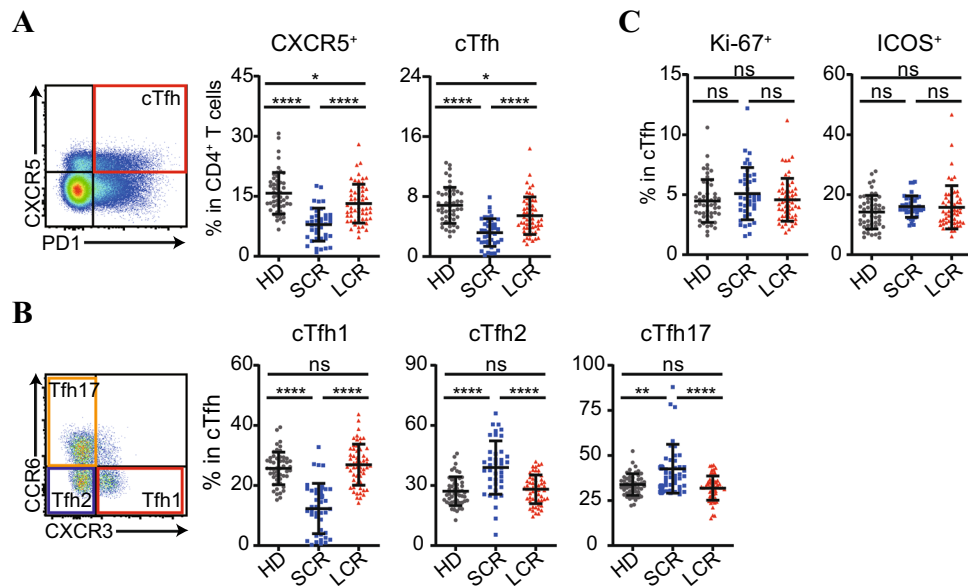


Fig. 4 Categorical subsets, proliferation and activation of circulating Tfh (cTfh) cells in PBMCs of HD, SCR and LCR cohorts. **A–C** PBMCs were co-stained with phenotypic markers and proliferation marker Ki-67 and were analyzed by flow cytometry. **A** Gating strategy of circulating Tfh cells (cTfh), and frequencies of CXCR5⁺ CD4⁺ T cells, CXCR5⁺ PD-1⁺ CD4⁺ circulating Tfh (cTfh) cells. **B** Gating strategies of cTfh subsets, and frequencies of CXCR3⁺

CCR6⁻ cells (cTfh1), CXCR3⁻ CCR6⁻ cells (cTfh2) and CXCR3⁻ CCR6⁺ (cTfh17) in cTfh cells. **C** Frequencies of proliferated Ki-67⁺ cTfh cells and activated ICOS⁺ cTfh cells. Results are shown as mean \pm S.D. One-way ANOVA was used to analyze the statistical difference between the three cohorts. ns, non-significant; * P < 0.05; ** P < 0.01; **** P < 0.0001.

ICOS and proliferation marker Ki-67 in cTfh cells of LCR were not different from that of HD (Fig. 4C). All data indicated the frequency and status of cTfh in LCR eventually returned to nearly the same as that in HD.

Sustained SARS-CoV-2 Specific IFN- γ ⁺ T Cell Responses

Although the general immunological status of T cells in the LCR cohort had recovered toward base line, the IFN- γ secreting potential was still remarkably reduced. We thus wondered whether the SARS-CoV-2 specific T cell responses declined after a long period of recovery. Therefore, ELISpot assay was performed under the stimulation of SARS-CoV-2 protein S1, S2, N or a negative control protein HIV-1 p24. We found that similar to the SCR cohort, SARS-CoV-2 specific IFN- γ ⁺ T cell responses could also be detected in the LCR cohort. In terms of antigen specificity, significantly higher levels of S2 and N specific IFN- γ ⁺ T cell responses could be observed in the SCR and LCR cohorts (Fig. 5). In contrast, level of S1 specific IFN- γ ⁺ T cell response was much lower than that of S2 and N specific T cell responses in the SCR and LCR cohorts. More importantly, the S1, S2, and N specific IFN- γ ⁺ T cell responses in LCR were all comparable to that of SCR. This suggested that, in the LCR cohort, SARS-CoV-2 specific IFN- γ ⁺ T cell responses were sustained.

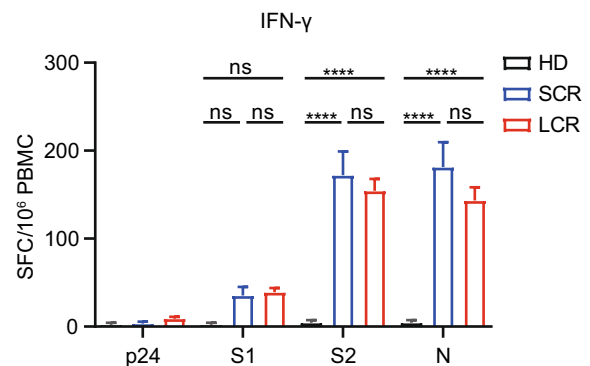


Fig. 5 SARS-CoV-2 specific T cell responses in PBMCs of HD, SCR and LCR cohorts. IFN- γ producing T cells were measured by ELISpot under the stimulation of the SARS-CoV-2 N protein, S1 protein, S2 protein or irrelevant HIV-1 p24 protein. Data were obtained from 18 subjects in the HD cohort, 27 subjects in the SCR cohort and 57 subjects in the LCR cohort. Results are expressed as mean \pm S.E.M. Statistical difference was calculated with two-way ANOVA. ns, non-significant; **** P < 0.0001.

Discussion

How long the T cell respond in convalescent COVID-19 patients against re-infection is still a big question. The protection time might greatly affect the prevalence of the pandemic (Kissler *et al.* 2020). Besides antibody response, T cell responses also play key roles in viral control. However, it is still unknown, after a long period of

recovery, whether the altered non-specific T cell responses would resume, and whether the SARS-CoV-2 specific T cell responses would be sustained. Hence, in the present study, we established a COVID-19 long-time clinically recovered (20–26 weeks) cohort (LCR), an age and sex matched short-time clinically recovered (4–9 weeks) cohort (SCR) and an uninfected healthy donor cohort (HD) (Fig. 1). We then analyzed the non-specific and SARS-CoV-2-specific T cell responses in PBMCs of those three cohorts.

Phenotypic analysis showed that the differentiation of CD8⁺ T or CD4⁺ T cells in LCR was comparable to HD, although the repression on activation marker PD-1 in CD4⁺ T cells, and the elevation of proliferation marker Ki-67 in CD8⁺ T cells still exist (Figs. 2, 6). Functional potential analysis under non-specific stimulation indicated that different from IL-4 and IL-17, repression on IFN- γ secretion by CD8⁺ T and CD4⁺ T cells were still prominent in LCR (Figs. 3, 6). Moreover, increase of IL-2 production became prominent. For CD4⁺ T cells, although the frequency of cTfh was not completely recovered, the composition, activation and proliferation levels of cTfh became similar to that of HD (Figs. 4, 6). In conclusion, our study showed that some non-specific T cell responses have recovered after 20–26 weeks of discharge.

In the process of our cohort establishment, potentially influential factors such as sex, age, and the presence of severe illness were all tracked as strictly as possible. Hence, we could still find the long-term alteration induced by COVID-19 by comparing the HD, SCR and LCR cohorts, although with the caveat that the individuals recruited for the SCR cohort were not the same people recruited for the LCR cohort, and the SARS-CoV-2

specific T cell response in HD and SCR were only tested in sub-cohorts. Another limitation is that all analyses have been performed on PBMCs. In SCR, the individuals were clinically recovered from COVID-19 for 4–9 weeks. At this time point, the changes in PBMCs may not necessarily reflect changes in the frequency at the actual site of action (the lung) or secondary lymphoid compartments. But in LCR, the individuals were clinically recovered from COVID-19 for 20–26 weeks, and so the immune systems became quiescent, leading to the realization that the differences in PBMCs between LCR and HD could actually reflect the long-term effects of COVID-19.

The loss of Bcl-6-expressing Tfh cells and germinal centers in COVID-19 patients suggest an underlying basis for the lower quality and lack of durability of humoral immune responses observed during natural infection with SARS-CoV-2 (Kaneko *et al.* 2020). Consistent with this observation, we found profoundly reduced cTfh in PBMCs, even in those individuals clinically recovered after 4 to 9 weeks. But in the LCR cohort, the reduced cTfh could be eventually recovered, though still at lower rates than that of HD.

A recent study of COVID-19 convalescent individuals highlighted that SARS-CoV-2 specific T cells sharply declined within one month of clinical recovery (Ni *et al.* 2020). Fortunately, compared with 4–9 weeks post clinical recovery, the SARS-CoV-2 specific T cell responses didn't decline after 20–26 weeks. We also found that SARS-CoV-2 N, S2 and S1 specific IFN- γ ⁺ T cell responses in LCR were comparable to those in SCR (Figs. 5, 6). The SARS-CoV-2 specific T cell responses were supposed to be negatively related with degrees of disease severity (Canete and Vinuesa 2020; Rydzynski Moderbacher *et al.* 2020). It

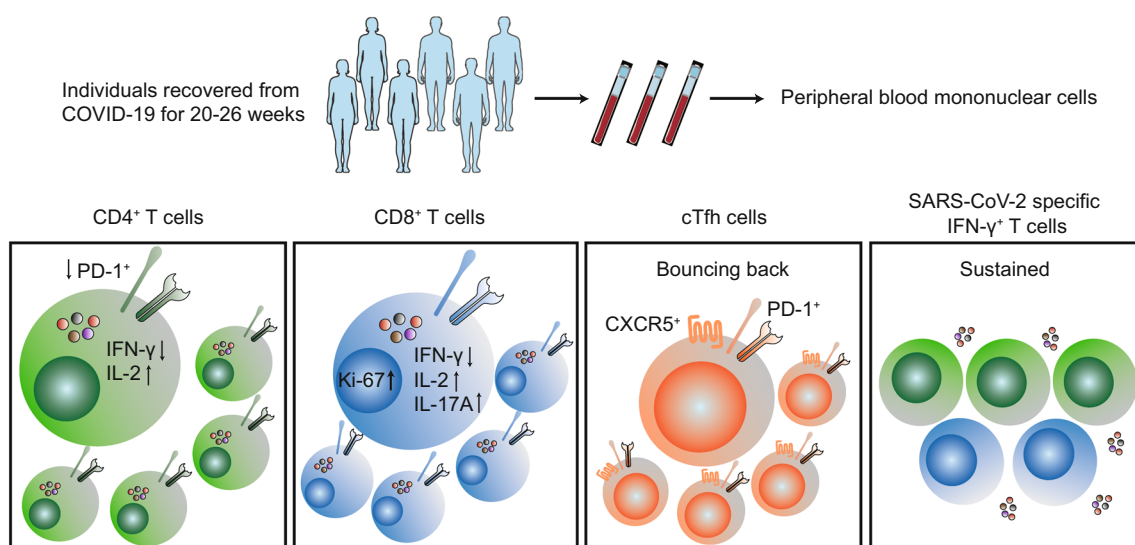


Fig. 6 Overview of alterations in peripheral T lymphocytes in individuals clinically recovered from COVID-19 for 20–26 weeks compared with healthy donors. \uparrow , increased frequency; \downarrow , decreased frequency.

is important to consider that T cell responses against N and S proteins of SARS-CoV can still be detected in PBMCs of recovered patients for years (Liu *et al.* 2017). We could speculate that the SARS-CoV-2 specific T cell responses in COVID-19 convalescent individuals might remain for a relatively long time, which may help to resist SARS-CoV-2 re-infection.

In another sense, CD4⁺ T cells and CD8⁺ T cells are both critical for the control of intracellular pathogen infections and tumors (Douek *et al.* 2002; Yang *et al.* 2016). Hence, attention should be paid to the fact that the long-term dysfunction of T cells might impair the immune surveillance and protection in COVID-19 clinically recovered individuals. The long-lasting dysfunction of lymphocytes is common in patients infected by viruses which could induce chronic infection such as HIV and HCV (Douek *et al.* 2002; Ahmed *et al.* 2019), but is rarely reported in patients infected by viruses which only induce acute infection. Moreover, it is surprising that, while the IFN- γ -producing potential was remarkably repressed, the expression of the IFN- γ upstream transcription factor T-bet in T cells did not change. In addition, there were no significant differences in the frequencies of T_H1, T_H2, T_H17 and T_H22 in both CD8⁺ T and CD4⁺ T cells. Hence, we can infer that COVID-19 might leave imprints on transcription, translation or stability of IFN- γ mRNA or protein. The “imprint” on the functional potential of T cells requires further study in the future.

Acknowledgements We would like to thank the COVID-19 recovered individuals and healthy donors involved in this study, and the medical staff at Wuhan Jinyintan Hospital. We also thank Professor Ningshao Xia for generously providing the recombinant SARS-CoV-2 specific N protein. This work was supported by National Natural Science Foundation of China (31970881).

Author contributions BZ, JY and MZ contributed to the conception, design, data acquisition, analysis, interpretation and drafted and revised the manuscript. QY, KH, JX, XL and YL contributed to the sample collection and acquisition of data. YC, JY, CH and HY contributed to the study concept and design, data analysis, interpretation and revised the manuscript. All authors gave final approval and agreed to be accountable for all aspects of the work.

Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interest.

Animal and Human Rights Statement This study was approved by the Medical Ethical Committee of Wuhan Jinyintan hospital (Number KY-2020-47.01). All COVID-19 recovered patients and healthy donors signed a written informed consent to use their biological samples and clinical data for research purposes.

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