JCI The Journal of Clinical Investigation

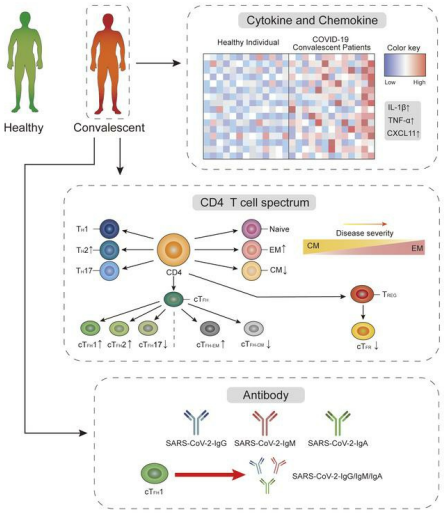
Peripheral CD4+ T cell subsets and antibody response in COVID-19 convalescent individuals

Fang Gong, Yaping Dai, Ting Zheng, Liang Cheng, Dan Zhao, Hao Wang, Min Liu, Hao Pei, Tengchuan Jin, Di Yu, Pengcheng Zhou

J Clin Invest. 2020. https://doi.org/10.1172/JCI141054.

Clinical Research and Public Health | In-Press Preview | Immunology

Graphical abstract



Peripheral CD4$^+$ T cell subsets and antibody response in COVID-19 convalescent individuals

Fang Gong[1,*], Yaping Dai[3,*], Ting Zheng[4,*], Liang Cheng[5], Dan Zhao[6], Hao Wang[2], Min Liu[1], Hao Pei[3], Tengchuan Jin[6], Di Yu[4,7], Pengcheng Zhou[2,8,#]

[1]Department of Laboratory Medicine, Affiliated Hospital of Jiangnan University, Wuxi, China
[2]Department of Immunology and Infectious Disease, The John Curtin School of Medical Research, The Australian National University, Canberra, Australian Capital Territory, Australia
[3]Department of Laboratory Medicine, The Fifth People's Hospital of Wuxi, Wuxi, Jiangsu, China
[4]Qilu University of Technology (Shandong Academy of Sciences), Shandong Analysis and Test Center, Laboratory of Immunology for Environment and Health, Jinan, China
[5]Department of Respiration, The Fifth People's Hospital of Wuxi, Wuxi, Jiangsu, China
[6]Hefei National Laboratory for Physical Sciences at Microscale, Laboratory of Structural Immunology, CAS Key Laboratory of Innate Immunity and Chronic Disease, Division of Life Sciences and Medicine, University of Science and Technology of China, China
[7]The University of Queensland Diamantina Institute, Translational Research Institute, Brisbane, Australia
[8]Lead Contact
*These authors contributed equally to this work

[#]Correspondence:
Pengcheng Zhou (pengcheng.zhou@anu.edu.au)
Department of Immunology and Infectious Disease,
The John Curtin School of Medical Research, The Australian National University,
Canberra, ACT, 2601, Australia

Conflict of interest statement: The authors declare no conflict of interest.

ABSTRACT

Background: Marked progress is achieved in understanding the physiopathology of COVID-19 that caused global pandemics. However, $CD4^+$ T cell population that is critical for antibody response in COVID-19 is poorly understood.

Methods: In this study, we provided a comprehensive analysis of peripheral $CD4^+$ T cells of 13 COVID-19 convalescent patients, as defined as confirmed free of SARS-CoV-2 for 2-4 weeks, using flow cytometry, magnetic chemiluminescence enzyme antibody immunoassay and correlated the data with clinical characteristics.

Results: We observed that relative to healthy individuals, convalescent patients displayed an altered peripheral $CD4^+$ T cell spectrum. Specifically, consistent with other viral infections, $cT_{FH}1$ cell associated with SARS-CoV-2 targeting antibodies, which was found to skew with disease severity as more severe individuals showed higher frequency of T_{EM} and $T_{FH\text{-}EM}$ cells but a lower frequency of T_{CM}, $T_{FH\text{-}CM}$ and T_{Naive} cells, relative to mild and moderate patients. Interestingly, higher frequency of $cT_{FH\text{-}EM}$ cells correlated with lower number of recorded admission blood oxygen level in convalescent patients. These observations might constitute residual effects by which COVID-19 can impact the homeostasis of $CD4^+$ T cells in the long-term and explain the highest ratio of class-switched virus-specific antibody producing individuals found in our severe COVID-19 cohort.

Conclusion: Together, our study demonstrated close connection between $CD4^+$ T cells and antibody production in COVID-19 convalescents.

Funding: This study was supported by Six Talent Peaks Project in Jiangsu Province and the National Natural Science Foundation of China (NSFC) grants 81970759.

Key words

COVID-19, SARS-CoV-2, $CD4^+$ T cells, T_{FH} cells, antibody response, humoral immunity

INTRODUCTION

Newly identified severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has infected over 13 million people and caused more than 500,000 deaths globally (1-3). In hospital, test-positive individuals of this virus are characterized as COVID-19 (coronavirus disease) severe, moderate or mild, with some of the patients develop acute respiratory distress syndrome (ARDS) and have low blood oxygen that requires intensive health care and ventilators. Increasing evidence has shown that patients recovered from COVID-19 develop protective neutralizing antibodies against SARS-CoV-2, rising hope for the development of effective antibody-based treatment as well as vaccinations towards this contagious disease. Despite the fact that there have been a few studies reviewed the broad immune defense towards COVID-19, the clear picture of adaptive immune cells that cooperatively impact this disease through antibody response is poorly studied.

A proportion of $CD8^+$ T cells in both healthy individual and COVID-19 patients can recognize antigen from SARS-CoV-2 (4-6). However, $CD8^+$ T cells often exhibit exhausted phenotypes in this disease (7, 8), together with markedly reduced cell counts in some severe patients. These facts raise concerns on the failure of $CD8^+$ T cells mediated cellular protection during the peak of the infection (9, 10). In the contrast, clear evidences have shown that antibody treatment using convalescent plasma were effective to some of the severe COVID-19 patients, suggesting the existence of protective neutralizing antibodies made by individuals recovered from this disease (11). Indeed, similar to patients infected by severe acute respiratory syndrome coronavirus (SARS) and Middle East respiratory syndrome (MERS) (12-15), most of COVID-19 patients develop virus-specific antibody response to SARS-CoV-2 (6, 16, 17). However, there are big gaps in understanding how T cells regulate the effective antibody production as well as the long-term humoral immune protection in COVID-19.

Human peripheral $CD4^+$ T cells can be characterized as naïve ($CCR7^+CD45RA^+$), central memory ($CCR7^+CD45RA^-$) and effector-memory ($CCR7^-CD45RA^-$) cells that respond differently during antigen re-exposure (18, 19). Patients recovered from SARS showed persistent memory $CD4^+$ T cells that could be potentiated by spike protein (20). Mouse experiments also demonstrated boosting memory $CD4^+$ T cells can protect mice from SARS and MERS infection (21). Thus, better understandings of these memory $CD4^+$

T cells in COVID-19 convalescents could help us develop long-term host protection to this disease.

$CD4^+$ T follicular helper (T_{FH}) cells are critical for high affinity antibody response and successful vaccination during infection (22-24). Different from other $CD4^+$ T cell lineage subsets, these cells are specialized in providing help to B cells for quality germinal center reaction (25). Human peripheral $CXCR5^+$ circulating T_{FH} (cT_{FH}) cells possess similar profile and functionality to their *bona fide* counterparts in secondary lymphoid organs (26). Case report with one recovered COVID-19 patient has showed the progressively increased frequency of $CXCR5^+ICOS^+PD\text{-}1^+$ peripheral blood T_{FH} cells up to 20 days from onset of infection (5). Recent single cell analysis also revealed the existence of T_{FH} cells in the bronchoalveolar lavage fluid (BALF) of severe COVID-19 patients (27). Although less characterized, majority of peripheral antigen specific $CD4^+$ T cells that were shown to correlate with antibody production in COVID-19 convalescent individuals may represent cT_{FH} cells (4, 6). cT_{FH} cells can be further defined as central memory like or effector memory like based on the expression of CCR7 and PD-1 (28-30). In particular, $CCR7^{lo}PD\text{-}1^+$ effector memory like cT_{FH} cells in the peripheral circulation can indicate the *bona fide* T_{FH} cell activity and foster antibody response against re-exposure of antigen (29). Correspondingly, $CXCR3^+$ subsets of cT_{FH} cells have been classified as $cT_{FH}1$ cells and positively correlate with neutralizing antibody responses during HIV infection and induce virus-specific B cell response upon influenza vaccination (31, 32). On the contrary, regulatory type of T follicular (T_{FR}) cells can suppress germinal center response required for high-affinity antibody formation (33-35). The immune-profile of these T_{FH} related cells in COVID-19 are largely unknown. Therefore, there is an urgent need to depicture them in this disease.

In this study, we provide a comprehensive analysis of $CD4^+$ T cells in COVID-19 convalescent patients, as defined as confirmed free of SARS-CoV-2 for 2-4 weeks, finding that relative to healthy individuals, convalescent patients display an altered peripheral $CD4^+$ T cell spectrum. Specifically, consistent with other viral infections, $cT_{FH}1$ cell associates with the titers of SARS-CoV-2 targeting antibody, which is found to skew with disease severity as more severe individuals showed higher frequency of T_{EM} and $T_{FH\text{-}EM}$ cells but a lower frequency of T_{CM}, $T_{FH\text{-}CM}$ and T_{Naive} cells, relative to mild and

moderate patients. Interestingly, higher frequency of $cT_{FH\text{-}EM}$ cells correlates with lower number of recorded admission blood oxygen level in convalescent patients. These observations may give rise to the highest ratio of virus-specific IgG or IgA producing individuals in the group of severe comparing to the groups with moderate and mild COVID-19.

RESULTS

Altered peripheral CD4$^+$ T cell spectrum in COVID-19 convalescent patients

To investigate the immune-profile of CD4$^+$ helper T cells, we have collected blood samples from 13 convalescent patients who visited the hospital for reexamination 2 to 4 weeks after being confirmed free of SARS-CoV-2. The clinical characteristics of these convalescent patients at study have been presented in Table 1, with their hospital COVID-19 diagnosis information in Supplementary Table 1. We have also compared the clinical characteristics between these convalescents and 13 healthy individuals who generously participated in our study (Table 2). Most of the clinical metadata are comparable including the similar median age (48 to 53, *P*=0.7345) between healthy individuals and COVID-19 convalescents (Table 2).

To characterize CD4$^+$ T cells, we first isolated peripheral blood mononuclear cell (PBMC) from patients and healthy individuals for subsequent antibody staining. Using multi-color flow cytometry, we have separated CD4$^+$ T cells into naïve (CD45RA$^+$CCR7$^+$), central memory (CD45RA$^-$CCR7$^+$) and effector memory (CD45RA$^-$CCR7$^-$) stage (18) (Figure 1A). Among them, we have seen comparable naïve CD4$^+$ T cells between healthy individuals and convalescents with COVID-19 (Figure 1B). Interestingly, we have noticed about 2-fold reduction of the frequency of central memory CD4$^+$ T cells, while approximately 1.5-fold increase of effector-memory CD4$^+$ T cells in convalescents (Figure 1B).

To evaluate the peripheral presence of different subsets of CD4$^+$ T cell, we have established the gating strategies based on the combination of signature surface molecules (Figure 1C). No difference has been observed on the overall frequency of circulating T_{FH} cells between healthy individuals and COVID-19 convalescents (Figure S1a). CCR7^{low}PD-1$^+$ $T_{FH\text{-}EM}$ cells can indicate the T_{FH} cell activity in the germinal centers of secondary lymphoid organ and can quickly differentiate into mature T_{FH} cells to potentiate antibody response (28, 29). Indeed, within cT_{FH} cells, the frequency of CCR7^{low}PD-1$^+$ effector-memory like circulating T_{FH} ($T_{FH\text{-}EM}$) cells are preferentially higher in convalescent patients comparing to healthy individuals, correspondingly lower frequency of CCR7^{high}PD-1$^-$ central-memory like circulating T_{FH} ($T_{FH\text{-}CM}$) cells has been found in COVID-19 convalescents (Figure 1D). Statistical analysis further confirmed these

notions (Figure 1E & F). These data suggest that the ongoing GC response may exist in convalescents post-confirmation of virus-free.

$CXCR3^+$ T_{FH} cells in peripheral circulation positively correlate with the development of protective antibody response against influenza (31). To study such connection in COVID-19 convalescents, we have compared the expression of CXCR3 and CCR6 within cT_{FH} cells to healthy individuals (Figure 1C). In line with the results from influenza vaccination (31), frequency of $CXCR3^+CCR6^-$ $cT_{FH}1$ cells are about 1.5-fold higher in COVID-19 convalescents than healthy individuals (Figure 1G). This observation suggests that although in recovering stage, patients with COVID-19 may have prolonged, $cT_{FH}1$ cells-mediated neutralizing antibody production. We also see the increased frequency of $CXCR3^-CCR6^-$ cT_{FH} 2 cells. $CXCR3^-CCR6^+$ $cT_{FH}17$ cells provide superior help to naïve B cells for antibody production (26). However, we have noticed a preferential loss of $CXCR3^-CCR6^+$ $cT_{FH}17$ cells in COVID-19 convalescents (Figure 1G). Together, these data have highlighted cT_{FH} cells were more activated in COVID-19 convalescent patients and may regulate prolonged or memory antibody protection against SARS-CoV-2.

Regulatory T (T_{REG}) cells and T_{FR} cells play important roles in constraining antibody response. In COVID-19 convalescents, we have found negligible difference on the frequency of peripheral T_{REG} cells but largely reduced frequency of $CD45RA^-CD127^-CD25^+CXCR5^{hi}PD\text{-}1^{hi}$ circulating T_{FR} cells (Figure 1H). T_H1, T_H2 and T_H17 cells are examined gating on $CD25^-CD45RA^-CXCR5^-$ $CD4^+$ T cells and through surface expression of CXCR3 and CCR6 (Figure 1C). There are about 2-fold increase of T_H2 cells in COVID-19 convalescent patients, but trivial changes on T_H1 and T_H17 cells (Figure 1I). In line with other reports, the overall expression of PD-1 on these subsets are higher in convalescents (Figure 1J), whereby increased PD-1 expression can lead to either cell exhaustion or increased help to B cells. Collectively, our date has suggested a widely altered spectrum of peripheral $CD4^+$ T cells in COVID-19 convalescents.

Increased production of inflammatory cytokines in convalescents

To understand the microenvironment where peripheral $CD4^+$ T cells receive constant stimuli and which may lead to the altered spectrum, we have measured 21 cytokines and chemokines that have large impacts on $CD4^+$ T cells. Although in recovering stage,

COVID-19 convalescents generally have a cytokine profile where inflammatory cytokine productions are mildly increased (Figure 2A). In particular, we have observed around 4-fold higher of IL-6 production (0.6192 to 2.233, mean; *P* = 0.0699) in COVID-19 convalescent patients (Figure 2B). Higher level of IL-1β (~1.8-fold, *P* = 0.0173) while comparable IFN-γ have been noticed in convalescents (Figure 2B). We have also noticed that around 46% of COVID-19 convalescents displayed higher TNF-α (~2-fold, *P* = 0.0243, t-test; *P* = 0.0456, MW test), and surprisingly, exhibited higher plasma level of CXCL11 (ITAC, interferon inducible T cell alpha chemoattractant), the ligand that has highest binding affinity to CXCR3 (36) (~5 fold, 9.426 to 52.41, mean; *P* = 0.038, t-test; *P* = 0.0338, MW test) (Figure 2B). It is possible that some convalescents may still have ongoing germinal center reaction in lymph nodes due to long-term retention of virus proteins by FDC, thus these subsets of patients show increased cytokine productions. There are trends of increased plasma level of IL-5 and IL-21 in convalescents, while most of other signature cytokines for T_H2, T_H17 and T_{REG} cells remain intact (Figure 2B, Figure S1b). Together, these findings have described the peripheral cytokine profile related to $CD4^+$ T cells and revealed that the plasma level of CXCL11 is preferentially higher in COVID-19 convalescents.

CXCR3 expressing $cT_{FH}1$ cells correlate with higher titer of SARS-CoV-2 specific antibody

To understand the antibody production in COVID-19 convalescent patients, we have utilized plasma to measure SARS-CoV-2 specific IgG, IgM and IgA. Viral nucleocapsid and spike proteins have been purified and used for the detection. In line with recent reports, we have observed higher viral-specific IgG, IgM and IgA in convalescents comparing to the healthy individual (Figure 3A). Negligible differences on antibody productions between genders or correlated to age have been noticed (Figure 3B, Figure S2a, S3a & S4a).

$cT_{FH}1$ cells shape memory B cell response and correlate with the quantity and avidity of neutralizing antibody reaction during HIV, influenza and ZIKV viral infections (31, 32, 37-39). To examine this correlation in COVID-19, we performed Pearson correlation coefficient analysis on data from convalescent patients. As shown in Figure 3C, $cT_{FH}1$

cells positively correlate with the magnitude of viral-specific IgG (R = 0.5614, P = 0.0459) while $cT_{FH}2$ cells do not show any correlation (R = 0.2953, P = 0.3273). Although not statistically significant, there is a trend of negative correlation between $cT_{FH}17$ cells and the magnitude of viral-specific IgG (R = -0.4352, P = 0.1372) (Figure 3C). Similar results have been found on viral-specific IgM (Figure 3D). We have also noticed a mild correlation between $cT_{FH}1$ cells and viral-specific IgA in patient blood (R = 0.5043, P = 0.0789), but not from $cT_{FH}2$ and $cT_{FH}17$ cells (Figure 3E). No correlations between cT_{FH} cells, $cT_{FH\text{-}EM}$/$cT_{FH\text{-}CM}$ cells and antibody titers are noticed (Figure S2b & c, S3b & c, S4b & c). Of note, we do not observe correlations between other $CD4^+$ T cell subsets including $CXCR3^+$ T_H1 cells and antibody titers (Figure S2d & e, S3d & e, S4d & e). Interestingly, we have found the trend of inverse correlation between cT_{FR} cells and the magnitude of SARS-CoV-2 specific IgG and IgM antibody titer, as well as statistical difference (R = -0.5649, P = 0.0443) on such correlation between cT_{FR} cells and SARS-CoV-2 specific IgA (Figure 3F). These results indicate that regulatory cells constraining antibody response may be the limiting factor of viral-specific antibody production in COVID-19 convalescents (Figure 3F). Taken together, these results have revealed $cT_{FH}1$ cells are vital for the titer of high-quality antibodies against SARS-CoV-2.

Peripheral $CD4^+$ T cells in COVID-19 convalescents recovering from different disease severities

To further investigate the connection between peripheral $CD4^+$ T cells and the clinical characteristics of COVID-19, 13 COVID-19 convalescents have been categorized into mild (N=4), moderate (N=4) and severe (N=5) group based on their diagnosis certificates during hospital admission, which are in line with the *Diagnosis and Treatment Protocol for COVID-19 (Trial Version 7)* and the WHO guidance. The representative chest CT images at both admission and convalescent from individuals in each group have been shown in Figure 4A. To briefly describe such categorization, we have observed more elderly patients with severe condition in our cohort (Figure 4B). Besides chest CT, acute respiratory distress syndrome often associates with reduction of blood oxygen level. We have retrospectively looked into the data of arterial oxygen tension (PaO_2) over inspiratory oxygen fraction (FiO_2) from each convalescent measured during hospital admission to

understand the blood oxygen levels at the time (One moderate convalescent without record of this measurement, total number 12). Statistical analysis has shown that PaO_2/FiO_2 index lower than 300 mmHg was consistently found in all convalescents in severe group (Figure 4C). Unfortunately, we do not have access to the measurement of PaO_2/FiO_2 in health individuals.

Previous disease severity may have long-term residual effects on the homeostasis of peripheral $CD4^+$ T cells in individuals recovered from COVID-19. To establish the connection between $CD4^+$ T cell and the disease severity, we studied the representation of peripheral $CD4^+$ T cell in each group of convalescents. We have found that $T_{Naïve}$ and T_{CM} cells remained low in moderate group and was further reduced in the severe group of convalescents, whilst T_{EM} was increased in the severe group (Figure 4D). Similar trends have been found on $T_{FH\text{-}CM}$ and $T_{FH\text{-}EM}$ in convalescents (Figure 4D). However, subpopulation of T_{FH} cells ($cT_{FH}1$, $cT_{FH}2$ and $cT_{FH}17$) are not significantly changed among different groups of severity (Figure 4D), despite their overall changes comparing to healthy individuals. Meantime, low frequency of T_{FR} cells has been observed in all convalescents, although such low frequency is not further reduced in severe group (Figure 4D). Frequency of T_{REG} cells have remained largely unaffected while wo do see more T_H2 cells in the severe group (Figure S5a). These results suggest that there are close connections between severity of COVID-19 and the homeostasis of T_{FH}, T_{FR} and T_H2 cells in convalescent stage. Residual effects from peak period of COVID-19 may also potentiate the generation of T_{EM} cells while the reduction of T_{CM} and T_{Naive} cells.

To understand whether blood oxygen level is one of the factors contributed to the residual effect that has impacted the homeostasis of peripheral $CD4^+$ T cell in COVID-19 convalescents, we have conducted the correlation analysis between PaO_2/FiO_2 index and peripheral $CD4^+$ T cells. No significant differences have been found on the correlation analysis between PaO_2/FiO_2 and the frequency of $T_{Naive,}$ T_{CM} and T_{EM} cells (Figure 4E). However, we have noticed that the frequency of $cT_{FH\text{-}CM}$ positively correlated with PaO_2/FiO_2 (R = 0.519, P = 0.08) while the frequency of $cT_{FH\text{-}EM}$ cells had a tight and negative correlation with PaO_2/FiO_2 (R = -0.721, P < 0.01) (Figure 4E). Of note, PaO_2/FiO_2 is not strongly correlated with the frequency of cT_{FR} cells, T_{FH} subpopulations ($cT_{FH}1$, $cT_{FH}2$ and $cT_{FH}17$) and other $CD4^+$ subsets (Figure 4E, Figure S5b). Notably, we have

considered other factors that may contribute to the residual effect, but do not find strong correlation for example between age and the frequency of peripheral $CD4^+$ T cells, except the reduction of T_{Naive} and the increase of T_{EM} cells were significantly correlated with the age factor in convalescent (Figure S5c).

Antibody response in different groups of COVID-19 convalescents

To understand the potential consequences of increased frequency of $cT_{FH\text{-}EM}$ cells in the severe group, we have evaluated the titers of IgG, IgM and IgA but do not see differences among patient groups (Figure 5A, Figure S5d). Utilizing the cutoff value (1, blue dash line in Figure 5A, Figure S5d) generated from large numbers of testing (40), we have classified the convalescents as positive (greater than 1) or negative individuals to each antibody type based on the antibodies they produced. Intriguingly, the IgM and/or IgG positive ratio in different groups of convalescents are clearly different in our cohort, where more IgM^+ patients have been found in the mild group and more IgG^+ patients have been observed in the severe group (Figure 5B). Similar observations have been noticed on IgA (Figure S5e). These data may imply that the activity of class-switching and the generation of memory B cells that requires activated T_{FH} cells are particularly high in those had severe COVID-19.

DISCUSSION

Emerging evidences have revealed that patients recovered from COVID-19 produce robust antibodies against SARS-CoV-2 that requires participation from T and B cells. Here, we have shown that in COVID-19 convalescents who were recently discharged from hospital, peripheral $CD4^+$ helper T cells are more activated as effector memory cells. Correspondingly, we have shown higher frequency of effector memory cT_{FH} cells in convalescents. One subset of cT_{FH} cells, $CXCR3^+$ $cT_{FH}1$ cells positively correlate with plasma virus-specific IgG and IgM titer in convalescent patients, which is in line with the observations in influenza, HIV and ZIKA virus infections. Interestingly, convalescents, who are diagnosed as in severe condition in hospital, exhibit higher frequency of T_{EM} and $T_{FH\text{-}EM}$ cells while lower frequency of T_{CM}, $T_{FH\text{-}CM}$ and T_{Naive} cells than those diagnosed as in moderate and mild conditions. Of note, the frequency of $T_{FH\text{-}EM}$ cells negatively correlates with blood oxygen level (PaO_2/FiO_2, mmHg), and these cells may contribute to the production of class-switched IgG antibody in COVID-19 convalescents. Thus, our study depictures the immune-profile of peripheral $CD4^+$ T cell subsets and demonstrates the close association between T_{FH} cells and the virus-specific antibody production in COVID-19 convalescents.

Memory $CD4^+$ T cells provide superior protection upon virus re-infection. Our data has suggested that 2 to 4 weeks post “virus-free”, most convalescing patients showed increased frequency of effector memory like $CD4^+$ T cells, which is in line with the report on preprints (41). This observation indicates that $CD4^+$ T cells might actively respond to the reformed host microenvironment post COVID-19 for a prolonged period of time. These responses might include clearing the latent SARS-CoV-2 in the reservoir cells or the formation of tissue resident memory (RM) response in the lung or other damaged tissues post COVID-19. In fact, $CD4^+$ T_{RM} response is well characterized in infections such as influenza or mouse LCMV infection (42, 43). We acknowledge the limitation that the study on memory cells should include more parameters such as Ki-67, CD127, CD62L or BCL-2 (44, 45). Nevertheless, in this study, patients have been strictly discharged from hospital only after being tested with at least two negative nucleic acid qPCR results for SARS-CoV-2 and blood samples have been collected from convalescents around 50 days after infection. Thus, due to the lack of antigen and the infection phase, we cautiously consider

that most of effector memory or central memory like $CD4^+$ T cells are presumed memory cells (44, 46).

One key observation from our data is the increased frequency of $cT_{FH\text{-}EM}$ and $cT_{FH}1$ cells in COVID-19 convalescent patients. It has thus inspired us to interrogate whether these increases are connected with antibody production or clinical characteristics. Indeed, $CXCR3^+$ $cT_{FH}1$ cells are positively associated with the magnitude of in SARS-CoV-2 specific antibody titers. Most of human SARS-CoV-2-specific IgG show strong activity in neutralizing virus (6, 47, 48). Our result implies that future immunomodulation of $cT_{FH}1$ cells might have profound impacts on the production of neutralizing antibody production in COVID-19 patients. Moreover, our data has suggested that CXCL11, the ligand with highest binding affinity to CXCR3, is highly accumulated in COVID-19 convalescents. This observation is in consistence with reports showing the other two CXCR3 ligands, CXCL9 and CXCL10, are highly produced in both active or recovered COVID-19 patients (49, 50), while CXCL11 is far less studied in this disease. There could be multiple possibilities that increased peripheral level of CXCL11 may be related to different T cell responses in COVID-19 convalescents. Both CXCL11 and CXCR3 are induced following IFN-γ and IFN-β (51), therefore they are likely related to the overall activated T_H1 response, where we have noticed the increased PD-1 expression in T_H1 cells. It is also possible that as a high-affinity chemoattractant, high CXCL11 may coordinate the distribution of circulating T_{FH} cells into tissues to form resident memory, whereby they could quickly response to antigen re-exposure at barriers, such as inducible bronchus-associated lymphoid tissue, and provide help to local resident memory B cells and $CD8^+$ T cells for protection (52, 53) (54-56). Single cell analysis has supported this speculation with the data showing the existence of infiltrated T_{FH} cells in the airway of patients in COVID-19 (27). Of note, although higher PD-1 expression has been observed in T_H1 cells, which may lead to their help function to B cells (57), we do not see the statistically increased frequency of these cells and their correlation to antibody production in convalescents.

Interestingly, our data has shown that the frequency of $cT_{FH\text{-}EM}$ cells is preferentially higher while the frequency of $cT_{FH\text{-}CM}$ cells is lower in convalescents compared to that of in healthy individuals, accompanied by similar results on T_{EM} and T_{CM} cells. Indeed, this

observation is correspondingly supported by a recent study on preprints (58). Based on the hospital admission diagnoses of illness, COVID-19 convalescents have been assigned into severe, moderate and mild groups. Utilizing this categorizing strategy, we have revealed that convalescents in severe group displayed highest frequency of $cT_{FH\text{-}EM}$ while lowest frequency of $cT_{FH\text{-}CM}$ cells comparing to that of frequencies in convalescents in mild or moderate group. Although we do not see the changes of antibody titers among these three groups where $cT_{FH\text{-}EM}$ cells might have impacts on, we do have noticed that the ratio of patients producing SARS-CoV-2 IgG or IgA antibody was higher in convalescents diagnosed with severe condition before. This result suggests that Ig class-switching to IgG and/or IgA and ongoing GC response that requires participation of activated T_{FH} cells in secondary lymphoid organ might be essential for patients to rejuvenate from severe COVID-19. Meanwhile, IgM antibody is found to preferentially produced by convalescents who had mild symptoms in hospital. This might suggest that the ability to produce virus specific IgM antibody early after infection could result in mild symptom and faster recovery. We acknowledge that our sample size is limited due to the availability and accessibility of patient samples. More studies are encouraged to elucidate these important connections in the future.

Blood oxygen level is utilized to estimate the disease severity and the requirement of ventilator. We have found that $cT_{FH\text{-}EM}$ cells negatively correlate with recorded PaO_2/FiO_2. This data not only supports the notion of increased frequency of $cT_{FH\text{-}EM}$ cells in convalescents experienced severe condition but indicates that low blood oxygen, which can cause hypoxia, may have large impacts on the homeostasis of $cT_{FH\text{-}EM}$ cells in patients post COVID-19. The metabolic profile of the microenvironment from both active and post COVID-19 individuals are largely unknown. Our data, however, has revealed that hypoxia might constitute the residual effects of COVID-19, which could regulate the frequency and duration of activated cT_{FH} cells and impact their relationship with antibody production. In fact, germinal center response is in favor of hypoxia (59). And hypoxia can trigger glycolysis that supports the effector memory T cells as well as the long-lived T_{FH} cells (60-62). Notably, we do not rule out other important possibilities that can give rise to higher frequency of $cT_{FH\text{-}EM}$ cells in severe patients after recovery. It has been reported that similar to patients infected by SARS-CoV-1, severe COVID-19 patients have higher

virus load and longer duration of viral shedding period than mild patients (63, 64). Higher titer and prolonged shedding of virus might enhance the activation and duration of antigen presentation to T_{FH} cells (65, 66), which could lead to the increased frequency of cT_{EM} and $cT_{FH\text{-}EM}$ cells in severe COVID-19 patients after recovery. While undetectable, there might also be latent SARS-CoV-2 virus in the reservoir cells in severe patients that could lead to prolonged activation of T cells (67, 68). These observations constitute important compartments of COVID-19 immunology (69), and are factors need to be considered in future antibody-based therapeutics and vaccination design to this contagious disease.

METHODS

COVID-19 convalescent patients

Blood samples from 13 laboratory-confirmed COVID-19 convalescent patients were collected from the Fifth People's Hospital of Wuxi from March to April 2020. The Fifth People's Hospital of Wuxi is the Wuxi city's designated hospital for treating COVID-19 patients. All enrolled convalescent patients were confirmed with both negative detection from virus-test and free of symptoms, then allowed to be discharged from hospital. All patient data were anonymized before study inclusion (**Supplementary Table 1**).

The illness of COVID-19 has been defined as mild, moderate, or severe based on the WHO interim guidance (WHO Reference Number: WHO/2019-nCoV/clinical/2020.4) and the *Diagnosis and Treatment Protocol for COVID-19 (Trial Version 7)*.

Healthy Individuals

13 healthy individuals were enrolled from Affiliated Hospital of Jiangnan University. All healthy individuals had no known history of any significant systemic diseases, including, but not limited to, autoimmune disease, diabetes, allergic disease, kidney or liver disease, or malignancy. Overall clinical characteristics of COVID-19 convalescent patients and healthy individuals are provided in **Table 1 & Table 2.**

Isolation of human PBMC

Blood samples from healthy individuals (n=13) and COVID-19 convalescent patients (n=13) were collected in EDTA-2K tubes (BD Biosciences). Blood was diluted with PBS (1:1) and then gently loaded to Ficoll-Paque Plus (GE) layer at the ratio of 1:1 followed by density gradient centrifugation (400 g, 20°C, 20 min) without brake. Plasma samples were aliquoted and stored at -80°C after density gradient centrifugation. Fetal bovine serum (FBS) containing 10% dimethyl sulfoxide was used to resuspend the cell after thorough wash. Cells were then cryopreserved in liquid nitrogen until further use.

Measurement of basic clinic parameters

Kappa light chain (KAP), Lambda light chain (LAM), Complement 3 (C3), Complement 4 (C4), Anti-streptolysin O (ASO), and C reactive protein (CRP) in plasma were tested using

IMMAGE® 800 Immunochemistry System (Beckman Coulter, Fullerton, CA, USA), according to the manufacturers' instructions. Briefly, plasma sample was mixed with antibodies specific to each protein to form immune complexes during antigen-antibody reaction. Increased rates of light scattered from particles in reaction solution were measured. The intensity of the scattered light is converted to the concentration of each protein in the sample. The result is evaluated by comparison with standards.

Measurement of cytokine and chemokine using MILLIPLEX assay

Aliquots of plasma samples were evaluated using a human high sensitivity T cell panel (21-plex) kit (MILLIPLEX, Merck), according to the manufacturer's instruction. Plasma sample was mixed with beads coated with capture antibodies specific for CX3CL1, GM-CSF, IFNγ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8 (CXCL8), IL-10, IL-12, IL-13, IL-17A, IL-21, IL-23, CXCL11, MIP-1α (CCL3), MIP-1β (CCL4), MIP-3α (CCL20), and TNFα and incubated overnight (16-18 hour) at 4°C. Beads were washed and incubated with biotin-labelled detection antibodies for 1 hour at room temperature (20-25°C), followed by a final incubation with streptavidin-phycoerythrin for 30 minutes at room temperature (20-25°C). After the final wash, beads were resuspended with Sheath Fluid until analyzed by Luminex MAGPIX. Analysis was performed using MILLIPLEX® Analyst 5.1.

Measurement of SARS-CoV-2 specific IgG, IgM and IgA antibody

The concentrations of anti-SARS-CoV-2 IgG/IgM in plasma samples were measured by magnetic chemiluminescence enzyme immunoassay (MCLIA) kits supplied by Bioscience Co., according to the manufacturer's protocols. The measurement was developed from a double-antibody sandwich immunoassay. There are three main components: alkaline phosphatase conjugated anti-human IgG/IgM antibody, the recombinant antigens containing the nucleoprotein and a peptide from the spike protein of SARS-CoV-2 conjugated with FITC, and anti-FITC antibody-conjugated magnetic particles. The tests were conducted on an automated magnetic chemiluminescence analyzer (Axceed 260, Bioscience) according to the manufacturer's instructions.

SARS-CoV-2 specific IgA detection kit using chemiluminescent method was developed by Kangrun Biotech (Guangzhou, China), in which the receptor binding domain of spike

protein was coated onto magnetic particles to catch SARS-CoV-2 specific IgA in patient samples. Secondary antibody that recognizes human IgA was used for detection. The detected chemiluminescent signal over background signal was calculated as relative light units (RLU). It has been validated in a large cohort of serum samples showing high sensitivities and specificities (40). Patient serum samples were collected by centrifugation and diluted 40 times using the dilution buffer before testing.

Cut-Off Index (S/CO) is the ratio of RLU Signal / Cut-Off value. The Cut-Off values were recommended by the company according to large numbers of testing. S/CO value greater than 1 suggests a positive result in antibody testing. The antibody level shown in the figure was measured with chemiluminescence values divided by the cutoff (S/CO) and calculated as $\log_2$ (S/CO + 1).

Antibody staining and flow cytometry

Before antibody staining, frozen PBMC were thawed and carefully washed. Cells were then resuspended in complete RPMI containing 10% FBS, 10 mM HEPES, 1× Penicillin-Streptomycin-Glutamate solution (PSG, Gibco), 1 mM sodium pyruvate, 55 μM 2-mercaptoethanol). Around $1X10^6$ cells were plated with FACs buffer, which was PBS containing 2% heat-inactivated fetal bovine serum (FBS, Gibco). Fc-receptor blocking antibodies (Human BD Fc Block™) were used to block non-specific staining on human lymphocytes for 15 min on ice.

For surface staining, cells were washed once with FACs buffer and incubated for 30 min at 25 °C in the dark with the following monoclonal antibodies at predetermined optimal dilutions and 7-Aminoactinomycin D (7-AAD) was used to exclude dead cells, CD8-FITC (1:200), CD279-PerCP Cy5.5 (1:50), CD25-PE CF594 (1:100), CD197-PE Cy7 (1:50), CD185-Alexa Fluor 647 (1:50), CD4-Alexa Fluor 700 (1:100), CD3-BV510 (1:100), TIM-3-PE (1:100), CD127-BV421 (1:100), CD196-PE (1:100), CD45RA-APC/Cyanine7 (1:200), CD183-BV421 (1:100). Following surface staining, cells were washed twice with FACs buffer and kept at 4 °C throughout the acquisition by NAVIOS flow cytometer (Beckman Coulter Co, Miami, Florida). Data was analyzed using FlowJo v10 software. Antibody information is presented in **Supplementary Table 2.**

Quantification and statistical analysis

Algorithm from 'Heatmap.2' (gplots package version 3.0.1.1) was used to generate heatmap of plasma level of proteins via R version 3.6.1. Statistical analysis on all experimental data was performed by unpaired and two-tailed Student's *t*-test, One-way ANOVA (groups over two) or two-tailed Pearson correlation coefficient analysis using GraphPad Prism 8.0 software. Two-tailed, non-parametric Mann–Whitney (MW) tests were used in highly skewed distributions. All values were expressed as mean and bar graph indicates the mean value, box plot represent min to max. Mann-Whitney *U* test was used to examine basic clinical characteristics of all participates. Differences were considered to be statistically different at $*P < 0.05$, $**P < 0.01$.

Study approval

This study was conducted in accordance with the Declaration of Helsinki 2000. All experimental procedures were approved by the ethics committee at Fifth People's Hospital of Wuxi (#2020-034-1). Written informed consent was waived by the ethics committee of the designated hospital (Fifth People's Hospital of Wuxi) for emerging infectious diseases. The medical ethical committee at the Affiliated Hospital of Jiangnan University has approved the collection of samples from 13 healthy individuals (IEC2020052601). Informed consent was obtained from all healthy subjects for being included in the study.

AUTHOR CONTRIBUTIONS

P.Z conceived and oversaw the study. P.Z designed the experiments. F.G, Y.D and D.Z performed the experiments, P.Z, F.G, T.Z, L.C, D.Z, H.W, T.J, Y.D, and M.L analyzed the data. P.Z wrote the manuscript. T.J, H.P and D.Y participated in scientific discussion. P.Z revised the manuscript and led the submission.

ACKNOWLEDGEMENT

We thank patients and healthy individuals for participation in our study. We thank Professor Carola G Vinuesa for constructive suggestions on the manuscript revision. This study was supported by Six Talent Peaks Project in Jiangsu Province (2017-WSN-186 to

F.G) and the National Natural Science Foundation of China (NSFC) grants 81970759 (T.Z & P.Z), Bellberry-Viertel Senior Medical Research fellowship (D.Y).

References

1. Dong E, Du H, and Gardner L. An interactive web-based dashboard to track COVID-19 in real time. *The Lancet infectious diseases.* 2020;20(5):533-4.
2. Wang C, Horby PW, Hayden FG, and Gao GF. A novel coronavirus outbreak of global health concern. *Lancet.* 2020;395(10223):470-3.
3. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med.* 2020;382(8):727-33.
4. Grifoni. A, Weiskopf. D, and Ramirez. SI. Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals. *Cell.* 2020.
5. Thevarajan I, Nguyen THO, Koutsakos M, Druce J, Caly L, van de Sandt CE, et al. Breadth of concomitant immune responses prior to patient recovery: a case report of non-severe COVID-19. *Nature medicine.* 2020.
6. Ni L, Ye F, Cheng ML, Feng Y, Deng YQ, Zhao H, et al. Detection of SARS-CoV-2-Specific Humoral and Cellular Immunity in COVID-19 Convalescent Individuals. *Immunity.* 2020.
7. Zheng HY, Zhang M, Yang CX, Zhang N, Wang XC, Yang XP, et al. Elevated exhaustion levels and reduced functional diversity of T cells in peripheral blood may predict severe progression in COVID-19 patients. *Cellular & molecular immunology.* 2020;17(5):541-3.
8. Zheng M, Gao Y, Wang G, Song G, Liu S, Sun D, et al. Functional exhaustion of antiviral lymphocytes in COVID-19 patients. *Cellular & molecular immunology.* 2020;17(5):533-5.
9. Xu Z, Shi L, and Wang Y. Pathological findings of COVID-19 associated with acute respiratory distress syndrome (vol 8, pg 420, 2020). *Lancet Resp Med.* 2020;8(4):E26-E.
10. Chen G, Wu D, Guo W, Cao Y, Huang D, Wang H, et al. Clinical and immunological features of severe and moderate coronavirus disease 2019. *The Journal of clinical investigation.* 2020;130(5):2620-9.
11. Shen C, Wang Z, Zhao F, Yang Y, Li J, Yuan J, et al. Treatment of 5 Critically Ill Patients With COVID-19 With Convalescent Plasma. *JAMA.* 2020.
12. Ko JH, Muller MA, Seok H, Park GE, Lee JY, Cho SY, et al. Serologic responses of 42 MERS-coronavirus-infected patients according to the disease severity. *Diagn Microbiol Infect Dis.* 2017;89(2):106-11.
13. Vijay R, and Perlman S. Middle East respiratory syndrome and severe acute respiratory syndrome. *Curr Opin Virol.* 2016;16:70-6.
14. Li G, Chen XJ, and Xu AL. Profile of specific antibodies to the SARS-associated coronavirus. *New Engl J Med.* 2003;349(5):508-9.
15. Hsueh PR, Huang LM, Chen PJ, Kao CL, and Yang PC. Chronological evolution of IgM, IgA, IgG and neutralisation antibodies after infection with SARS-associated coronavirus. *Clin Microbiol Infec.* 2004;10(12):1062-6.
16. Long QX, Liu BZ, Deng HJ, Wu GC, Deng K, Chen YK, et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. *Nature medicine.* 2020.
17. Cao Y, Su, B., Guo, X., Sun, W., Deng, Y., Bao, L., Zhu, Q., Zhang, X., Zheng, Y., Geng, C., Chai, X., He, R., Li, X., Lv, Q., Zhu, H., Deng, W., Xu, Y., Wang, Y., Qiao, L., Tan, Y., Song, L., Wang, G., Du, X., Gao, N., Liu, J., Xiao, J., Su, X.-d., Du, Z., Feng, Y., Qin, C., Qin, C., Jin, R., Xie, X.S. Potent neutralizing antibodies against SARS-CoV-2 identified by high-throughput single-cell sequencing of convalescent patients' B cells. *Cell.* 2020.
18. Sallusto F, Lenig D, Forster R, Lipp M, and Lanzavecchia A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature.* 1999;401(6754):708-12.
19. Sallusto F, Geginat J, and Lanzavecchia A. Central memory and effector memory T cell subsets: Function, generation, and maintenance. *Annual review of immunology.* 2004;22:745-63.
20. Yang LT, Peng H, Zhu ZL, Li G, Huang ZT, Zhao ZX, et al. Long-lived effector/central memory T-cell responses to severe acute respiratory syndrome coronavirus (SARS-CoV) S antigen in recovered SARS patients. *Clin Immunol.* 2006;120(2):171-8.
21. Zhao JC, Zhao JX, Mangalam AK, Channappanavar R, Fett C, Meyerholz DK, et al. Airway Memory CD4(+) T Cells Mediate Protective Immunity against Emerging Respiratory Coronaviruses. *Immunity.* 2016;44(6):1379-91.
22. Vinuesa CG, Linterman MA, Yu D, and MacLennan IC. Follicular Helper T Cells. *Annual review of immunology.* 2016;34:335-68.
23. Crotty S. T follicular helper cell differentiation, function, and roles in disease. *Immunity.* 2014;41(4):529-42.
24. Locci M, Havenar-Daughton C, Landais E, Wu J, Kroenke MA, Arlehamn CL, et al. Human Circulating PD-1(+)CXCR3(-)CXCR5(+) Memory Tfh Cells Are Highly Functional and Correlate with Broadly Neutralizing HIV Antibody Responses. *Immunity.* 2013;39(4):758-69.

25. Ueno H, Banchereau J, and Vinuesa CG. Pathophysiology of T follicular helper cells in humans and mice. *Nature immunology.* 2015;16(2):142-52.
26. Morita R, Schmitt N, Bentebibel SE, Ranganathan R, Bourdery L, Zurawski G, et al. Human Blood CXCR5(+)CD4(+) T Cells Are Counterparts of T Follicular Cells and Contain Specific Subsets that Differentially Support Antibody Secretion (vol 34, pg 108, 2011). *Immunity.* 2011;34(1):135-.
27. Bost P, Giladi A, Liu Y, Bendjelal Y, Xu G, David E, et al. Host-viral infection maps reveal signatures of severe COVID-19 patients. *Cell.* 2020.
28. Chevalier N, Jarrossay D, Ho E, Avery DT, Ma CS, Yu D, et al. CXCR5 Expressing Human Central Memory CD4 T Cells and Their Relevance for Humoral Immune Responses. *Journal of immunology.* 2011;186(10):5556-68.
29. He J, Tsai LM, Leong YA, Hu X, Ma CS, Chevalier N, et al. Circulating precursor CCR7(lo)PD-1(hi) CXCR5(+) CD4(+) T cells indicate Tfh cell activity and promote antibody responses upon antigen reexposure. *Immunity.* 2013;39(4):770-81.
30. Gong F, Zheng T, and Zhou P. T Follicular Helper Cell Subsets and the Associated Cytokine IL-21 in the Pathogenesis and Therapy of Asthma. *Frontiers in immunology.* 2019;10:2918.
31. Bentebibel SE, Lopez S, Obermoser G, Schmitt N, Mueller C, Harrod C, et al. Induction of ICOS(+)CXCR3(+)CXCR5(+) T-H Cells Correlates with Antibody Responses to Influenza Vaccination. *Science translational medicine.* 2013;5(176).
32. Koutsakos M, Wheatley AK, Loh L, Clemens EB, Sant S, Nussing S, et al. Circulating TFH cells, serological memory, and tissue compartmentalization shape human influenza-specific B cell immunity. *Science translational medicine.* 2018;10(428).
33. Chung Y, Tanaka S, Chu F, Nurieva RI, Martinez GJ, Rawal S, et al. Follicular regulatory T cells expressing Foxp3 and Bcl-6 suppress germinal center reactions. *Nature medicine.* 2011;17(8):983-8.
34. Linterman MA, Pierson W, Lee SK, Kallies A, Kawamoto S, Rayner TF, et al. Foxp3+ follicular regulatory T cells control the germinal center response. *Nature medicine.* 2011;17(8):975-82.
35. Canete PF, Sweet RA, Gonzalez-Figueroa P, Papa I, Ohkura N, Bolton H, et al. Regulatory roles of IL-10-producing human follicular T cells. *The Journal of experimental medicine.* 2019.
36. Cole KE, Strick CA, Paradis TJ, Ogborne KT, Loetscher M, Gladue RP, et al. Interferon-inducible T cell alpha chemoattractant (I-TAC): A novel non-ELR CXC chemokine with potent activity on activated T cells through selective high affinity binding to CXCR3. *Journal of Experimental Medicine.* 1998;187(12):2009-21.
37. Liang HB, Tang JY, Liu ZH, Liu YH, Huang YY, Xu YF, et al. ZIKV infection induces robust Th1-like Tfh cell and long-term protective antibody responses in immunocompetent mice. *Nature communications.* 2019;10.
38. Martin-Gayo E, Cronin J, Hickman T, Ouyang Z, Lindqvist M, Kolb KE, et al. Circulating CXCR5(+)CXCR3(+)PD-1(lo) Tfh-like cells in HIV-1 controllers with neutralizing antibody breadth. *JCI Insight.* 2017;2(2):e89574.
39. Baiyegunhi O, Ndlovu B, Ogunshola F, Ismail N, Walker BD, Ndung'u T, et al. Frequencies of Circulating Th1-Biased T Follicular Helper Cells in Acute HIV-1 Infection Correlate with the Development of HIV-Specific Antibody Responses and Lower Set Point Viral Load. *Journal of virology.* 2018;92(15).
40. Ma H, Zeng W, He H, Zhao D, Jiang D, Zhou P, et al. Serum IgA, IgM, and IgG responses in COVID-19. *Cellular & molecular immunology.* 2020:1-3.
41. Weiskopf D, Schmitz KS, Raadsen MP, Grifoni A, Okba NM, Endeman H, et al. Phenotype of SARS-CoV-2-specific T-cells in COVID-19 patients with acute respiratory distress syndrome. *medRxiv.* 2020.
42. Teijaro JR, Turner D, Pham Q, Wherry EJ, Lefrançois L, and Farber DL. Cutting edge: Tissue-retentive lung memory CD4 T cells mediate optimal protection to respiratory virus infection. *The Journal of Immunology.* 2011;187(11):5510-4.
43. Teijaro JR, Verhoeven D, Page CA, Turner D, and Farber DL. Memory CD4 T cells direct protective responses to influenza virus in the lungs through helper-independent mechanisms. *Journal of virology.* 2010;84(18):9217-26.
44. Mahnke YD, Brodie TM, Sallusto F, Roederer M, and Lugli E. The who's who of T‐cell differentiation: human memory T‐cell subsets. *European journal of immunology.* 2013;43(11):2797-809.
45. Mueller SN, Gebhardt T, Carbone FR, and Heath WR. Memory T cell subsets, migration patterns, and tissue residence. *Annual review of immunology.* 2013;31:137-61.
46. Kaech SM, and Cui W. Transcriptional control of effector and memory CD8+ T cell differentiation. *Nature reviews Immunology.* 2012;12(11):749-61.

47. Robbiani DF, Gaebler C, Muecksch F, Lorenzi JC, Wang Z, Cho A, et al. Convergent Antibody Responses to SARS-CoV-2 Infection in Convalescent Individuals. *bioRxiv.* 2020.
48. Poh CM, Carissimo G, Bei W, Amrun SN, Lee CY-P, Chee RS-L, et al. Potent neutralizing antibodies in the sera of convalescent COVID-19 patients are directed against conserved linear epitopes on the SARS-CoV-2 spike protein. *bioRxiv.* 2020.
49. Yang Y, Shen C, Li J, Yuan J, Wei J, Huang F, et al. Plasma IP-10 and MCP-3 levels are highly associated with disease severity and predict the progression of COVID-19. *The Journal of allergy and clinical immunology.* 2020.
50. Liao M, Liu Y, Yuan J, Wen Y, Xu G, Zhao J, et al. Single-cell landscape of bronchoalveolar immune cells in patients with COVID-19. *Nature medicine.* 2020:1-3.
51. Rani MS, Foster GR, Leung S, Leaman D, Stark GR, and Ransohoff RM. Characterization of β-R1, a gene that is selectively induced by interferon β (IFN-β) compared with IFN-α. *Journal of Biological Chemistry.* 1996;271(37):22878-84.
52. Moyron-Quiroz JE, Rangel-Moreno J, Kusser K, Hartson L, Sprague F, Goodrich S, et al. Role of inducible bronchus associated lymphoid tissue (iBALT) in respiratory immunity. *Nature medicine.* 2004;10(9):927-34.
53. Kelsen SG, Aksoy MO, Georgy M, Hershman R, Ji R, Li X, et al. Lymphoid follicle cells in chronic obstructive pulmonary disease overexpress the chemokine receptor CXCR3. *American journal of respiratory and critical care medicine.* 2009;179(9):799-805.
54. Allie SR, Bradley JE, Mudunuru U, Schultz MD, Graf BA, Lund FE, et al. The establishment of resident memory B cells in the lung requires local antigen encounter. *Nature immunology.* 2019;20(1):97-108.
55. Laidlaw BJ, Zhang N, Marshall HD, Staron MM, Guan T, Hu Y, et al. CD4+ T cell help guides formation of CD103+ lung-resident memory CD8+ T cells during influenza viral infection. *Immunity.* 2014;41(4):633-45.
56. Son YM, Cheon IS, Wu Y, Li C, Wang Z, Chen Y, et al. Tissue-resident CD4+ T helper cells assist protective respiratory mucosal B and CD8+ T cell memory responses. *bioRxiv.* 2020.
57. Caielli S, Veiga DT, Balasubramanian P, Athale S, Domic B, Murat E, et al. A CD4(+) T cell population expanded in lupus blood provides B cell help through interleukin-10 and succinate. *Nature medicine.* 2019;25(1):75-+.
58. Mathew D, Giles JR, Baxter AE, Greenplate AR, Wu JE, Alanio C, et al. Deep immune profiling of COVID-19 patients reveals patient heterogeneity and distinct immunotypes with implications for therapeutic interventions. *bioRxiv.* 2020.
59. Cho SH, Raybuck AL, Stengel K, Wei M, Beck TC, Volanakis E, et al. Germinal centre hypoxia and regulation of antibody qualities by a hypoxia response system. *Nature.* 2016;537(7619):234-8.
60. Xu Y, Chaudhury A, Zhang M, Savoldo B, Metelitsa LS, Rodgers J, et al. Glycolysis determines dichotomous regulation of T cell subsets in hypoxia. *The Journal of clinical investigation.* 2016;126(7):2678-88.
61. Zeng H, Cohen S, Guy C, Shrestha S, Neale G, Brown SA, et al. mTORC1 and mTORC2 kinase signaling and glucose metabolism drive follicular helper T cell differentiation. *Immunity.* 2016;45(3):540-54.
62. Künzli M, Schreiner D, Pereboom TC, Swarnalekha N, Litzler LC, Lötscher J, et al. Long-lived T follicular helper cells retain plasticity and help sustain humoral immunity. *Science Immunology.* 2020;5(45).
63. Liu Y, Yan L-M, Wan L, Xiang T-X, Le A, Liu J-M, et al. Viral dynamics in mild and severe cases of COVID-19. *The Lancet Infectious Diseases.* 2020.
64. Wang Y, Zhang L, Sang L, Ye F, Ruan S, Zhong B, et al. Kinetics of viral load and antibody response in relation to COVID-19 severity. *The Journal of clinical investigation.* 2020.
65. Fazilleau N, Eisenbraun MD, Malherbe L, Ebright JN, Pogue-Caley RR, McHeyzer-Williams LJ, et al. Lymphoid reservoirs of antigen-specific memory T helper cells. *Nature immunology.* 2007;8(7):753-61.
66. Deenick EK, Chan A, Ma CS, Gatto D, Schwartzberg PL, Brink R, et al. Follicular helper T cell differentiation requires continuous antigen presentation that is independent of unique B cell signaling. *Immunity.* 2010;33(2):241-53.
67. Baumjohann D, Preite S, Reboldi A, Ronchi F, Ansel KM, Lanzavecchia A, et al. Persistent antigen and germinal center B cells sustain T follicular helper cell responses and phenotype. *Immunity.* 2013;38(3):596-605.
68. Vinuesa CG. HIV and T follicular helper cells: a dangerous relationship. *The Journal of clinical investigation.* 2012;122(9):3059-62.
69. Vabret N, Britton GJ, Gruber C, Hegde S, Kim J, Kuksin M, et al. Immunology of COVID-19: current state of the science. *Immunity.* 2020.

Figure 1

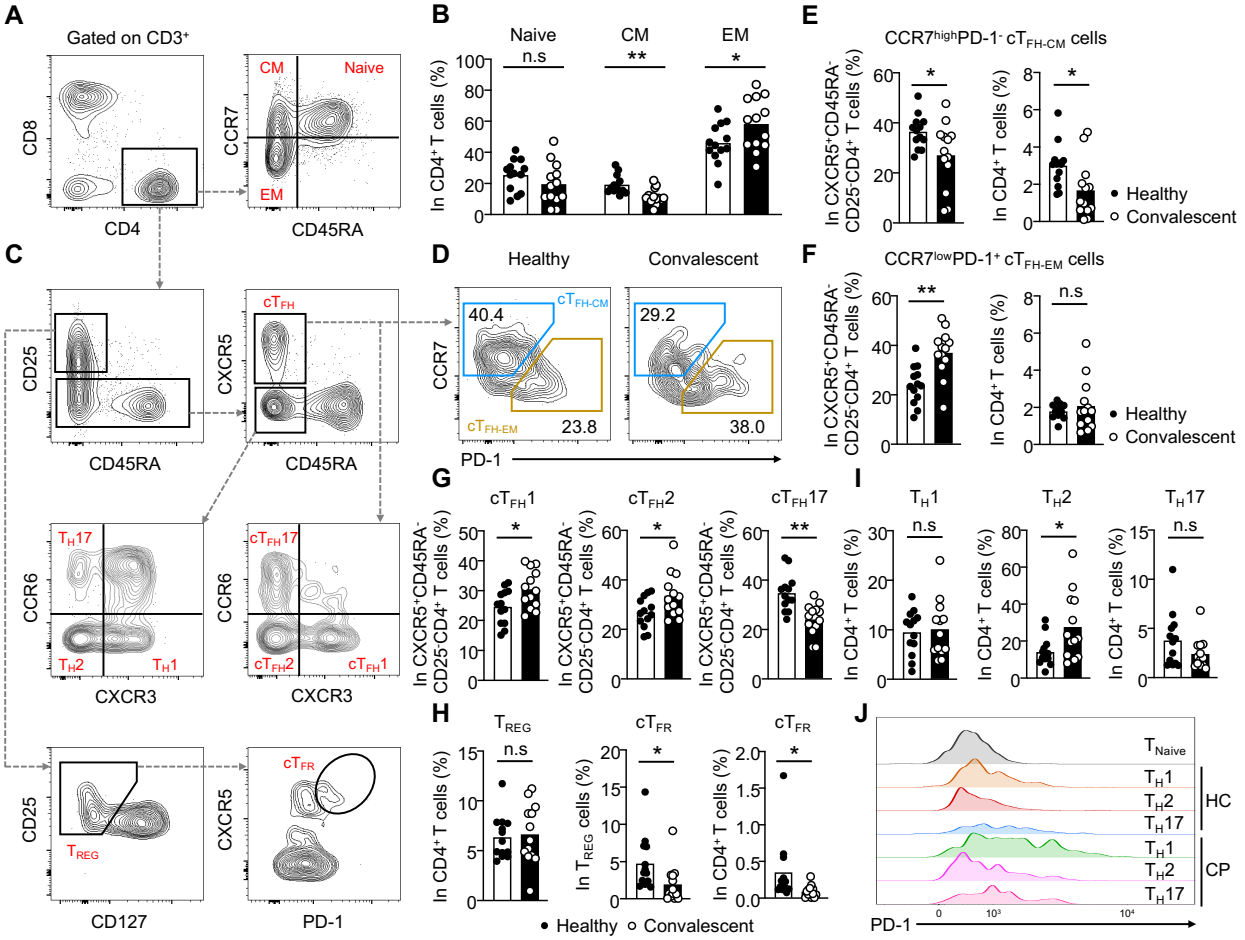


Figure 1. Peripheral CD4⁺ T cell subsets in COVID-19 convalescent patients.

Blood samples were collected from COVID-19 convalescent patients (n=13) and healthy individuals (n=13). PBMC were isolated for antibody staining and FACs phenotyping of $CD4^+$ T cells (A-J). (A) Gating strategies on naïve $CD4^+$ T cells ($CD45RA^+CCR7^+$), central-memory $CD4^+$ T cells ($CD45RA^-CCR7^+$) and effector-memory $CD4^+$ T cells ($CD45RA^-CCR7^-$). (B) Statistical analysis of the frequency of $CD4^+$ T_{Naive}, $CD4^+$ T_{CM} and $CD4^+$ T_{EM} cells between healthy individuals and COVID-19 convalescent patients. (C) Gating strategies on different peripheral circulating $CD4^+$ T cell subsets, including $CD25^-CD45RA^-CXCR5^+$ circulating T follicular helper (cT_{FH}) cells, $CCR7^{high}PD\text{-}1^-$ central-memory cT_{FH} ($cT_{FH\text{-}CM}$) cells, $CCR7^{low}PD\text{-}1^+$ effector-memory cT_{FH} ($cT_{FH\text{-}EM}$) cells, $CXCR3^+CCR6^-$ cT_{FH} ($cT_{FH}1$) cells, $CXCR3^-CCR6^-$ cT_{FH} ($cT_{FH}2$) cells and $CXCR3^-CCR6^+$ cT_{FH} ($cT_{FH}17$) cells. Within $CD3^+CD8^-CD4^+$ circulating T cells, T_H1 cells were defined as

$CD25^-CD45RA^-CXCR3^+CCR6^-$ cells, T_H2 cells as $CD25^-CD45RA^-CXCR3^-CCR6^-$ cells and T_H17 cells as $CD25^-CD45RA^-CXCR3^-CCR6^+$ cells. Regulatory T (cT_{REG}) cells were defined as $CD25^+CD45RA^-CD127^-$ cells and circulating T follicular regulatory (cT_{FR}) cells as $CD25^+CD45RA^-CD127^-CXCR5^{high}PD\text{-}1^{high}$ cells. (D) FACs plot showing the representative $cT_{FH\text{-}CM}$ and $cT_{FH\text{-}EM}$ cells between healthy individuals and COVID-19 convalescent patients. Quantifications on the frequency of these cells within cT_{FH} cells and $CD4^+$ T cells were present respectively in (E & F). (G) Frequency of $cT_{FH}1$, $cT_{FH}2$ and $cT_{FH}17$ cells within cT_{FH} cells in healthy individuals and COVID-19 convalescents (H) Statistical analysis showing the differences of the frequency of T_{REG} and cT_{FR} cells between healthy individuals and COVID-19 convalescent patients, and the same analysis on T_H1, T_H2 and T_H17 cells (I). (J) Histogram showing the PD-1 expression on T_H1, T_H2 and T_H17 cells between healthy individuals and COVID-19 convalescent patients; HC, healthy control individuals (n=13), CP, COVID-19 convalescent patients (n=13). Each dot represents an individual subject. Bars represent the mean values. n.s, not significant; *P<0.05 and ** P<0.01 by unpaired and two-tailed Student's t-test.

Figure 2

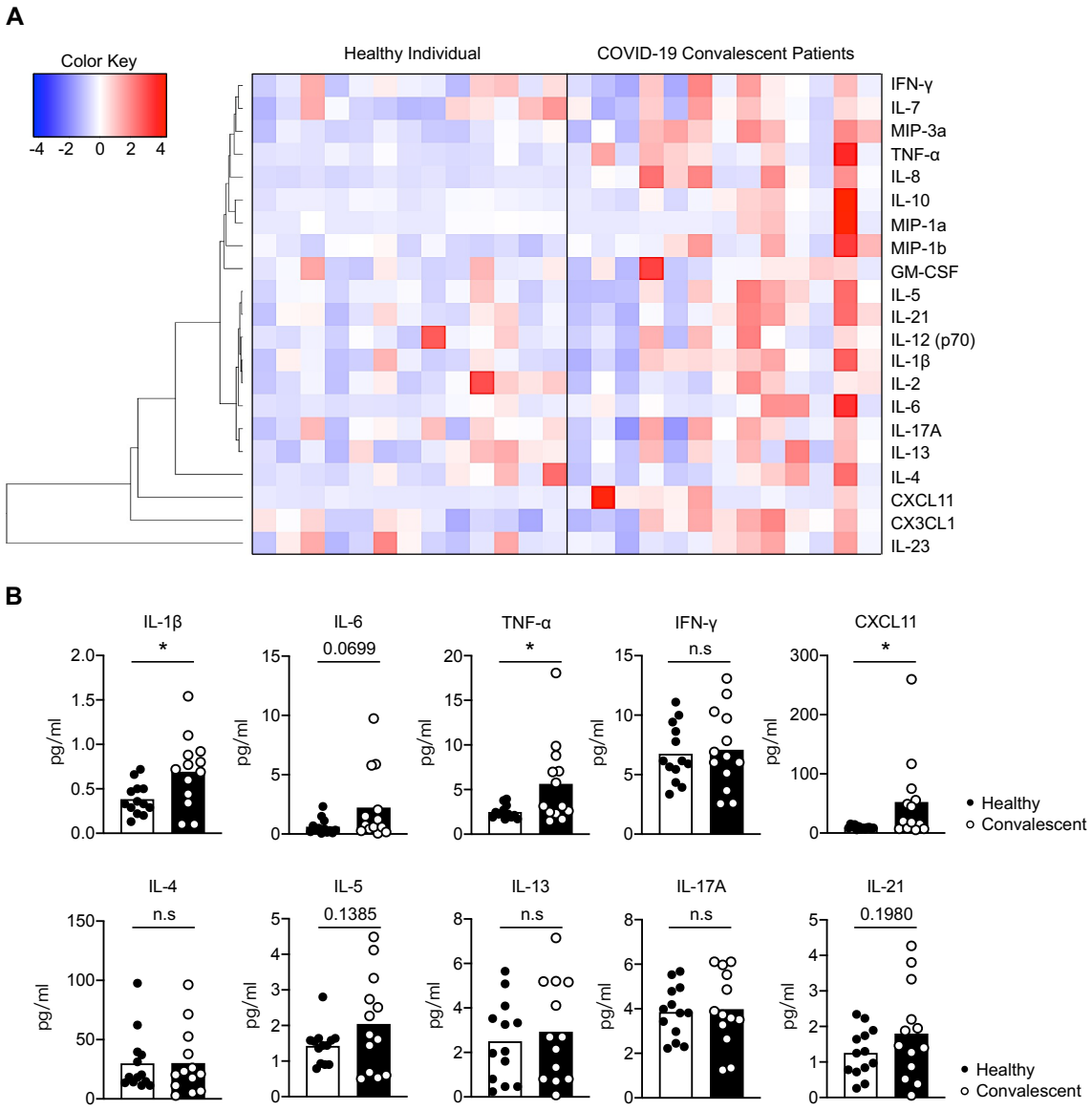


Figure 2. Peripheral cytokines and chemokines related to CD4⁺ T cells in COVID-19 convalescent patients.

Blood samples were collected from COVID-19 convalescent patients (n=13) and healthy individuals (n=13). Plasma were obtained after processing the blood to examine cytokines and chemokines using multiplex assay (Luminex xMAP). (A) 21 cytokines and chemokines related to CD4⁺ T cells were detected. Unsupervised clustering was applied to generate the heatmap of cytokine profile between healthy individuals and COVID-19

convalescent patients. (B) Statistical analysis of pro-inflammatory cytokines including IL-1β, IL-6, TNF-α, IFN-γ and CXCL11. Plasma level of IL-4, IL-5, IL-13, IL-17A and IL-21 were measured and the differences between healthy individuals and COVID-19 convalescent patients were analyzed. Each dot represents an individual subject. Bars represent the mean values. n.s, not significant; *P<0.05 and ** P<0.01 by unpaired and two-tailed Student's *t*-test. Non-parametric Mann–Whitney tests were used to determine the difference in highly skewed distributions such as IL-6, TNF-α and CXCL11.

Figure 3

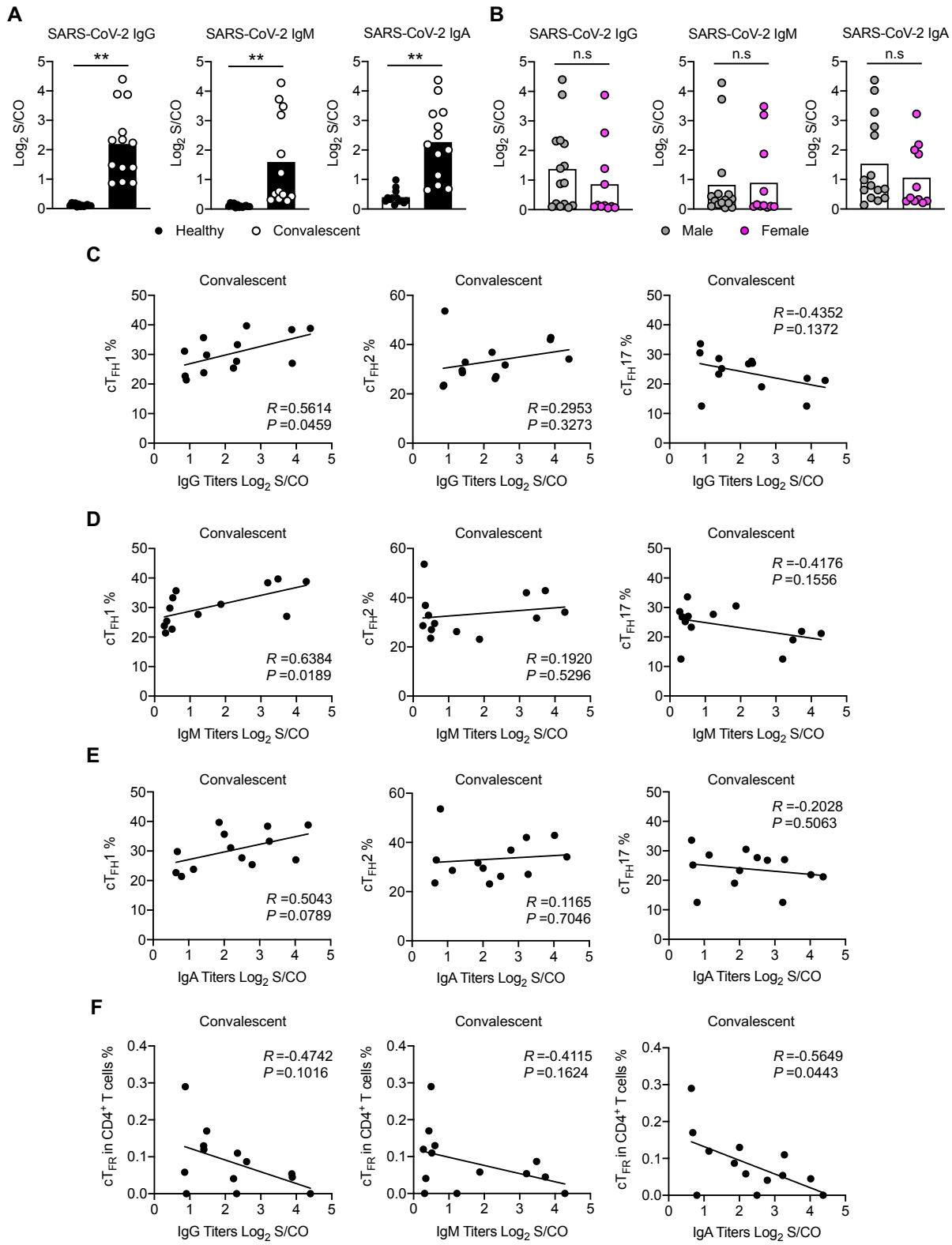

A
SARS-CoV-2 IgG
SARS-CoV-2 IgM
SARS-CoV-2 IgA
**
Log2 S/CO
Healthy
Convalescent
B
SARS-CoV-2 IgG
SARS-CoV-2 IgM
SARS-CoV-2 IgA
n.s
Log2 S/CO
Male
Female
C
Convalescent
cTFH1 %
cTFH2 %
cTFH17 %
IgG Titers Log2 S/CO
R =0.5614
P =0.0459
R =0.2953
P =0.3273
R =-0.4352
P =0.1372
D
IgM Titers Log2 S/CO
R =0.6384
P =0.0189
R =0.1920
P =0.5296
R =-0.4176
P =0.1556
E
IgA Titers Log2 S/CO
R =0.5043
P =0.0789
R =0.1165
P =0.7046
R =-0.2028
P =0.5063
F
cTFR in CD4+ T cells %
R =-0.4742
P =0.1016
R =-0.4115
P =0.1624
R =-0.5649
P =0.0443

Figure 3. $cT_{FH}1$ cells correlate with higher titer of SARS-CoV-2 specific antibody

Blood samples were collected from COVID-19 convalescent patients (n=13) and healthy individuals (n=13). Plasma were obtained after processing the blood to detect the antibodies specific to SARS-CoV-2 using chemiluminescent immunoassays (CLIA). (A) IgG, IgM and IgA were measured. (B) Statistical analysis of the IgG, IgM and IgA antibody production between male and female participants including both healthy individuals and COVID-19 convalescent patients. (C) Correlation analysis on $cT_{FH}1$ cells (%), $cT_{FH}2$ cells (%), $cT_{FH}17$ cells (%) and SARS-CoV-2 specific IgG antibody titer. (D) Correlation between $cT_{FH}1$ cells (%), $cT_{FH}2$ cells (%), $cT_{FH}17$ cells (%) and SARS-CoV-2 specific IgM antibody titer. (E) Correlation analysis on $cT_{FH}1$ cells (%), $cT_{FH}2$ cells (%), $cT_{FH}17$ cells (%) and SARS-CoV-2 specific IgA antibody titer. (F) Correlation between cT_{FR} cells (%) and SARS-CoV-2 specific IgG, IgM and IgA antibody titer. Each dot represents an individual subject. Bars represent the mean values. Measured chemiluminescence values divided by the cutoff (S/CO) were used to present the antibody level. n.s, not significant; **P*<0.05 and ** *P*<0.01 by unpaired and two-tailed Student's *t*-test or two-tailed Pearson correlation coefficient.

Figure 4

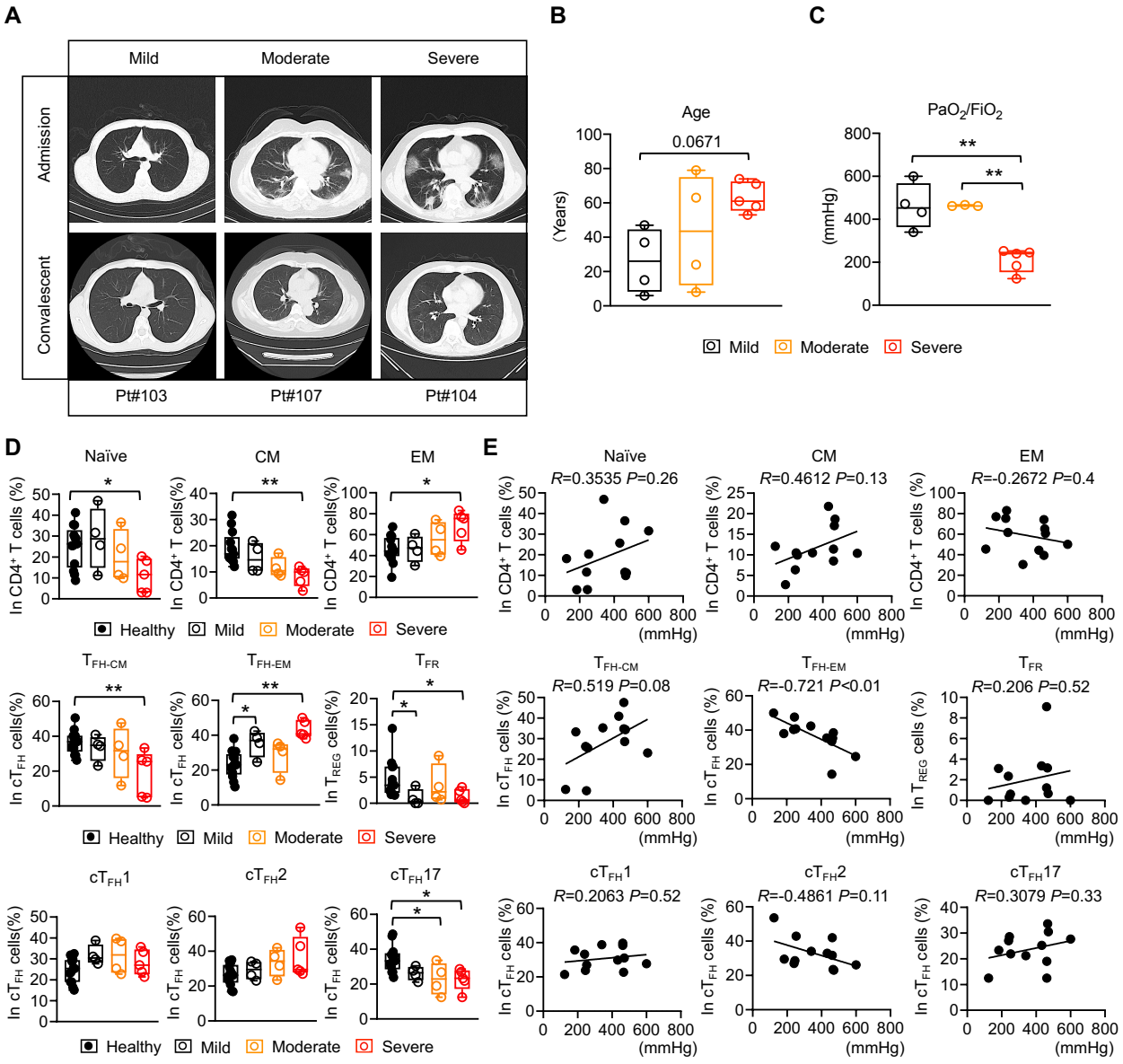


Figure 4. Peripheral CD4⁺ T cells in different groups of COVID-19 convalescents

COVID-19 convalescents have been categorized into mild (N=4), moderate (N=4) and severe (N=5) group based on their diagnosis certificates during admission at hospital. (A) Representative chest CT images of patients during admission and convalescent. (B) Age of convalescent patients in different groups. (C) Blood oxygen level indicated by PaO_2/FiO_2 in convalescent patients of different groups. (D) Statistics showing the

peripheral $CD4^+$ T cell subsets in health individuals and different groups of COVID-19 convalescents. Healthy individuals (n=13) (E) Correlation of peripheral $CD4^+$ T cell subsets and PaO_2/FiO_2 in convalescent patients. Each dot represents an individual subject. Box plot show min to max. *P<0.05 and ** P<0.01 by One-way ANOVA test (B, C, D) or two-tailed Pearson correlation coefficient (E). Two-tailed, non-parametric Mann–Whitney tests were used in highly skewed distributions (D, $T_{Naïve}$, $T_{FH\text{-}CM}$, $T_{FH\text{-}EM}$, T_{FR}).

Figure 5

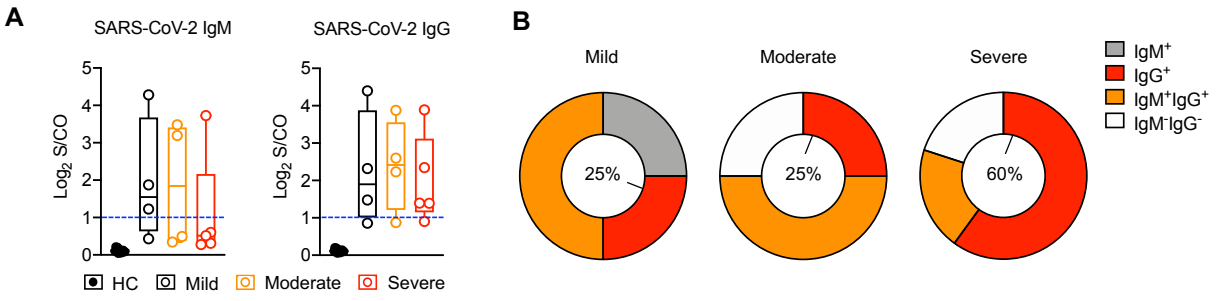


Figure 5. Antibody response in different groups of COVID-19 convalescents

(A) Antibody titer of SARS-CoV-2 specific IgG and IgM from convalescent patients in different groups. (B) Ratio of IgM^+ (grey), IgG^+ (red), IgM^+ IgG^+ (orange) and IgM^- IgG^- (white) individual (based on the produced antibody type) in mild, moderate and severe group; percentages in the central circle represent the ratio of IgG^+ individual in each group.

Table 1 Clinical and pathological characteristics of the COVID-19 convalescent patients

	COVID-19 (n=13)
Age (years)	53 (19.5-67)
Gender (%)	
Male (%)	69.2% (9/13)
Female (%)	30.8% (4/13)
SARS-CoV-2 PCR Positivity	100%
Disease severity	
Mild	30.8% (4/13)
Moderate	30.8% (4/13)
Severe	38.4% (5/13)
Signs and symptoms at admission	
Cough	46.2% (6/13)
Fatigue	7.7% (1/13)
Fever	53.8% (7/13)
Diarrhea	7.7% (1/13)
Muscular soreness	15.4% (2/13)
Dizziness	7.7% (1/13)
Chest congestion	15.4% (2/13)
Days since discharge from hospital	28 (27-33)
Past Medical History	
No known disease history	53.8% (7/13)
Hypertension	30.8% (4/13)
Diabetes mellitus	23.1% (3/13)
Gastric carcinoma	7.7% (1/13)
Blood transfusion	7.7% (1/13)

Table 2 Comparison of laboratory parameters between healthy individuals and COVID-19 convalescent patients

	Healthy donors (n=13)	COVID-19 (n=13)	*p* value
Age, median (IQR), years	48.0 (33.5-56.0)	53.0 (19.5-67.0)	0.7345
Gender, Male/Female, n (%)	6(46.2)/7(53.8)	9 (69.2)/4 (30.8)	0.4283
Anti-SARS-CoV-2 IgM, median (IQR), S/CO	0.07 (0.05-0.11)	0.52 (0.31-9.16)	**<0.0001
Anti-SARS-CoV-2 IgG, median (IQR), S/CO	0.08 (0.06-0.11)	3.70 (1.25-9.37)	**<0.0001
Anti-SARS-CoV-2 IgA, median (IQR), S/CO	0.25 (0.21-0.41)	3.54 (0.97-8.51)	**<0.0001
Hemoglobin, median (IQR), g/L	142.0 (138.0-153.5)	135.0 (119.5-150.0)	0.2693
Platelet, median (IQR), 10^9/L	261.0(201.0-328.5)	231.0 (194.5-270.0)	0.3425
White blood cell, median (IQR), 10^9/L	5.20 (4.15-5.85)	5.31 (4.91-6.98)	0.2273
Neutrophil, median (IQR), 10^9/L	2.80 (2.40-3.75)	3.06 (2.54-4.49)	0.3554
Lymphocyte, median (IQR), 10^9/L	1.80 (1.40-2.05)	1.86 (1.35-2.01)	0.7912
Eosinophil, median (IQR), 10^9/L	0.10 (0-0.15)	0.11 (0.03-0.21)	0.2845
Total bilirubin, median (IQR), μmol/L	11.0 (9.0-15.8)	9.0 (7.5-18.0)	0.4537

ALT, median (IQR), U/L	16.0 (12.5-25.0)	21.5 (15.8-26.3)	0.4543
AST, median (IQR), U/L	20.0 (17.5-21.5)	23.0 (17.8-27.5)	0.2921
Urea nitrogen, median (IQR), mmol/L	4.7 (3.6-5.4)	4.6 (3.4-22.2)	0.6504
Creatinine, median (IQR), μmol/L	55.4 (45.4-76.1)	65.0 (42.3-156.0)	0.5532
Complement 3, median (IQR), g/L	0.93 (0.71-1.01)	0.91 (0.77-1.06)	0.5360
Complement 4, median (IQR), g/L	0.23 (0.17-0.26)	0.26 (0.21-0.32)	0.1639
KAP, median (IQR), g/L	10.10 (9.01-11.65)	10.00 (7.34-13.95)	0.9703
LAM, median (IQR), g/L	5.27 (4.70-6.17)	6.03 (5.19-10.23)	0.0811
CRP, median (IQR), mg/L	1.57 (1.15-3.24)	3.50 (2.32-5.44)	*0.0225
ASO, median (IQR), IU/mL	74.0 (38.8-136.0)	53.0 (35.9-111.5)	0.5457

ALT, alanine aminotransferase; AST, aspartate transaminase; KAP, Kappa light chain; LAM, Lambda light chain; CRP, C reactive protein; ASO, Anti-streptolysin O.