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LETTER

Severe COVID-19 is associated with deep and sustained multifaceted cellular immunosuppression

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Dear Editor,

SARS-CoV-2 infection is associated with marked lymphopenia that correlates with morbidity and mortality [1, 2]. Here, we present the first report on serial immunophenotypic and functional changes in 13 consecutively recruited patients infected with SARS-CoV-2 virus during their first week of ICU stay (Supplementary Table 1) with 10 healthy donors used as controls.

Patients uniformly exhibited deep global and persisting T, NK and B cell lymphopenia from ICU admission (D0) to day 7 (D7) (Fig. 1a to d). On D0, median absolute lymphocyte count was dramatically reduced at 0.72 [0.65–0.88] G/L as were CD4 and CD8 T cell counts at 0.29 [0.19–0.43] and 0.08 [0.05–0.1] G/L (Fig. 1a, e, f), such CD4 T cell levels reflecting profound immunosuppression in HIV-infected patients. Few CD4 T cells transiently expressed CTLA-4 during the first 3 days (Fig. 1g) while expression of PD-1 was observed at D0 and increased until D7 (Fig. 1h). CD8 T cells significantly and persistently expressed PD-1 from D0 to D7 while CTLA-4 expression remained unchanged (Fig. 1i, j).

Being heterogeneous at D0 (Fig. 1k), percentages of regulatory T cells (Tregs) increased during time. Few of them over-expressed CTLA-4 while PD-1 expression was strongly and stably increased until D7 (Fig. 1l, m). Total granulocytes were moderately increased with a transient egression of immature granulocytes in 4/10 patients at day 4–5 (Supplementary Figure 1). Monocyte counts increased during the first week. Nevertheless, HLA-DR expression was strongly down-regulated by a threefold factor at D0. Strikingly this decrease persisted unabated until D7, possibly impairing antigen presentation, and was associated with increased PD-L1 expression (Fig. 1n, o and Supplementary Figure 4d).

Being either an exhaustion or an activation marker, PD-1 is an inducer of CD8 T cell apoptosis when activated. Therefore, functional evaluation of T-lymphocytes was performed in three patients and controls for comparison. Meanwhile production of TNF-α and IL-2 was normal, CD4 T cell IFN-γ production was decreased (Supplementary Figure 2), indicating a CD4 exhaustion process. In contrast, CD8 T cells could be involved in anti-viral immune response since they produced higher levels of IFN-γ and TNF-α (Supplementary Figure 3). Consistently, percentages of effector CD4 T cells were decreased while those of effector memory and activated CD8 T cells were increased (Supplementary Figure 4a to 4c). Circulating levels of IL-6 and IL-8 were moderately but significantly and sustainly increased over time, reflecting the known SARS-CoV-2 related sub-acute inflammatory response of innate immune cells [4] (Supplementary Figure 5).

Although our results warrant further confirmation in larger cohort, they strongly suggest a multifaceted devastating effect of the virus to cause depletion of virtually all classes of adaptive immune cells and to cause upregulation of potent T cell killing and immunosuppressive mechanisms in critically-ill COVID-19 patients. Since T cells are essential for definitive viral clearance, these results call into question therapies (e.g., anti-IL-6, corticosteroids, JAK inhibitors) that aim to block the ability of the patient to mount an effective immune response to

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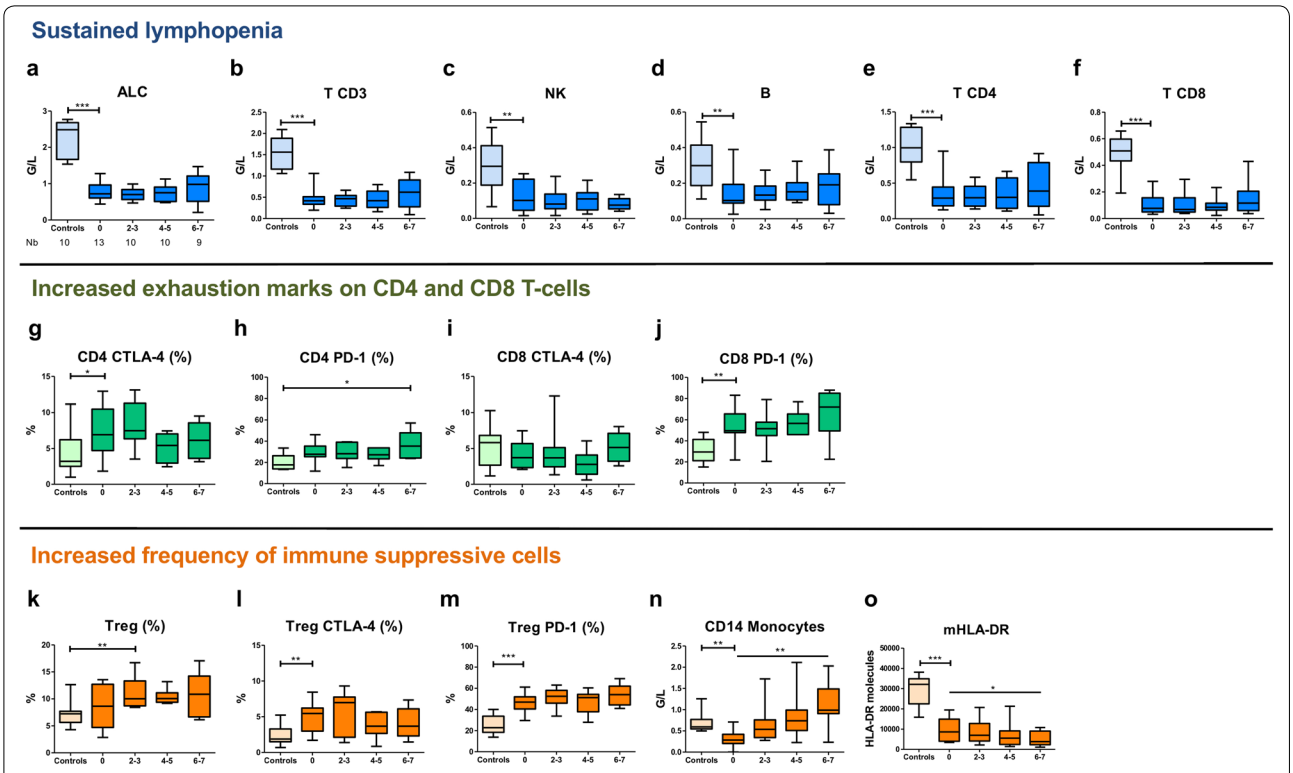

Fig. 1 Over time, ICU COVID-19 patients showed a profound and sustained lymphopenia correlated with increased percentages of CD4 and CD8 expressing exhaustion marks and increased frequency of immune suppressive cells. Box plot represent results for 10 healthy subjects (controls) and for the following time point and number of COVID-19 ICU patients. The number of patients is given below the horizontal axis of panel a. Boxes give the median with the first and the third quartile. Whiskers represent min to max. Lines with bracket and plain lines indicate a Mann–Whitney and ANOVA (Kruskall–Wallis test) comparison with controls or during the ICU stay respectively. Test *p* values are represented by *, ** and *** for $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$ respectively. Upper panel **a**, **b**, **c**, **d**, **e**, **f**: absolute lymphocytes count (ALC) (**a**), CD3 T-lymphocytes (**b**), Natural Killer (NK) cells (**c**), B lymphocytes (**d**), CD4 (**e**) and CD8 T cells (**f**). Light blue boxes represent controls and darker blue boxes represent patients. Middle panel **g**, **h**, **i**, **j**: percentages of CD4 (**g** and **h**) and CD8 (**i** and **j**) T cells expressing CTLA-4 (**g** and **i**) and PD-1 (**h** and **j**). Light green boxes represent controls and darker green boxes represent patients. Lower panel **k**, **l**, **m**, **n**, **o**: percentages of CD4 +/CD25 +/CD127low regulatory T cells (T-reg) [3] (**k**) expressing CTLA-4 (**l**) and PD-1 (**m**) and CD14 monocyte counts (**n**) with quantification of the mHLA-DR at their surface membrane (**o**). Light orange boxes represent controls and darker orange boxes represent patients

the invading SARS-CoV-2. Knowing that almost all anti-inflammatory therapies have also chronically failed in sepsis, consideration to therapies that boost host immunity in selected severe ARDS ICU patients (e.g., IL-7, IFN-γ or checkpoint inhibitors) may be appropriate [5, 6].

Electronic supplementary material

The online version of this article (https://doi.org/10.1007/s00134-020-06127-x) contains supplementary material, which is available to authorized users.

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Authors' contributions

TD, BF included patients. RJ, RF, JF analyzed the data. RJ, TD drafted the manuscript. JF, BF, and RF reviewed the manuscript. All authors read and approved the final version of the manuscript.

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