# Persistence of a SARS-CoV-2 T-cell response in patients with long COVID and lung sequelae after COVID-19



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*To the Editor:*

Most individuals fully recover after suffering coronavirus disease 2019 (COVID-19), but a subset of patients present persistent post-disease abnormalities, including 1) long COVID, defined as the persistence (>2 months) of symptoms 3 months after the onset of COVID-19 that cannot be explained by an alternative diagnosis [1]; and 2) chronic pulmonary sequelae, defined as abnormal lung function and/or lung structure [2–5]. The mechanisms underlying long COVID or pulmonary sequelae remain unknown, but both have been associated with persistent inflammation [6, 7], including alterations in the blood $CD8^+$ T-cells and a sustained virus-specific T-cell response [8–10]. Additionally, recent studies suggest that the persistence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA in some organs may drive chronic activation of B-cells and inflammation [6, 11, 12].

In a previous study on convalescent COVID-19 patients studied at the short term (6 months after hospital discharge) with pulmonary sequelae, we reported the persistence of a virus-specific T-cell response [13]. Whether this is also associated with long COVID and pulmonary sequelae at a later stage (12 months after discharge) or with the change in the diffusing capacity of the lungs for carbon monoxide ($D_{LCO}$) from 6 to 12 months, has not been explored so far. To investigate it, in this prospective observational study we included 40 adults at 12 months post-discharge: 11 with long COVID [1]; 17 with pulmonary sequelae ($D_{LCO}$ <80% predicted) [3, 4]; and 12 who had suffered a COVID-19 episode, but at 12 months were fully recovered (controls). All of them had been hospitalised in our institution because of a PCR-confirmed COVID-19 pneumonia. In this study we included patients followed in the clinic after hospital discharge because of 1) respiratory symptoms, according to the Spanish Society of Pulmonology and Thoracic Surgery (SEPAR) consensus for follow-up of post-COVID-19 patients [11]; and/or 2) complained of fatigue, chest pain, arthralgia, myalgia, headache, neurocognitive dysfunction or autonomic dysfunction. Symptoms were evaluated using the 36-item short form (SF-36) and fatigue structured questionnaires [1, 14, 15]. Forced spirometry and $D_{LCO}$ were measured following international recommendations [16, 17]. To address the unavoidable limitation that lung function had not been determined before hospitalisation because of the acute COVID-19 episode, only patients without any previously known pulmonary disease were included in the study. The Strengthening the Reporting of Observational Studies in Epidemiology guidelines were used in the reporting of this observational study; the ethical review board of our institution approved it (HCB/2020/0422); and all patients signed their informed consent.

In this study we used the same methodology described in our previous study at 6 months [13]. Briefly, we used flow cytometry in peripheral blood mononuclear cells (PBMCs) to quantify different $CD4^+$ and $CD8^+$ lymphocyte subpopulations at 12 months. In each patient, PBMCs were obtained and stimulated for 10 days at 0.5 $\mu g \cdot mL^{-1}$ with a specific SARS-CoV-2 peptide pool of the S (spike) or N (nucleocapsid), purchased from Miltenyi Biotec (USA; 130-126-701 and 130-126-699, respectively). At day 10, cells were restimulated with 2.5 $\mu g \cdot mL^{-1}$ individual virus-specific peptide pools and (1:100) FastImmune (BD, USA) for 2 h, followed by the addition of 10 $\mu g \cdot mL^{-1}$ Brefeldin A (Sigma, Germany) for four additional hours. Stimulated PBMC were then analysed by flow cytometry using the same staining and intracellular determination of interferon-γ. The expansion of specific populations in response to this stimulation is presented as fold change (*i.e.* frequency of the population in stimulated PBMCs divided by frequency in unstimulated cells).

Shareable abstract (@ERSpublications)
**1 year after an acute COVID-19 episode, patients with either lung sequelae or long COVID show a stronger SARS-CoV-2-specific T-cell response than fully recovered individuals, suggesting persistent cell stimulation by residual viral reservoirs** https://bit.ly/40bPZm7

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**TABLE 1** Main characteristics of the study groups and key cell population findings at basal frequencies and under coronavirus disease 2019 (COVID-19)-specific S and N peptides

| | Recovered | Pulmonary sequelae | p-value | Recovered | Long COVID | p-value |
|---|---|---|---|---|---|---|
| **Participants** | 12 | 17 | | 12 | 11 | |
| **Age (years)** | 55.6±13.9 | 55.6±11.4 | 0.999 | 55.6±13.9 | 48.5±13.8 | 0.237 |
| **Males** | 6 (50.0) | 10 (58.8) | 0.927 | 6 (50.0) | 9 (81.8) | 0.193 |
| **$D_{LCO}$ at 12 months (% predicted)** | 91.7 (83.1–101) | 71.2 (67.4–75.6) | **<0.001** | 91.7 (83.1–101) | 89.7 (87.8–91.7) | 0.877 |
| **Lung sequelae at 12 months (yes)** | 0 (0.00) | 17 (100) | **<0.001** | 0 (0.00) | 0 (0.00) | 0.1 |
| **$D_{LCO}$ change (from 6 to 12 months)#** | 11.8±18.5 | −7.12±15.3 | **0.03** | 11.8±18.5 | −16.02±13.4 | 0.131 |
| **Extrapulmonary symptoms (yes)** | 0 (0.00) | 11 (64.7) | **0.001** | 0 (0.00) | 11 (100) | **<0.001** |
| **WHO severity** | 5.00 (3.75–5.00) | 5.00 (3.00–6.00) | 0.7 | 5.00 (3.75–5.00) | 1.00 (1.00–2.00) | **0.003** |
| **ICU admission (yes)** | 7 (58.3) | 8 (47.1) | 0.825 | 7 (58.3) | 0 (0.00) | **0.005** |
| **ICU (days)** | 2.50 (0.00–5.25) | 0.00 (0.00–14.0) | 0.655 | 2.50 (0.00–5.25) | 0.00 (0.00–0.00) | **0.004** |
| **Corticoids** | 2 (18.2) | 5 (29.4) | 0.79 | 2 (18.2) | 0 (0.00) | 0.129 |
| **Vaccine doses** | | | 0.774 | | | **0.015** |
| 0 | 6 (50.0) | 9 (52.9) | | 6 (50.0) | 0 (0.00) | |
| 1 | 5 (41.7) | 5 (29.4) | | 5 (41.7) | 7 (63.6) | |
| 2 | 1 (8.33) | 3 (17.6) | | 1 (8.33) | 4 (36.4) | |
| **Basal frequencies of T-cell subpopulations** | | | | | | |
| CD4 $HLA^+$ (% of CD4) | 4.72 (3.38–8.00) | 4.43 (3.45–5.08) | 0.492 | 4.72 (3.38–8.00) | 2.68 (2.16–5.46) | 0.085 |
| CD4 effector $HLA^+$ (% of CD4 effector) | 9.70 (5.37–13.6) | 7.79 (5.36–11.3) | 0.842 | 9.70 (5.37–13.6) | 9.34 (4.30–11.8) | 0.735 |
| CD4 CM $HLA^+$ (% of CD4 CM) | 3.24 (2.28–3.97) | 2.64 (2.05–3.30) | 0.177 | 3.24 (2.28–3.97) | 1.63 (1.19–2.73) | 0.069 |
| CD4 EM $HLA^+$ (% of CD4 EM) | 13.5 (4.75) | 10.9 (4.37) | 0.143 | 14.2 (10.2–17.2) | 6.65 (5.70–7.60) | **0.006** |
| CD4 naïve $HLA^+$ (% of CD4 naïve) | 1.54 (1.19–3.54) | 2.39 (1.89–2.87) | 0.4 | 1.54 (1.19–3.54) | 1.14 (0.62–2.52) | 0.196 |
| CD4 Th1 (% of CD4) | 1.90 (1.37–3.91) | 1.39 (1.08–2.40) | 0.215 | 1.90 (1.37–3.91) | 0.83 (0.62–1.97) | **0.019** |
| CD4 $Th17^-$ (% of CD4) | 6.62 (3.10) | 6.22 (3.28) | 0.74 | 6.76 (5.16–8.55) | 3.01 (2.57–5.48) | 0.11 |
| CD8 $HLA^+$ (% of CD8) | 12.3 (5.34–23.0) | 9.33 (6.57–17.0) | 0.4 | 12.3 (5.34–23.0) | 5.43 (3.01–10.4) | **0.027** |
| CD8 effector $HLA^+$ (% of CD8 effector) | 18.4 (6.73–31.6) | 11.2 (7.09–19.7) | 0.376 | 18.4 (6.73–31.6) | 4.79 (3.86–13.4) | **0.031** |
| CD8 CM $HLA^+$ (% of CD8 CM) | 6.32 (4.43–10.2) | 4.63 (4.03–6.97) | 0.241 | 6.32 (4.43–10.2) | 3.63 (2.54–6.24) | **0.049** |
| CD8 EM $HLA^+$ (% of CD8 EM) | 14.1 (8.57–25.5) | 11.8 (9.09–13.8) | 0.413 | 14.1 (8.57–25.5) | 6.40 (4.96–12.3) | **0.042** |
| CD8 naïve $HLA^+$ (% of CD8 naïve) | 3.18 (1.84–5.80) | 2.83 (2.04–5.35) | 0.929 | 3.18 (1.84–5.80) | 0.99 (0.62–3.32) | **0.036** |
| **Responders to SARS-CoV-2 peptides** | | | | | | |
| N SARS-CoV-2 $CD4^+$ | 5 (41.66) | 8 (47.06) | 0.806 | 5 (41.66) | 6 (54.54) | 0.545 |
| N SARS-CoV-2 $CD8^+$ | 6 (50) | 10 (58.82) | 0.629 | 6 (50) | 5 (45.45) | 1 |
| S SARS-CoV-2 $CD4^+$ | 6 (50) | 12 (70.59) | 0.144 | 6 (50) | 6 (54.54) | 1 |
| S SARS-CoV-2 $CD8^+$ | 4 (33.33) | 9 (52.94) | 0.119 | 4 (33.33) | 7 (63.63) | **0.05** |
| Response to N or S | 6 (50) | 14 (82.36) | **0.012** | 6 (50) | 8 (72.72) | 0.22 |
| **Type of specific T-response to N SARS-CoV-2 peptides** | | | | | | |
| CD4 Th1 (% of CD4) | 1.05 (0.59–2.29) | 1.55 (1.10–2.51) | 0.132 | 1.05 (0.59–2.29) | 4.24 (1.21–5.24) | **0.031** |
| CD4 Th17 (% of CD4) | 4.40 (3.04–7.27) | 3.94 (2.28–8.44) | 0.626 | 4.40 (3.04–7.27) | 5.88 (4.55–6.61) | 0.389 |
| **Type of specific T-response to S SARS-CoV-2 peptides** | | | | | | |
| CD4 Th1 (% of CD4) | 0.71 (0.48–1.32) | 0.97 (0.80–1.94) | **0.046** | 0.71 (0.48–1.32) | 2.12 (1.10–2.73) | **0.041** |
| CD4 Th17 (% of CD4) | 4.59 (4.08–6.99) | 4.10 (2.42–9.60) | 0.535 | 4.59 (4.08–6.99) | 7.84 (5.85–10.2) | 0.166 |

Data are presented as n, mean±SD, n (%) or median (95% CI), unless otherwise stated. The Shapiro test was performed for each variable and the appropriate statistic test (t-test or Mann–Whitney) was performed accordingly to their distribution using the "compareGroups" R package. The difference in the percentage of responders was evaluated with a binomial statistic test. Bold type represents statistical significance. $D_{LCO}$: diffusing capacity of the lung for carbon monoxide; WHO: World Health Organization; ICU: intensive care unit; HLA: human leukocyte antigen; CM: central memory; EM: effector memory; Th1/17: type 1/17 T-helper cell; SARS-CoV-2: severe acure respiratory syndrome coronavirus 2. #: change in the $D_{LCO}$ volume between the lung function test at 6 and at 12 months ($D_{LCO}$ at 12 months minus $D_{LCO}$ at 6 months).

We studied 12 patients fully recovered after COVID-19 (controls), 11 with long COVID and 17 with pulmonary sequelae (table 1). By design, there were significant differences in $D_{LCO}$ at 12 months, and in $D_{LCO}$ change from 6 to 12 months post-discharge, as well as in the presence of extrapulmonary symptoms. At hospitalisation, patients with long COVID presented lower World Health Organization severity scores and required less intensive care unit admission. There were no differences regarding treatment with systemic corticosteroids during the acute COVID-19 episode.

Additionally, table 1 shows that, under basal conditions and compared to controls, patients with long COVID showed reduced expression of human leukocyte antigen-DR in all $CD8^+$ T-lymphocyte and T-effector memory $CD4^+$ T-lymphocyte subpopulations, as well as a reduction of the type 1 T-helper cell (Th1) response.

In response to stimulation with SARS-CoV-2 N or S peptides (table 1), we observed that 1) 50% of controls presented a positive *in vitro* T-cell response against SARS-CoV-2 12 months post-discharge; 2) the proportion of responders was higher in those with lung sequelae (82.36%; p=0.012) and long COVID (72.72%; p=0.22), without differences in the proportion of responders between patients with lung sequelae and long COVID; 3) $CD4^+$ Th1 cells increased in patients with long COVID in response to stimulation with both S and N SARS-CoV-2 peptides, while patients with lung sequelae responded to the S peptide only; and 4) we found a negative correlation between the ratio of $CD8^+CD28^-$ T-cells in response to SARS-CoV-2 N (r= −0.46; p=0.028) or S peptide (r= −0.49; p=0.019) stimulation at 12 months and the change in the $D_{LCO}$ from 6 to 12 months.

The main and novel findings of this study are, at discharge compared to fully recovered individuals (controls), 12 months after the acute COVID-19 episode, 1) patients with long COVID showed abnormal $CD8^+$ and $CD4^+$ T-lymphocyte subpopulation distributions at baseline, and an enhanced Th1 response to S and N peptides after SARS-CoV-2-specific stimulation; 2) patients with long-term lung sequelae also present a higher T-cell response to SARS-CoV-2, characterised by S-specific $CD4^+$ Th1-cells; and 3) the long-term worsening of $D_{LCO}$ from 6 to 12 months after the acute COVID-19 event correlates with an increase of SARS-CoV-2-specific T-cells with exhaustion features ($CD8^+CD28^-$). Our results are in line with previous work describing altered T-cell frequencies in patients with long COVID, but to our knowledge this is the first study describing a persistent virus-specific T-cell response in patients with lung sequelae.

In conclusion, collectively these observations suggest that patients with long COVID and those with lung sequelae have an exhausted SARS-CoV-2-specific T-cell response ($CD8^+CD28^-$), suggesting a nonresolved stimulation potentially driven by persistence of viral reservoirs [6, 12]. Further work is needed to understand the specific stimulation mechanism underlying the persistent T-cell response in these patients.

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