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

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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 (≥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 2×10^6 cells·mL^{-1} in X-Vivo plus 2% AB serum (Lonza, Belgium) with the S and N peptide pools (at 0.5 µg·mL^{-1}) for 10 days. At day 10, cells were re-stimulated with 2.5 µg·mL^{-1} individual virus-specific peptide pools and (1/100) FastImmune (BD, USA) for 2 h following the addition of 10 µg·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 Cytofix/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

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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±SD age of 60.0±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-γ 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±10.5	60.2±9.4	59.8±12.3	0.9	55.4±9.6	62.9±10.4	0.13
Males	14 (61%)	8 (62%)	6 (60%)		6 (67%)	8 (57%)	
BMI, kg·m^{-2}	30.2±6.3	30.5±5.3	29.9±7.6	0.69	30.0±8.3	30.4±4.9	0.61
Previous lung disease[#]	1 (4%)	1 (8%)	0		1 (8%)	0	
WHO disease severity score	4.5±1.4	3.6±0.8	5.7±1.2	**<0.001**	3.6±0.7	5.19±1.4	**0.01**
D_{LCO} at 6 months, % pred	82.2±17.2	86.8±17.2	76.3±16.2	0.12	99.1±14.9	71.4±6.5	**<0.001**
Sequelae	14 (61%)	6 (46%)	8 (80%)		0 (0%)	14 (100%)	
FEV_1 at 6 months, % pred	95.4±13.1	99.4±13.7	90.3±10.4	0.14	104.9±12.4	89.3±9.6	**<0.001**
FVC at 6 months, % pred	91.5±14.2	95.2±14.1	86.8±13.6	0.12	104.1±12.7	83.5±8.0	**<0.001**
FEV_1/FVC at 6 months, %	78.6±5.1	77.7±5.2	79.9±5.1	0.42	77.0±4.8	79.7±5.2	0.21
Response to SARS-CoV-2 S peptides							
CD4 IFN-γ, %	5.1±4.4	3.2±3.4	7.5±4.6	**0.05**	4.0±3.3	5.8±5.0	0.61
CD8 IFN-γ, %	2.1±1.7	2.2±2.0	2.0±1.3	0.50	2.0±2.1	2.2±1.5	0.56
FC CD4 TEMRA, %	6.3±8.8	8.9±10.8	2.8±3.1	**0.03**	10.7±12.4	3.4±3.6	**0.02**
FC CD8 TEMRA, %	0.8±0.5	0.9±0.6	0.7±0.2	**0.03**	1.1±0.6	0.7±0.2	**0.05**
FC CD4 TCM, %	0.6±1.0	0.7±1.3	0.5±0.2	0.46	0.4±0.2	0.8±1.2	0.21
FC CD8 TCM, %	2.5±4.8	1.9±3.1	1.1±0.7	0.39	0.9±0.4	2.0±3.0	0.38
FC CD4 TEM, %	2.7±1.8	2.9±2.3	2.4±1.1	0.80	3.2±2.4	2.4±1.4	0.41
FC CD8 TEM, %	1.5±2.4	1.0±0.4	1.4±0.4	**0.01**	1.0±0.4	1.3±0.4	0.06
FC CD4 Th1, %	4.7±4.2	3.2±1.7	6.6±5.7	0.08	4.1±3.9	5.0±4.5	0.31
FC CD4 Th17, %	2.9±4.9	3.7±6.4	1.8±0.9	0.66	3.7±7.5	2.4±2.3	0.21
FC CD4 Th1/17, %	1.2±0.9	1.3± 1.0	1.1±0.6	0.85	1.3±1.1	1.1±0.7	0.66
Response to SARS-CoV-2 N peptides							
CD4 IFN-γ, %	4.3±4.0	3.0±3.5	5.9±4.2	0.08	3.8±3.4	4.6±4.4	0.78
CD8 IFN-γ, %	8.0±9.0	7.5±9.5	8.8±8.7	0.85	13.2±10.9	4.7±5.8	**0.01**
FC CD4 TEMRA, %	4.2±4.1	5.4±4.7	2.6±2.5	0.07	6.0±4.1	3.0±3.7	**0.02**
FC CD8 TEMRA, %	0.7±0.6	0.8±0.7	0.5±0.2	**0.04**	0.9±0.8	0.5±0.2	0.28
FC CD4 TCM, %	0.7±1.1	0.8±1.4	0.5±0.2	0.71	0.4±0.2	0.9±1.4	0.41
FC CD8 TCM, %	2.3±5.1	1.9±3.2	0.9±0.7	0.62	0.8±0.3	1.9±3.1	0.26
FC CD4 TEM, %	2.6±1.8	2.8±2.2	2.4±1.1	0.99	3.2±2.4	2.2±1.2	0.15
FC CD8 TEM, %	1.5±2.4	1.2±0.5	2.3±1.3	**0.04**	1.6±1.2	1.8±1.0	0.31
FC CD4 Th1, %	5.6±5.1	3.6±2.0	8.1±6.7	**0.04**	4.2±3.7	6.4±5.7	0.27
FC CD4 Th17, %	2.1±2.8	2.7±3.8	1.4±0.6	0.66	2.8±4.2	1.7±1.6	0.28
FC CD4 Th1/17, %	0.8±0.4	0.8±0.4	0.8±0.5	0.80	0.9±0.6	0.8±0.3	0.99

Data are presented as mean±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_1: 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-γ 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-γ 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-γ 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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