JCI insight

Severe immunosuppression and not a cytokine storm characterize COVID-19 infections

Kenneth E. Remy, … , Lyle L. Moldawer, Richard S. Hotchkiss

JCI Insight. 2020. https://doi.org/10.1172/jci.insight.140329.

Research In-Press Preview COVID-19

COVID-19-associated morbidity and mortality have been attributed to a pathologic host response. Two divergent hypotheses have been proposed: a hyper-inflammatory 'cytokine-storm'-mediated injury versus failure of host protective immunity resulting in unrestrained viral dissemination and organ injury. A key explanation for the inability to address this controversy has been the lack of diagnostic tools to evaluate immune function in COVID-19 infections. ELISpot, a highly sensitive, functional immunoassay was employed in 27 COVID-19, 51 septic, 18 critically-ill non-septic (CINS), and 27 healthy controls to evaluate adaptive and innate immune status by quantitating T cell IFN-ɣ and monocyte TFN-α production. Circulating T cell subsets were profoundly reduced in COVID-19 patients. Additionally, stimulated blood mononuclear cells produced less than 40% to 50% of the IFN-ɣ and TNF-α observed in septic and CINS patients, consistent with markedly impaired immune effector cell function. Approximately 25% of COVID-19 patients had increased IL-6 levels greater than 1,000 pg/mL that were not associated with elevations in other canonical pro-inflammatory cytokines. Collectively, these findings support the hypothesis that COVID-19 suppresses host functional adaptive and innate immunity. Importantly, Interleukin-7 administered ex vivo restored T cell IFN-ɣ production in COVID-19 patients. Thus, ELISpot may functionally characterize host immunity in COVID-19 and inform prospective therapies.

Severe Immunosuppression and not a Cytokine Storm Characterize COVID-19 Infections

Kenneth E. Remy[1,2,3], Monty Mazer[1,3], David A. Striker[4], Ali H. Ellebedy[5], Andrew H. Walton[3], Jacqueline Unsinger[3], Teresa M. Blood[3], Philip A. Mudd[6], Daehan J. Yi[1], Daniel A. Mannion[1,7], Dale F. Osborne[3], R. Scott Martin[4], Nitin J. Anand[4], James P. Bosanquet[4], Jane Blood[3], Anne M. Drewry[3], Charles C. Caldwell[8], Isaiah R. Turnbull[9], Scott C. Brakenridge[10], Lyle L. Moldwawer[10], Richard S. Hotchkiss[2,3,9]

[1]Department of Pediatrics, Washington University School of Medicine, St. Louis, MO, USA.

[2]Department of Internal Medicine, Washington University School of Medicine, St. Louis, MO, USA.

[3]Department of Anesthesiology, Washington University School of Medicine, St. Louis, MO, USA.

[4]Department of Critical Care, Missouri Baptist Medical Center, St. Louis, USA.

[5]Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO, USA.

[6]Department of Emergency Medicine, Washington University School of Medicine, St. Louis, MO, USA.

[7]Saint Louis University School of Medicine, St. Louis, MO, USA.

[8]Department of Surgery, University of Cincinnati College of Medicine, Cincinnati, OH, USA.

[9]Department of Surgery, Washington University School of Medicine, St. Louis, MO, USA.

[10]Department of Surgery, Sepsis and Critical Illness Research Center, University of Florida College of Medicine, Gainesville, FL, USA.

Corresponding Author: Richard S. Hotchkiss, MD*:* Departments of Anesthesiology, Medicine, and Surgery, Washington University School of Medicine, St Louis, 660 South Euclid Avenue, St Louis, MO, 63110-1093, USA. (314) richardshotchkiss@wustl.edu

Word count*:* 8210

Conflict of Interest: RSH, KER, LLM, and SCB are investigators on a clinical trial of IL-7 conducted by RevImmune in sepsis and COVID-19.

Abstract

COVID-19-associated morbidity and mortality have been attributed to a pathologic host response. Two divergent hypotheses have been proposed: a hyper-inflammatory *'cytokine-storm'*-mediated injury versus failure of host protective immunity resulting in unrestrained viral dissemination and organ injury. A key explanation for the inability to address this controversy has been the lack of diagnostic tools to evaluate immune *function* in COVID-19 infections. ELISpot, a highly sensitive, *functional* immunoassay was employed in 27 COVID-19, 51 septic, 18 critically-ill non-septic (CINS), and 27 healthy controls to evaluate adaptive and innate immune status by quantitating T cell IFN-ɣ and monocyte TFN-α production.

Circulating T cell subsets were profoundly reduced in COVID-19 patients. Additionally, stimulated blood mononuclear cells produced less than 40-50% of the IFN-ɣ and TNF-α observed in septic and CINS patients, consistent with markedly impaired immune effector cell function. Approximately 25% of COVID-19 patients had increased IL-6 levels >1,000 pg/mL that were not associated with elevations in other canonical pro-inflammatory cytokines. Collectively, these findings support the hypothesis that COVID-19 suppresses host *functional* adaptive and innate immunity. Importantly, Interleukin-7 administered ex-vivo restored T cell IFN-ɣ production in COVID-19 patients. Thus, ELISpot may functionally characterize host immunity in COVID-19 and inform prospective therapies.

Introduction

One of the most remarkable realities about the current SARS-CoV-2 infection outbreak (COVID-19) is that despite intense worldwide investigations, the decisive pathophysiologic processes that are responsible for patient morbidity and mortality remain unknown. Currently, the predominant paradigm is that an over-exuberant immune response mediated by excessive pro-inflammatory cytokines drives excessive lung injury and a pro-coagulant state(1-7). Accordingly, death is assumed to be primarily due to inflammatory lung injury, disturbances in micro- and macro-circulation, and resultant respiratory failure or vascular coagulopathy(8-14). This concept of a '*cytokine storm'*-mediated death in COVID-19 patients has been popularized in both the lay press and in many leading scientific publications(6, 15). Based upon this theory, a number of anti-cytokine and anti-inflammatory therapies are being tested in COVID-19 including anti-IL-6(R) antibodies, IL-1 receptor antagonists, and JAK-STAT inhibitors, with early trial results failing to demonstrate significant efficacy(2, 3, 9, 15-18).

Paradoxically, a second and diametrically opposed theory for COVID-19-induced morbidity and mortality is an '*immunologic collapse'* of the host's protective system(15, 19-21). This collapse of host protective immunity manifests itself as a failure to control unrestrained viral replication and dissemination with direct host cytotoxicity. Support for this contrasting theory is based upon the observed progressive and profound lymphopenia, often to numbers seen in patients with AIDS(22). Unlike the *'cytokine storm'* which is often considered episodic, multiple recent studies show that lymphopenia is incessant in critically-ill COVID-19 patients and correlates with increased secondary infections and death(11, 13). Postmortem studies of patients dying

of COVID-19 have also described a devastating loss of immune cells in spleen and secondary lymphoid organs(23). Multiple lymphocyte subsets are lost, including CD4 T, CD8 T, and NK cells that play vital antiviral roles, and in B cells that are essential for making antibodies that neutralize the virus(4, 21, 24-26).

Personalized medicine approaches require a better understanding on which of these immune endotypes predominate because the appropriate intervention is diametrically different depending upon whether the patient is suffering from hyper-inflammation or profound immunosuppression. For example, anti-IL-6(R) antibodies, IL-1 receptor antagonists and JAK-STAT inhibitors are currently undergoing clinical testing in COVID-19 patients(27-32) and carry the potential to further compromise the patient's ability to eradicate the virus. Conversely, treatment with immune stimulants such as checkpoint inhibitors, IL-7, interferon-γ or GM-CSF, currently either proposed or in active clinical trials in COVID-19(15, 33), could exacerbate a dysfunctional and robust inflammatory response, and worsen organ injury.

Two distinct and key questions must be addressed in critically-ill COVID-19 patients: (1) what is their primary immune endotype, i.e., hyper-inflammatory versus immunosuppressive, and (2) how do these evolve over time with regards to disease progression or resolution. A better understanding of the COVID-19 patient's immune status would be instrumental in guiding proper immunotherapy.

There have been many efforts to immune endotype patients using genomic or proteomic biomarkers of immunity(34, 35). While these methods have been helpful in predicting outcomes in sepsis and other disorders(36, 37), in general they have not

been able to either provide an accurate assessment of the *functional state* of host immunity as it varies over time, or have been used to determine response to therapy. Enzyme-linked immunosorbent spot (ELISpot) is a highly sensitive, functional immunoassay that measures the number of cytokine-secreting cells at the single-cell level in response to ex vivo stimulation(38, 39). A key advantage of ELISpot is that the assay has excellent dynamic range. ELISpot can detect as few as one in 100,000 cytokine-secreting cells. Furthermore, ELISpot can test simultaneously the integrity and robustness of the two disparate arms of immunity, i.e., innate (blood monocytes and low-density granulocytes) and adaptive cellular immunity (blood lymphocytes) by focusing on the responses of individual cell populations to cell-specific agonists.

The purpose of this study was to determine whether critically ill COVID-19 patients have an exaggerated pro-inflammatory "*cytokine storm*" versus an immunosuppressive immunological endotype of COVID-19 patients, and determine whether their immune function changes during the progression of their disease. To provide a comprehensive evaluation, we utilized conventional flow cytometry to quantitate the effect of COVID-19 to cause depletion of immune effector cells. In addition to quantitating circulating pro- and anti-inflammatory cytokines, we evaluated adaptive and innate immune system via serial ELISpot assays of T cell interferon-gamma (IFN-ɣ) and monocytes TNF-α production respectively.

Results

Demographic and Clinical Characteristics

We enrolled 27 COVID-19, 51 septic, 18 critically-ill non-septic (CINS) patients in a prospective observational cohort study (Table 1) evaluating innate and adaptive immune function in SARS-CoV-2 infection over two weeks after intensive care unit (ICU) admission. Primary diagnosis for COVID-19, septic, and CINS are included in Supplemental tables 1, 2, and 4. Twenty-seven healthy subjects served as controls.

COVID-19 patients were hospitalized in the ICU with a mean of 6 (1-14) days after onset of symptoms. Twenty-three out of 27 COVID-19 patients were intubated and received invasive mechanical ventilation on average 1 (0-5) day from ICU admission. The mean SOFA and APACHE II scores were the equivalent in the COVID-19 and sepsis cohorts (7 and 18 respectively). The 30-day mortality was greater in the COVID-19 group than in patients with sepsis (37% vs. 22%; p=0.14) but did not reach statistical significance. All non-surviving COVID-19 patients died later than two weeks after onset of symptoms and at least after day six of ICU admission (Figure 1A,B).

The absolute lymphocyte counts (ALC) for COVID-19 patients was 900 cells/mm^3 and non-survivors had persistent lymphopenia throughout their course of illness compared with COVID-19 survivors (Figure 1C, Table 1). Ten of the 27 COVID-19 patients (37%) had evidence of secondary infections during the first 30 days after enrollment. Thirty percent of patients with secondary infection were non-survivors and one patient had co-infection with coronavirus 229E at admission.

Plasma Cytokines

To evaluate the inflammatory response over time, plasma cytokines were measured in COVID-19, septic, CINS patients, and healthy control subjects (Table 2). COVID-19 and septic patients were followed for up to 4 serial time points during their ICU admission. The mean number of sample time points was 2.2 for the COVID-19 patients and 3 for septic patients. A single time point was used for healthy controls and CINS patients. Of note, for COVID-19 patients, the blood sample for cytokine analysis was obtained within the first 24 hours from clinical deterioration (endotracheal intubation) after admission to the ICU in order to try to capture the early hyper-inflammatory phase of infection. Although several key pro-inflammatory cytokines including IL-1β, IFN-ɣ, and TNF-α were modestly increased in COVID-19 patients compared to healthy control subjects, the increases were close to the lower limit of detection of the assay (Table 2). There was considerable variation in the plasma IL-6 levels in COVID-19 patients with a range from 6 to over 5,000 pg/μL (Fig. 2). IL-6 concentrations were elevated compared to healthy controls.

COVID-19 induces profound suppression of T cell IFN-ɣ production

In order to determine the presence and magnitude of functional immunosuppression during COVID-19 infection, quantitation of the number of IFN-ɣ and TNF-α producing cells in overnight cell culture was assessed in isolated peripheral blood mononuclear cells (PBMCs) by ELISpot analysis after admission. PBMCs were stimulated and incubated overnight with anti-CD3/anti-CD28 to activate T cells, and IFN-ɣ producing cell numbers were quantified. Data are expressed as positive secreting cells per thousand lymphocytes plated. Representative ELISpot figures for IFN-ɣ producing cells of representative COVID-19, septic, CINS patients and healthy controls

are shown in Figure 3. Quantitatively, the number of cells producing IFN-ɣ in patients with COVID-19 infection was significantly reduced compared to all the other groups; p = 0.004. Stimulated healthy controls had nearly three-fold more IFN-ɣ producing cells than COVID-19 patients (mean 14.4 ± 2.5 vs. 4.8 ± 1). CINS had three-fold greater levels of stimulated IFN-ɣ production than COVID-19 patients (mean 15.7 ± 2 vs. 4.8 ± 1) Additionally, the mean number of IFN-ɣ producing cells in septic patients was two-fold greater than in COVID-19 patients (mean 12 ± 2 vs. 4.8 ± 1) (Figure 4A, Supplemental Figure 1).

COVID-19 induces profound suppression of monocyte TNF-α

PBMCs were also stimulated overnight with lipopolysaccharide (LPS) to activate monocytes and the numbers of TNF-α producing cells were measured for COVID-19, septic, or CINS patients. Data for TNF-α cytokine producing cells are expressed as secreting cells per thousand myeloid cells plated. Representative ELISpot figures for the mean number of TNF-α producing cells of three different COVID-19, septic, CINS patients and healthy controls are shown in Figure 5.

Importantly, there was considerable patient heterogeneity in TNF-α production determined by ELISpot assay. A subset of COVID-19 patients had LPS-stimulated TNF-α production that was comparable to that occurring in other critically-ill patients, while a large number of COVID-19 patients had reduced production (Figure 4B). None of the COVID-19 patients had increased TNF-α production in response to LPS stimulation.

Quantitatively, the number of cells producing TNF-α was reduced 3-fold and 2-fold in patients with COVID-19 compared to CINS and septic patients, respectively; p =

0.009; mean CINS: 272 ± 64; septic 168 ± 22; COVID-19: 80 ± 14. Compared with healthy volunteers, stimulated PBMCs from COVID-19 patients had half as many TNF-α producing cells (healthy 177.5 ± 27). (Figure 4B, supplemental figure 2).

Both innate and adaptive immune cells from COVID-19 patients who experienced mortality within 30 days of ICU admission were among the most phenotypically suppressed samples. Although not statistically significant, COVID-19 non-survivors had quantitatively low ELISpot IFN-ɣ and TNF-α production (Figure 4, red dots).

Sustained immune suppression over time in COVID-19 patients

COVID-19 patients were followed over time with serial ELISpot assays and the mean number of IFN-ɣ and TNF-α producing cells remained suppressed and did not improve over the time course of their disease (IFN-ɣ p=0.54, TNF-α p=0.42) (Figure 6). Although non-survivors maintained lower numbers of IFN-ɣ and TNF-α producing cells than survivors this did not reach statistical significance.

Profound depletion of CD4 and CD8 T cells in COVID-19

Flow cytometric analysis of samples was performed in all COVID-19 patients (days 1-3, 4-7, 8-11, and 12-15) and in CINS patients (days 1-3 patients as previously described[33,34]. ALC was profoundly depressed in COVID-19 patients over the entire duration of the study compared to non-septic patients (first comparison days 1-3; p=0.01) (Figure 7A). Next, we evaluated CD3, CD4, CD8 T cell, NK, and monocyte absolute cell numbers (Figure 7B-F). The CD3, CD4, CD8 T cell numbers were severely depressed compared to the normal range (pink shaded area) reported for healthy individuals at the Clinical and Diagnostic CLIA-CLA Laboratories at Barnes Jewish

Hospital, and remained suppressed for the duration of the study. Although CINS patient samples were not followed sequentially, their initial values were reduced similarly to the low levels found in the COVID-19 patients.

Interleukin-7 increases T cell IFN-ɣ production in COVID-19

The potential efficacy for interleukin (IL)-7 as an immunoadjuvant therapy to restore COVID-19 induced T cell exhaustion, patient derived PBMCs were co-cultured with IL-7 for ELISpot analysis. The mean number of IFN-ɣ producing T cells from COVID-19 patients nearly doubled from 101 ± 21 to 201 ± 36 ($p<0.0001$) following ex vivo administration of IL-7 (Figure 8A). Although there was an increase in LPS-induced TNF-α producing cells in some samples, and a mean increase of 101% overall after IL-7 co-incubation, these changes were not statistically significant (Figure 8B). The effect of IL-7 to increase the number of IFN-ɣ producing T cells was also observed in septic and CINS patients (Supplemental Figures 3 and 4).

Discussion

Currently, the prevailing paradigm that guides the therapeutic approach to COVID-19 is that patients are dying from the effects of *'cytokine storm'*-mediated inflammation with resultant lung and other organ injury(6, 7, 40-43). Based upon this theory of unbridled inflammation, COVID-19 patients are currently being treated with a variety of drugs that block pro-inflammatory cytokines or inhibit the inflammatory signaling cascade. *The results from the present study strongly suggest that the primary endotype of COVID-19 is one of immunosuppression rather than hyperinflammation. Therefore, the approach to broadly inhibit the host inflammatory response may be misguided, and may actually worsen clinical trajectories in some COVID-19 patients due to further impairment of an already compromised host protective immune response*. Circulating cytokines in COVID-19 patients, at least early in their clinical course, did not show widespread elevations. Most COVID-19 patients had either no elevations or only mild increases in the major pro-inflammatory cytokines including TNF-α, IL-1α, IL-1β, IFN-ɣ, etc. (Table 2). There were modest elevations in plasma IL-6 in COVID-19 patients, with only 6 patients reaching IL-6 concentrations above 1,000 pg/μL, as typically seen during overwhelming bacterial sepsis or the cytokine release syndrome(44, 45). There were two additional COVID-19 patients who had IL-6 levels close to 1000 pg/mL as well as four patients whose IL-6 levels were above the level of detection for the assay. Of the aforementioned patients, sustained elevation of IL-6 was detected in some, while others had variable fluctuations in their IL-6 levels over time. In addition to macrophages, IL-6 can be made by many different types of cells including pulmonary epithelial cells, infected with coronaviruses(1, 46). Thus, the increase in IL-6,

and IL-8 concentrations that occur in COVID-19 infection may be more a reflection of viral-induced epithelial cell production or cell injury rather that evidence for a systemic hyper-inflammatory response.

In addition, there is no evidence of exaggerated TNF-α production in response to ex vivo LPS stimulation of PBMCs when compared to septic and critically ill non-septic patients, nor do the patients have elevated plasma TNF-α levels. Rather, the findings show a predominant endotype of immunosuppression, manifesting as both a profound and sustained loss of CD4 and CD8 T cells, as well as a reduced responsiveness of remaining lymphocytes to T cell receptor activation. These cells and their responsiveness are essential to containing and eliminating viral pathogens(47). The key finding in the present study is that we can unequivocally demonstrate that not only is there a loss in the numbers of immune cells, but there is also an accompanying critical defect in the responsiveness of surviving lymphocytes and monocytes.

A novel aspect of the present study is the use of ELISpot assays performed on freshly obtained blood samples to evaluate individual immune cell responsiveness to agonists. The ELISpot method provides an improved readout of cell function with enhanced sensitivity and increased dynamic range compared to flow cytometric techniques(15, 38). The ELISpot assay shows that stimulated PBMCs from COVID-19 patients will activate less than half of the IFN-ɣ producing lymphocytes seen in similar assays for by critically-ill non-septic patients ($p<0.0001$). Similar declines were seen in LPS-stimulated TNF-α production by monocytes from COVID-19 patients. Interestingly, COVID-19 patients that died appeared to have the most profound suppression of TNF-α

and IFN-ɣ production (Figure 4) and the immune suppression is sustained through at least the first three weeks of ICU admission (Figure 6).

Both clinical and pathological findings suggest that immunosuppression is a critical pathophysiologic phenomenon of COVID-19. Zhou et al reported that 50% of COVID-19 patients who die develop secondary hospital-acquired infections(48). Autopsy studies of COVID-19 patients demonstrate inclusion bodies, pathologic findings consistent with viral persistence within cells present in lung, kidney, and other organs(23, 49, 50). A recent autopsy investigation of 12 patients who died of COVID-19 showed that 11 of the patients had up to 500,000 viral copies/1x10^6 RPPH1 copies detected in lung tissue by SARS-CoV2 specific RT-qPCR(51). Ten of their 12 patients had superimposed bronchopneumonia with both focal and diffuse distribution. *Collectively, these studies suggest that there is an inability of the host to mount an adequate immune defense leading to viral dissemination and organ injury and rendering the patient more susceptible to subsequent hospital-acquired infections.*

One important implication of the massive depletion and impaired function of lymphocytes is that immune adjuvants that enhance host immunity should be strongly considered as potential therapeutic interventions in patients with COVID-19. Decades of mechanistic immunologic studies have invariably demonstrated that an intact T cell-mediated adaptive immune response is required for eliminating and suppressing viral infections(52). Support for this potential immune therapeutic approach is provided by studies showing that checkpoint inhibitors and common γ-chain cytokines, which stimulate CD4 and CD8 T cells, have been effective in a number of serious viral infections including hepatitis C, JC virus induced progressive multi-focal

leukoencephalopathy, and HIV(47, 53). Several of these agents (NKG2D-ACE2 CAR-NK cells, anti-PD1, IL-7) are either in active clinical trials or in the planning stages in COVID-19 (*NCT04324996; NCT04356508, NCT04379076*).

Of particular relevance regarding potential immune adjuvant therapy for COVID-19 are the ELISpot results showing that ex vivo IL-7 increased IFN-ɣ production of stimulated T cells nearly two-fold (Figure 8). A clinical trial of IL-7 in patients with sepsis showed that IL-7 was well-tolerated, reversed sepsis-induced lymphopenia, and increased CD4 and CD8 T cells by 2-3 fold(54).

Another important implication of the present study is that ELISpot may be used to phenotype COVID-19 patients to determine appropriate immuno-modulatory drug therapies. Results of the ELISpot analysis showed that some COVID-19 patients displayed ex vivo cytokine production comparable to results from CINS patients (Figure 4). Therefore, use of immunostimulant therapies to restore protective immunity in these patients might not be indicated. Conversely, COVID-19 patients who had severe reduction in T cell or monocyte cytokine production might benefit from agents to boost their host immunity. We would expect the ELISpot assay could be used clinically to evaluate the progression of immune dysfunction and to evaluate the effect of different immune therapies to restore innate and adaptive immunity in an immunosuppressed patient.

Limitations

There are several limitations to the present study. Most of the COVID-19 patients had symptoms of infection several days prior to hospitalization (Figure 1). Although an

early and excessive hyper-inflammatory phase may have already occurred prior to hospitalization, we deem this unlikely because significant systemic inflammatory reactions typically induce hypotension and dyspnea that would have led patients to seek immediate care. A second limitation to this study is that it does not exclude a subset of COVID-19 who do have cytokine-storm mediated hyper-inflammation with accompanying lung and organ injury. Thus, anti-cytokine therapy or drugs to negatively modulate the inflammatory response may be beneficial in this subset of patients. However, the present results definitively show that a markedly immunosuppressive phenotype predominates in COVID-19 patients. The ongoing clinical trials of anti-cytokine agents and immunosuppressive therapies will likely resolve whether COVID-19 patients actually have damaging hyper-inflammatory responses. Ultimately, in order to eradicate the virus, patients need a competent and active immune system, and research should focus on such therapies to restore this vital function.

Finally, the present results, which are based on blood measurements, do not exclude the possibility that damaging inflammation occurs locally within the lung and other organs that is not detected by levels of circulating cytokines or ELISpot analysis of peripheral blood mononuclear cells. Direct examination of samples obtained by bronchoalveolar lavage would help address this issue of potential compartmentalized response to COVID-19 infection.

Conclusions

We conclude that the major immunologic abnormality in COVID-19 is a profound defect in host immunity and not hyper-cytokinemia induced organ injury. The defect in

host immunity includes both a profound depletion in the number of effector immune cells as well as severe functional defects in T cell and monocyte function. Based upon these findings, immuno-adjuvant therapies to enhance host immunity should be considered. Evaluating patient innate and adaptive immunity using functional assays such as the ELISpot may be useful in guiding immuno-modulatory therapies. IL-7 reverses T cell exhaustion in COVID-19 and should be considered as a potential therapy in this highly lethal disorder.

Methods

Study design

A prospective observational cohort study among patients with COVID-19 in a mixed medical and surgical ICU between March 2020 and May 2020 at Missouri Baptist Medical Center and Barnes-Jewish Hospital (St. Louis, MO). Blood sampling and data collection and analysis were approved by the Institutional Review Boards at each respective hospital (protocols # MOBAP 1132, WUSTL 201211101, WUSTL 201603006, WUSTL 202003085, and 201808049). Informed consent was obtained from healthy control subjects and patients or their legally authorized representatives. Additionally, comparison with sepsis or critically ill non-septic patients (CINS) were utilized from previously obtained sampling (2018-20).

Patient demographic data, including clinical course, relevant laboratory testing, onset of symptoms prior to admission to the hospital, morbidity, mortality, and medical management data were collected and de-identified. Complete blood counts were recorded at the time closest to blood sampling for immune functional testing. For the COVID-19 patients, the first study blood sample was obtained within the first 24 hours from clinical deterioration (endotracheal intubation) after admission to the ICU in order to try to capture the early hyper-inflammatory phase of infection. COVID-19 patients had two blood draws weekly for maximum of four blood draws, and septic patients had the option for a redraw at one week if the patient remained in the ICU.

Inclusion criteria

We included hospitalized patients, 18 years old or greater, who were COVID-19 positive via either nasopharyngeal- or tracheal aspirate-derived SARS-CoV-2 RNA

using an FDA-approved clinical PCR test. COVID-19 testing results were available from 6-30 hours after hospital admission. Patients with sepsis were defined as previously described(54) including the presence of two or more criteria for systemic inflammatory response syndrome (SIRS), two or greater point increase on the sequential organ failure assessment (SOFA) score and a clinically or microbiologically suspected infection were included in the study. CINS patients included patients admitted to the medical or surgical ICU following major surgical procedures, major traumatic injury or with non-infectious causes of organ failure, requiring intensive care management who were not suspected of having infection. Healthy control subjects had no ongoing infections or auto-immune disease, and no past history of cancer or solid organ transplant.

Exclusion criteria

No screened patients were excluded from the COVID-19 cohort. For the critically-ill groups, to minimize confounding effects of immunosuppressive medications or underlying immunologic disease, patients with the following criteria were excluded: (1) patients with active cancer and/or undergoing chemotherapy or radiation treatment within past 6 weeks, (2) HIV, (3) known history of acute or chronic lymphocytic leukemia, (4) pregnancy, (5) organ or bone marrow transplantation, (6) use of current high-dose corticosteroid regimens that were greater than or equivalent to 300 mgs/day of hydrocortisone or other immunosuppressive medications, (7) current use of immune-modifying biological agents including inhibitors of TNF-α or other cytokines, viral hepatitis, or systemic autoimmune diseases, or (8) participation in another interventional trial within the past four weeks

Specific Laboratory Studies

Plasma Cytokine Measurements

Cytokine quantitation was performed on plasma obtained from patients (frozen at -80°C prior to use), and subsequently analyzed using a human MagPix™ multiplex cytokine panel (Invitrogen) and analyzed on a Luminex FLEXMAP™ 3D instrument according to the manufacturer's instructions.

ELISpot quantitation of IFN-ɣ and TNF-α production

Quantitation of the number of IFN-ɣ and TNF-α producing cells was assessed on isolated peripheral blood mononuclear cells (PBMCs) by ELISpot analysis, as per the manufacturer's instruction (CTL Immunospot, R&D Systems) and as previously described(38, 39). Patient PBMCs were harvested from whole blood via Ficoll-Paque™, counted using the Vi-Cell™ counter from Beckman Coulter (Brea, CA, USA), and incubated overnight plated in 96-well ELISpot culture plates with CLT media or RPMI 1640 media (Sigma-Aldrich, St. Louis, MO) supplemented with human AB serum, non-essential amino acids, penicillin/streptomycin, and L-glutamine. Septic and CINS subject samples are plated in duplicate and COVID-19 subject samples are plated in triplicate; these results were averaged for each patient sample. ELISpot plates were used for capture of both IFN-ɣ and TNF-α. For R&D kits, when used, capture antibody was prepared and placed in wells as per manufacturers recommendations. CTL kits came with capture antibody precoated. Cells plated in IFN-ɣ plates were plated at a standardized density of 2.5 x 10^4 and 5 x 10^4. PBMCs per well were stimulated with anti-CD3 (clone HIT3a; BioLegend) and anti-CD28 (clone CD28.2; BioLegend)

antibodies at 1 µg/mL. Cells plated in TNF-α wells were plated at a standardized density of 2.5 x 10^3 and 5 x 10^3 PBMCs per well and 5 x 10^3 were stimulated with 100 ng/ml LPS (from *Salmonella abortus equi* S-form, ALX-581-009, Enzo Life Sciences, Farmingdale, New York). Anti-CD3 with anti-CD28 or LPS were used as stimulants to evaluate the baseline function of T cells and monocytes, respectively, to assess ability to produce and secrete IFN-ɣ or TNF-α. ELISpot plates were made by Merck Millipore and obtained through Fisher Scientific (Hampton, NH; catalog number M8IPS4510). Spots were detected using a colorimetric reagent kit (Strep-AP and BCIP-NBT, R&D Systems, Minneapolis, MN, USA; catalog number SEL002). Following development, images were captured and analyzed on Cellular Technologies Ltd (Cleveland, OH) ImmunoSpot 7.0 plate reader and software.

The immuno-adjuvant, IL-7 was obtained from R&D Systems (catalog numbers 207-IL-200). Additional ELISpot wells were prepared as mentioned above with the addition of IL-7 at a final concentration of 50 ng/ml.

Flow cytometry

Flow cytometric analysis of samples was performed as previously described(39, 55). Briefly, whole blood or PBMCs were stained for 30’ at room temperature, and red blood cells lysed (in the case of whole blood) using Red Blood Cell Lysis Buffer (BioLegend, San Diego, CA). Samples were acquired on an Attune NxT cytometer (ThermoFisher) and data analyzed using FlowJo 10.6.2 (BD Biosciences, San Jose, CA). Absolute cell counts were ascertained by use of counting beads in LUCID DURAclone staining tubes (Beckman Coulter, Indianapolis, IN). The gating strategy used is reflected in Supplemental Figures 5 and 6.

The following antibodies (clones) were used in this work: CD3 (HIT3a)-FITC, CD14 (M5E2)-PerCP/Cy5.5, CD4 (RPA-T4)-APC/Cy7, CD8 (SK1)-APC, CD56 (5.1H11)-BV711, CD14 (M5E2)-BV650 (BioLegend), CD3 (UCHT1)-FITC, CD4 (13b8.2)-PacificBlue, and CD8 (B9.11)-KromeOrange (Beckman Coulter).

Statistical Methods

All statistical analyses were performed using GraphPad Prism 8.4 (San Diego, CA) and SPSS statistics v25. Mean percentage change in spot number was calculated by dividing the difference between the control and treatment sample by the value of the control. Statistical analysis of ELISpot data comparing unstimulated results to stimulated results was performed using paired analysis with the nonparametric Wilcoxon signed rank test. In this test, each patient sample is compared with its own unstimulated control, and these changes are compared for the entire group to determine statistical significance. Mann–Whitney U tests were used to compare the mean ELISpot results between different cohorts under similar stimulations. Comparisons of differences in continuous variables within a group (isotype control vs treatments) were done using paired Student t-tests, one-way ANOVA and multivariate analysis. P-values of <0.05 were considered significant.

ELISpot results were corrected for number of cells plated in the following method: The number of spots counted using the CTL ELISpot analyzer represents the number of cells secreting the relevant cytokine. PBMC IFN-ɣ spots were corrected as the number of spots per lymphocyte percent in PBMC fraction based on flow cytometry data. PBMC TNF-α spots were corrected as the number of spots per myeloid cell percentage in the PBMC fraction. For COVID-19 samples, flow cytometry was performed on the PBMC

fraction, and neutrophil contamination were included in the correction fraction. Spot number for IFN-ɣ and TNF-α were reported per thousand cells plated. For samples that did not have flow cytometry data available, complete blood count and differential was used.

Study Approval

Blood sampling and data collection and analysis were approved by the Institutional Review Boards at Washington University School of Medicine and at Missouri Baptist Hospital, St Louis, MO 63011. (protocols # WUSTL 201211101, WUSTL 201603006, WUSTL 202003085, and 201808049; and # MOBAP 1132). Informed consent was obtained from healthy control subjects and patients or their legally authorized representatives.

Author Contributions:

KER, SCB, MM, LLM, AHE, PAM, and RSH designed the study, supervised experiments, analyzed and interpreted the data; KER, SCB, LLM, MM, AW, JU, and RSH wrote/edited the manuscript; MM, TMB, JU, and AW performed all statistical analysis; MM, PM, DJY, AW, JU, DAM, and DO performed relevant experiments; JB and TMB provided study coordination; DAS, RSM, NJA, JPB, AMD, CCC, and IRT provided analysis and edited the manuscript; JU, MM, TMB, AW provided figure generation. All authors reviewed and approved the final version of the manuscript.

Acknowledgements:

The authors would like to acknowledge the nurses and staff at Barnes Jewish Hospital and Missouri Baptist Medical Center. We would also like to acknowledge the patients and their families during this difficult pandemic. Additional support is provided by the CTSA-WUSTL KL2 TR002346, CTSA-UF-FSU UL1 TR001427 NIH GM129763, GM126928, GM008721, GM133756, GM111153, AG056444, and AI139813.

References

1. Chen X, Zhao B, Qu Y, et al. Detectable serum SARS-CoV-2 viral load (RNAaemia) is closely correlated with drastically elevated interleukin 6 (IL-6) level in critically ill COVID-19 patients. *Clin Infect Dis*. Apr 17 2020;doi:10.1093/cid/ciaa449
2. Guo YR, Cao QD, Hong ZS, et al. The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak - an update on the status. *Mil Med Res*. Mar 13 2020;7(1):11. doi:10.1186/s40779-020-00240-0
3. McGonagle D, Sharif K, O'Regan A, Bridgewood C. Interleukin-6 use in COVID-19 pneumonia related macrophage activation syndrome. *Autoimmun Rev*. Apr 3 2020:102537. doi:10.1016/j.autrev.2020.102537
4. Qin C, Zhou L, Hu Z, et al. Dysregulation of immune response in patients with COVID-19 in Wuhan, China. *Clin Infect Dis*. Mar 12 2020;doi:10.1093/cid/ciaa248
5. Sarzi-Puttini P, Giorgi V, Sirotti S, et al. COVID-19, cytokines and immunosuppression: what can we learn from severe acute respiratory syndrome? *Clin Exp Rheumatol*. Mar-Apr 2020;38(2):337-342.
6. Tveito K. Cytokine storms in COVID-19 cases? *Tidsskr Nor Laegeforen*. Mar 24 2020;140Cytokinstormer ved covid-19? doi:10.4045/tidsskr.20.0239
7. Zhang C, Wu Z, Li JW, Zhao H, Wang GQ. The cytokine release syndrome (CRS) of severe COVID-19 and Interleukin-6 receptor (IL-6R) antagonist Tocilizumab may be the key to reduce the mortality. *Int J Antimicrob Agents*. Mar 28 2020:105954. doi:10.1016/j.ijantimicag.2020.105954
8. Chatterjee P, Nagi N, Agarwal A, et al. The 2019 novel coronavirus disease (COVID-19) pandemic: A review of the current evidence. *Indian J Med Res*. Mar 30 2020;doi:10.4103/ijmr.IJMR_519_20
9. Ciotti M, Angeletti S, Minieri M, et al. COVID-19 Outbreak: An Overview. *Chemotherapy*. Apr 7 2020:1-9. doi:10.1159/000507423
10. Du RH, Liu LM, Yin W, et al. Hospitalization and Critical Care of 109 Decedents with COVID-19 Pneumonia in Wuhan, China. *Ann Am Thorac Soc*. Apr 7 2020;doi:10.1513/AnnalsATS.202003-225OC
11. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. Feb 15 2020;395(10223):497-506. doi:10.1016/S0140-6736(20)30183-5
12. Kang YJ. Mortality rate of infection with COVID-19 in Korea from the perspective of underlying disease. *Disaster Med Public Health Prep*. Mar 31 2020:1-6. doi:10.1017/dmp.2020.60
13. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. *JAMA*. Feb 7 2020;doi:10.1001/jama.2020.1585
14. Du Y, Tu L, Zhu P, et al. Clinical Features of 85 Fatal Cases of COVID-19 from Wuhan: A Retrospective Observational Study. *Am J Respir Crit Care Med*. Apr 3 2020;doi:10.1164/rccm.202003-0543OC
15. Remy KE, Scott C Brakenridge, Bruno Francois, Thomas Daix, Clifford S Deutschman, Guillaume Monneret, Robin Jeannet, Pierre-Francois Laterre, Richard S Hotchkiss, Lyle L Moldawer. Immunotherapies for COVID-19: lessons learned from sepsis. *The Lancet Respiratory Medicine*. 2020;
16. Devaux CA, Rolain JM, Colson P, Raoult D. New insights on the antiviral effects of chloroquine against coronavirus: what to expect for COVID-19? *Int J Antimicrob Agents*. Mar 12 2020:105938. doi:10.1016/j.ijantimicag.2020.105938
17. Roback JD, Guarner J. Convalescent Plasma to Treat COVID-19: Possibilities and Challenges. *JAMA*. Mar 27 2020;doi:10.1001/jama.2020.4940
18. Shah P, Zampella JG. Use of Systemic Immunomodulatory Therapies During the Coronavirus Disease 2019 (COVID-19) Pandemic. *J Am Acad Dermatol*. Mar 31 2020;doi:10.1016/j.jaad.2020.03.056
19. Diao B, Wang C, Tan Y, Chen X, Liu Y, Ning L, Chen L, Li M, Liu Y, Wang G, Yuan Z, Feng Z, Zhang Y, Wu Y, Chen Y. Reduction and Functional Exhaustion of T Cells in Patients with Coronavirus Disease 2019 (COVID-19). *Frontiers in Immunology.* May 1, 2020.

20. Zheng HY, Zhang M, Yang CX, et al. Elevated exhaustion levels and reduced functional diversity of T cells in peripheral blood may predict severe progression in COVID-19 patients. *Cell Mol Immunol*. Mar 17 2020;doi:10.1038/s41423-020-0401-3
21. Zheng M, Gao Y, Wang G, et al. Functional exhaustion of antiviral lymphocytes in COVID-19 patients. *Cell Mol Immunol*. Mar 19 2020;doi:10.1038/s41423-020-0402-2
22. Sereti I, Dunham RM, Spritzler J, et al. IL-7 administration drives T cell-cycle entry and expansion in HIV-1 infection. *Blood*. Jun 18 2009;113(25):6304-14. doi:10.1182/blood-2008-10-186601
23. Hanley B, Lucas SB, Youd E, Swift B, Osborn M. Autopsy in suspected COVID-19 cases. *J Clin Pathol*. Mar 20 2020;doi:10.1136/jclinpath-2020-206522
24. Wang F, Nie J, Wang H, et al. Characteristics of peripheral lymphocyte subset alteration in COVID-19 pneumonia. *J Infect Dis*. Mar 30 2020;doi:10.1093/infdis/jiaa150
25. Xiong Y, Liu Y, Cao L, et al. Transcriptomic characteristics of bronchoalveolar lavage fluid and peripheral blood mononuclear cells in COVID-19 patients. *Emerg Microbes Infect*. Dec 2020;9(1):761-770. doi:10.1080/22221751.2020.1747363
26. Zheng Y, et al. Study of the lymphocyte change between COVID-19 and non-COVID-19 pneumonia cases suggesting other factors besides uncontrolled inflammation contributed to multi-organ injury [preprint]. https://doi.org/10.1101/2020.02.19.20024885. Posted on MeddRXiv Feb 19, 2020.
27. Peterson D, Damsky W, King B. Calm before the storm: understanding the role of JAK inhibitors in COVID-19. *J Am Acad Dermatol*. Apr 25 2020;doi:10.1016/j.jaad.2020.04.097
28. Russell B, Moss C, George G, et al. Associations between immune-suppressive and stimulating drugs and novel COVID-19-a systematic review of current evidence. *Ecancermedicalscience*. 2020;14:1022. doi:10.3332/ecancer.2020.1022
29. Spinelli FR, Conti F, Gadina M. HiJAKing SARS-CoV-2? The potential role of JAK inhibitors in the management of COVID-19. *Sci Immunol*. May 8 2020;5(47)doi:10.1126/sciimmunol.abc5367
30. Aouba A, Baldolli A, Geffray L, et al. Targeting the inflammatory cascade with anakinra in moderate to severe COVID-19 pneumonia: case series. *Ann Rheum Dis*. May 6 2020;doi:10.1136/annrheumdis-2020-217706
31. Phase I / II Clinical Study of Immunotherapy Based on Adoptive Cell Transfer as a Therapeutic Alternative for Patients With COVID-19 in Colombia. https://clinicaltrials.gov/ct2/show/NCT04344548?term=covid+nk+cells&draw=2&rank=3
32. A Phase I/II Study of Universal Off-the-shelf NKG2D-ACE2 CAR-NK Cells for Therapy of COVID-19. https://clinicaltrials.gov/ct2/show/NCT04324996?term=covid+nk&draw=2&rank=1
33. Jamilloux Y, Henry T, Belot A, et al. Should we stimulate or suppress immune responses in COVID-19? Cytokine and anti-cytokine interventions. *Autoimmun Rev*. May 4 2020:102567. doi:10.1016/j.autrev.2020.102567
34. Hayashi N, Yamaguchi S, Rodenburg F, et al. Multiple biomarkers of sepsis identified by novel time-lapse proteomics of patient serum. *PLoS One*. 2019;14(9):e0222403. doi:10.1371/journal.pone.0222403
35. Sharma NK, Salomao R. Sepsis Through the Eyes of Proteomics: The Progress in the Last Decade. *Shock*. Jan 2017;47(1S Suppl 1):17-25. doi:10.1097/SHK.0000000000000698
36. Wong HR, Cvijanovich N, Allen GL, et al. Genomic expression profiling across the pediatric systemic inflammatory response syndrome, sepsis, and septic shock spectrum. *Crit Care Med*. May 2009;37(5):1558-66. doi:10.1097/CCM.0b013e31819fcc08
37. Oberholzer A, Souza SM, Tschoeke SK, et al. Plasma cytokine measurements augment prognostic scores as indicators of outcome in patients with severe sepsis. *Shock*. Jun 2005;23(6):488-93.

38. Mazer M, Unsinger J, Drewry A, et al. IL-10 Has Differential Effects on the Innate and Adaptive Immune Systems of Septic Patients. *J Immunol*. Oct 15 2019;203(8):2088-2099. doi:10.4049/jimmunol.1900637
39. Thampy LK, Remy KE, Walton AH, et al. Restoration of T Cell function in multi-drug resistant bacterial sepsis after interleukin-7, anti-PD-L1, and OX-40 administration. *PloS one*. 2018;13(6):e0199497. doi:10.1371/journal.pone.0199497
40. Jose RJ, Manuel A. COVID-19 cytokine storm: the interplay between inflammation and coagulation. *Lancet Respir Med*. Apr 27 2020;doi:10.1016/S2213-2600(20)30216-2
41. Mehta P, McAuley DF, Brown M, et al. COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet*. Mar 28 2020;395(10229):1033-1034. doi:10.1016/S0140-6736(20)30628-0
42. Ruscitti P, Berardicurti O, Iagnocco A, Giacomelli R. Cytokine storm syndrome in severe COVID-19. *Autoimmun Rev*. May 3 2020:102562. doi:10.1016/j.autrev.2020.102562
43. Wright DJM. Prevention of the cytokine storm in COVID-19. *Lancet Infect Dis*. May 7 2020;doi:10.1016/S1473-3099(20)30376-5
44. Waage A, Brandtzaeg P, Halstensen A, Kierulf P, Espevik T. The complex pattern of cytokines in serum from patients with meningococcal septic shock. Association between interleukin 6, interleukin 1, and fatal outcome. *J Exp Med*. Jan 1 1989;169(1):333-8. doi:10.1084/jem.169.1.333
45. Tanaka T, Narazaki M, Kishimoto T. Immunotherapeutic implications of IL-6 blockade for cytokine storm. *Immunotherapy*. Jul 2016;8(8):959-70. doi:10.2217/imt-2016-0020
46. Matsukura S, Kokubu F, Noda H, Tokunaga H, Adachi M. Expression of IL-6, IL-8, and RANTES on human bronchial epithelial cells, NCI-H292, induced by influenza virus A. *J Allergy Clin Immunol*. Dec 1996;98(6 Pt 1):1080-7. doi:10.1016/s0091-6749(96)80195-3
47. Swain SL, McKinstry KK, Strutt TM. Expanding roles for CD4(+) T cells in immunity to viruses. *Nat Rev Immunol*. Jan 20 2012;12(2):136-48. doi:10.1038/nri3152
48. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet*. Mar 28 2020;395(10229):1054-1062. doi:10.1016/S0140-6736(20)30566-3
49. Liu Q, Wang RS, Qu GQ, et al. Gross examination report of a COVID-19 death autopsy. *Fa Yi Xue Za Zhi*. Feb 2020;36(1):21-23. doi:10.12116/j.issn.1004-5619.2020.01.005
50. Wichmann D, Sperhake JP, Lutgehetmann M, et al. Autopsy Findings and Venous Thromboembolism in Patients With COVID-19: A Prospective Cohort Study. *Ann Intern Med*. May 6 2020;doi:10.7326/M20-2003
51. Menter T, Haslbauer JD, Nienhold R, et al. Post-mortem examination of COVID19 patients reveals diffuse alveolar damage with severe capillary congestion and variegated findings of lungs and other organs suggesting vascular dysfunction. *Histopathology*. May 4 2020;doi:10.1111/his.14134
52. Vardhana SA, Wolchok JD. The many faces of the anti-COVID immune response. *J Exp Med*. Jun 1 2020;217(6)doi:10.1084/jem.20200678
53. Hotchkiss RS, Opal SM. Activating Immunity to Fight a Foe - A New Path. *N Engl J Med*. Mar 26 2020;382(13):1270-1272. doi:10.1056/NEJMcibr1917242
54. Francois B, Jeannet R, Daix T, et al. Interleukin-7 restores lymphocytes in septic shock: the IRIS-7 randomized clinical trial. *JCI Insight*. Mar 8 2018;3(5)doi:10.1172/jci.insight.98960
55. Drewry AM, Ablordeppey EA, Murray ET, et al. Monocyte Function and Clinical Outcomes in Febrile and Afebrile Patients With Severe Sepsis. *Shock*. Oct 2018;50(4):381-387. doi:10.1097/SHK.0000000000001083

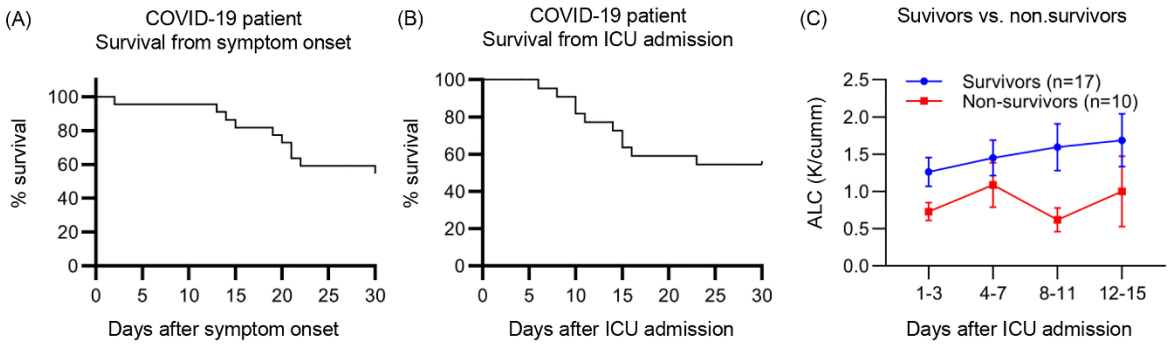

Figure 1. COVID-19 patient survival.

Survival is plotted as a function from symptom onset (A) and ICU admission (B). Difference in absolute lymphocyte count (ALC) over time between survivors and non-survivors (C). Total number of patients n=27; number of survivors n=17, number of non-survivors n=10.

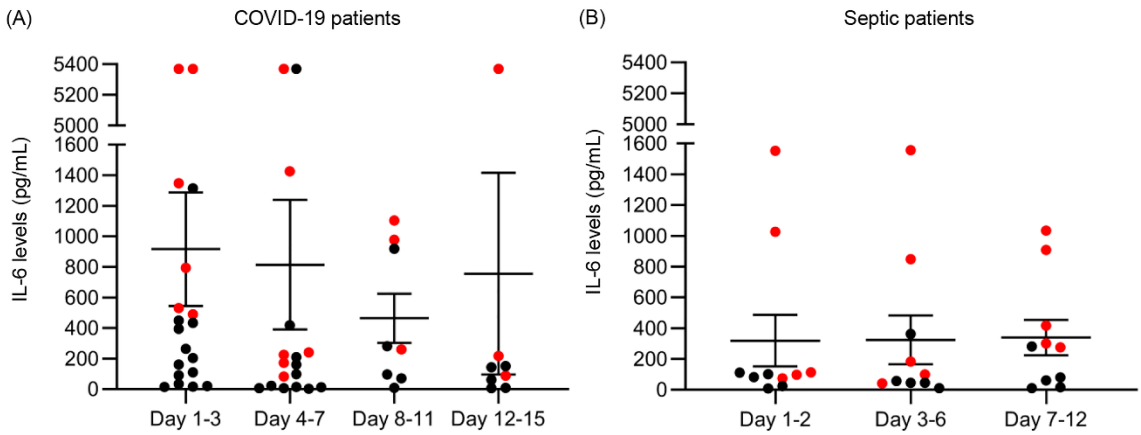

Figure 2. Plasma IL-6 levels in patients with COVID-19 and sepsis

Dot plot representing plasma IL-6 levels for COVID-19 patients (A) and patients with sepsis (B) at various time points during their ICU admission. Data bars represent mean +/- SEM. Red dots represent non-survivors. Septic patients n=10; COVID-19 days 1-3 n=19, days 4-7 17, days 8-11 n=8, days 12-15 n=8.

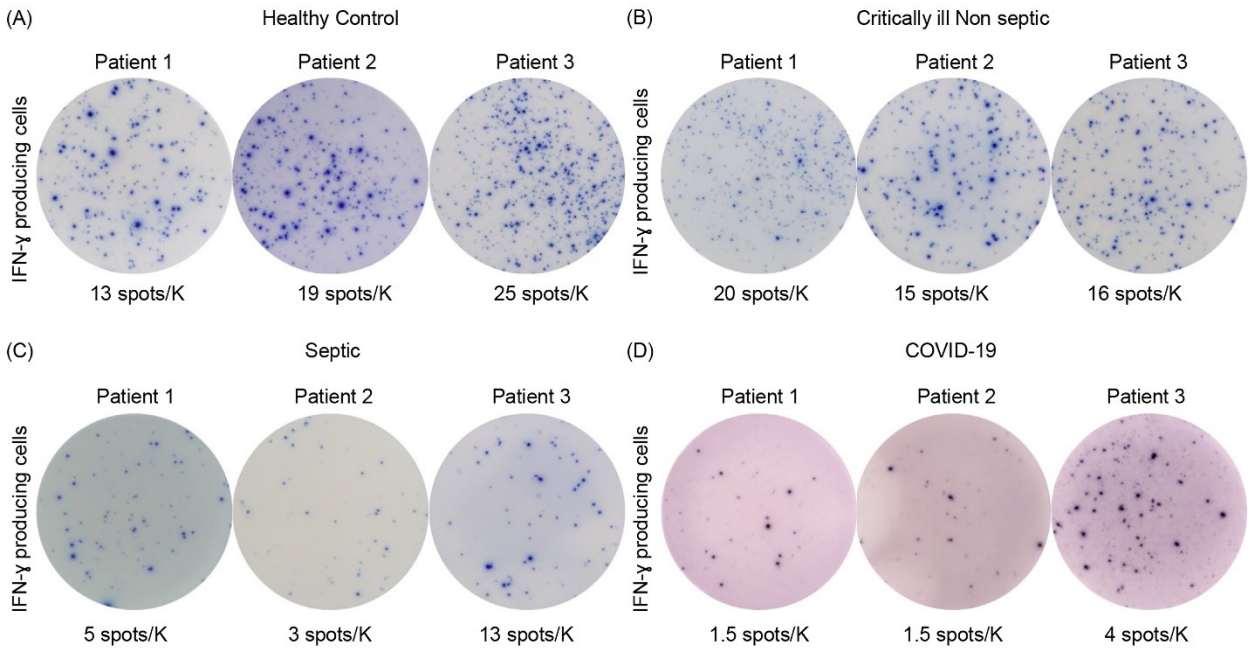

Figure 3. Adaptive immune suppression in COVID-19 patients

Representative ELISpot photomicrographs displaying IFN-ɣ production following overnight stimulation with anti-CD3/anti-CD28 antibodies. (A) Healthy volunteers, (B) Critically-ill non-septic (CINS), and (C) septic non-COVID-19 patients. (D) Three representative COVID-19 positive samples. Number of spots demonstrates the number of cytokine-producing T cells. Counts are presented as corrected number of spots per thousand lymphocytes plated as fraction of the 2.5 x 10^4 PBMCs plated in each well. Note the reduction in IFN-ɣ production in both septic and COVID-19 patients compared to critically-ill non-septic patients. Note also a degree of heterogeneity in IFN-ɣ production in COVID-19 and septic patients. Each photomicrograph was captured with the same magnification and each image is to scale. ELISpots were performed on fresh whole blood; PBMC fraction and were run in duplicate for the control samples and triplicate for COVID-19 samples.

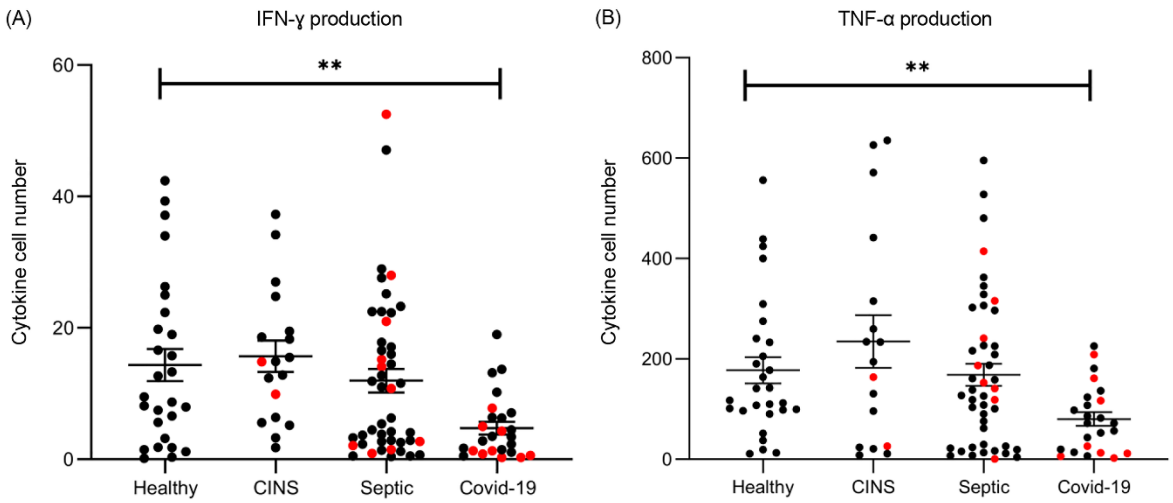

Figure 4. Functional immune cytokine production measured by ELISpot in COVID-19, critically-ill non-septic (CINS), healthy and septic patients

Comparison graphs for ex vivo cytokine production using ELISpot, comparing healthy controls, CINS, septic and COVID-19. (A) Number of spots per 1000 lymphocytes plated following overnight culture stimulated with anti-CD3/anti-CD28 for IFN-ɣ samples and (B) number of spots per 1000 myeloid cells placed stimulated with LPS for TNF-α production. Each dot represents an individual patient. Red dots represent non-survivors. Data bars represent mean +/- SEM. Healthy n = 27 for IFN-ɣ, 28 for TNF-α, CINS n = 18, Septic n= 46, COVID-19 n = 25 for IFN-ɣ, 24 for TNF-α. ANOVA analysis comparing all groups for IFN-γ production showed that there was a difference between COVID-19 and the other groups with $p = 0.003$; and for TNF-α groups there was a statistically significant difference as well with $p = 0.009$.

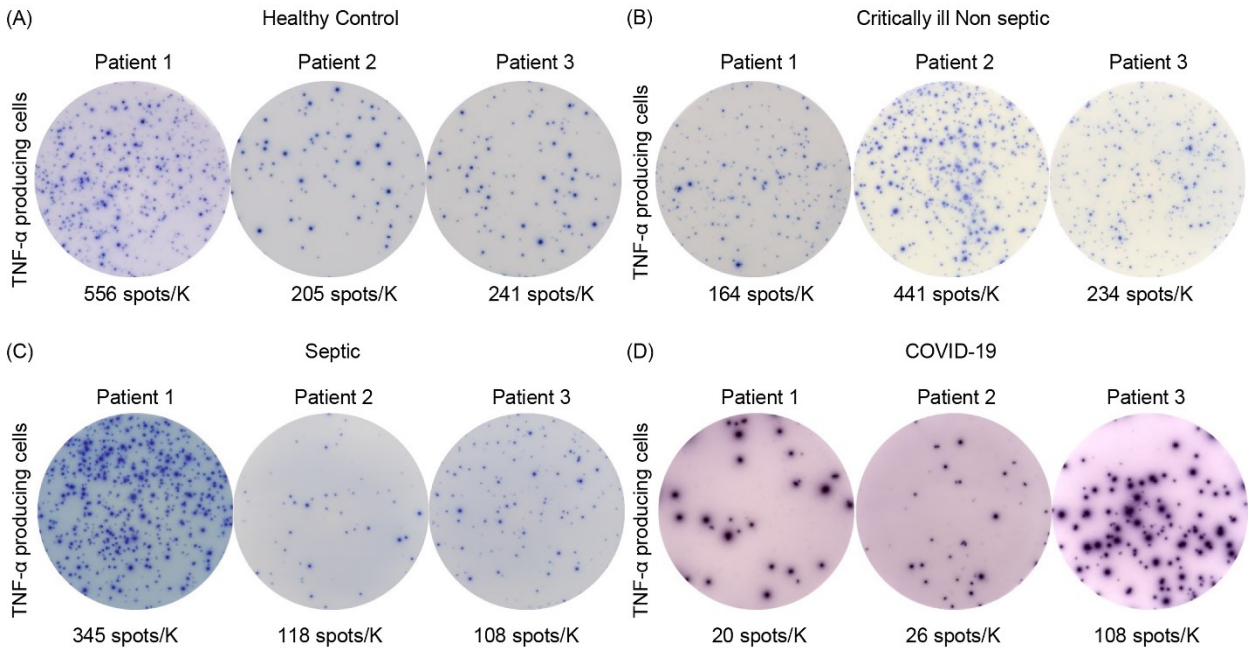

Figure 5. Suppressed innate immune TNF-α response in COVID-19

Representative ELISpot photomicrographs displaying baseline innate immune (monocyte) function with LPS stimulated TNF-α production in PBMCs. Comparison between different patient types include (A) Healthy controls, (B) CINS, (C) Septic patients, (D) COVID-19. Number of spots demonstrates the number of cytokine-producing monocytes and counts are presented as corrected number of spots per thousand monocytes cells plated as fraction of the 2.5×10^3 PBMCs plated in each well. COVID-19 patients had suppressed TNF-α production when compared with controls. Each photomicrograph was captured with the same magnification and each image is to scale. ELISpots were performed on fresh whole blood; PBMC fraction and were run in duplicate for the control samples and triplicate for COVID-19 samples.

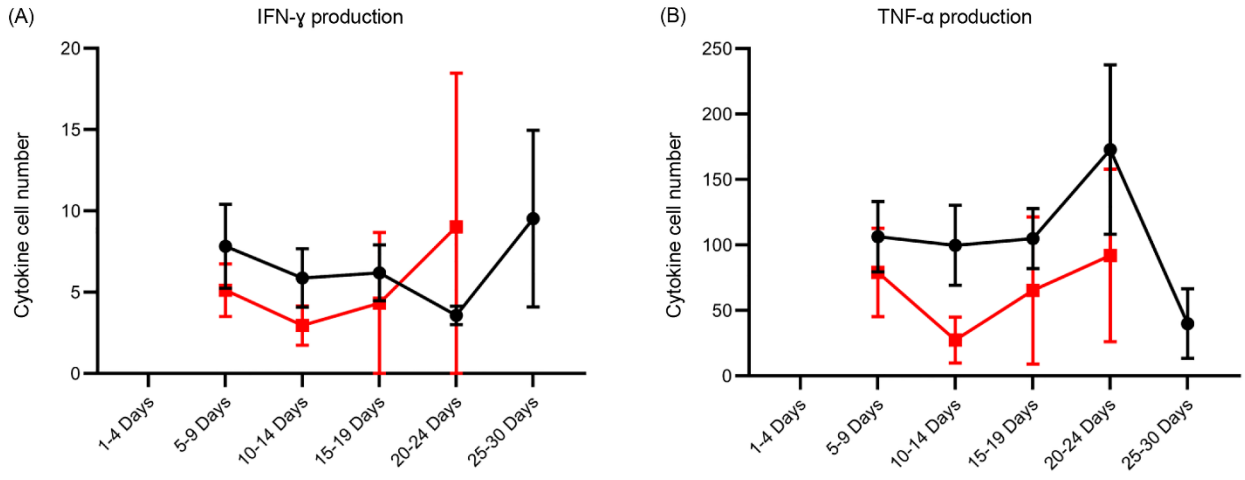

Figure 6. Number of cytokine-producing cells in COVID-19 patients serially over time

Time course analysis of ELISpot results comparing COVID-19 survivors vs. non-survivors (red) from onset of illness throughout their ICU admission for (A) IFN-ɣ, and (B) TNF-α. There is no statistical significance between survivors and non-survivors using a modified T-test. Day of illness data collected via chart review. Data bars represent mean +/- SEM. For each timepoint there are the following number of samples: IFN-ɣ survivors: 0, 8, 10, 10, 8, 4. IFN-ɣ non-survivors: 0, 4, 7, 3, 3, 0. TNF-α survivors: 0, 8, 7, 9, 6, 3. TNF-α non-survivors: 0, 5, 5, 4, 3, 0.

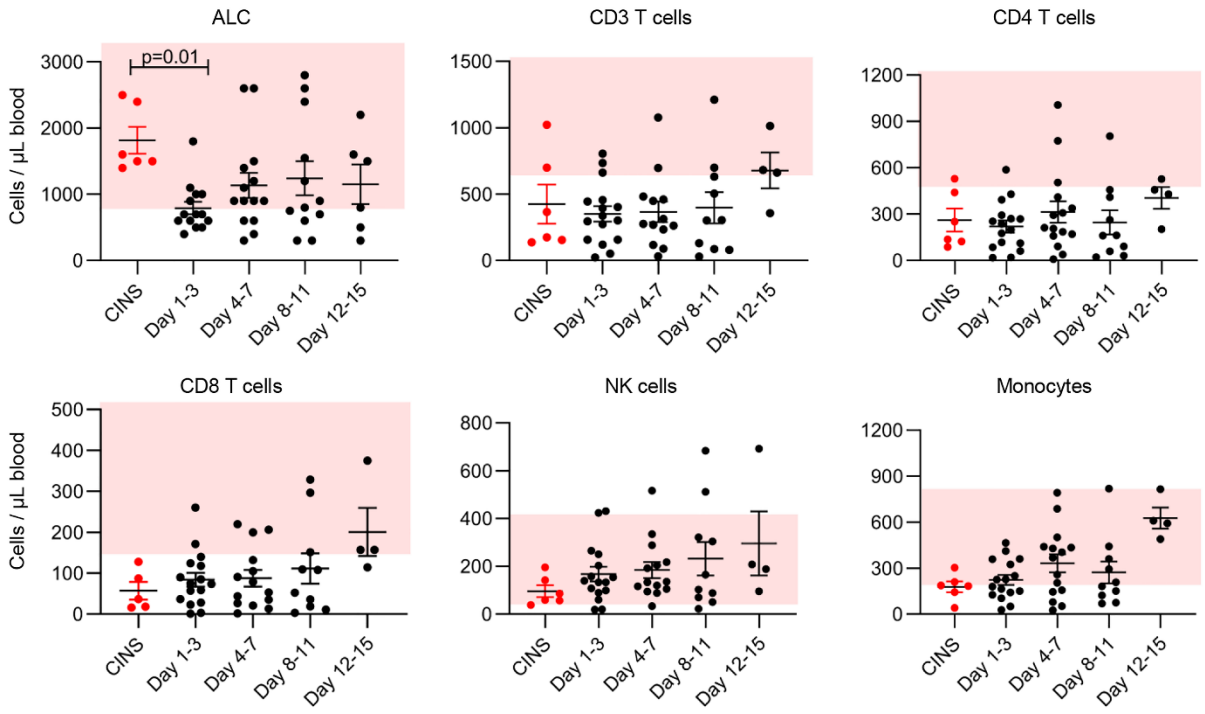

Figure 7. **COVID-19 induces profound depletion of circulating immune effector cells**

Absolute numbers of various white blood cell types (displayed as cells/µL) were determined in COVID-19 positive and critically-ill non-septic (CINS) patients (red dots). The absolute lymphocyte count (ALC) was determined by Barnes Jewish Hospital Clinical Laboratory as part of patient clinical labs. The CD3 T, CD4 T, CD8 T, NK, and monocyte quantification was performed using flow cytometry as described in Methods. Pink shading represents normal reference values for healthy individuals at Barnes-Jewish Hospital Laboratories. Analysis by ANOVA with Dunnett's multiple comparison tests showed a significant decrease in ALC from CINS to COVID-19 days 1-3; p = 0.01. ALC for CINS n=6; ALC for COVID-19 days 1-3 n=15, days 4-7 n=14, days 8-11 n=12, days 12-15 n=6. Cell counts for CD3, CD4, CD8, NK, and monocytes CINS n=6, COVID-19 days 1-3 n=15, days 4-7 n=14, days 8-11 n=10, days 12-15 n=4.

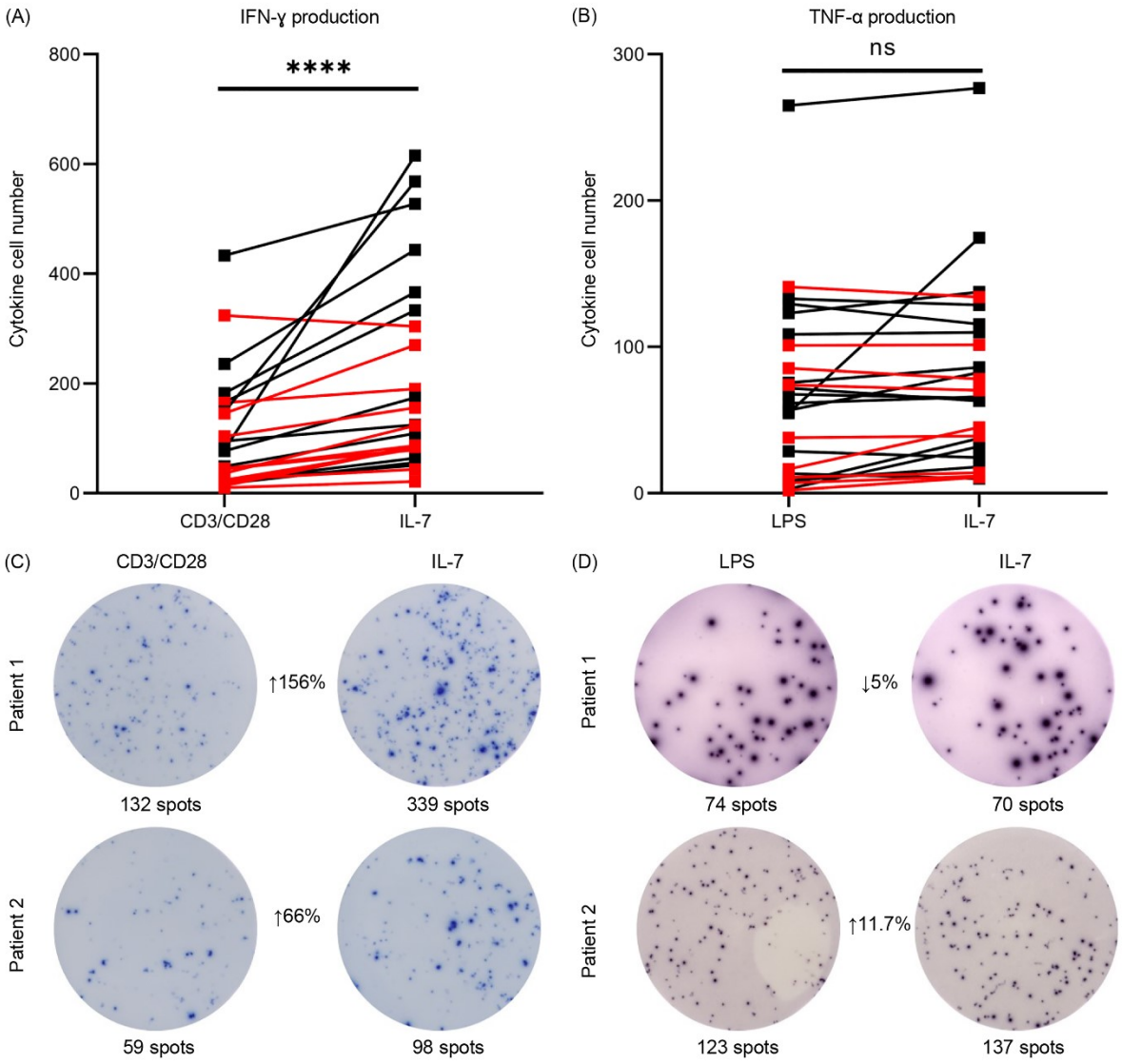

Figure 8. IL-7 restores adaptive immune function in patients with COVID-19

Line plot demonstrating change in number of cytokine producing cells using ELISpot between control (anti-CD3/anti-CD28 antibody or LPS) samples and stimulation with IL-7 for IFN-ɣ (A), and TNF-α (B). Each dot represents and individual patient. Red lines represent values for patients who died. IL-7 caused a significant increase in the number of IFN-ɣ producing T cells in COVID-19 patients. IL-7 did not increase monocyte TNF-α production. Panels (C), (D) are representative photomicrographs demonstrating ELISpot change from control sample to IL-7 stimulated for IFN-ɣ and TNF-α. Paired samples were analyzed using a paired rank sum Wilcoxon test. IFN-ɣ n= 25, TNF-α n=25.

Table 1. Patient Demographics

	COVID ICU Patients (n=27)	Septic Patients (n=51)	Critically Ill Non-Septic Patients (n=18)	Healthy Controls (n=27)
Age, mean (range)	57 (25-86)	56 (18-89)	59 (23 - 80)	56 (25-79)
Sex:				
Female	12 (44%)	27 (53%)	6 (33%)	13 (48%)
Male	15 (56%)	24 (47%)	12 (67%)	14 (52%)
Race:				
African American	19 (70%)	11 (22%)	2 (11%)	7 (26%)
Caucasian	8 (30%)	40 (78%)	16 (89%)	20 (74%)
Comorbidities:				
Hypertension	17 (63%)	9 (18%)	10 (56%)	
Diabetes	11 (41%)	19 (37%)	0	
Obesity	8 (30%)	4 (8%)	2 (11%)	
Respiratory Disease	8 (30%)	11 (22%)	8 (44%)	
Cardiovascular Disease	7 (26%)	18 (35%)	8 (44%)	
Neurologic Disease	7 (26%)	10 (20%)	0	
Hyperlipidemia	6 (22%)	6 (12%)	3 (17%)	
Thyroid Disease	3 (11%)	3 (6%)	0	
Cancer	3 (11%)	6 (12%)	1 (6%)	
Kidney Disease	2 (7%)	7 (14%)	1 (6%)	
Autoimmune disease	2 (7%)	0	0	
Hepatic Disease	1 (4%)	6 (12%)	0	
Substance Abuse	0	7 (14%)	1 (6%)	
GI Disease	0	2 (4%)	1 (6%)	
Number of Days from Symptoms to ED, mean (range)	6 (1-14)*			
Number of Days from ED to Intubation, mean (range)	1 (0-5)**			
Number of Days from ICU to First Blood Draw, mean (range)	3 (0-8.5)			
ALC at ICU admission, mean (range)	0.9 (0.4-2.3)			

APACHE II Score, mean (range)	18 (6-36)***	18 (7-29)		
SOFA Score, mean (range)	7 (2-14)***	7 (0-18)		
Subjects with Secondary Infections	10 (37%)	23 (45%)		
30-Day Mortality	10 (37%)	11 (22%)	2 (11%)	

Table 2. Plasma cytokines comparing COVID-19 with Septic, CINS and healthy controls

		COVID-19 d1-3	COVID-19 d4-7	COVID-19 d8-11	COVID-19 d12-15	Septic d1-2	Septic d3-6	Septic d7-11	Healthy Control	CINS
IL-1β	Mean (SEM)	9.5 (5.4)	6.0 (4)	1.3 (0.4)	3 (1)	3.0 (1.2)	2.3 (0.4)	3.6 (0.7)	2 (0.8)	0.8 (0.3)
	n	19	17	8	8	10	10	10	10	2
IL-6	Mean (SEM)	916.4 (381)	814.1 (437)	464.4 (172)	755.6 (705)	319.2 (176)	324.8 (167)	338.9 (121)	45.4 (46)	137.3 (110)
	n	19	17	8	8	10	10	10	10	4
IL-7	Mean (SEM)	16.5 (6)	39 (12)	47.1(26)	11.8 (5.5)	62.3 (39)	65.4 (43)	65 (34)	38.7 (8)	5.5 (0.6)
	n	19	17	8	8	10	10	10	10	4
IL-8	Mean (SEM)	118 (21)	327.9 (177)	174.9 (62)	795.3 (493.5)	105.9 (55)	93.7 (33)	114.8 (47)	14.5 (1)	73 (26)
	n	19	17	8	8	10	10	10	10	4
IL-10	Mean (SEM)	116.6 (65)	95.4 (59)	27.2 (12)	67 (35.2)	881.9 (886)	677.9 (668)	685.1 (658)	226.6 (143)	25.5 (6)
	N	19	17	8	8	10	10	10	10	4
IL-12	Mean (SEM)	145.2 (48)	112.1 (21)	109.6 (43)	102.9 (37)	81.1 (33)	51.4 (11)	67.5 (15)	48.4 (6)	44.4 (15)
	n	19	17	8	8	10	10	10	10	4
MCP-1	Mean (SEM)	1394.6 (282)	1177.3 (257)	1573.4 (632)	738.4 (241)	507.9 (116)	491.3 (117)	686.6 (141)	512.3 (53)	414.8 (83)
	n	19	17	8	8	10	10	10	10	4
IL-1RA	Mean (SEM)	333.4 (81)	497.7 (154)	323.6 (110)	646.8 (239)	113.7 (36)	98.4 (31)	169.3 (54)	32.1 (4)	108.5 (39)
	n	19	17	8	8	10	10	10	10	4
IFN-γ	Mean (SEM)	5.9 (2)	2.1 (1)	0.5 (0.1)	0.9 (0.2)	2.4 (0.6)	2.3 (0.2)	2.5 (0.3)	4.4 (0.7)	9
	n	19	17	8	8	10	10	10	10	1
TNF-α	Mean (SEM)	4.4 (0.5)	4.3 (1)	2.6 (1)	4.6 (3)	6.4 (2)	5.2 (1.6)	7 (2.5)	1.4 (0.2)	6.6 (4)
	n	19	17	8	8	10	10	10	10	4