Unique Immunological Profile In Patients With COVID-19

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Abstract

The relationship between SARS-CoV-2 and host immunity is unknown. We show here that patients with COVID-19 had an altered immune phenotype, with an expansion of adaptive FceRIg^{neg} NK cells, and inflammatory CD14^{+}CD16^{+} monocytes. T cells were reduced and overexpressed the Tim-3 exhaustion molecule. Low frequencies of CD8 T cells and NKG2A^{+} NK cells, and expansion of mature CD57^{+} NK cells were associated with poor prognosis. These findings unveil a unique immunological profile in COVID-19 patients.

Introduction, Results And Discussion

Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) is responsible for a pandemic thus far responsible for nearly 1 million cases of Coronavirus Disease-19 (COVID-19) with a case/fatality rate of 4.5% [1]. The infection usually causes mild symptoms, but may be responsible for severe interstitial pneumonia, myocarditis, acute kidney injury, acute respiratory distress syndrome (ARDS), multiorgan failure and death [2]. Laboratory tests indicate that patients with severe progression of COVID-19 show signs of secondary haemophagocytic lymphohistiocytosis (sHLH), a hyperinflammatory syndrome characterised by a potentially fatal cytokine storm with multiorgan failure, which may be triggered by viral infections [3]. Akin to sHLH, COVID-19 is characterized by lymphopenia, and increased serum ferritin, D-dimer, C-reactive protein (CRP), and lactic- dehydrogenase (LDH), which are also considered predictors of poor outcome [4].

Moreover, several serum cytokine concentrations are increased during COVID-19, supporting the hypothesis that virally driven hyperinflammation plays a key pathogenetic role [2].

Despite clear evidence of ongoing overexuberant inflammation, there are no systematic studies addressing phenotypic and functional alterations of innate and adaptive immune cells, that are likely exposed to a variety of stimuli in COVID-19 patients at presentation. The lack of a comprehensive immunological analysis prompted us to assess the phenotypic and functional status of NK cells, γδT cells, monocytes and CD4 and CD8 T cells in patients presenting with clinically moderate to severe interstitial pneumonia emerging in the setting of COVID-19. Patient clinical details and laboratory findings, as well as peripheral blood mononuclear cells (PBMC) flow cytometric analysis are reported in Supplementary Information, Patients and Methods.

The frequency of NK cells was significantly higher in COVID-19 patients compared to healthy controls, being significantly enriched in mature (CD56^{dim}CD57^{+}) NK cells (Fig.1a). Interestingly, there was a relative expansion of CD57^{+}/FcεRIγ^{neg} adaptive NK cells compared with non-COVID-19 disease controls and healthy controls (Fig.1a) suggesting a SARS-CoV-2-related expansion of this population, whereas the proportion of CD56^{bright} NK cells was reduced. An increase in CD16^{+} NK cells was also evident compared with healthy controls (Fig 1a). Notably, the frequency of CXCR6-expressing NK cells was low in COVID-19 patients (Fig.1a), most likely since these cells home to the lungs where they concentrate, their ligand

CXCL16 being produced in large amounts by alveolar macrophages [5]. Additional changes in NK cells included significant reductions in the frequencies of Siglec-7, DNAM-1, NKG2D, NKp30 (Fig.1a), the latter being particularly evident in the adaptive subset (Fig.1c). Importantly, the frequency of PD-1 positive NK cells was significantly higher in the adaptive compared with conventional NK cells in patients with COVID-19 (Fig. 1c). No changes were noted in bulk NK cell expression of NKG2C, NKG2A, GITR, TRAIL, CD69, PD-1, TIGIT. The trend noted for TIM-3 was not statistically significant (Suppl. Fig.1a). Of note, although no significant changes in degranulation activity or IFNγ production were observed using K562 as target cells (Suppl. Fig.1b), there was an increased ability of NK cells to exert antibody-dependent cell- mediated cytotoxicity (ADCC), a function exquisitely performed by adaptive NK cells [6] (Fig. 1d). The proportions of $CD56^{bright}$, NKG2A and NKp46 positive NK cells were significantly lower and the proportion of mature $CD57^{+}$ cells significantly higher in patients who succumbed compared with those who survived (Fig.2a). The relative frequencies of total $CD3^{+}$, $CD4^{+}$ and $CD8^{+}$ T cells were significantly lower than healthy controls, although no apparent differences were noted with disease controls (Fig.2b). Patients who died showed a significantly lower frequency of CD8 T cells compared with those who survived (Fig.2a). Moreover, both CD4 and CD8 T cells from COVID-19 patients overexpressed Tim-3 compared with healthy controls, suggesting a pan T-cell exhaustion profile (Fig.2c). No differences were found in CD45RO, HLA-DR, GITR expression or Treg population frequency (Suppl. Fig.2). Importantly, there was a clear relative expansion of CD14/CD16 double positive monocytes, a phenotype associated with an inflammatory profile (Fig. 2d) [7]. There were no statistically significant changes in the frequency of γδT cells (Fig. 2e). Negative correlations were found between laboratory indicators of severe or progressive disease. Thus, NK cells expressing the activating receptors NKp30 and NKp46, as well as $CD45RO^{+}$ and $Tim\text{-}3^{+}$ CD4 T cells, correlated negatively with LDH (Fig. 2f). A negative correlation was also present between $NKp46^{+}$ NK cells and CRP (Fig. 2g).

Information on PBMC phenotype and function are virtually lacking in patients with COVID-19. Here we had the opportunity to evaluate patients admitted to hospital because of moderate to severe COVID-19 interstitial pneumonia and compared them to a small group of SARS-CoV-2 negative pneumonia and healthy We showed that patients with COVID-19 had a relative expansion of mature adaptive NK cells endowed with ADCC function, which was increased in this setting in line with findings in other viral infections, particularly cytomegalovirus [6]. Other phenotypic features were compatible with a dysfunctional NK cell phenotype, namely the reduced frequencies of Siglec-7-, NKG2D- and NKp30-expressing cells [8,9]. A recent study addressed the kinetics and breadth of immune responses associated with clinical resolution of COVID-19 in a single patient with relatively mild disease [10]. Antibody-secreting cells appeared at the time of viral clearance together with follicular helper T cells and activated CD4 and CD8 T cells. In contrast, in our patients with moderate to severe interstitial pneumonia, some of whom sadly succumbed, Tim-3 positive exhausted CD4 and CD8 T cells largely prevailed at presentation and lower frequencies of $CD8^{+}$ T cells were linked to poor prognosis. A recent study found lower frequencies of CD8 T cells and NK cells with a relative enrichment of NKG2A-expressing cells which returned to normal after clinical recovery, suggesting rescue of impaired T and NK cell function [11]. Interestingly, although no difference in the frequency of NKG2A- expressing NK cells was found between patients and

controls in the present study, NKG2A$^+$ NK cells were lower in patients who did not survive, suggesting that loss of this inhibitory receptor somehow unleashed NK cells in patients with fatal outcome.

Our study provides important novel insights into the pathogenetic mechanisms of COVID- 19, characterized by a rapid expansion of phenotypically mature NK cells persisting at high frequency in patient with poor prognosis. The simultaneously reduced frequency of CD4$^+$ and CD8$^+$ T cells expressing the Tim-3 exhaustion marker unveils a multifaceted behavior of the two arms of immunity in this clinical setting. The relative enrichment of inflammatory monocytes lends support to the hypothesis that COVID-19 resembles in part to the macrophage-activation syndrome which is thought to be closely related to hemophagocytic lymphohistiocytosis (HLH) [12], an uncommon life-threatening disorder of severe hyperinflammation caused by uncontrolled proliferation of activated lymphocytes and macrophages that secrete high levels of inflammatory cytokines. Of note patterns similar to cytokine storm syndromes have been described for COVID-19 and SARS [2].

It is difficult at this early stage to precisely frame COVID-19 within an immunologically coherent clinical entity. Indeed, several peculiarities have emerged that contribute to the uniqueness of its immune profile. Understanding the dynamics and the quality of immune responses to SARS-CoV-2 will provide invaluable translational information to design effective treatments for this potentially deadly disease.

Methods

Provided as supplemental file.

Declarations

Author Contributions: SV and DL designed and performed experiments and critically contributed to drafting the manuscript; BO, SM, AC performed experiments and critically read the manuscript; SL, MV, SR, MS, RB recruited patients, prepared the database and critically read the manuscript, MUM designed and discussed the experiments and wrote the manuscript.

Competing Interests: none.

Ethics: The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Institutional Review Board and Ethical Committee of Fondazione IRCCS Policlinico San Matteo (Protocol number 20200033215). All patients provided written or, in case they were unable to sign, verbally witnessed informed consent as per the above study protocol.

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Figures

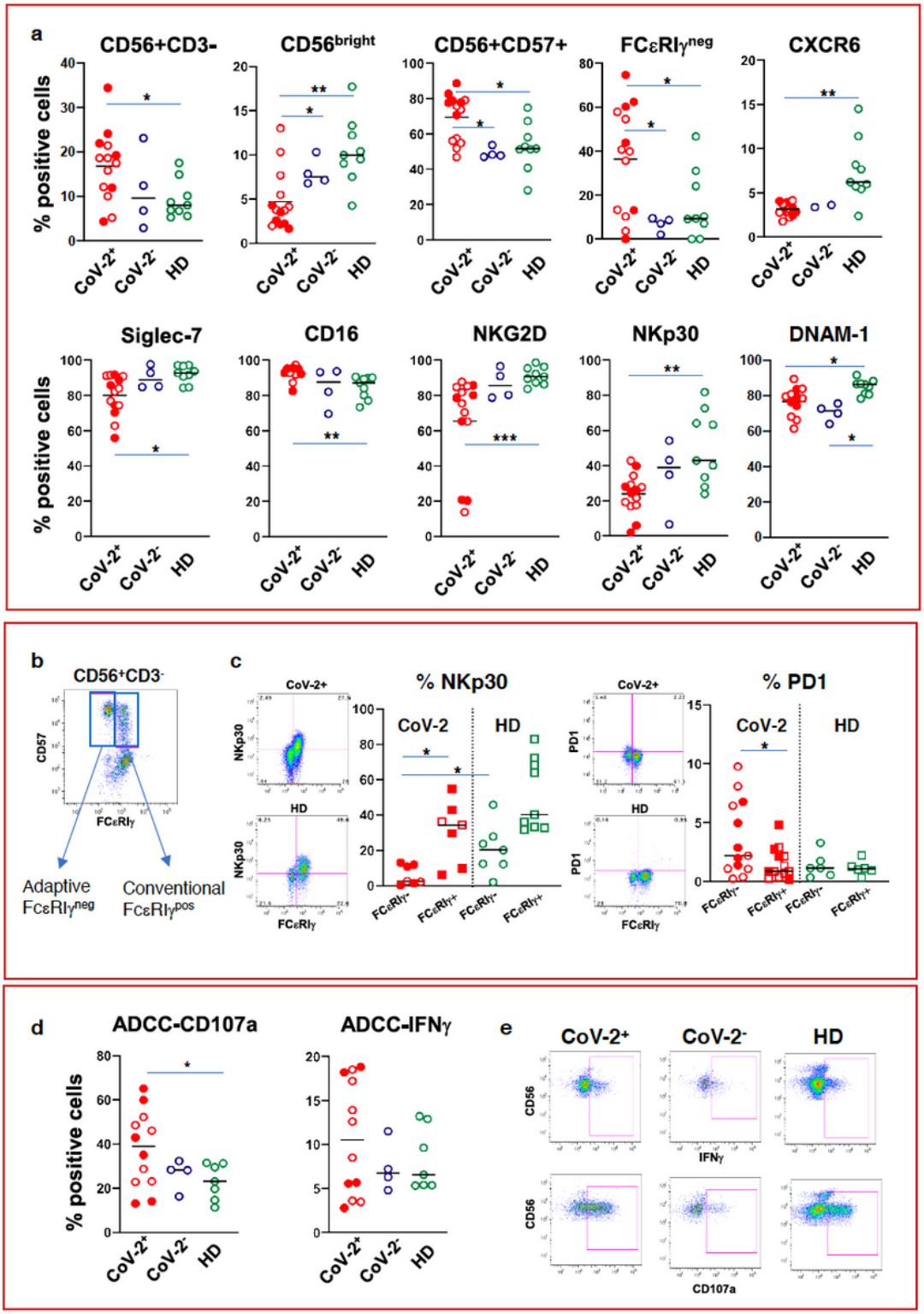

Figure 1

NK cell characterization in SARS-CoV-2 infection. a) Frequency of NK cells and expansion of mature CD57+ and adaptive (FcεRIγneg) NK cells in COVID-19 patients. Reduced frequency of CXCR6, Siglec-7, NKG2D and NKp30, and increased proportion of CD16+ cells. b) Dot plot showing gating on CD57+FcεRIγneg adaptive and CD57+FcεRIγpos conventional NK cells. c) Representative dot plots and graphs showing NKp30 reduction and PD1 increase in adaptive compared with conventional NK cells in

COVID-19 patients. Representative dot plots are gated on total CD57+ NK cells. Circles indicate adaptive NK; squares, conventional NK. d) Increased NK degranulation and IFNγ expression in COVID- 19 patients. e) Representative IFNγ and CD107a dot plots in patients and controls. Full red symbols indicate patients who subsequently died. Middle bars represent medians. The One Way Anova test was used to compare three groups. *p<0.05, **p<0.01, *** p<0.001.

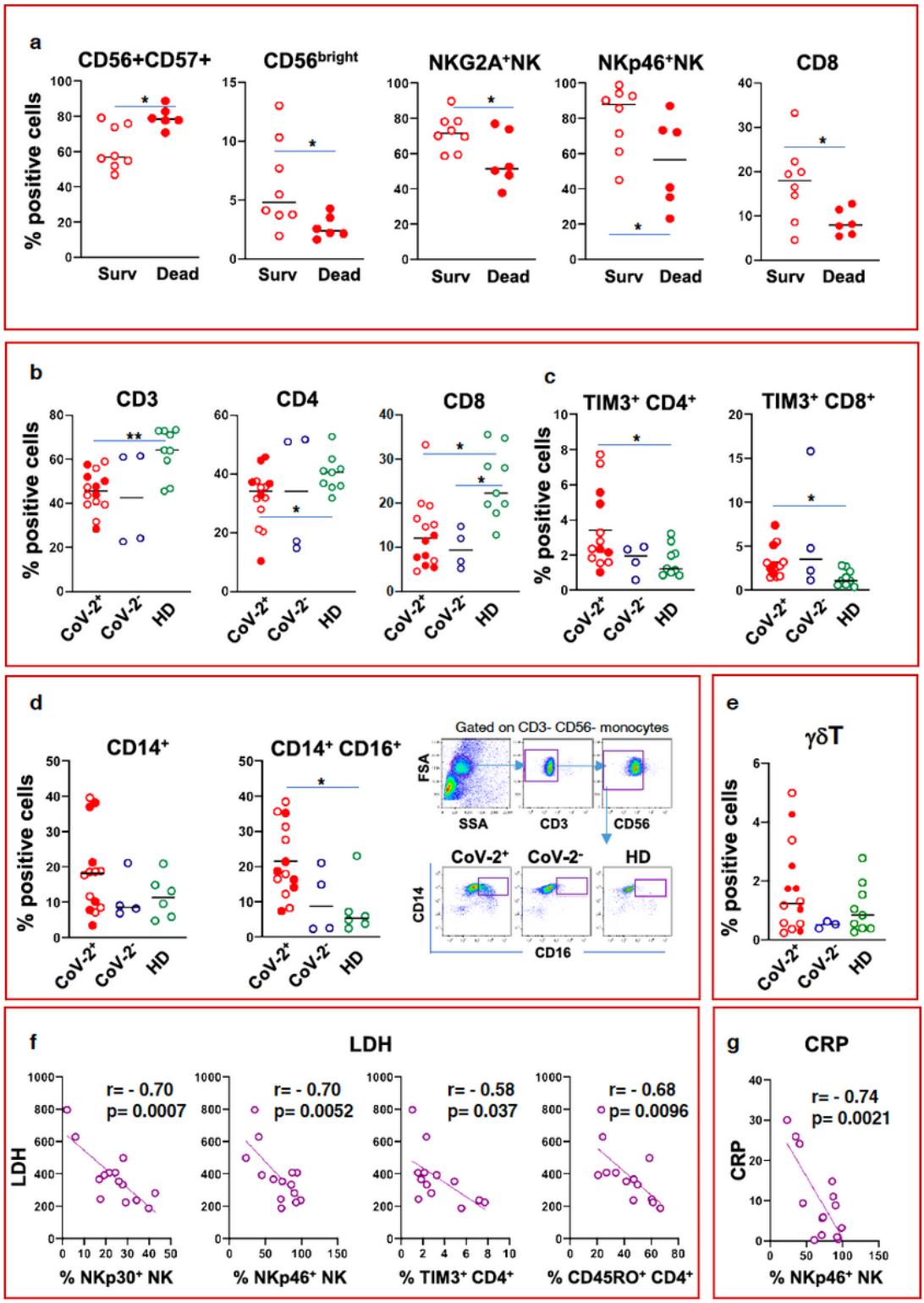

Figure 2

a) Expansion of mature CD57+ NK cells and reduction of CD56bright NK cells, NKG2A+ and NKp46+ NK cells and CD8 T cells in patients who survived and in those who succumbed. b) Frequencies of total CD3+, CD4+ and CD8+ T cells were reduced in COVID-19 patients compared to HD. c) Tim-3 expressing CD4 and CD8 T cells were increased in COVID-19 patients. d) Expansion of CD14+CD16+ double positive monocytes in COVID-19 patients and representative dot plots. e) No differences were observed in the proportion of γδT cells. f & g) Correlations of NK and CD4 T receptor molecules with LDH and CRP. Middle bars represent median values. The Mann-Whitney test was used to compare survivors versus dead patients. The One Way Anova test was used to compare three groups. The Pearson test was used to examine correlations. * $p<0.05$, ** $p<0.01$.

Supplementary Files

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