Longitudinal Analysis of Human Memory T-Cell Response according to the Severity of Illness up to 8 Months after SARS-CoV-2 Infection

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Conflicts of Interest

No conflicts of interest declared.

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Flow cytometric analyses showed frequent and functional memory T-cell response to SARS-CoV-2 for 8 months post-symptom onset. Memory $CD4^+$ T-cell response tended to be greater in severe patients than in mild or asymptomatic patients.

ABSTRACT

Background. Understanding the memory T-cell response to severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is crucial for assessing the longevity of protective immunity after SARS-CoV-2 infection or coronavirus disease-2019 (COVID-19) vaccination. However, the longitudinal memory T-cell response up to 8 months post-symptom onset (PSO) according to the severity of illness is unknown.

Methods. We analyzed peripheral blood mononuclear cells (PBMCs) from healthy volunteers or patients with COVID-19 who experienced asymptomatic, mild, or severe illness at 2, 5, and 8 months PSO. SARS-CoV-2 spike, nucleocapsid, and membrane protein-stimulated PBMCs were subjected to flow cytometry analysis

Results. A total of 24 patients—seven asymptomatic and nine with mild and eight with severe disease—as well as six healthy volunteers were analyzed. SARS-CoV-2-specific $OX40^{+}CD137^{+}$ $CD4^{+}$ T cells and $CD69^{+}CD137^{+}$ $CD8^{+}$ T cells persisted at 8 months PSO. Also, antigen-specific cytokine-producing or polyfunctional $CD4^{+}$ T cells were maintained for up to 8 months PSO. Memory $CD4^{+}$ T-cell responses tended to be greater in patients who had severe illness than in those with mild or asymptomatic disease.

Conclusions. Memory response to SARS-CoV-2, based on the frequency and functionality, persists for 8 months PSO. Further investigations involving its longevity and protective effect from reinfection are warranted.

Keywords: Memory response, T-cell, SARS-CoV-2, 8 months, severity

Introduction

Although 1 year has elapsed the first report of coronavirus disease-19 (COVID-19), caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), the pandemic is ongoing [1, 2]. Recent reports on COVID-19 vaccines with high efficacy raise hope for pandemic control [3, 4]. However, evaluations of the duration of SARS-CoV-2-specific immune responses after SARS-CoV-2 infection or vaccination are needed [5, 6].

The cell-mediated immune response is important in both the acute and convalescent phases of COVID-19. Delayed clinical deterioration in severe COVID-19 after an early peak in the viral load suggests the importance of the immune response in the progression of COVID-19 [7], and hyperactivated and uncontracted T cells and their infiltration of vital organs have been implicated.[8, 9] In addition, the memory T-cell response after the acute phase of COVID-19 is crucial for protection against recurrence or progression of the disease [6, 10].

Understanding the memory T-cell response to SARS-CoV-2 is critical to further examine the longevity of protective immunity after SARS-CoV-2 infection or vaccination [11]. The T-cell response at 2 months post-symptom onset (PSO) of COVID-19 has been reported [12, 13], and was stronger in patients who experienced severe illness than in those who had mild disease. However, there are few studies of the memory T-cell response after the first 2 months PSO. Although Dan *et al.* reported that SARS-CoV-2-specific $CD4^+$ or $CD8^+$ T cells declined with a half-life of 3–5 months after analyzing samples within and beyond 6 months PSO [14], most of the patients had mild COVID-19 and the evaluation was performed at a single time point. Therefore, the longitudinal memory T-cell response to SARS-CoV-2 up to 8 months PSO is unclear, especially depending on the severity of illness.

We examined the longitudinal memory T-cell response to SARS-CoV-2 at 2, 5, and 8 months PSO, stimulated by various SARS-CoV-2 antigens, in patients who experienced asymptomatic, mild, or severe illness.

Materials and Methods

Study design and participants

We analyzed peripheral blood mononuclear cells (PBMCs) of patients with COVID-19, which had been collected at 2, 5, and 8 months (± 2, 4, and 4 weeks, respectively) after diagnosis (asymptomatic patients) or disease onset. All patients were laboratory-confirmed with reverse-transcription polymerase chain reaction and were treated or monitored at Seoul National University Hospital or at a community treatment center in Daegu, Republic of Korea [15]. All asymptomatic patients were diagnosed during contact tracing in the midst of Daegu metropolitan city outbreak, March 2020 [16]. The Institutional Review Board of Seoul National University Hospital approved the study (No. H-2004-158-1118), and all participants provided written informed consent in accordance with the Declaration of Helsinki.

The asymptomatic patients were defined as those with a body temperature of < 37.5℃and without symptoms during their stay in the community treatment center despite undergoing a comprehensive medical interview twice daily [17]. Severe cases were defined as radiological pneumonia and an oxygen saturation of ≤ 93% in ambient air during their illness [18]. Others were classified as mild cases. Information on clinical characteristics—age, gender, the day of onset or diagnosis of COVID-19, maximal oxygen demand, and antiviral or anti-inflammatory drugs—was collected from the electronic medical records.

We analyzed PBMCs of SARS-CoV-2-seronegative healthy volunteers (healthy controls, HCs) during the pandemic (HC [2020]) who had neither been diagnosed of COVID-19 nor had received COVID-19 exposure notification [17]. Additionally, we analyzed PBMCs of those who had been infected with Middle East respiratory syndrome coronavirus (MERS-CoV) 5 years ago and donated their PBMCs in 2019 (HC [MERS]) as the second control group.

Collection of PBMCs and antigen stimulation

PBMCs were purified from heparinized peripheral whole blood using a Ficoll–Histopaque gradient (1.077 g/mL; GE Healthcare Life Sciences, Piscataway, NJ). They were stored in liquid nitrogen until analysis in freezing medium comprising 50% fetal bovine serum, 10% dimethyl sulfoxide (DMSO), and 40% RPMI-1640 (all reagents from Thermo Fisher Scientific, Waltham, MA) [19].

After thawing, the PBMCs (1×10^6 cells/mL) were stimulated with 2 µg/mL SARS-CoV-2 spike glycoprotein peptide pool, 2 µg/mL SARS-CoV-2 NCAP (nucleocapsid) peptide pool, or 2 µg/mL SARS-CoV-2 VME1 (membrane protein) peptide pool (all peptide pools purchased from JPT Peptide Technologies, Berlin, Germany), respectively, for 16–18 hours in the presence of 10 µg/mL anti-human CD28/CD49d antibodies (Abs) (BD Biosciences, San Jose, CA) for co-stimulation. PBMCs stimulated with 4 µg/mL CEF peptide pool (Mabtech AB, Hamburg, Germany), composed of well-defined peptides derived from cytomegalovirus (C), Epstein-Barr virus (E) and influenza virus (F), were used as the positive control. DMSO was used as the negative control. A fluorescein isothiocyanate–anti-human CD4 Ab (clone RPA-T4, BD Bioscience) was applied concomitantly with antigen stimulation. PBMCs were treated with BD Golgistop™ (Monensin, BD Biosciences) for the final 4 hours of the antigen stimulation.

Intracellular cytokine staining and flow cytometry

After stimulation, dead cells were stained with Fixable Viability Dye eFluor 506 (Thermo Fisher Scientific). Surface antigens were stained with Alexa Fluor® 700–anti-human CD8 (clone, RPA-T8), BUV395–anti-human CD137 (clone, 4B4-1), phycoerythrin (PE)-CF594–anti-human OX40 (clone, ACT35), and PE–anti-human CD69 (clone, FN50,) Abs. After fixation and permeabilization with a Cytofix/Cytoperm kit (BD Biosciences), PBMCs were incubated with PE-Cy7–anti-human interferon (IFN)-γ (clone, B27), eF450–anti-human tumor necrosis factor (TNF)-α, and allophycocyanin (APC)–anti-human interleukin (IL)-2 (clone, MQ1-17H12) Abs (all from BD Biosciences, except for APC–anti-IL-2 Ab, BioLegend, San Diego, CA). Brilliant Stain Buffer (BD Biosciences) was added to each sample.

Stained PBMCs were analyzed using an LSR II flow cytometer (BD Biosciences) and FACSDiva software with a minimum target event count of 500,000 cells. Data were analyzed using FlowJo software version 9.9.6 (TreeStar, Ashland, OR).

The frequencies of SARS-CoV-2-specific T cells (activation-induced markers, AIM^+ T cells; $OX40^+$ $CD137^+$ $CD4^+$ T cells or $CD69^+$ $CD137^+$ $CD8^+$ T cells) [20] or SARS-CoV-2-specific cytokine-producing cells (IFN-γ, TNF-α, and IL-2) among $CD137^+$ T cells were evaluated. T cells expressing two or more of IFN-γ, TNF-α, and IL-2 were determined by sequential gating and were regarded as polyfunctional cells (Supplementary Figure S1) [21].

The percentages of target populations in the unstimulated specimens (DMSO control) were subtracted from that in the antigen stimulated specimens to account for a nonspecific response [21]. If there was no available unstimulated specimen at the same time point, the mean percentages of samples at other time points from the same patient were used. The responses to the three SARS-CoV-2 antigens were calculated by summing the final value of the response to each antigen [20].

Statistical analyses

To compare the clinical characteristics of the asymptomatic, mild, and severe patients, the Kruskal-Wallis rank sum test or linear-by-linear association was performed. Data are expressed as means ± standard errors of the mean (SEMs) and as dot plots. When comparing the proportions of activated or cytokine-producing T cells between COVID-19 patients and HCs (2020), the Mann-Whitney *U* test with the Benjamini-Hochberg method for multiple comparisons was used. The Kruskal-Wallis rank-sum test with Dunn's *post hoc* test for multiple comparisons was used to compare frequencies according to disease severity.

$P < 0.05$ was considered indicative of statistical significance. All statistical analyses were two-tailed and performed using PASW for Windows (version 25.0; IBM Corp., Armonk, NY) and GraphPad Prism 8 (GraphPad Software, La Jolla, CA). Graphs were generated using Prism 8.

Results

Participants

A total of 24 patients—seven asymptomatic and nine with mild and eight with severe disease—were analyzed (Table 1). No patient had evidence of immunodeficiency or a history of re-exposure to COVID-19 or confirmed patients during the follow-up period. The median (range) ages of the asymptomatic, mild, and severe patients were 25 (20–28), 48 (24–69), and 63 (39–76) years, respectively (P = 0.001). Regarding anti-inflammatory treatment, one mild and three severe patients received baricitinib [22], and two severe patients received a therapeutic dose of steroid. The demographics, disease severity, treatment, details on availability of samples from each patient, and timing of sample collections are shown in Supplementary Table S1.

The HC (2020) group comprised six healthy volunteers who donated their blood in September 2020. Their median (range) age was 35 (28–47) years, and five (83.3%) were male (Table 1). The HC (MERS) group comprised seven blood samples from MERS survivors obtained in October 2019. Their median (range) age at the time of donation was 60 (38–64) years, and five (85.7%) were male.

Distribution of SARS-CoV-2-specific memory $CD4^+$ or $CD8^+$ T cells

The frequency of SARS-CoV-2-specific ($OX40^+CD137^+$) $CD4^+$ T cells (Figure 1A) in the patients with COVID-19, especially in those with severe disease, was higher at 8 months PSO than those in the HCs (2020) (Figure 1B and Supplementary Figure S2). The proportion of Ag-specific memory $CD4^+$ T cells responding to spike protein was similar to that to the nucleocapsid and membrane proteins. The responses to the three antigens were significantly higher in patients with mild and severe disease than those of HCs (2020) (Figure 1C), especially in symptomatic patients, up to 8 months PSO. The frequency of SARS-CoV-2-specific $CD4^+$ T cells was significantly higher in the patients with severe disease than in the asymptomatic patients at 2 and 5 months PSO (Figure 1B). A similar albeit nonsignificant trend was detected in comparison with the patients with mild disease (Figure 1B). The

frequency of SARS-CoV-2-specific memory $CD4^+$ T cells tended to decline over time in all severity groups, and the significance of the differences among the groups decreased.

The SARS-CoV-2-specific ($CD69^+CD137^+$) memory $CD8^+$ T-cell response in patients with COVID-19 was also distinct when compared to the HCs (2020) at 8 months PSO (Figure 2 and Supplementary Figure S3), similar to those of memory $CD4^+$ T cells. However, the level of SARS-CoV-2-specific $CD8^+$ T cells was not significantly different among the severity of COVID-19 patients (Figure 2B). Paired dot-plots of SARS-CoV-2-specific memory $CD4^+$ or $CD8^+$ T-cell responses through 2, 5, and 8 months PSO according to the severity showed similar trends (Supplementary Figure S4A). Heatmap shows more robust memory responses in severe patients than in mild or asymptomatic patients, especially in $CD4^+$ T cells (Supplementary Figure S5).

Additionally, there was no significant difference among healthy subjects and patients with COVID-19 in the CEF peptide-specific memory response (positive control) (Supplementary Figure S2 and 3), suggesting that the magnitude of the CEF peptide-specific memory response is not markedly affected by SARS-CoV-2 infection. Collectively, a broad (covering various SARS-CoV-2 antigens) memory T-cell response was induced after recovery from COVID-19 and persisted up to 8 months PSO. The magnitude of the $CD4^+$ T-cell memory response tended to be greater in the patients with severe compared to those with mild disease.

Functionality of memory T cells responding to SARS-CoV-2 antigens

To assess the functional competence of SARS-CoV-2-specific ($CD137^+$) memory $CD4^+$ T cells, we measured cytokine production by $CD4^+$ T cells responding to spike, nucleocapsid, and membrane proteins (Figure 3A–C) from patients who were asymptomatic and from those with mild and severe disease. The levels of IFN-γ, TNF-α, and IL-2 in memory $CD4^+$ T cells at 2 and 5 months PSO in patients with COVID-19 tended to be higher than those in HCs (2020) (Figure 3D–F and Supplementary Figure S1). The proportion of IL-2-producing memory $CD4^+$ T cells responding to spike protein from patients with mild and severe disease was higher than that from HCs (2020) even

at 8 months PSO (mean [± SEM], 213 [± 76] in mild disease *vs.* 23 [± 9] in HCs [2020]; adjusted *P* = 0.0261; 293 [± 75] in severe disease *vs.* 23 [± 9] in HCs [2020]; adjusted *P* = 0.0648).

IFN-γ, TNF-α, and IL-2-production by Ag-specific memory $CD4^+$ T cells in patients with severe disease was significantly increased compared to that of asymptomatic patients at 2 months PSO (Figure 3D). Similar to the proportion of Ag-specific memory $CD4^+$ T cells (Figure 1), the functionality of Ag-specific memory $CD4^+$ T cells declined over time, and the significance of the differences among patients with asymptomatic, mild, and severe disease decreased. However, the proportions of cytokine-producing Ag-specific memory $CD8^+$ T cells were not significantly different according to disease severity (Figure 4A–F). Therefore, the functionality of memory $CD4^+$ T cells responding to SARS-CoV-2 antigens was greatest in symptomatic patients. The similar trends are also observed in paired dot-plots or heatmaps of cytokine productions stimulated by spike protein (Supplementary Figure S4B and S5).

Longitudinal analysis of polyfunctional memory T-cell responses

To evaluate further the functionality of memory $CD4^+$ T cells responding to SARS-CoV-2 antigens, we examined the frequencies of polyfunctional T cells.[20] IFN-γ^+TNF-α^+, IFN-γ^+IL-2^+, TNF-α^+IL-2^+, or triple-positive cells among $CD4^+$ T cells responding to spike protein were more dominant in patients with mild or severe disease up to 5 months PSO compared to the HCs (2020) (Figure 5A–D). The proportion of polyfunctional $CD4^+$ T cells also tended to be higher in patients with severe disease than in those with mild disease or in asymptomatic patients (Figure 5).

Discussion

We analyzed longitudinal memory T-cell responses up to 8 months PSO, in terms of frequency and functionality, to SARS-CoV-2 antigens in patients with COVID-19 according to disease severity. SARS-CoV-2-specific memory $CD4^+$ or $CD8^+$ T cells slowly decline up to 8 months PSO. Memory T-cell responses tended to be stronger in symptomatic than in asymptomatic patients, especially in those

with severe disease. The spike, nucleocapsid, and membrane proteins stimulated similar memory T-cell response patterns.

After the early reports on efficacy of COVID-19 vaccines [3, 4], several countries have initiated national vaccination programs. However, the correlation between protection against COVID-19 and the longevity of the immunity induced by vaccination is unclear [5]. Our findings on the memory T-cell response of patients with COVID-19 of differing severities could be used as reference data for studies of the cellular immunogenicity of COVID-19 vaccines.

SARS-CoV and MERS-CoV induce long-term cell-mediated immune responses [23, 24]. However, the degree and longevity of the memory response to SARS-CoV-2 according to disease severity was unknown. Our results show that a memory T-cell response persists up to 8 months PSO, particularly in patients with severe COVID-19. Further long-term and larger studies are warranted to characterize the magnitude and duration of the protective effect.

Although we could not explore the relationship between the numbers of AIM^+ T cells and the magnitude of antibody response [25], decreased, but persistent cellular response to COVID-19 up to 8 months PSO were similar to the humoral response [14, 26]. Since Dan *et al* reported that circulating follicular helper (Tfh) memory $CD4^+$ T cells which enhance B-cell function were maintained until 8 months PSO [14], similar kinetics might be mediated by circulating Tfh memory $CD4^+$ T cells. In addition, increased memory T-cell response with disease severity was also similar to the humoral response [27]. Such a severity-dependent response may be attributed to the delayed but strong type I IFN response in the acute phase of severe COVID-19 [28], because the type I IFN response contributes to the memory formation in response to viral infection [29]. Similar responses were observed when PBMCs were stimulated with SARS-CoV-2 nucleocapsid and membrane proteins, in agreement with previous reports [12, 20].

IFN-γ and TNF-α production by activated $CD4^+$ T cells in patients with COVID-19 was robust up to 2 or 5 months PSO; however, it decreased at 8 months PSO despite the abundance of SARS-CoV-2-specific T cells. In contrast, IL-2 production was greater in symptomatic patients up to 8 months PSO.

Notably, IFN-γ enzyme-linked immunospot assay of the cell-mediated immune response during the late convalescent phase could yield a false-negative result.

Pre-existing SARS-CoV-2-reactive T cells might have been induced by seasonal coronaviruses [13, 20]. One could accurately examine the degree of COVID-19-specific T-cell responses if they had pre-COVID-19 PBMCs, which is impractical. To minimize this concern, we subtracted the frequencies in unstimulated samples to determine the SARS-CoV-2-specific response. In addition, the responses in terms of all three cytokines in $CD4^+$ T cells were robust at 2 months but decreased over time. These kinetics imply that the responses measured in this study were COVID-19-specific.

We analyzed two control groups to compensate for confounding by seasonal coronavirus- and/or MERS-CoV-reactive T cells. Interestingly, the frequency of SARS-CoV-2 spike-specific $CD4^+$ T cells in HCs (2020), which represents HC during the COVID-19 pandemic, was considerably higher than that of HCs (MERS) (Figure 1B). Cytokine production by Ag-specific memory $CD4^+$ T cells from HCs (2020) was lower than that of HCs (MERS) and similar to that of asymptomatic patients (Figure 5). The mechanism underlying this frequency-functionality discordance is unclear. The composition of a truly HC group in the COVID-19 era necessitates further research.

The memory response was less prominent in $CD8^+$ T cells than in $CD4^+$ T cells, as reported previously [30]. However, the possibility of suboptimal stimulation of $CD8^+$ T cells by the 15-mer peptides pool could not be excluded because major histocompatibility complex (MHC) class Ⅰhas a shorter binding groove (typically 8–10 residues) than MHC class II [31]. Moreover, Tarke *et al.* recently reported that epitope pools could be helpful to optimize detection of T cell responses because of HLA binding-related immunodominance. Therefore, further evaluation using either a shorter peptide or epitope megapool is warranted [32].

This study had several limitations. First, since we could analyze a small number of samples, the results of statistical analyses should be interpreted with caution. Similarly, we could not draw a meaningful severity-specific decay rate of SARS-CoV-2-specific T cells in this study. Second, the age distribution differed among the severity groups. Further validation using a larger, age-matched

cohort is therefore needed. Third, baricitinib or steroid treatment in severe group could have affected memory response [33]. Lastly, we could not account initial viral load in the present study. Although inoculum size of SARS-CoV-2 might affect severity of COVID-19 [34], viral load itself could influence establishment or longevity of memory T-cell response.

In conclusion, a memory T-cell response, in terms of frequency and functionality, persisted up to 8 months PSO, particularly in symptomatic patients. Further studies of the protective effect of the memory T-cell response to SARS-CoV-2 and the kinetics at ≥ 8 months are needed.

Author contributions

C.K.K., M.K., H.-R.K. and M.-d.O. conceived and designed the project. C.K.K., M.K., H.-R.K., and M.-d.O. analyzed the data. C.K.K., P.G.C., W.B.P., and N.J K. collected the human PBMCs, C.K.K., M.K., S.L., G.K., C.-H.L., I.S.K., K.J., D.-S.L., H.M.S., and H.-R.K. performed flow cytometric analysis. C.K.K, M.K., H.-R.K., and M.-d.O. wrote the manuscript with the help from all authors. M.-d.O. and H.-R.K. had full access to all data in the study and took responsibility for the integrity of the data, as well as for the manuscript.

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Figure legends

Figure 1. SARS-CoV-2-specific $CD4^+$ T cells up to 8 months post-symptom onset (PSO). (A) Representative gating strategy for SARS-CoV-2-specific ($OX40^+CD137^+$) $CD4^+$ T cells. (B) SARS-CoV-2-specific $CD4^+$ T cells according to the severity of illness and months PSO responding to different antigens. (C) SARS-CoV-2-specific $CD4^+$ T cells responding to all three antigens. Comparisons between healthy control (2020) group and each severity groups were performed by Mann-Whitney *U* test with Benjamini-Hochberg method for multiple comparison. Frequencies among three different severity groups were compared with Kruskal-Wallis test with Dunn's *post hoc* test for multiple comparison. * $P < 0.05$; ** $P < 0.01$; NS, not significant

Figure 2. SARS-CoV-2-specific $CD8^+$ T cells up to 8 months post-symptom onset (PSO). (A) Representative gating strategy for SARS-CoV-2-specific ($CD69^+CD137^+$) $CD8^+$ T cells. (B) SARS-CoV-2-specific $CD8^+$ T cells according to the severity of illness and months PSO responding to different antigens. (C) SARS-CoV-2-specific $CD8^+$ T cells responding to all three antigens. Comparisons between healthy control (2020) group and each severity groups were performed by Mann-Whitney *U* test with Benjamini-Hochberg method for multiple comparison. Frequencies among three different severity groups were compared with Kruskal-Wallis test with Dunn's *post hoc* test for multiple comparison. * $P < 0.05$; ** $P < 0.01$; NS, not significant

Figure 3. SARS-CoV-2-specific cytokine-producing $CD4^+$ T cells up to 8 months post-symptom onset (PSO). (A–C) Representative gating strategy for the expression levels of IFN-γ, TNF-α, and IL-2 in $CD137^+$ $CD4^+$ T cells, respectively. (D–F) IFN-γ, TNF-α, and IL-2 productions in $CD137^+$ $CD4^+$ T cells according to the severity of illness and months PSO responding to different antigens. Comparisons between healthy control (2020) group and each severity groups were performed by Mann-Whitney *U* test with Benjamini-Hochberg method for multiple comparison. Frequencies among three

different severity groups were compared with Kruskal-Wallis test with Dunn's *post hoc* test for multiple comparison. * $P < 0.05$; ** $P < 0.01$; NS, not significant

Figure 4. SARS-CoV-2-specific cytokine-producing $CD8^+$ T cells up to 8 months post-symptom onset (PSO). (A–C) Representative gating strategy for the expression levels of IFN-γ, TNF-α, and IL-2 in $CD137^+$ $CD8^+$ T cells, respectively. (D–F) IFN-γ, TNF-α, and IL-2 productions in $CD137^+$ $CD8^+$ T cells according to the severity of illness and months PSO responding to different antigens. Comparisons between healthy control (2020) group and each severity groups were performed by Mann-Whitney *U* test with Benjamini-Hochberg method for multiple comparison. Frequencies among three different severity groups were compared with Kruskal-Wallis test with Dunn's *post hoc* test for multiple comparison. * $P < 0.05$; ** $P < 0.01$; NS, not significant

Figure 5. Polyfunctional SARS-CoV-2-specific $CD4^+$ T cells up to 8 months post-symptom onset (PSO). (A–D) IFN-γ^+TNF-α^+, IFN-γ^+IL-2^+, TNF-α^+IL-2^+, or IFN-γ^+TNF-α^+IL-2^+ $CD4^+$ T cells according to the severity of illness and months PSO responding to different antigens, respectively. Comparisons between healthy control (2020) group and each severity groups were performed by Mann-Whitney *U* test with Benjamini-Hochberg method for multiple comparison. Frequencies among three different severity groups were compared with Kruskal-Wallis test with Dunn's *post hoc* test for multiple comparison. * $P < 0.05$; ** $P < 0.01$; NS, not significant

Table 1. Clinical characteristics of completely asymptomatic, mild, and severe patients with COVID-19

	HC [2020] (*n* = 6)	HC [MERS] (*n* = 7)	Completely asymptomatic (*n* = 7)	Mild (*n* = 9)	Severe (*n* = 8)	*P-value*
Age, median years (range)	35 (28–47)	60 (38–64)	25 (20–28)	48 (24–72)	63 (39–76)	0.001
Male gender, *n* (%)	5 (83.3)	6 (85.7)	5 (71.4)	4 (44.4)	6 (75.0)	0.364
Maximal oxygen demand, median (range)	*n.a.*	*n.a.*	*n.a.*	*n.a.*	HFNC (NP to MV)	*n.a.*
Treatment	*n.a.*	*n.a.*				
Lopinavir/ritonavir			0 (0)	2 (22.2)	2 (25.0)	0.213
Remdesivir			0 (0)	1 (11.1)	7 (87.5)	< 0.001
Baricitinib			0 (0)	1 (11.1)	3 (37.5)	0.054
Steroid			0 (0)	0 (0)	2 (25.0)	0.079
Days of sample collection from the onset of COVID-19, median (range)	*n.a.*	*n.a.*				

For 2 months	57 (55–61)	62 (40–68)[a]	65 (39–73)	0.281
For 5 months	133 (131–137)	134 (102–169)	127 (99–157)	0.365
For 8 months	231 (229–235)	235 (192–253)[a]	196 (185–198)[a]	0.004

HC, healthy control; MERS, Middle East respiratory syndrome; *n.a.*, not applicable; HFNC, high flow nasal canula; NP, nasal prong; MV, mechanical ventilation

[a]one missing value

Figure 1

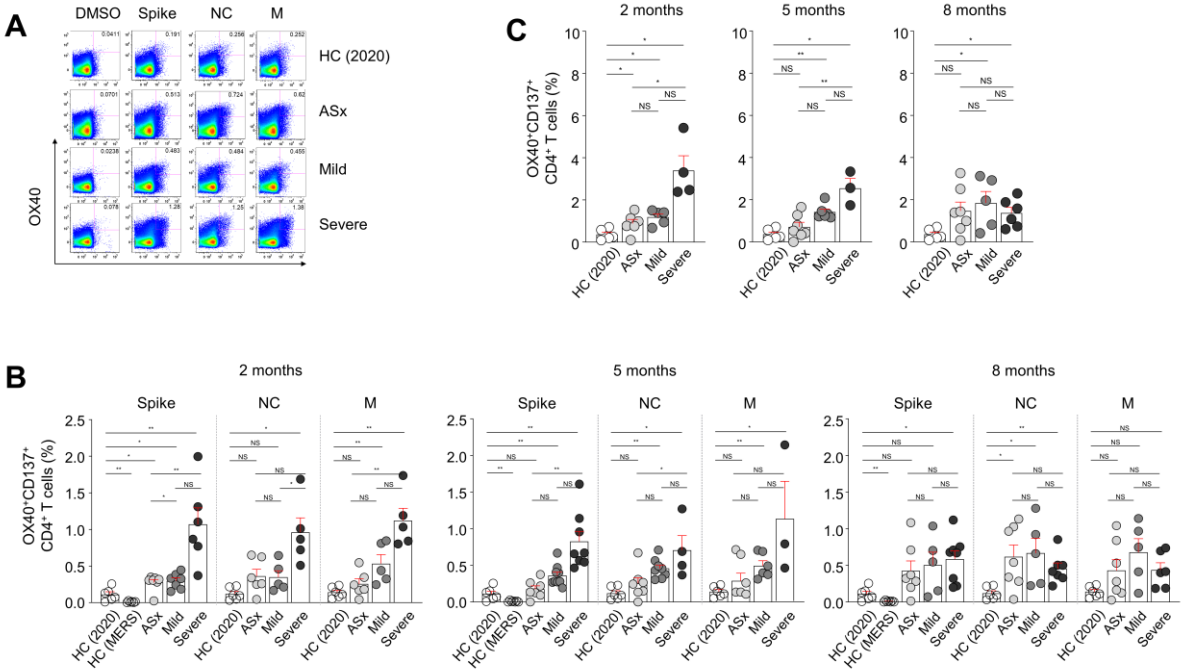


Figure 2

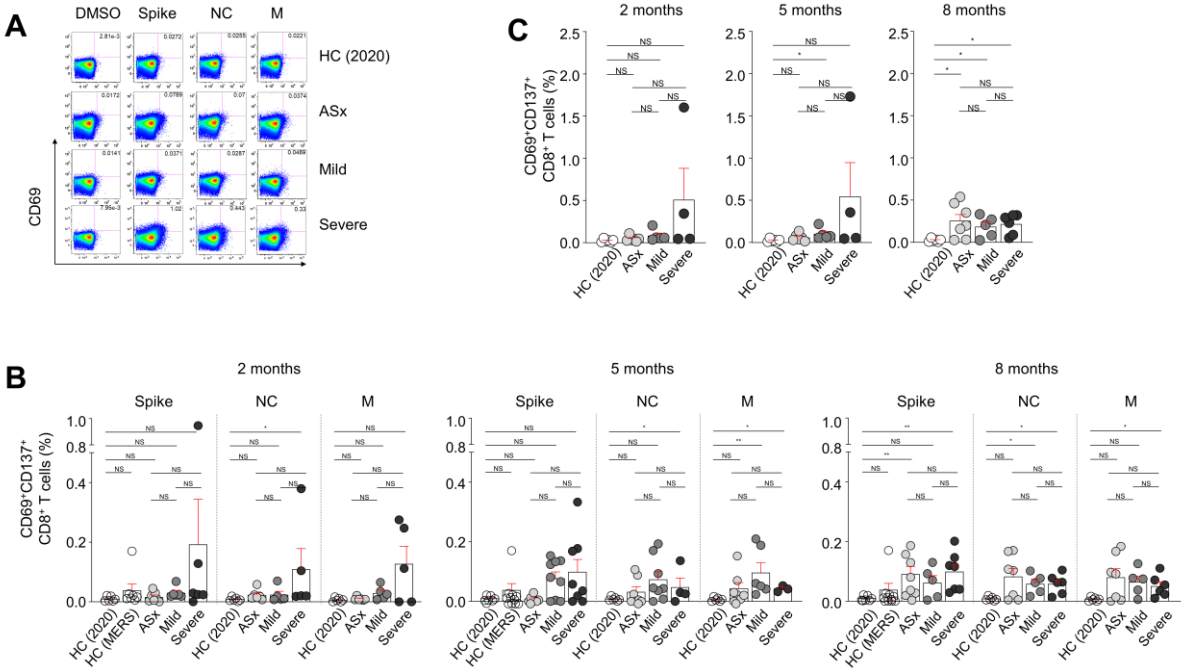


Figure 3

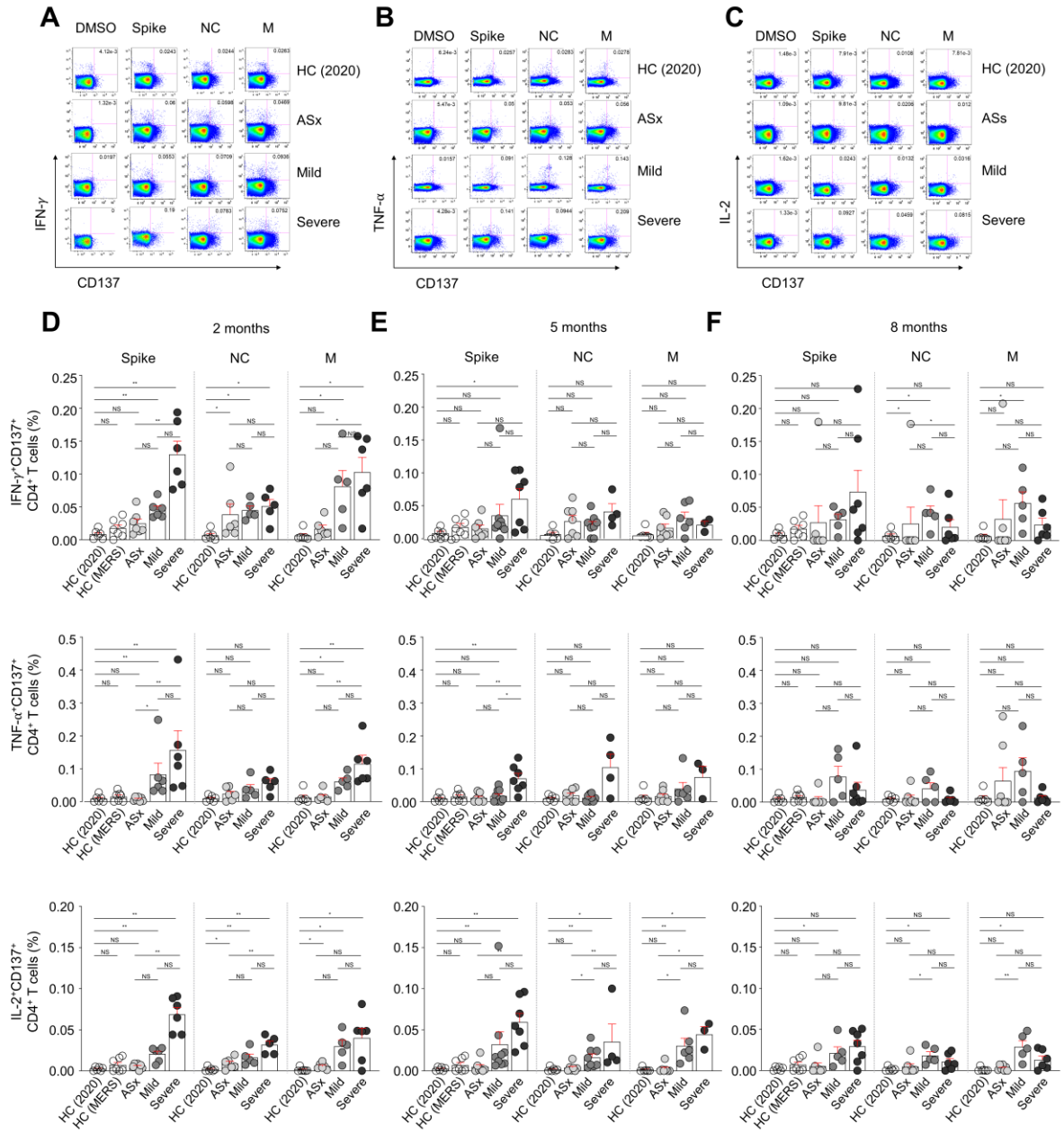

A
DMSO
Spike
NC
M
HC (2020)
ASx
Mild
Severe
IFN-γ
CD137
B
TNF-α
C
ASs
IL-2
D
2 months
E
5 months
F
8 months
IFN-γ+CD137+ CD4+ T cells (%)
TNF-α+CD137+ CD4+ T cells (%)
IL-2+CD137+ CD4+ T cells (%)
HC (MERS)

Figure 4

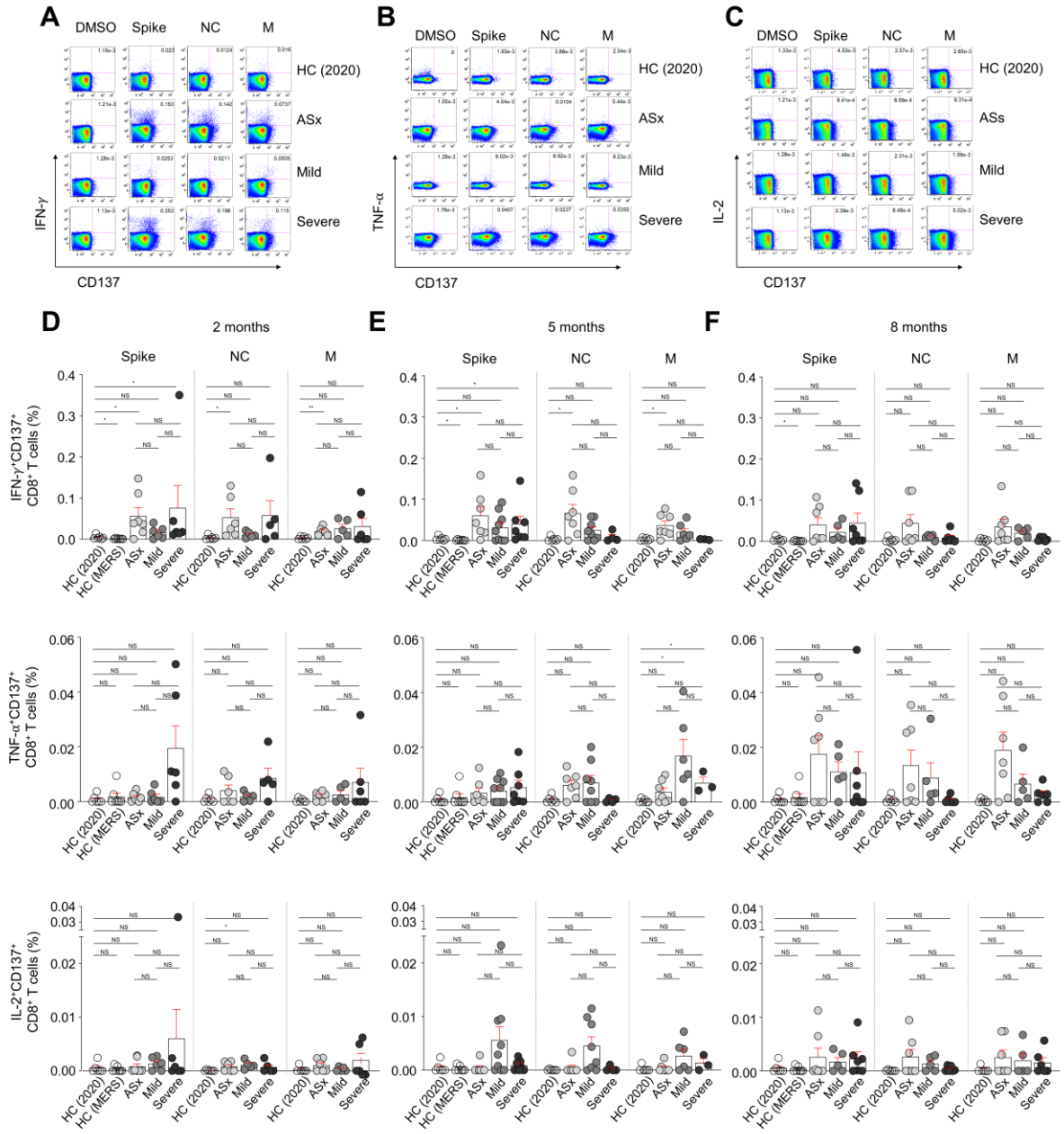
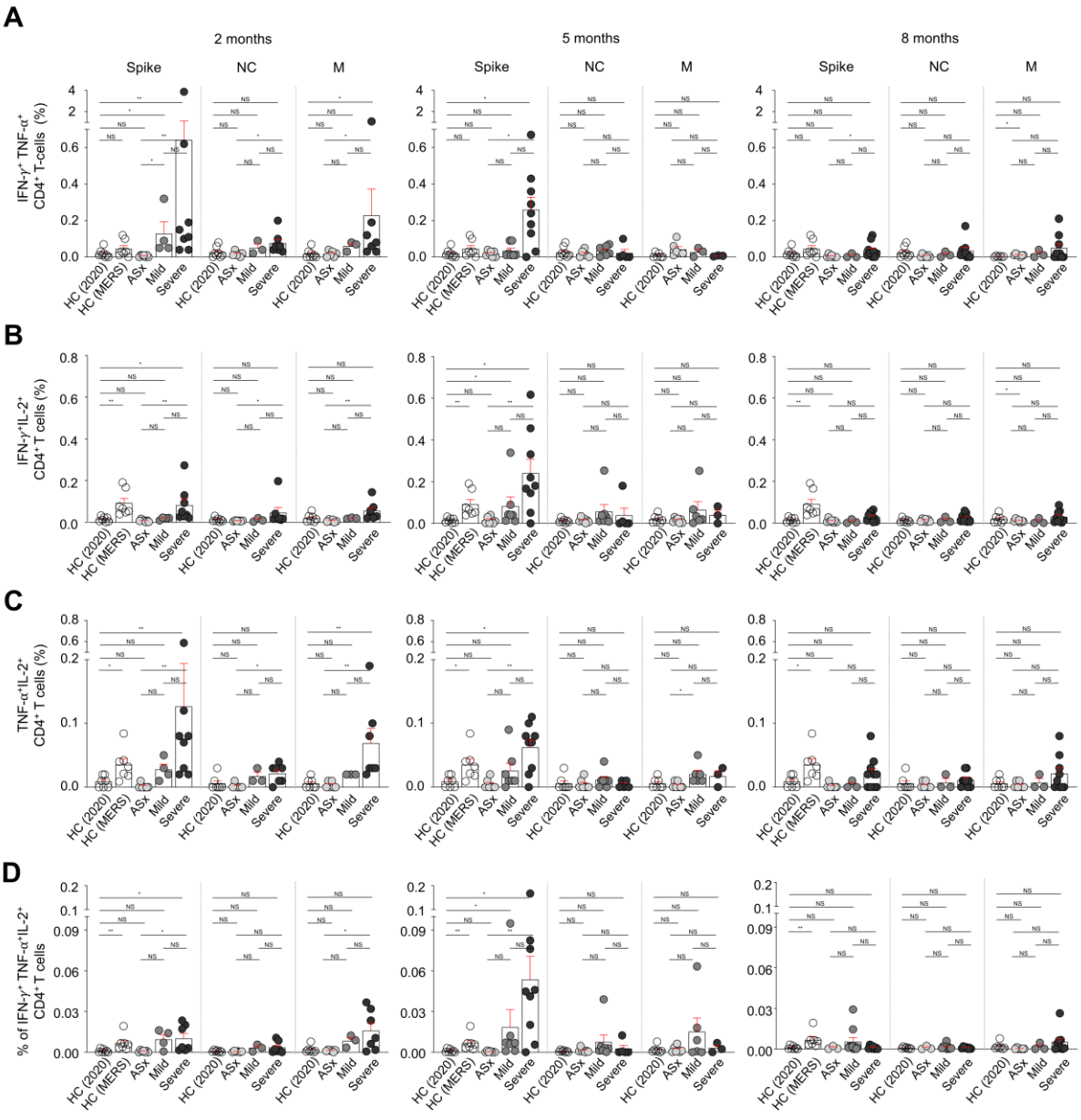

A
DMSO
Spike
NC
M
HC (2020)
ASx
Mild
Severe
IFN-γ
CD137
B
TNF-α
C
ASs
IL-2
D
2 months
E
5 months
F
8 months
IFN-γ+CD137+ CD8+ T cells (%)
TNF-α+CD137+ CD8+ T cells (%)
IL-2+CD137+ CD8+ T cells (%)
HC (MERS)

Figure 5


A
2 months
5 months
8 months
Spike
NC
M
IFN-γ+ TNF-α+ CD4+ T-cells (%)
B
IFN-γ+IL-2+ CD4+ T cells (%)
C
TNF-α+IL-2+ CD4+ T cells (%)
D
% of IFN-γ+ TNF-α+IL-2+ CD4+ T cells
HC (2020)
HC (MERS)
ASx
Mild
Severe
NS