Early View

Research letter

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

Tamara Cruz, Núria Mendoza, Lúdia Perea, Núria Albacar, Azucena Gonzalez, Fernanda Hernandez-Gonzalez, Manel Juan, Alvar Agustí, Jacobo Sellares, Oriol <SNM>Sibila Rosa

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**SARS-CoV-2 T-CELL RESPONSE IN COVID-19 CONVALESCENT PATIENTS WITH
AND WITHOUT LUNG *SEQUELAE***

Tamara Cruz^{1,2}, Núria Mendoza¹, Lúdia Perea^{1,2}, Núria Albacar^{1, 2,3}, Azucena Gonzalez⁴, Fernanda Hernandez-Gonzalez^{1,2,3}, Manel Juan⁴, Alvar Agustí^{1,2,3}, Jacobo Sellares^{1,2,3}, Oriol Sibila^{1,2, 3 *}, Rosa Faner^{1,2,3*}

¹ Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS). C/Roselló 149, 08036, Barcelona, Spain.

² Centro de Investigación Biomédica en Red de Enfermedades Respiratorias (CIBER), Spain

³ Respiratory Insitute, Hospital Clínic, University of Barcelona. C/Villaroel 170, 08036, Barcelona, Spain

⁴ Immunology Department, Hospital Clínic, University of Barcelona. C/Villaroel 170, 08036, Barcelona, Spain

*CO-LAST AUTHORS

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Corresponding author: Dra. Rosa Faner CIBERES, IDIBAPS-CELLEX. C/Casanova 143, P2A, Barcelona 08036, Spain. Tel.: +34 93 227 1715; Fax: +34 93 227 1716; e-mail: rfaner@clinic.cat

Take home message: 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.

To the editor:

Patients infected by SARS-CoV-2 may develop pneumonia (COVID19) and require hospital admission and, eventually, critical care (1). This has been related with a weaker innate immune response with impaired production of type I interferons (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 characterized 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 (DLCO), three 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 Nucleocapside (N) proteins, two well recognized viral antigens, in COVID-19 convalescent patients with normal and abnormal DLCO 6 months after hospital discharge.

Methods

This prospective, observational study included 25 adults who were hospitalized in our institution because of PCR-confirmed COVID-19 pneumonia and were studied at 6 months after hospital discharge. Participants were categorized according to their intensive care unit (ICU) admission (or not) during the acute COVID-19 episode, or by their DLCO value 6 months after discharge (normal

($\geq 80\%$ ref.) or abnormal ($< 80\%$ ref.)). The study was approved by the Ethical Review Board of our hospital (HCB/2020/0422), and all patients signed their informed consent.

Demographic, clinical and biological characteristics were recorded on hospital admission and 6 months after discharge. At the latter time point, spirometry and DLCO were 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 FBS (Gibco, US) 10% DMSO. The pools of peptides covering the S and N proteins of SARS-CoV-2 were purchased from Miltenyi Biotec, US (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 in X-Vivo plus 2% AB serum (Lonza, Belgium) with the S and N peptides pools (at 0.5 $\mu\text{g/mL}$) for 10 days. At day 10, cells were re-stimulated with 2.5 $\mu\text{g/mL}$ individual virus-specific peptide pools and (1:100) FastImmune (BD, US) for 2h following the addition of 10 $\mu\text{g/mL}$ Brefeldin A (Sigma, Germany) for 4h. Cells were stained with CD8-BV650, CD4-BV711, CD3-APC-R700, CD45-APC-H7, CD45RA-FITC, CD197-PECF594, CD196-PECy7, CXCR3-APC and IFN γ -PE (BD, US) using the Cytotfix/Cytoperm (BD, US). All samples were acquired using a LSRFortessaSORP (BD, US) and analyzed by (FlowJo LLC, USA). Lymphocyte subpopulations were analyzed as the proportion of CD4 or CD8. The expansion of specific populations is presented as fold change variations (i.e., FC= frequency of the population in stimulated PBMCs divided per 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 GraphPad Prism 7 (La Jolla, Calif). A limitation of the study is the lack of lung function 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 was not related to low levels of DLCO (Table 1).

Results

We studied 23 patients (61% males) with a mean 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 DLCO <80% ref. six 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 interferon gamma (IFN γ) producing cells after stimulation) was found in 70% of the patients, and a CD8 response in 43%. On the other hand, 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 TH1 (CXCR3) and TH17 (CD196) polarization, whereas the S and N peptides expanded TEM and T memory central (TMC) CD8 cells (Table 1).

The CD4 TEMRA and IFN γ producing cells against the S peptide and the CD8 TEM y 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 DLCO at convalescence and presented a reduced proportion of CD4 TEMRA cells (Table 1).

Discussion

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 DLCO 6 months after discharge is related to a decrease in SARS-CoV-2 specific IFN γ producing CD8 T cells. Also, upon antigen stimulation, these patients presented a reduced expansion of cells with 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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Table 1. Clinical characteristics and T-cells response of COVID19 patients 6 months after hospital discharge.

	All n= 23 (Mean ± SD)	NO ICU n= 13 (Mean ± SD)	ICU n = 10 (Mean ± SD)	p value	DLCO >80% n = 9 (Mean ± SD)	DLCO<80% n = 14 (Mean ± SD)	p value
Age	60.0 ± 10.5	60.2 ± 9.4	59.8 ± 12.3	0.9	55.4 ± 9.6	62.9 ±10.4	0.13
Gender (m) n(%)	14 (61%)	8 (62%)	6 (60%)		6 (67%)	8 (57%)	
BMI	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* n(%)	1 (4%)	1 (8%)	0		1 (8%)	0	
Severity WHO	4.5 ± 1.4	3.6 ± 0.8	5.7 ± 1.2	<0.001	3.6 ± 0.7	5.19 ±1.4	0.01
DLCO at 6 months	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 n(%)	14 (61%)	13 (57%)	10 (43%)		0 (0%)	14 (100%)	
FEV1 at 6 months	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	91.5 ± 14.2	95.2 ± 14.1	86.8 ± 13.6	0.12	104.1 ± 12.7	83.5 ±8.0	<0.001
FEV1/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
SARS S peptide							
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
SARS N peptide							
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 $\pm$ 9.0	7.5 $\pm$ 9.5	8.8 $\pm$ 8.7	0.85	13.2 $\pm$ 10.9	4.7 $\pm$ 5.8	0.01
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	0.02
FC % CD8 TEMRA	0.7 $\pm$ 0.6	0.8 $\pm$ 0.7	0.5 $\pm$ 0.2	0.04	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	0.04	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	0.04	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

Abbreviations:

FC = fold change

TCM = T central memory

TEM = T effector memory

TEMRA = T effector memory re-expressing CD45RA

DLCO = carbon monoxide lung

* previous lung disease, refers to a patient with a diagnosis of asthma prior de COVID19 episode.