

1 Aberrant pathogenic GM-CSF⁺ T cells and inflammatory CD14⁺CD16⁺ monocytes 2 in severe pulmonary syndrome patients of a new coronavirus

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20 Pathogenic human coronavirus infections, such as severe acute respiratory syndrome CoV
21 (SARS-CoV) and Middle East respiratory syndrome CoV (MERS-CoV), cause high
22 morbidity and mortality^{1,2}. Recently, a severe pneumonia-associated respiratory syndrome
23 caused by a new coronavirus was reported at December 2019 (2019-nCoV) in the city Wuhan,
24 Hubei province, China³⁻⁵, which was also named as pneumonia-associated respiratory
25 syndrome (PARS)⁶. Up to 9th of February 2020, at least 37, 251 cases have been reported
26 with 812 fatal cases according to the report from China CDC. However, the immune
27 mechanism that potential orchestrated acute mortality from patients of 2019-nCoV is still
28 unknown. Here we show that after the 2019-nCoV infection, CD4⁺T lymphocytes are rapidly
29 activated to become pathogenic T helper (Th) 1 cells and generate GM-CSF etc. The cytokines
30 environment induces inflammatory CD14⁺CD16⁺ monocytes with high expression of IL-6 and
31 accelerates the inflammation. These aberrant and excessive immune cells may enter the
32 pulmonary circulation in huge numbers and play an immune damaging role to causing lung
33 functional disability and quick mortality. Our results demonstrate that excessive non-effective
34 host immune responses by pathogenic T cells and inflammatory monocytes may associate
35 with severe lung pathology. Therefore, we suggest that monoclonal antibody that targets the
36 GM-CSF or interleukin 6 receptor may potentially curb immunopathology caused by
37 2019-nCoV and consequently win more time for virus clearance.
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39 Coronavirus, including SARS and MERS, has caused two large-scale pandemic in the last
40 two decades^{1,2}. Although viral evasion of host immune responses and virus-induced
41 cytopathic effects are believed to be critical in disease severity, studies from humans who died
42 of SARS and animal models suggested that an excessive and aberrant host immune response
43 resulting in an exuberant immunopathology and lethal disease⁷⁻⁹. Similarly, patients infected
44 with 2019-nCoV, that have been reported recently, have increased plasma concentrations of

inflammation related cytokines, including interleukins (IL) 2, 7, and 10, granulocyte-colony stimulating factor (G-CSF), interferon- γ -inducible protein 10 (IP10), monocyte chemoattractant protein 1 (MCP1), macrophage inflammatory protein 1 alpha (MIP1A), and tumour necrosis factor α (TNF- α), especially in moribund patients¹⁰. Importantly, 2019-nCoV infected patients have developed characteristic pulmonary ground glass changes on imaging and lymphocytes decreasing^{11,12}. These phenomena suggest severe pulmonary inflammation and cytokine storm also exist in 2019-nCoV infection. At present, symptomatic treatments with organ support to moribund patients are the mainstays of clinical managements. It is urgent to identify the immunopathology mechanism to delay the pulmonary immune injury.

In patients infected with SARS-CoV, it has been reported that the severity of pulmonary immune injury correlated with extensive infiltration of neutrophils and macrophages in the lungs^{13,14}, accompanied with increased numbers of neutrophils and monocytes and lower CD8⁺ and CD4⁺ T cell counts in the peripheral blood samples¹⁵⁻¹⁷. To identify the immune characteristic of patients infected with 2019-nCoV, peripheral blood samples from patients with severe pneumonia were collected for immune analysis. Consistent with previous clinical characteristics reports¹⁸, these hospitalized patients with confirmed 2019-nCoV infection involved from The First Affiliated Hospital of University of Science and Technology of China commonly have fever symptoms. The patients in intensive care unit (ICU) have significantly decreased concentrations of haemoglobin and albumin, but increased concentrations of C-reactive protein, alanine aminotransferase, aspartate aminotransferase and lactate dehydrogenase (Extended Data Table 1). The number of total leukocytes in peripheral blood had no significant differences between patients of 2019-CoV and healthy controls, whereas the number of lymphocytes decreased significantly in ICU patients. Specifically, monocytes from both ICU and non-ICU patients significantly decreased compared with healthy controls. The number of T cells also significantly decreased from both ICU and non-ICU patients. The CD4⁺ T cells from both patients in ICU and non-ICU decreased remarkably, whereas CD8⁺ T cells decreased more significantly in ICU patients. Other kinds of leukocytes, including granulocyte, B cells and NK cells have no significantly change in numbers between patients of 2019-nCoV and healthy controls (Extended Data Figure. 1).

To demonstrate the status of these aberrant altered T cells, several lymphoid antigens have been analyzed on T cells. These CD4⁺ T cells in patients infected with 2019-nCoV have higher expression of CD69, CD38, and CD44 compared with healthy controls (Fig.1a, b), indicating their activated status. OX40 have been reported to play a major role in promoting clonal expansion and inducing production of several cytokines in T cells¹⁹. In patients infected with 2019-nCoV, OX40 expression increased remarkably on CD4⁺ T cells, especially in severe ICU patients (Fig.1a, b). CD8⁺ T cells in patients infected with 2019-nCoV also showed activated phenotype with higher expression of CD69, CD38 and CD44 (Fig.1c, d). 41BB (CD137; TNFRS9) is an activation-induced co-stimulatory molecule, which is important to priming immune responses of cytotoxic CD8⁺ T cells²⁰. In ICU patients infected with 2019-nCoV, the expression of 41BB increased significantly compared to healthy controls (Fig.1c, d). It has been reported that co-expression of Tim-3 and PD-1 may represent a subset of T cells with more severe exhaustion in virus infections^{21,22}. It is worth noting that much

higher percentage of co-expression Tim3⁺PD-1⁺ T subset exist both in CD4⁺ and CD8⁺ T cells from patients of 2019-nCoV (Fig.1e-h), especially in ICU patients, suggesting the exhausted status in T cells in these patients infected 2019-CoV.

To further identify the key pathogenic cytokines and the main source of these cytokines, interferon- γ (IFN γ), TNF- α , granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-6 have been selected to analyzed through intracellular cytokine staining, for these inflammatory mediators have been proven to be critical as the primary cause of inflammatory cytokine storm in patients infected with SARS-CoV or MERS-CoV^{23,24}. Without re-stimulation with PMA or incubation with monensin, high percentage of GM-CSF⁺ and IL-6⁺ expressions could be found in CD4⁺T cells from patients infected with 2019-nCoV in both ICU and non-ICU patients compared to healthy controls (Fig.2a, c). ICU patients with more severe pneumonia showed correlated higher percentage of GM-CSF⁺ and IL-6⁺CD4⁺ T cells (Fig.2a, c). Pathogenic Th1 cells with both IFN- γ and GM-CSF expression have been reported in central nervous system inflammation²⁵. Importantly, aberrant pathogenic Th1 cells with co-expressing IFN γ and GM-CSF exist only in ICU patients infected 2019-nCoV, whereas little was found in non-ICU patients and healthy controls, indicating this pathogenic Th1 cells which have correlative evidence from patients with severe disease, play a critical role for hyper-inflammatory responses in 2019-nCoV pathogenesis (Fig.2b, d). Meanwhile, TNF- α were not significant up-regulated in CD4⁺T cells from patients of 2019-nCoV (Extended Data Figure 2a-c). CD8⁺ T cells from ICU patients also showed expression of GM-CSF compared to those from non-ICU patients and healthy controls. IL-6 and TNF- α were not found in CD8⁺ T cells (Extended Data Figure 2d, e). Neither NK cells nor B cells were the secreting source of GM-CSF and IL-6 (Extended Data Figure 2f-i).

GM-CSF has been recently been implicated in the pathogenesis of inflammatory and autoimmune diseases, in a mechanism that controls diverse pathogenic capabilities of inflammatory myeloid cells. Among these myeloid cells, monocyte is the pathogenic GM-CSF responsive cells that require GM-CSF to initiate tissue damage in both mouse and human^{26,27}. To identify whether inflammatory monocyte exist in patients infected 2019-nCoV, phenotype and subpopulation of monocytes have been analysis. There was little CD14⁺CD16⁺ inflammatory monocyte subset in healthy controls. By contrast, significant higher percentage of CD14⁺CD16⁺ inflammatory monocyte exist in peripheral blood of patient infected 2019-nCoV. The percentage of CD14⁺CD16⁺ monocyte was much higher in severe pulmonary syndrome patients from ICU (Fig.3a, c). Moreover, these monocyte from patients infected 2019-nCoV also showed capability to secrete GM-CSF. Importantly, significantly higher expression of IL-6 secreted from these inflammatory monocyte especially in ICU patients, which let the cytokine storm even worse (Fig.3b, d). Meanwhile, the number of GM-CSF⁺ monocytes and IL-6⁺ monocytes increased rapidly (Fig.3e), suggesting the potential high risk of inflammatory cytokine storm caused by monocytes that may migrate to the lung and further derive into macrophage or monocyte derived dendritic cells. Thus, in patients infected with 2019-nCoV, GM-CSF potentially links the severe pulmonary syndrome-initiating capacity of pathogenic Th1 cells (GM-CSF⁺IFN γ ⁺) with the inflammatory signature of monocytes (CD14⁺CD16⁺ with high expression of IL-6) and their progeny. These activated

immune cells may enter the pulmonary circulation in large numbers and played an immune damaging role in severe pulmonary syndrome patients (Fig.4).

The study provides the detailed immunopathology report on 2019-nCoV, suggesting excessive activated immune response caused by pathogenic GM-CSF⁺ Th1 cells and inflammatory CD14⁺CD16⁺ monocytes may connect pulmonary immunopathology leading to deleterious clinical manifestations and even acute mortality after 2019-nCoV infections. Consistent with the situation with SARS-CoV or MERS-CoV^{12,28}, it is remarkable that children always experience mild-moderate clinical illness, elderly individuals exhibit worse outcomes after infection with 2019-nCoV, further indicating that mature excessive immune response towards these pathogenic human coronavirus infections play a key role in inducing severe pulmonary syndrome and even organ failure. However, many urgent questions remain to be answered. Evidence from alveolar washing fluid and lung autopsy from patients infected 2019-nCoV are further needed to verify whether and how these aberrant pathogenic immune cells play a fatal immune damage to cause organ functional disability and mortality. Specific new drugs targeted 2019-nCoV may take long time to evaluate and develop. At this critical moment, several marketed drugs to target cytokine storm and reduce immunopathology could be considered²⁹. Blocking inflammatory cytokines may temporarily weaken the anti-infection immunity, yet such strategy is already the lesser of the evils. Other strategies towards blocking the over-activated immune response, such as glucocorticoid treatment showed more side-effect and disappointed outcome towards 2019-CoV¹⁸. Therefore, we suggest that monoclonal antibody that targets the GM-CSF or interleukin 6 receptor may potentially prevent or curb immunopathology caused by 2019-nCoV and consequently win more time for virus clearance.

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Fig. 1

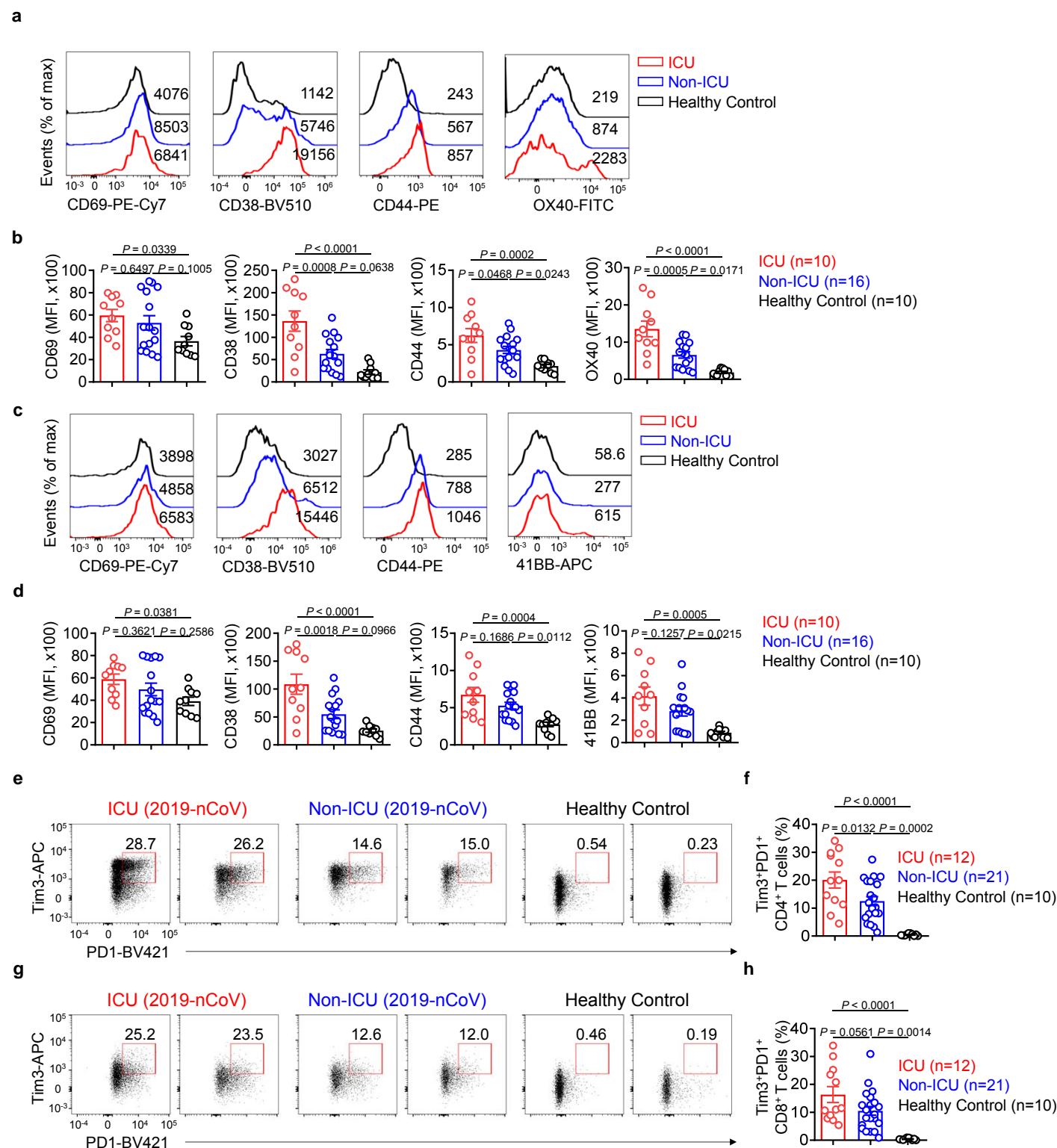


Figure 1. Activated T cells in severe pulmonary syndrome patients of 2019-nCoV.

(a, b) Representative density plots and MFI statistics calculated for CD69, CD38, CD44 and OX40 expressions in gated CD45⁺CD3⁺CD4⁺ T cells (Gating strategy showing in Extended Data Figure 2a) isolated from peripheral blood in healthy controls, ICU and non-ICU patients of 2019-nCoV. (c, d) Representative density plots and MFI statistics calculated for CD69, CD38, CD44 and 41BB expressions in gated CD45⁺CD3⁺CD8⁺ T cells isolated from peripheral blood in healthy controls, ICU and non-ICU patients of 2019-nCoV. (e, f) Representative density plots and percentage statistics calculated for Tim-3 and PD-1 co-expressions in gated CD45⁺CD3⁺CD4⁺ T cells isolated from peripheral blood in healthy controls, ICU and non-ICU patients of 2019-nCoV. (g, h) Representative density plots and percentage statistics calculated for Tim-3 and PD-1 co-expressions in gated CD45⁺CD3⁺CD8⁺ T cells isolated from peripheral blood in healthy controls, ICU and non-ICU patients of 2019-nCoV. Data represent the mean \pm SEM. One-way ANOVA. $P < 0.05$ was considered statistically significant.

Fig.2

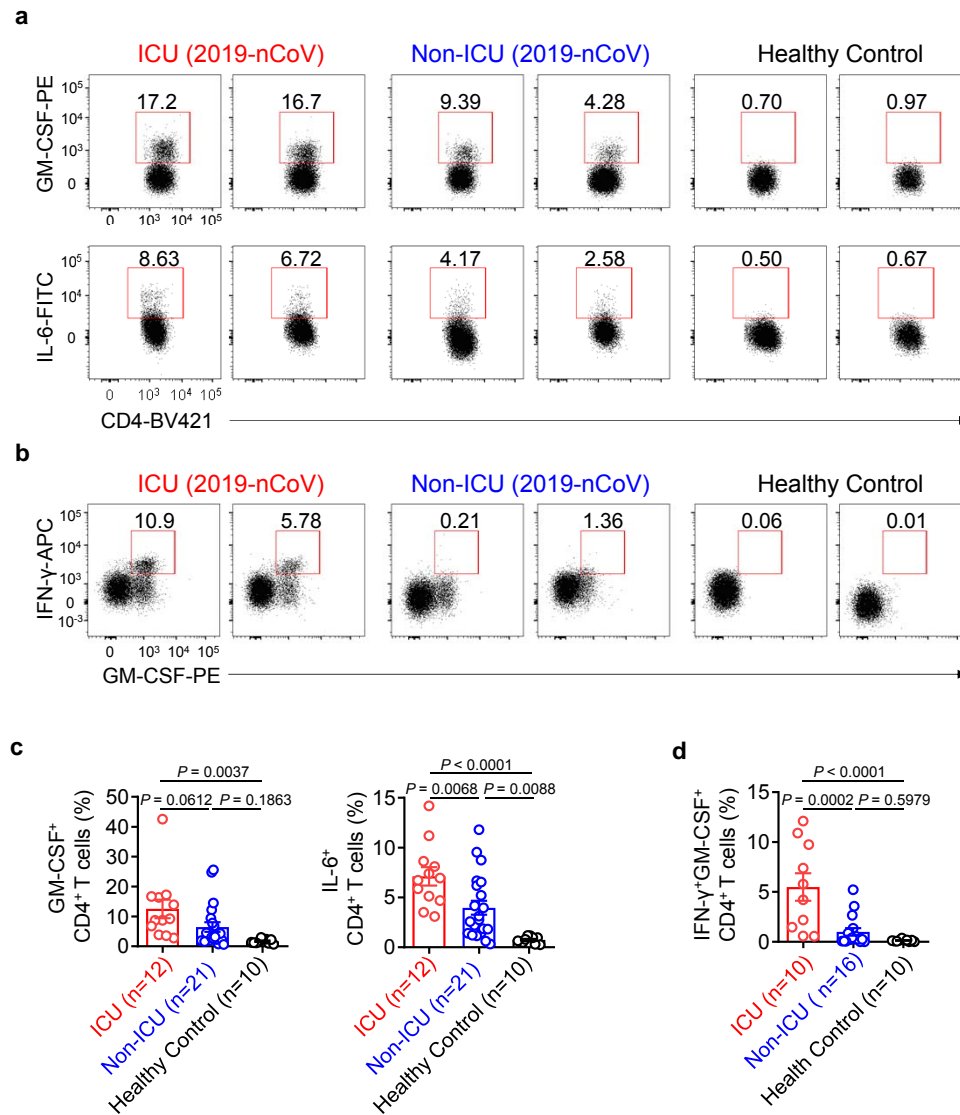


Figure 2. Pathogenic Th1 cells with high expression of GM-CSF in severe pulmonary syndrome patients of 2019-nCoV.

(a) Representative density plots showing an analysis of GM-CSF and IL-6 expressions in gated CD45⁺CD3⁺CD4⁺ T cells (Gating strategy showing in Extended Data Figure 1a) isolated from peripheral blood in healthy controls, ICU and non-ICU patients of 2019-nCoV. (b) Representative density plots showing an analysis of co-expression of GM-CSF and IFN-γ in gated CD45⁺CD3⁺CD4⁺ T cells isolated from peripheral blood in healthy controls, ICU and non-ICU patients of 2019-nCoV. (c) Statistics calculated by the percentage of GM-CSF⁺ or IL-6⁺ cells from CD4⁺ T cells. (d) Statistics calculated by the percentage of GM-CSF and IFN-γ co-expressing CD4⁺ T cells. Data represent the mean ± SEM. One-way ANOVA. $P < 0.05$ was considered statistically significant.

Fig.3

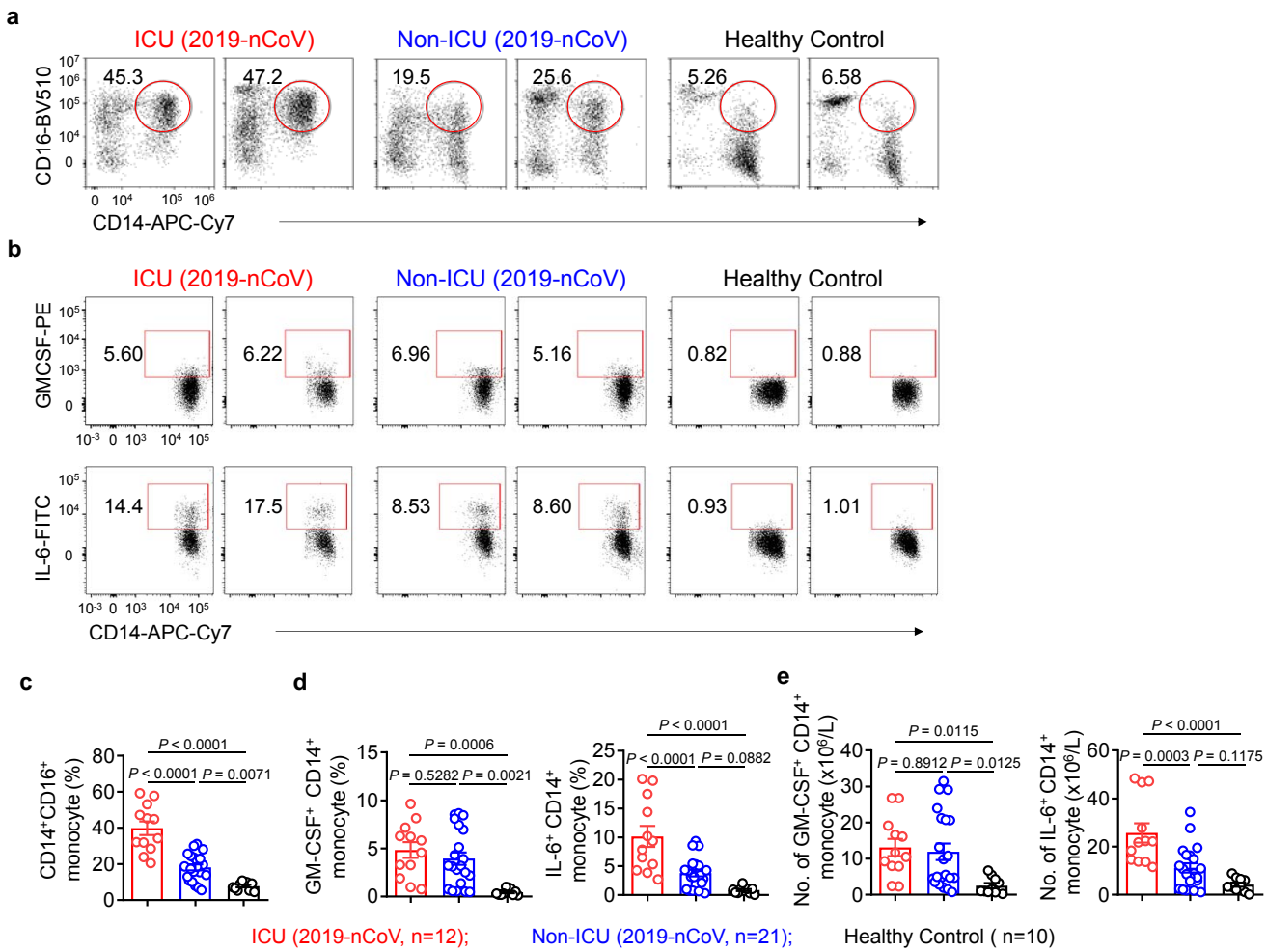


Figure 3. Inflammatory monocytes with high expression of IL-6 in severe pulmonary syndrome patients of 2019-nCoV.

(a) Representative density plots showing an analysis of CD14 and CD16 expressions in gated CD45⁺ monocytes (Gating strategy showing in Extended Data Figure 1a) isolated from peripheral blood in healthy controls, ICU and non-ICU patients of 2019-nCoV. (b) Representative density plots showing an analysis of GM-CSF and IL-6 expressions in gated CD45⁺CD14⁺ monocyte cells isolated from peripheral blood in healthy controls, in ICU and non-ICU patients of 2019-nCoV. (c) Statistics calculated by the percentage of CD14⁺CD16⁺ subsets from monocytes. (d) Statistics calculated by the percentage of GM-CSF⁺ or IL-6⁺ cells from CD14⁺ monocytes. (e) Statistics calculated by the cell number of GM-CSF⁺ CD14⁺ or IL-6⁺CD14⁺ monocytes. Data represent the mean \pm SEM. One-way ANOVA. $P < 0.05$ was considered statistically significant.

Fig.4

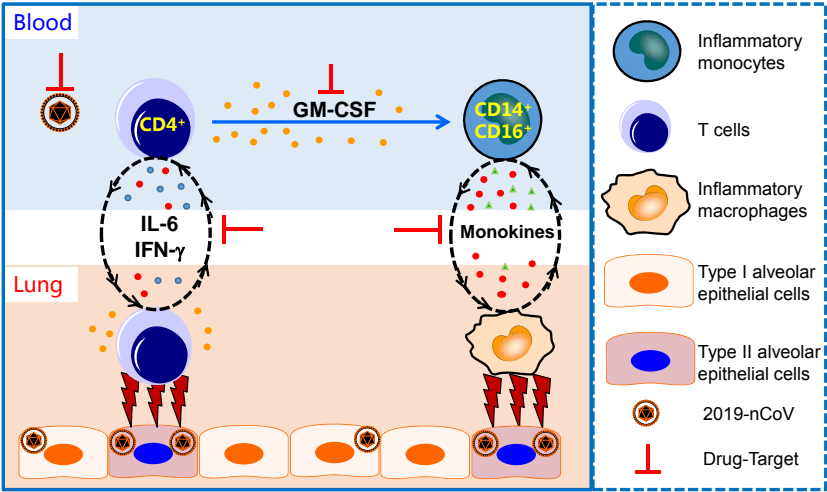


Figure 4. Pathogenic Th1 cells and inflammatory monocytes have positive correlations with severe pulmonary syndrome in patients infected 2019-nCoV.

Pathogenic CD4⁺Th1 (GM-CSF⁺IFN γ ⁺) cells were rapidly activated to produce GM-CSF and other inflammatory cytokines to form a cascade signature of inflammatory monocytes (CD14⁺CD16⁺ with high expression of IL-6) and their progeny. These activated immune cells may enter the pulmonary circulation in large numbers and played an immune damaging role in severe pulmonary syndrome patients. The monoclonal antibodies that targets the GM-CSF or interleukin 6 receptor may potentially prevent or curb immunopathology caused by 2019-nCoV.