



# SARS-CoV-2 T-cell response in COVID-19 convalescent patients with and without lung sequelae

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Received: 27 Dec 2021  
Accepted: 10 Jan 2022

To the Editor:

Patients infected by SARS-CoV-2 may develop pneumonia (COVID-19), and require hospital admission and, eventually, critical care [1]. This has been related to a weaker innate immune response with impaired production of type I interferons (IFNs) [2]. In this setting, an antigen-specific T-cell response is needed for the elimination of SARS-CoV-2, as well as to develop long-lasting memory to respond to potential future SARS-CoV-2 infections [3, 4]. However, this response needs to be contained once the virus is eradicated to avoid further damaging the host.

Several studies have characterised the SARS-CoV-2 T-cell response in patients recovering from COVID-19 and showed that the intensity of the T-cell response relates to the severity of the acute pneumonia episode [5, 6]. Moreover, the severity of the disease is a risk factor for potential lung sequelae in COVID-19 survivors [7]. We recently reported that up to 57% of COVID-19 survivors present lung function abnormalities, particularly reduced carbon monoxide lung diffusion capacity ( $D_{LCO}$ ), 3 months after hospital discharge [8]. The relationship between the persistence of the specific T-cell response elicited during the acute COVID-19 episode and lung function abnormalities during follow-up is unknown.

To investigate these questions, we contrasted the *in vitro* T-cell response against the SARS-COV-2 spike (S) and the nucleocapsid (N) proteins, two well recognised viral antigens, in COVID-19 convalescent patients with normal and abnormal  $D_{LCO}$  6 months after hospital discharge.

This prospective, observational study included 25 adults who were hospitalised in our institution because of PCR-confirmed COVID-19 pneumonia and were studied at 6 months after hospital discharge. Participants were categorised according to their intensive care unit admission (or not) during the acute COVID-19 episode or by their  $D_{LCO}$  6 months after discharge (normal ( $\geq 80\%$  pred) or abnormal ( $< 80\%$  pred)). The study was approved by the Ethical Review Board of our hospital (HCB/2020/0422), and all patients provided signed informed consent.

Demographic, clinical and biological characteristics were recorded on hospital admission and 6 months after discharge. At the latter time point, spirometry was performed and  $D_{LCO}$  was measured (Medisoft, Sorinnes, Belgium) following international recommendations [9]. Likewise, blood was collected in EDTA tubes, and peripheral blood mononuclear cells (PBMCs) were isolated (Lymphoprep Abbott, Norway) and cryopreserved in fetal bovine serum (Gibco, US) and 10% dimethylsulfoxide. Pools of peptides covering the S and N proteins of SARS-CoV-2 were purchased from Miltenyi Biotec, USA (130-126-701 and 130-126-699 respectively). PBMCs from each donor were thawed, washed and: 1) an aliquot was stained with the antibodies listed below, to obtain the basal cell proportions; and 2) another aliquot was stimulated at  $2 \times 10^6$  cells·mL<sup>-1</sup> in X-Vivo plus 2% AB serum (Lonza, Belgium) with the S and N peptide pools (at  $0.5 \mu\text{g}\cdot\text{mL}^{-1}$ ) for 10 days. At day 10, cells were re-stimulated with  $2.5 \mu\text{g}\cdot\text{mL}^{-1}$  individual virus-specific peptide pools and (1/100) FastImmune (BD, USA) for 2 h following the addition of  $10 \mu\text{g}\cdot\text{mL}^{-1}$  brefeldin A (Sigma, Germany) for 4 h. Cells were stained with CD8-BV650, CD4-BV711, CD3-APC-R700, CD45-APC-H7, CD45RA-FITC, CD197-PECF594, CD196-PECy7, CXCR3-APC and IFNg-PE (BD) using Cytotfix/Cytoperm (BD). All samples were acquired using a LSRFortessa SORP (BD) and analysed by FlowJo (FlowJo LLC, USA). Lymphocyte subpopulations were analysed as the proportion of CD4 or CD8.



Shareable abstract (@ERSpublications)

**A specific T-cell response persists in the majority of COVID-19 patients 6 months after hospital discharge. This response is more prominent in those who required critical care during the acute COVID-19 episode but is reduced in patients with lung sequelae.** <https://bit.ly/3fBuVA4>

**Cite this article as:** Cruz T, Mendoza N, Perea L, *et al.* SARS-CoV-2 T-cell response in COVID-19 convalescent patients with and without lung sequelae. *ERJ Open Res* 2022; 8: 00706-2021 [DOI: 10.1183/23120541.00706-2021].



The expansion of specific populations is presented as fold change variations (*i.e.* the frequency of the population in stimulated PBMCs divided by the frequency of the population in unstimulated cells). Groups were compared using Mann–Whitney tests and analyses were performed using R version 3.6.1 or Prism 7 (GraphPad, La Jolla, CA, USA). A limitation of the study is the lack of lung function data prior the COVID-19 episode but only one patient in our study had a diagnosis of a lung condition (asthma) prior the COVID episode and this was not related to low  $D_{LCO}$  (table 1).

We studied 23 patients (61% males) with a mean $\pm$ SD age of 60.0 $\pm$ 10.5 years; 10 of them (43%) needed critical care during the acute COVID-19 episode and 14 of them (61%) had  $D_{LCO}$  <80% pred 6 months after discharge. Table 1 presents their main clinical and functional characteristics, and the study results.

A CD4 T-cell response to the S protein of SARS-CoV-2 (*i.e.* >2% of IFN- $\gamma$  producing cells after stimulation) was found in 70% of the patients and a CD8 response in 43%. Conversely, 57% of the patients responded with CD4 and 70% with CD8 T-cells to the N protein of SARS-CoV-2. Overall, a T-cell specific response to either the S or N proteins was observed in 20 of the 23 patients studied (87%). Both the S and N peptides induced expansion of CD4 T-effector memory re-expressing CD45RA (TEMRA) and T-effector memory (TEM) cells with a T-helper (Th)1 (CXCR3) and Th17 (CD196) polarisation, whereas the S and N peptides expanded TEM and T-central memory CD8 cells (table 1).

TABLE 1 Clinical characteristics and T-cell response of COVID-19 patients 6 months after hospital discharge

	All (n=23)	No ICU (n=13)	ICU (n=10)	p-value	$D_{LCO}$ >80% pred (n=9)	$D_{LCO}$ <80% (n=14)	p-value
Age, years	60.0 $\pm$ 10.5	60.2 $\pm$ 9.4	59.8 $\pm$ 12.3	0.9	55.4 $\pm$ 9.6	62.9 $\pm$ 10.4	0.13
Males	14 (61%)	8 (62%)	6 (60%)		6 (67%)	8 (57%)	
BMI, kg·m <sup>-2</sup>	30.2 $\pm$ 6.3	30.5 $\pm$ 5.3	29.9 $\pm$ 7.6	0.69	30.0 $\pm$ 8.3	30.4 $\pm$ 4.9	0.61
Previous lung disease <sup>#</sup>	1 (4%)	1 (8%)	0		1 (8%)	0	
WHO disease severity score	4.5 $\pm$ 1.4	3.6 $\pm$ 0.8	5.7 $\pm$ 1.2	<b>&lt;0.001</b>	3.6 $\pm$ 0.7	5.19 $\pm$ 1.4	<b>0.01</b>
$D_{LCO}$ at 6 months, % pred	82.2 $\pm$ 17.2	86.8 $\pm$ 17.2	76.3 $\pm$ 16.2	0.12	99.1 $\pm$ 14.9	71.4 $\pm$ 6.5	<b>&lt;0.001</b>
Sequelae	14 (61%)	6 (46%)	8 (80%)		0 (0%)	14 (100%)	
FEV <sub>1</sub> at 6 months, % pred	95.4 $\pm$ 13.1	99.4 $\pm$ 13.7	90.3 $\pm$ 10.4	0.14	104.9 $\pm$ 12.4	89.3 $\pm$ 9.6	<b>&lt;0.001</b>
FVC at 6 months, % pred	91.5 $\pm$ 14.2	95.2 $\pm$ 14.1	86.8 $\pm$ 13.6	0.12	104.1 $\pm$ 12.7	83.5 $\pm$ 8.0	<b>&lt;0.001</b>
FEV <sub>1</sub> /FVC at 6 months, %	78.6 $\pm$ 5.1	77.7 $\pm$ 5.2	79.9 $\pm$ 5.1	0.42	77.0 $\pm$ 4.8	79.7 $\pm$ 5.2	0.21
<b>Response to SARS-CoV-2 S peptides</b>							
CD4 IFN- $\gamma$ , %	5.1 $\pm$ 4.4	3.2 $\pm$ 3.4	7.5 $\pm$ 4.6	<b>0.05</b>	4.0 $\pm$ 3.3	5.8 $\pm$ 5.0	0.61
CD8 IFN- $\gamma$ , %	2.1 $\pm$ 1.7	2.2 $\pm$ 2.0	2.0 $\pm$ 1.3	0.50	2.0 $\pm$ 2.1	2.2 $\pm$ 1.5	0.56
FC CD4 TEMRA, %	6.3 $\pm$ 8.8	8.9 $\pm$ 10.8	2.8 $\pm$ 3.1	<b>0.03</b>	10.7 $\pm$ 12.4	3.4 $\pm$ 3.6	<b>0.02</b>
FC CD8 TEMRA, %	0.8 $\pm$ 0.5	0.9 $\pm$ 0.6	0.7 $\pm$ 0.2	<b>0.03</b>	1.1 $\pm$ 0.6	0.7 $\pm$ 0.2	<b>0.05</b>
FC CD4 TCM, %	0.6 $\pm$ 1.0	0.7 $\pm$ 1.3	0.5 $\pm$ 0.2	0.46	0.4 $\pm$ 0.2	0.8 $\pm$ 1.2	0.21
FC CD8 TCM, %	2.5 $\pm$ 4.8	1.9 $\pm$ 3.1	1.1 $\pm$ 0.7	0.39	0.9 $\pm$ 0.4	2.0 $\pm$ 3.0	0.38
FC CD4 TEM, %	2.7 $\pm$ 1.8	2.9 $\pm$ 2.3	2.4 $\pm$ 1.1	0.80	3.2 $\pm$ 2.4	2.4 $\pm$ 1.4	0.41
FC CD8 TEM, %	1.5 $\pm$ 2.4	1.0 $\pm$ 0.4	1.4 $\pm$ 0.4	<b>0.01</b>	1.0 $\pm$ 0.4	1.3 $\pm$ 0.4	0.06
FC CD4 Th1, %	4.7 $\pm$ 4.2	3.2 $\pm$ 1.7	6.6 $\pm$ 5.7	0.08	4.1 $\pm$ 3.9	5.0 $\pm$ 4.5	0.31
FC CD4 Th17, %	2.9 $\pm$ 4.9	3.7 $\pm$ 6.4	1.8 $\pm$ 0.9	0.66	3.7 $\pm$ 7.5	2.4 $\pm$ 2.3	0.21
FC CD4 Th1/17, %	1.2 $\pm$ 0.9	1.3 $\pm$ 1.0	1.1 $\pm$ 0.6	0.85	1.3 $\pm$ 1.1	1.1 $\pm$ 0.7	0.66
<b>Response to SARS-CoV-2 N peptides</b>							
CD4 IFN- $\gamma$ , %	4.3 $\pm$ 4.0	3.0 $\pm$ 3.5	5.9 $\pm$ 4.2	0.08	3.8 $\pm$ 3.4	4.6 $\pm$ 4.4	0.78
CD8 IFN- $\gamma$ , %	8.0 $\pm$ 9.0	7.5 $\pm$ 9.5	8.8 $\pm$ 8.7	0.85	13.2 $\pm$ 10.9	4.7 $\pm$ 5.8	<b>0.01</b>
FC CD4 TEMRA, %	4.2 $\pm$ 4.1	5.4 $\pm$ 4.7	2.6 $\pm$ 2.5	0.07	6.0 $\pm$ 4.1	3.0 $\pm$ 3.7	<b>0.02</b>
FC CD8 TEMRA, %	0.7 $\pm$ 0.6	0.8 $\pm$ 0.7	0.5 $\pm$ 0.2	<b>0.04</b>	0.9 $\pm$ 0.8	0.5 $\pm$ 0.2	0.28
FC CD4 TCM, %	0.7 $\pm$ 1.1	0.8 $\pm$ 1.4	0.5 $\pm$ 0.2	0.71	0.4 $\pm$ 0.2	0.9 $\pm$ 1.4	0.41
FC CD8 TCM, %	2.3 $\pm$ 5.1	1.9 $\pm$ 3.2	0.9 $\pm$ 0.7	0.62	0.8 $\pm$ 0.3	1.9 $\pm$ 3.1	0.26
FC CD4 TEM, %	2.6 $\pm$ 1.8	2.8 $\pm$ 2.2	2.4 $\pm$ 1.1	0.99	3.2 $\pm$ 2.4	2.2 $\pm$ 1.2	0.15
FC CD8 TEM, %	1.5 $\pm$ 2.4	1.2 $\pm$ 0.5	2.3 $\pm$ 1.3	<b>0.04</b>	1.6 $\pm$ 1.2	1.8 $\pm$ 1.0	0.31
FC CD4 Th1, %	5.6 $\pm$ 5.1	3.6 $\pm$ 2.0	8.1 $\pm$ 6.7	<b>0.04</b>	4.2 $\pm$ 3.7	6.4 $\pm$ 5.7	0.27
FC CD4 Th17, %	2.1 $\pm$ 2.8	2.7 $\pm$ 3.8	1.4 $\pm$ 0.6	0.66	2.8 $\pm$ 4.2	1.7 $\pm$ 1.6	0.28
FC CD4 Th1/17, %	0.8 $\pm$ 0.4	0.8 $\pm$ 0.4	0.8 $\pm$ 0.5	0.80	0.9 $\pm$ 0.6	0.8 $\pm$ 0.3	0.99

Data are presented as mean $\pm$ SD, unless otherwise stated. ICU: intensive care unit;  $D_{LCO}$ : diffusing capacity of the lung for carbon monoxide; BMI: body mass index; WHO: World Health Organization; FEV<sub>1</sub>: forced expiratory volume in 1 s; FVC: forced vital capacity; S: spike; IFN: interferon; FC: fold change; TEMRA: T-effector memory re-expressing CD45RA; TCM: T-central memory; TEM: T-effector memory; Th: T-helper; N: nucleocapsid. #: refers to a patient with a diagnosis of asthma prior to the COVID-19 episode. Statistically significant p-values are shown in bold.

The CD4 TEMRA and IFN- $\gamma$  producing cells against the S peptide, and the CD8 TEM and TEMRA against the S and N peptides, were increased in patients requiring critical care during the acute COVID-19 episode (table 1). The CD8 IFN- $\gamma$  response was reduced in patients with abnormal  $D_{LCO}$  at convalescence, who also presented a reduced proportion of CD4 TEMRA cells (table 1).

This study shows that a T-cell specific response persists in the majority (87%) of COVID-19 patients 6 months after hospital discharge. This response is more prominent in those who required critical care during the acute COVID-19 episode, suggesting that the severity of the acute episode determined a more robust virus-specific T-cell expansion.

We also observed that the presence of reduced  $D_{LCO}$  6 months after discharge is related to a decrease in SARS-CoV-2 specific, IFN- $\gamma$  producing CD8 T-cells. In addition, upon antigen stimulation, these patients presented a reduced expansion of cells with the TEMRA phenotype, suggesting a tighter control of the differentiation from memory cells towards the effector or the requirement of a costimulatory signal [10]. Further studies are required to elucidate these mechanisms and implications of these observations.

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Provenance: Submitted article, peer reviewed.

Acknowledgements: Authors thank all participants in the study for the willingness to contribute to medical research, and all field workers for their dedication and quality of their daily work. We are indebted to the HCB-IDIBAPS Biobank for the biological human samples and data procurement and to the Fundació Glòria Soler for its support to the COVIDBANK collection.

Conflict of interest: T. Cruz has nothing to disclose. N. Mendoza has nothing to disclose. L. Perea has nothing to disclose. N. Albacar has nothing to disclose. A. Gonzalez has nothing to disclose. F. Hernandez-Gonzalez has nothing to disclose. M. Juan declares research funding unrelated to the present work from ISC-III, the Spanish National Health Service and Fundació La Caixa, in the 36 months prior to manuscript submission. A. Agustí has nothing to disclose. J. Sellares has nothing to disclose. O. Sibila reports funding to their institution from Menarini, Fons Mecenatge HCB-IDIBAPS, SEPAR and AGAUR (PANDEMIES 2020), in support of the present study. R. Faner declares research grants unrelated to the present work from GlaxoSmithKline LLC, Menarini, AstraZeneca, ISC-III and the Spanish National Health Service; consulting fees from GlaxoSmithKline; and payment or honoraria from Chiesi, all in the 36 months prior to manuscript submission.

Support statement: This work has been financed by *ad hoc* patronage funds for research on COVID-19, from donations from citizens and organisations to the Hospital Clinic de Barcelona-Fundació Clínic per a la recerca Biomedica, "bizum solidario" by Fundación BBVA, and research grants from Menarini, Sociedad Española de Neumología y Cirugía Torácica and Agencia de Gestio d'Ajuts Universitaris i de Recerca (AGAUR, PANDEMIES 2020). R. Faner is Miguel Servet Fellow. T. Cruz is a Sara Borrell Fellow. Funding information for this article has been deposited with the Crossref Funder Registry.

## References

- 1 Guan WJ, Ni ZY, Hu Y, *et al.* Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med* 2020; 382: 1708–1720.
- 2 Sa Ribero M, Jouvenet N, Dreux M, *et al.* Interplay between SARS-CoV-2 and the type I interferon response. *PLoS Pathog* 2020; 16: e1008737.
- 3 Diao B, Wang C, Tan Y, *et al.* Reduction and functional exhaustion of T cells in patients with coronavirus disease 2019 (COVID-19). *Front Immunol* 2020; 11: 827.
- 4 Cao X. COVID-19: immunopathology and its implications for therapy. *Nat Rev Immunol* 2020; 20: 269–270.

- 5 Peng Y, Mentzer AJ, Liu G, *et al.* Broad and strong memory CD4<sup>+</sup> and CD8<sup>+</sup>T-cells induced by SARS-CoV-2 in UK convalescent individuals following COVID-19. *Nat Immunol* 2020; 21: 1336–1345.
- 6 Sherina N, Piralla A, Du L, *et al.* Persistence of SARS-CoV-2-specific B and T cell responses in convalescent COVID-19 patients 6–8 months after the infection. *Med (N Y)* 2021; 2: 281–295.e284.
- 7 Sonnweber T, Sahanic S, Pizzini A, *et al.* Cardiopulmonary recovery after COVID-19: an observational prospective multicentre trial. *Eur Respir J* 2021; 57: 2003481.
- 8 Sibila O, Albacar N, Perea L, *et al.* Lung function sequelae in COVID-19 patients 3 months after hospital discharge. *Arch Bronconeumol* 2021; 57: Suppl. 2, 59–61.
- 9 Graham BL, Steenbruggen I, Miller MR, *et al.* Standardization of spirometry 2019 update. An Official American Thoracic Society and European Respiratory Society technical statement. *Am J Respir Crit Care Med* 2019; 200: e70–e88.
- 10 Kaech SM, Wherry EJ, Ahmed R. Effector and memory T-cell differentiation: implications for vaccine development. *Nat Rev Immunol* 2002; 2: 251–262.