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Cellular Immunology 365 (2021) 104363

Contents lists available at ScienceDirect

Cellular Immunology

journal homepage: www.elsevier.com/locate/ycimm

Short communication

Functional responsiveness of memory T cells from COVID-19 patients

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ARTICLE INFO

Keywords:
SARS-CoV-2
Central memory T cells, Effector memory T cells, Spike S1 glycoprotein
Dendritic cell
Helper T cells
Cytotoxic T lymphocytes

ABSTRACT

The presence of memory T cells in COVID-19 patients has been acknowledged, however the functional potency of memory responses is critical for protection. In this study, naïve, effector, effector memory, and central memory $CD4^+$ and $CD8^+$ T cells obtained from the COVID-19 survivors were re-exposed to autologous monocyte-derived DCs that were loaded with SARS-CoV-2 spike glycoprotein S1. Proliferation capacity, CD25, 4-1BB, and PD-1 expression, and IFN-γ, IL-6, granzyme, granulysin, and FasL secretion were enhanced in $CD4^+$ and $CD8^+$ effector memory and central memory T cells. Albeit being at heterogeneous levels, the memory T cells from the individuals with COVID-19 history possess functional capacities to reinvigorate anti-viral immunity against SARS-CoV-2.

1. Introduction

Despite the emerging data on coronavirus disease 2019 (COVID-19), the immune response to the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) remains to be better defined. The strength and duration of the humoral and cellular responses against SARS-CoV-2 have been associated with neutralizing antibodies and memory T cells [1,2]. Especially, the spike glycoprotein-S1 bears significant immunodominance since the neutralizing antibodies block its attachment to the angiotensin-converting enzyme 2 (ACE2) and hinder the viral entry [3]. Besides, approximately 50% of the patients displayed S1-specific T cell responses [4,5].

Nevertheless, the heterogeneity observed amongst the COVID-19 cases has a confounding effect. While the majority of the patients develop anti-viral immunity, even the convalescent individuals may not be protected from the re-infection which is potentially due to the insufficient magnitude and/or stability of T cell and antibody production [6–8]. Intriguingly, in severe SARS-CoV-2 infections, the interaction between $CD4^+$ helper T (Th) cells and B cells is blunted in the germinal center, which potentially dampens the longevity of antibody responses [9]. Together with the Th activities, the robustness of $CD8^+$ cytotoxic T cells is also pivotal for a successful anti-viral immunity [10]. Previously, the presence of $CD4^+$ or $CD8^+$ memory T cells was reported in COVID-19, nevertheless the functional capacities of these cells need to be addressed thoroughly [11–13]. In this study, the functional responsiveness of naïve, effector, central memory, and effector memory $CD4^+$ or $CD8^+$ T cells, which were obtained from the patients with COVID-19 history, against monocyte-derived dendritic cells (DCs) bearing SARS-CoV-2 S1 antigen is confirmed.

2. Material and methods

2.1. Patients and sample collection

At two different time points, peripheral blood samples were freshly collected from the patients recovered from COVID-19 (Table 1) and peripheral blood mononuclear cells were separated by 1.077 g/mL Ficoll density gradient (Sigma-Aldrich). All protocols were approved by the local ethical committees and the Republic of Turkey Ministry of Health. İnformed consent was obtained from the patients. Patients with positive RT-PCR test and/or seropositivity were enrolled in the study. The clinical symptoms were categorized as mild (the non-hospitalized patients), moderate (the patients who had moderate pneumonia) and severe (the patients who had severe pneumonia and were hospitalized for more than 5 days). Blood samples from healthy donors [n = 10 (6 females, 4 males), median age 33 (min 28–max 55)] without SARS-CoV-2 history and seropositivity were used controls.

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https://doi.org/10.1016/j.cellimm.2021.104363
Received 20 January 2021; Received in revised form 31 March 2021; Accepted 13 April 2021
Available online 17 April 2021
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Table 1
Patient data.

	COVID-19 patients
Number (n =)	10
Age median (range)	37 (17–63)
Gender (female/male)	5/5
Clinical score (n =)	
Mild	6
Moderate	2
Severe	2
Anti-S1 Ig titer median (range)	8.4 RU/mL (1.9–9.5)
Timing of blood collection[a] median (range)	1 months (1–5)

RU, relative units.
[a] After the date of diagnosis.

2.2. Establishment of monocyte-derived DC and T cell co-cultures

DCs were generated from the monocytes (CD14 MACS, Miltenyi) according to a previously published protocol [14]. Antigen-loading with the recombinant S1 protein (S1; 10 μg/mL, Abcam) or HIV Gag antigen (10 μg/mL, TUBITAK Marmara Research Center) [15] or the tetanus toxoid (TT; 10 μg/mL, Turk Ilac) was simultaneously initiated with the maturation of monocyte-derived DCs with LPS (1 μg/mL, Sigma-Aldrich). Mature monocyte-derived DCs generated in the absence of a specific antigen were used as controls. At the end of 7-day-long incubation, the monocyte-derived DCs were characterized as a $CD11b^{hi}CD14^{lo}CD1a^{+}CD83^{+}$ population.

From the same COVID-19 patient, autologous naïve T (T_N), terminally-differentiated effector T (T_{EMRA}), central memory T (T_{CM}), and effector memory T (T_{EM}) cells were purified (≥96%) by FACS (FACSAria II; Becton Dickinson) as CD3-untouched, CD19- and CD56-negative lymphocytes according to the differential expression of CD45RA, CD45RO, and CCR7 markers (Fig. 1A).

The monocyte-derived DCs ($5x10^4$) were co-cultured with the purified subtypes of T cells (10^5) for 96 h in a round-bottom 96-well plate well containing 200 μL RPMI 1640 media completed with 10% FBS, 1% penicillin/streptomycin (Biological Industries), 5 ng/mL IL-2 (BioLegend). Prior to co-culturing, the T cell subtypes were labelled with 5 μM CFSE (BioLegend). As a positive control, anti-CD3 antibody (HIT3a, 25 ng/mL; BioLegend) was added into the co-cultures. The experimental setup is summarized in Fig. 1A.

2.3. Immunophenotyping and function-related assays by flow cytometry

Immunophenotyping was performed with monoclonal antibodies anti-human-CD4 (OKT4), -CD8 (RPA-T8), -CD56 (MEM-188), -CD19 (SJ25C1), -CD45RA (HI100), -CD45RO (UCHL1), -CCR7 (G043H7), -CD25 (M-A251), -CD38 (HIT2), -4-1BB (4B4-1), -PD-1 (NAT105), -CD14 (M5E2), -CD11b (ICRF44) (BioLegend); -CD1a (REA736), -CD83 (REA714) (Miltenyi). Median fluorescence intensity (MFI) values were determined on $CD4^{+}$ and $CD8^{+}$ T cells, and according to CFSE dilution. The change in the MFI were calculated by comparing the data from the co-cultures with the antigen-loaded DCs and the control DCs.

The percentage of T cells with CFSE dilution was assessed for proliferation. The antigen-specific proliferation capacity of T cells was calculated as the change in proliferation wherein the data from the co-cultures with control monocyte-derived DCs were used as normalizer. The supernatants collected were used in a multiplex ELISA (LEGENDplex, BioLegend). All flow cytometric analyses were performed on a FACSAria II sorter.

2.4. Statistical analysis

The results are presented as median ± SEM. Kruskal-Wallis test and Bonferroni correction were used for the statistical analyses. A P value <0.05 was considered statistically significant.

3. Results

Following the incubation with the S1-DC, a small percentage of proliferating T cells was identified (range %, $CD4^{+}$, T_N 0.49–6.8, T_{EMRA} 3.29–7.7, T_{CM} 0.38–20.2, T_{EM} 0.2–16.7; range %, $CD8^{+}$, T_N 0.64–5.6, T_{EMRA} 2.16–10.3, T_{CM} 0.8–15, T_{EM} 1.6–19.4). Both $CD4^{+}$ and $CD8^{+}$ T_{CM} and T_{EM} cells exhibited a considerably increased frequency of proliferation than the naïve or effector T cell populations (Fig. 1B). $CD4^{+}$ T_{CM} cells and $CD8^{+}$ T_{EM} cells displayed the highest proliferative activity. The $CD4^{+}$ memory T cell proliferation could be induced in ~90% of the COVID-19 patients, however the $CD8^{+}$ memory T cell proliferation was only evidenced in ~60% of the patients (Fig. 1B). The monocyte-derived DCs were also loaded with an irrelevant viral antigen, HIV Gag; expectedly, no significant response was obtained in the T cells from COVID-19 survivors (Fig. 1C). Similarly, the T cells from the healthy individuals did not respond to S1-DC. On the other hand, the monocyte-derived DC presenting the TT antigen served as a positive control for T cell memory responses (Fig. 1C). In addition, the surface expression of certain activation-related markers, especially CD25, PD-1 and 4-1BB, was significantly upregulated on S1-responsive T cells (Fig. 1D). The S1-DC-stimulated T_{EM} cells secreted the highest levels of IFN-γ and IL-6, which is largely produced by type 1 $CD4^{+}$ T cells and $CD8^{+}$ cytotoxic T cells. The markers of cytotoxic response, granzyme A, granulysin, Fas ligand (FasL), and especially granzyme B were also elevated in the co-cultures harboring T_{EM} cells from COVID-19 patients (Fig. 1E). The co-culture supernatants from which the soluble factors were measured contained the mediators secreted by T cells and DCs; thus, as stated in the literature, moderate amounts of the cytokines such as TNF-α, IL-6, IL-4 and IFN-γ may also be produced by the DC [16,17]. In some cases, the non-memory T cells responded to S1-DC, upregulated the CD38 and 4-1BB expression, and the secretion of IL-4 and TNF-α (Fig. 1). A heterogeneity was also noted between the T cell parameters studied and the time of blood sampling after recovery, the severity of clinical symptoms, or the level of anti-S1 antibodies (*data not shown*).

4. Discussion

In the COVID-19 survivors, the circulating S1-specific T_{EM} and T_{CM} cells retained functional responsiveness and displayed augmented effector capacities such as activation, proliferation, and secretion of immune mediators. Even though the proliferative response in the memory $CD8^{+}$T cells was not as potent as in the $CD4^{+}$ memory T cells, our data suggest that these cells can quickly advance to an effector state when exposed to SARS-CoV-2 S1 antigen. The T_{EM} cells are critical gatekeepers since they tend to locate into the tissues prone to invasion by the pathogenic microorganism, whereas the T_{CM} cells are recruited into the secondary lymphoid organs for accelerating the immune reactions inaugurated by the antigen-presenting DCs [18]. Albeit covering a limited number of COVID-19 cases, in our study, the majority of the patients harbored T_{EM} and T_{CM} cells that functionally responded to S1 protein in terms of at least one parameter tested. These preliminary findings may indicate a probable disparity between functional competence of T cells and COVID-19 severity. A previous study reported a higher frequency of S1-specific T cells than the T cells specific for N and M proteins [8]. Correspondingly, the functional responsiveness of T cells to other SARS-CoV-2 antigens remains to be better elucidated. Recently published seminal work demonstrated the presence of long-term memory in the T cells from COVID-19 patients [19,20]. By using a distinct experimental approach wherein the autologous monocyte-derived DCs were used as a feasible element for testing the T cell response, our study confirmed the function and character of T cells previously mentioned in the individuals with COVID-19 history. Accumulating evidence on the immunity established in the COVID-19 survivors would provide a better understanding of disease pathogenesis, therapeutic approaches, and vaccine development.

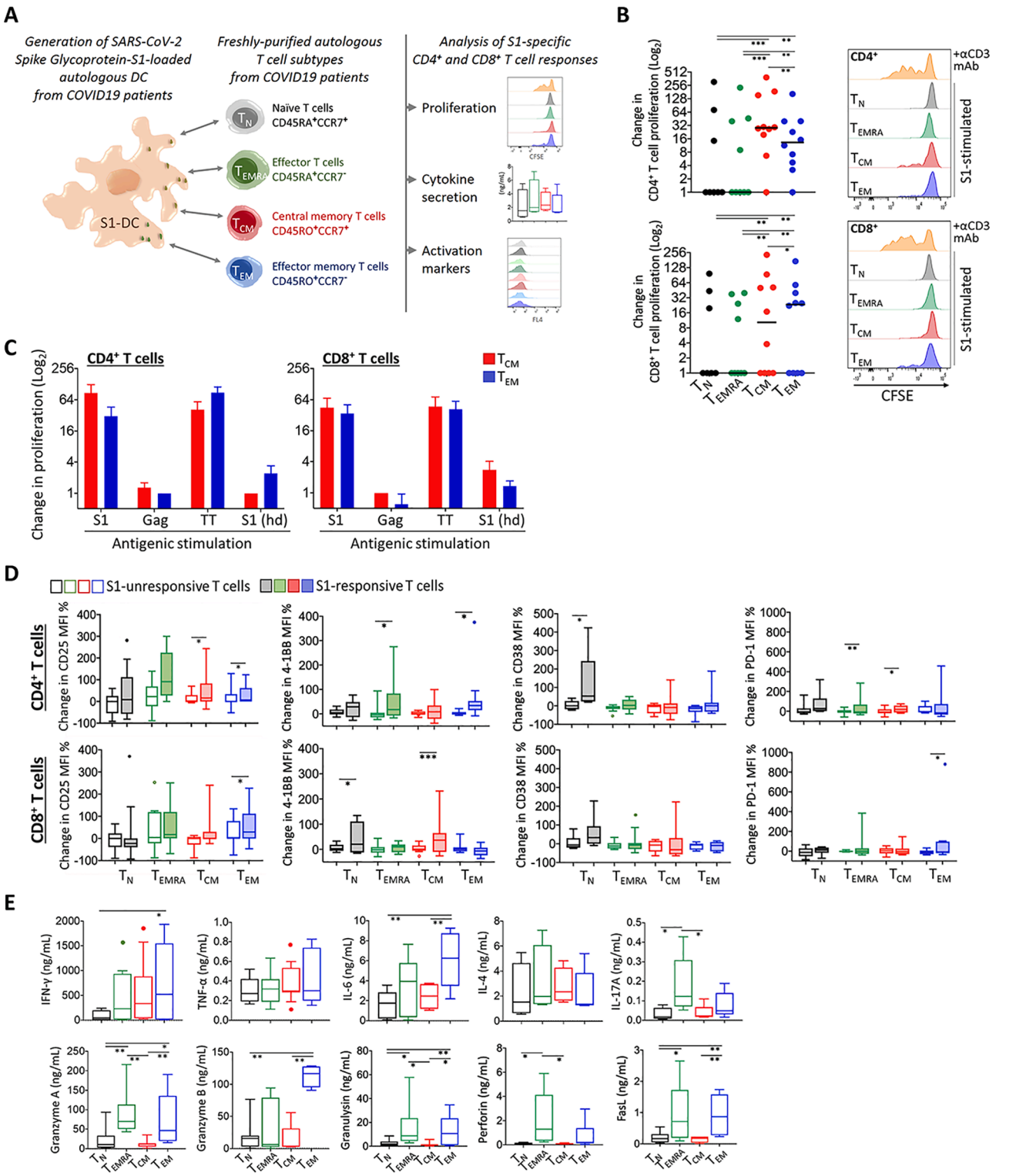

Fig. 1. Assessment of functional responses in T cells from COVID-19 survivors. A) Graphical outline of the experimental setup is shown. Monocyte-derived dendritic cells were generated from the individuals with COVID-19 history and loaded with SARS-CoV-2 Spike Glycoprotein-S1 (S1-DC); then, autologous naïve T (T_N), terminally-differentiated effector T (T_{EMRA}), central memory T (T_{CM}), and effector memory T (T_{EM}) cells were purified and co-cultured with these DCs. T cell proliferation, expression of activation markers and cytokine secretion were measured after 96 h incubation. B) Change in $CD4^+$ and $CD8^+$ T cell proliferation was plotted for each patient in comparison to that obtained with control co-cultures with monocyte-derived DCs without specific antigen loading. Representative flow cytometry histograms are given on the right side. The co-cultures stimulated with an anti-CD3 monoclonal antibody served as a technical positive control for T cell proliferation. C) The patient-derived T_{CM} and T_{EM} cells' proliferation response against the S1-DC was compared with those obtained with the HIV Gag or with the tetanus toxoid (TT) antigen-loaded DCs. T cells and S1-DCs obtained from healthy individuals (S1(hd)) were also used as controls. D) Changes in $CD4^+$ and $CD8^+$ T cell activation marker levels were shown in comparison to those obtained with control co-cultures with monocyte-derived DCs without specific antigen loading. E) Amount of T cell-associated cytokines secreted into the co-culture supernatants was assessed. ($^*p < 0.05$, $^{**}p < 0.01$).

CRediT authorship contribution statement

Ece Tavukcuoglu: Investigation, Visualization, Formal analysis, Writing - review & editing. **Utku Horzum:** Investigation, Visualization, Formal analysis, Writing - review & editing. **Ahmet Cagkan Inkaya:** Conceptualization, Resources. **Serhat Unal:** Conceptualization, Resources. **Gunes Esendagli:** Conceptualization, Writing - review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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