SARS-CoV-2-Induced ARDS Associates with MDSC Expansion, Lymphocyte Dysfunction, and Arginine Shortage

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Abstract

Purpose

The SARS-CoV-2 infection can lead to a severe acute respiratory distress syndrome (ARDS) with prolonged mechanical ventilation and high mortality rate. Interestingly, COVID-19-associated ARDS share biological and clinical features with sepsis-associated immunosuppression since lymphopenia and acquired infections associated with late mortality are frequently encountered. Mechanisms responsible for COVID-19-associated lymphopenia need to be explored since they could be responsible for delayed virus clearance and increased mortality rate among intensive care unit (ICU) patients.

Methods

A series of 26 clinically annotated COVID-19 patients were analyzed by thorough phenotypic and functional investigations at days 0, 4, and 7 after ICU admission.

Results

We revealed that, in the absence of any difference in demographic parameters nor medical history between the two groups, ARDS patients presented with an increased number of myeloid-derived suppressor cells (MDSC) and a decreased number of CD8pos effector memory cell compared to patients hospitalized for COVID-19 moderate pneumonia. Interestingly, COVID-19-related MDSC expansion was directly correlated to lymphopenia and enhanced arginase activity. Lastly, T cell proliferative capacity in vitro was significantly reduced among COVID-19 patients and could be restored through arginine supplementation.

Conclusions

The present study reports a critical role for MDSC in COVID-19-associated ARDS. Our findings open the possibility of arginine supplementation as an adjuvant therapy for these ICU patients, aiming to reduce immunosuppression and help virus clearance, thereby decreasing the duration of mechanical ventilation, nosocomial infection acquisition, and mortality.

Keywords

Covid-19 · ARDS · MDSC · Lymphocytes · Arginine · Cross infection · Immunosuppression

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Introduction

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) which can lead to severe pneumonia with acute respiratory distress syndrome (ARDS), responsible for hospitalizations in intensive care unit (ICU) with an ICU mortality rate reaching 30 to 60% [1, 2]. Among the factors associated with this severe outcome, older age, comorbid conditions, and an elevated body mass index have been constantly reported [1–4]. Furthermore, lymphopenia and diminished viral clearance illustrated by persistent SARS-CoV-2 in the lung have been associated with COVID-19 severity and poor prognosis [5–7]. Viral clearance and the resolution of infection involve a complex immune response initiated by resident cells of the respiratory tract along with innate immune cells and is ultimately resolved by adaptive immune cells. Like SARS-CoV-1, SARS-CoV-2 uses ACE2 as a receptor for bronchial epithelium cellular entry and activates inflammation through multiple pathways, directly related to cytotoxicity and dependent on innate immune cells [8, 9]. Although local immune responses have not yet been explored in COVID-19, it is known in severe respiratory viral infections that T cells crucially contribute to virus clearance from infected lungs and thus to a favorable outcome [5, 10]. In addition to delaying virus clearance, lymphopenia and/or functional T cell defects also favor secondary infections by opportunistic pathogens inducing prolonged mechanical ventilation responsible for late mortality [1, 11]. While sepsis is more often associated with bacterial or fungal infections [12], COVID-19 can thus feature “viral sepsis” with a pathologic host response characterized by an important cytokine release, and critically ill associated immunosuppression rather than “typical” ARDS [13, 14]. Lymphopenia, a crucial component of severe COVID-19, has in fact been noted in other severe respiratory viral infections, notably H1N1 influenza [15]. More broadly, immunosuppression in critically ill patients has been shown to be associated with increased lymphocyte apoptosis, decreased lymphocyte proliferation, and the striking emergence of myeloid-derived suppressor cells (MDSC), all associated with poorer outcome and nosocomial infections in ICU [16–18]. The underlying mechanisms of this MDSC-associated immunosuppression in infectious diseases are still not fully understood. However, various aspects of MDSC-mediated T cell immunosuppression have been reported in other conditions where MDSC are amplified, such as cancers and obesity [19]. Moreover, during acute viral illness, MDSC have been associated with chronicity in viral infections and poor clinical outcome [20]. Whether and how MDSC, including monocytic (M-MDSC) and granulocytic (G-MDSC) subsets, might be involved in the pathogenesis of SARS-CoV-2 has not yet been fully explored although emerging data reported that MDSC might influence disease severity [21, 22].

The present study explores the potential relationship between MDSC expansion, lymphopenia, and lymphocyte dysfunction in the first week following admission to ICU among COVID-19-associated ARDS patients.

Materials and Methods

Study Design, Patients, and Samples

This study was performed in the department of infectious diseases and the intensive care unit (ICU) at Rennes University Hospital. The study design was approved by our ethic committee (CHU Rennes, n°18.101-2), and informed consent was obtained from the patients. From late March 2020 to May 2020, 26 consecutive patients with moderate and severe acute respiratory distress syndrome (ARDS) or moderate pneumonia related to SARS-CoV2 infection were prospectively enrolled and compared with 13 healthy donors. Pregnant women, patients younger than 18 years old, patients with malignancy, HIV-infected patients, and patients with preexisting immune disorders or receiving immunosuppressive agents were excluded. The presence of SARS-CoV-2 in respiratory specimens (nasal and pharyngeal swabs or sputum) was detected by real-time reverse transcription-polymerase chain reaction (RT-PCR) methods. COVID-19 moderate pneumonia was defined as cases exhibiting fever and respiratory symptoms with radiological findings of pneumonia. Severe and moderate ARDS were defined in accordance with international guidelines [23]. The following data were recorded: age, reason for admission, patient’s preexisting condition (current smoking, diabetes, hypertension, cardiovascular disease, pulmonary disease, liver disease), obesity (defined as a body mass index > 30 kg/m²), ICU and hospital length of stay, mortality, the need for invasive mechanical ventilation, the need for prone position ventilation, the need for renal replacement therapy, and the need for vasoconstrictor agent during ICU stay. The occurrence of nosocomial infections was also recorded [17, 24]. Initial clinical laboratory measurement included a complete blood count and a serum biochemical test. Lymphopenia was defined as an absolute lymphocyte count under 1.5 cells/μL × 10³. The Simplified Acute Physiology Score (SAPS II) and the Sequential Organ Failure Assessment (SOFA) score at admission to ICU were used to assess severity [25, 26]. A senior radiologist who was blinded to the other clinical information reviewed the CT chest scans and classified lung injury as absent or minimal (<10%), moderate (10–15%), extensive (25–50%), or severe (50–75%). Blood samples were collected within the first 24 h following admission, 4 days (day 4) after admission and 7 days (day 7) after admission when patients were still hospitalized. The time lapse between sampling and initiation of laboratory procedures was less than 1 h. Peripheral blood mononuclear
cells (PBMC) were isolated by Ficoll density gradient, and plasma samples were stored at −80 °C until use.

**Flow Cytometry**

Quantification of monocytic populations was performed on fresh whole blood. After staining, erythrocytes were lysed twice with EasyLyse (Dako, Glostrup, Denmark) before washing in PBS. Monocyte populations have been determined based on CD14 and CD16 expression. M-MDSC were defined as CD14 pos HLA-DR low cells. Expression of PD-L1 has been determined on three monocyte populations (CD14 pos CD16 neg, CD14 pos CD16 low, and CD14 low CD16 pos). G-MDSC and early-MDSC (e-MDSC) were defined on fresh PBMC isolated after Ficoll density gradient and were defined as CD45 pos Lin neg HLA-DR low CD15 pos CD11b pos for G-MDSC and as CD45 pos CD3 neg Lin neg HLA-DR low CD15 pos CD33 pos for e-MDSC. Gating schemes for the monocyte populations and representative staining panels of the MDSC populations are represented in the supplementary Figs. 1 and 2. Precision Count Beads (Biolegend, San Diego, CA) were added after staining to calculate the absolute number of cell subpopulations. B cells, NK cells, and T cells were analyzed on whole blood by using CD19, CD3, CD56, and CD16. Frequencies of cells were defined by flow cytometry, and absolute counts were calculated relative to the lymphocyte count defined on a cell counter before processing. Naïve (N), central memory (CM), effector memory (EM), and effector memory expressing CD45RA (EMRA) were defined based on CCR7 and CD45RA expression, on CD4 and CD8 cells. On each cell types, the expression of HLA-DR and PD-1 was analyzed. All antibodies and fluorochromes are summarized in supplementary Table 1. A Fortessa X20 flow cytometer (Becton Dickinson, San Jose, CA, USA) was used to run all samples. Data were analyzed using Kaluza 2.0 software (Beckman Coulter, Brea, CA, USA).

**Apoptotic Analysis**

Whole blood was incubated with CD3 BV421 (Becton Dickinson). Erythrocytes were then lysed twice using EasyLyse (Dako, Glostrup, Denmark). To evaluate the proportion of apoptotic T cells, PE-conjugated active caspase-3 apoptosis kit (Becton Dickinson) has been used. Samples were run in a Fortessa X20 flow cytometer (Becton Dickinson) and data were analyzed using Kaluza 2.0 software (Beckman Coulter).

**T Cell Proliferation**

PBMC isolated after Ficoll density gradient were labeled with carboxyfluorescein succinimidyl ester (CFSE, 200 nM; interchim, Montluçon, France). The quantification of T cells was obtained by staining PBMC with an anti-CD3 APC antibody (Becton Dickinson). PBMC were seeded in 96-well round-bottom plates at a concentration of 1 × 10^5 T cells per well. Cells were cultured in RPMI 1640 supplemented with 10% human AB serum (Biowest, Nuaillé, France) and anti-CD3 and anti-CD28 monoclonal antibodies (0.6 μg/mL, Sanquin, Amsterdam, Netherlands). When indicated, cells were cultured in presence of the following chemical inhibitors or their controls: (i) L-arginine (1 mM, Sigma-Aldrich, St Louis, MO, USA) or with control D-arginine; (ii) coptisine (an IDO inhibitor) (50 nM, Sigma-Aldrich, St Louis, MO, USA) or vehicle; and (iii) PD-L1 blocking antibodies (10 μM, ebioscience, San Diego, CA, USA) or IgG. After 4 days of culture, cells were harvested and labeled with FVS 780 (Becton Dickinson) for cell viability, CD2 PC7 and CD8 APC (Beckman Coulter), CD4 BV496 and CD14 BV605 (Becton Dickinson). CFSE dilution was assessed on FVS neg viable T cells by flow cytometry on Fortessa X20 (Becton Dickinson) and results were analyzed with ModFit LT software (Verity Software, Topsham, ME).

**Cytokine and Amino Acid Quantifications**

Plasma interleukin (IL)-6, IL-10, chemokine (C-C motif) ligand 2 (CCL2), chemokine (C-X-C motif) ligand 9 (CXCL9), CXCL10, and granulocyte-colony stimulating factor (G-CSF) were measured using a Milliplex map magnetic bead kit (EMD Millipore). Indoleamine 2,3-dioxygenase (IDO) activity was evaluated by measuring kynurenine and tryptophan levels and arginase activities by measuring ornithine and arginine concentrations by liquid chromatography coupled with tandem mass spectrometry (LC MS-MS).

**Statistical Analysis**

Continuous variables were expressed as median (interquartile range, IQR) and compared using the nonparametric Mann-Whitney U test or the Kruskal-Wallis test or the Wilcoxon matched-pairs for matched samples as appropriate. Dunn’s correction tests were used if multiple comparisons were requested. Qualitative data were compared using chi-square test or Fisher exact test when required. Correlations between two continuous variables were investigated using the Pearson’s rank test. Tests were two-sided with significance set at α less than 0.05. All data were analyzed with GraphPad Prism 8.4 (GraphPad Software, La Jolla, CA).

**Results**

**Study Population**

Twenty-six COVID-19 patients were included in this prospective study (13 with ARDS and 13 with moderate pneumonia).
Representative CT scans are shown in Fig. 1 and their demographic and baseline characteristics in Table 1. Importantly, there was no difference in demographic characteristics, including comorbidities classically associated with COVID-19 severity, nor was there any difference in duration of symptoms before inclusion between the two groups of patients, thus making it possible to focus our analysis on the biological parameters related to COVID severity. Complete clinical characteristics at day 0, day 4 and day 7 are summarized in the supplementary Table 2.

Of note, patients admitted in ICU received selected digestive decontamination as already described [27]. In addition, the patients received 3rd-generation cephalosporin for 1 week. None received corticosteroids or experimental treatments.

**Lymphopenia, Accumulation of Circulating MDSC, and Susceptibility to Nosocomial Infections**

To determine the systemic impact of SARS-COV-2 on immune cells, a complete blood count was performed first. An increase in leukocyte counts at day 4 and day 7 along with an increased number of circulating neutrophils was noted among COVID-19 ARDS patients compared to patients with moderate pneumonia (Fig. 2A). By contrast, lymphopenia was observed at admission for both groups of COVID-19 patients and persisted until day 7 only for patients with ARDS (Fig. 2A). This persistent lymphopenia was specifically related to a T cell count decrease whereas NK and B cell counts were not significantly affected (Fig. 2A). Conversely, circulating monocytes were early and transiently reduced specifically among ARDS patients. Platelet counts were not affected by clinical status. By flow cytometry, we defined CD4 and CD8 effector memory T cells to be decreased at day 4 and/or day 7 while other CD4 and CD8 subsets were unaffected (Fig. 2B and Supplementary Fig. 3). As in previous reports [6], all of our patients exhibited lymphopenia at the time of admission, predominantly due to a CD8 effector memory T cell defect (CD8 EM). This anomaly persisted during the first week after admission among ARDS patients.

In parallel, M-MDSC and G-MDSC were both enriched among moderate and severe COVID patients at day 0 and remained high until day 7 only in ARDS patients thus

| Table 1 | Demographic and baseline characteristics |
|-----------------|----------------------------------------|
|                | All patients (n = 26) | ARDS COVID (n = 13) | Moderate COVID (n = 13) | P value |
| Demographic characteristics | | | | |
| Age, years      | 55 (47–67) | 58 (53–67) | 54 (45–67) | 0.19 |
| Sex             | 18 (69)   | 10 (77)   | 8 (61)    | 0.67 |
| Men             | 8 (31)    | 3 (23)    | 5 (39)    | |
| Women           | 0         | 0         | 0         | > 0.99 |
| Coexisting conditions | | | | |
| Any             | 15 (58)   | 7 (54)    | 8 (61)    | > 0.99 |
| Diabetes        | 4 (15)    | 3 (23)    | 1 (8)     | 0.59 |
| Hypertension    | 9 (35)    | 5 (38)    | 4 (31)    | 0.22 |
| Cardiovascular disease | 3 (11) | 3 (23) | 0 | > 0.99 |
| Obesity (BMI > 30) | 9 (35) | 4 (31) | 5 (38) | > 0.99 |
| Clinical and biological baseline characteristics | | | | |
| Days from illness onset to dyspnea | 10 (8–12) | 10 (7–13) | 10 (8–11) | 0.989 |
| Ratio of PaO₂ to F₉O₂ | 214 (139–362) | 140 (110–202) | 357 (250–444) | < 0.0001 |
| SAPS II score on day 1 | 26 (17–35) | 33 (19–39) | 22 (13–28) | 0.11 |
| SOFA score on day 1 | 2 (1–9.5) | 9 (2–10) | 1 (0–1) | < 0.0001 |
| Outcomes | | | | |
| Secondary infections | 7 (27) | 7 (54) | 0 | 0.007 |
| Vasoconstrictive agents | 7 (27) | 7 (54) | 0 | 0.007 |
| CT analysis scoring | | | | |
| Moderate (10–15%) | 4 (15) | 0 | 4 (31) | 0.003 |
| Extent (25–50%) | 15 (58) | 6 (46) | 9 (69) | |
| Severe (50–75%) | 7 (27) | 7 (54) | 0 | |

Data are presented as median (IQR), n (%). P values comparing ARDS and moderate pneumonia cases are tested by Mann-Whitney U test (continuous variables) or chi-square test (categorical variables). COVID-19 coronavirus disease; BMI body mass index; IQR interquartile range; PaO₂ arterial oxygen tension; SAPS II Simplified Acute Physiology Score II; SOFA Sequential Organ Failure Assessment; CT computed tomography.
mirroring T cell lymphopenia (Fig. 2C). Interestingly, M-MDSC and G-MDSC were inversely correlated with T cell count suggesting that they could contribute to T cell dysfunction. Of note, circulating early-stage MDSC (e-MDSC) were not affected by the ARDS status (data not shown). Among the ARDS patients, seven were diagnosed with ventilator-associated pneumonia (VAP) related to infection with *Aspergillus fumigatus* (*n* = 5), herpes simplex virus (*n* = 4), and *Enterococcus faecalis* (*n* = 1). Noteworthy, VAP occurred after several days in ICU since median duration of mechanical ventilation at infection was 6 days (2–10). This subgroup of ARDS patients with secondary infections had a more marked lymphopenia already detectable on the day of admission and a higher M-MDSC count at day 7 (Fig. 2D). Conversely, G-MDSC were not associated with secondary infection. Altogether, these data indicate that COVID-19 was significantly associated with lymphopenia at admission, mostly affecting CD8 EM cells and to a lesser extent CD4 EM cells and that T cell defect was correlated with M-MDSC expansion and persists in patients with ARDS until day 7.

**Cytokines Analysis**

Since cytokine release syndrome (CRS) is a major challenge in COVID-19 [5], various plasma cytokine levels were evaluated in all our COVID-19 patients compared to Healthy Donors (HD) (Fig. 3). Several factors were increased in all COVID-19 patients, but to significantly higher levels in the ARDS group: this included proinflammatory IL-6 and anti-inflammatory IL-10, together with chemokines CCL2, CXCL10, and CXCL9. Finally, only ARDS patients showed increased G-CSF.

**COVID-19 Is Associated with a Significant Decrease of Arginine Concentration; Its Supplementation Restores In Vitro T Cells’ Ability to Proliferate**

The MDSC increase prompted us to evaluate the activity of enzymes known to support their immunosuppressive activity. Indoleamine 2,3-dioxygenase (IDO) is known to be expressed in M-MDSC [19]. Accordingly, enhanced IDO activity was found among COVID-19 patients, and it was predominant among patients with ARDS and correlated with lymphopenia (Fig. 4A). Additionally, we measured arginase activity, which is expressed by e-MDSC, G-MDSC, and M-MDSC [28]. Our results showed a prolonged decrease in plasma arginine levels along with enhanced arginase activity among COVID patients, particularly in the most severe forms. As for IDO, a correlation between arginase activity and lymphopenia was observed (Fig. 4A). Both IDO and arginase activity could negatively have
SARS-CoV-2-induced acute respiratory distress syndrome (ARDS) is associated with lymphopenia and an accumulation of circulating myeloid-derived suppressor cells (MDSC) leading to a higher susceptibility to nosocomial infections. Blood count from 13 patients hospitalized for SARS-CoV-2 moderate pneumonia (MP) and 13 patients hospitalized for SARS-CoV-2 ARDS (ARDS) 24 h after their admission (D0), 4 days after (D4), and 7 days after (D7) and lymphocytes subsets defined by flow cytometry from 13 healthy donors (HD), 13 patients hospitalized for SARS-CoV-2 MP, and 13 patients hospitalized for SARS-CoV-2 ARDS. A CD4 and CD8 effector memory (EM) T cell numeration by flow cytometry. C Peripheral monocytic-MDSC (M-MDSC) and granulocytic-MDSC (G-MDSC) recruitment among ARDS patients, moderate COVID cases, and HD. D Two groups were defined according to the presence or absence of a nosocomial infection. Lymphocyte count, M-MDSC, and G-MDSC recruitment according to the acquisition of nosocomial infection. Nosocomial infections as defined by the Centers for Disease Control and Prevention were screened among patients hospitalized for a SARS-CoV-2 infection over 28 days after their admission. Box and whiskers plot features are as follows: central line in the box is the median, bottom line of the box is first quartile (25%), and top line of box is third quartile (75%). Bottom of whiskers is minimum value; top of whiskers is maximum value. Groups were compared using Kruskal Wallis test with Dunn’s multiple comparison test (A, B, and C) or Mann-Whitney U test (A and D) as appropriate. Pearson correlation coefficients (rho) and P values are indicated for each correlation (C). *P < 0.05; **P < 0.01; ***P < 0.001
a negative impact on T cell functions, and T cell proliferation is in particular dependent on arginine supply [28, 29]. Functional assessment of COVID-19 patient T cells accordingly showed a decreased ability to proliferate in vitro, which predominated for CD8 T cells, although no difference was found between both groups of COVID-19 patients (ARDS), the moderate COVID cases (MP), and the healthy donors (HD). Box and whiskers plot features are as follows: central line in the box is the median, bottom line of the box is first quartile (25%), and top line of box is third quartile (75%). Bottom of whiskers is minimum value; top of whiskers is maximum value. Groups were compared using Kruskal Wallis test with Dunn’s multiple comparison test. *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001

Discussion

Severe forms of COVID-19 with ARDS have been associated with biological and clinical markers of acquired immunosuppression such as opportunistic bacterial infection, virus reactivation, and invasive aspergillosis [1, 11, 30]. This immunosuppression constantly involves lymphopenia and an unbalanced innate vs adaptive immune response, thus delaying pathogen clearance and promoting lung injury. However, the basis for such a dysregulation remain poorly understood [5, 21, 31]. We found that COVID-19 was significantly associated with lymphopenia at admission, mostly affecting CD8 EM cells, and that T cell defects were correlated with M-MDSC expansion and decreased arginine level.

Reduced T cell number persisted during the first week after admission among ARDS patients, confirming that lymphopenia is a marker of severity in COVID-19 [6, 32]. CD8 T cells are essential for controlling the spread of the virus and for limiting pulmonary damage and mortality, by eliminating infected epithelial cells, thus decreasing virus replication [29, 33]. Autopsies of some patients with COVID-19 pneumonia have evidenced interstitial inflammatory infiltrates dominated by lymphocytes, suggesting that lymphopenia may result from the migration of T cells to injured tissues [34]. Whereas infected T cells by SARS-CoV-2 has been
documented, it is nevertheless unclear whether a direct cytotoxic effect might contribute to lymphopenia, unlike MERS-CoV infection, in which the virus directly binds its receptor to T cells [8, 9]. Our findings suggest that the diminished ability of lymphocytes to proliferate and the enhanced apoptosis are among contributing factors of change in lymphocyte count at
Wallis test with Dunn whiskers is maximum value. Groups were compared using Kruskal median, bottom line of the box is first quartile (25%), and top line of box and whiskers plot features are as follows: central line in the box is the proportion proliferated T cells were determined by flow cytometry. Box vehicle (NT), PD-L1 binding antibody (aPD-L1), or its isotype (IgG). The (L-Arg) or with control D-arginine (D-arg), IDO inhibitor (Coptisine) or ARDS were stimulated with anti-CD3/anti-CD28 monoclonal antibodies after CFSE labelling. The proportion of proliferated T-cells were determined by flow cytometry. C Proportion of apoptotic T cells was determined by flow cytometry using a Caspase-3 staining among 26 patients hospitalized for a SARS-CoV-2 infection at admission (D0), 4 days after (D4), and 7 days after (D7) and from 11 HD. Results are expressed by the percentage of Caspase3pos T cells. D PBMC obtained from 7 patients hospitalized for a SARS-CoV-2 infection at admission (D0), 4 days after (D4), and 7 days after (D7) and from 11 HD. Results are expressed by the percentage of Caspase3pos T cells. A Plasma arginine concentration; its supplementation restores the ability of T-cells to proliferate in vitro. A Plasma arginine, ornithine, kynurenine, and tryptophan concentrations were measured by liquid chromatography coupled with tandem mass spectrometry among 13 healthy donors (HD), 13 patients hospitalized for SARS-CoV-2 moderate pneumonia (MP), and 13 patients hospitalized for SARS-CoV-2 ARDS (ARDS) 24 h after their admission (D0), 4 days after (D4), and 7 days after (D7). Arginase activity was calculated using the ornithine/arginine ratio and IDO activity was calculated using the kynurenine/tryptophan ratio. B PBMC obtained at admission (D0), 4 days after admission (D4), and 7 days after (D7) from 14 patients hospitalized for a SARS-CoV-2 infection and from 7 HD were stimulated with anti-CD3/anti-CD28 monoclonal antibodies after CFSE labelling. The proportion of proliferated T-cells were determined by flow cytometry. C Proportion of apoptotic T cells was determined by flow cytometry using a Caspase-3 staining among 26 patients hospitalized for a SARS-CoV-2 infection at admission (D0), 4 days after (D4), and 7 days after (D7) and from 11 HD. Results are expressed by the percentage of Caspase3pos T cells. D PBMC obtained from 7 patients hospitalized for a SARS-CoV-2 ARDS were stimulated with anti-CD3/anti-CD28 monoclonal antibodies after CFSE labelling. Culture media were enriched with either L-arginine (L-Arg) or with control D-arginine (D-arg), IDO inhibitor (Coptisine) or vehicle (NT), PD-L1 binding antibody (aPD-L1), or its isotype (IgG). The proportion proliferated T cells were determined by flow cytometry. Box and whiskers plot features are as follows: central line in the box is the median, bottom line of the box is first quartile (25%), and top line of box is third quartile (75%). Bottom of whiskers is minimum value; top of whiskers is maximum value. Groups were compared using Kruskal Wallis test with Dunn’s multiple comparison test (A), Mann-Whitney U test (A, B, and C) or Wilcoxon test (D) as appropriate. Pearson correlation coefficients (rho) and P values are indicated for each correlation (a). *P < 0.05; **P < 0.01; ***P < 0.001.
adjuvant therapeutic interventions are needed to limit the dysfunctional immune response observed among COVID-19 patients and to decrease the late mortality observed in ICU due to persistent pulmonary inflammation and nosocomial infections acquisition [40]. Arginine administration has been found to maintain immune homeostasis, particularly with respect to T cells and macrophage functions and a reversal of the alteration in T cell function associated with trauma or surgery has been demonstrated among patients receiving arginine-enriched diet [41, 42]. Although there are no convincing results in patients with sepsis [43], restoring arginine availability could restore normal T cell function including their ability to proliferate, as we found in the present study.

Our study has several limitations. Firstly, only a few patients were included and we did not conduct sample size calculation. This methodological issue could weaken our conclusions. Secondly, we did not provide mechanistic information on how arginine could restore T cell function. Although the immunosuppressive effects of MDSC mediated through arginine depletion and lymphocyte mitochondrial dysfunction have been extensively studied in malignancies [28], mechanistic approaches should be performed in COVID-19 patients to strengthen our conclusions and develop therapeutic trials. Lastly, since T cell subpopulations differ in their proliferation rates and metabolic profile when activated, providing data regarding the capacity of purified COVID-19 T cell subsets to proliferate would be mandatory to definitely conclude on the qualitative versus quantitative T cell alterations [28, 44, 45].

**Conclusion**

To summarize, COVID-19 is responsible for MDSC expansion and T cell dysfunction, mainly CD8 T cells, through arginine depletion. This dysfunction is sustained in ARDS patients, the most severe form of COVID-19. Restoring arginine availability could be of value to restore lymphocyte function and prevent patients from acquiring nosocomial infections during their ICU stay.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s10875-020-00920-5.

**Author Contributions** Conception and design was provided by FR, ML, MG, MR, MC, KT, and JMT. Experiments, data analysis, and interpretation were carried out by FR, ML, MG, KP, AG, MaL, CV, DR, SLG, JD, CM, CB, MR, MC, KT, and JMT. Patient inclusions were performed by AM, BP, CC, YLT, MRe, ALB, AB, BCR, TL, and YL. CT scan analyses were performed by MLed. Drafting and revision of the manuscript were carried out by FR, ML, MG, MR, MC, KT, and JMT.

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**Compliance with Ethical Standards**

**Conflict of Interest** The authors declare that they have no competing interests.

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