COVID-19 and myeloid cells: complex interplay correlates with lung severity

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COVID-19 spans a wide range of symptoms, sometimes with profound immune system involvement. How immune cell subsets change during the disease course and with disease severity needs further study. While myeloid cells have been shown to initiate and maintain responses to pneumonia and lung inflammation, often playing a role in resolution, their involvement with COVID-19 remains unknown. In this issue of the *JCI*, Sánchez-Cerrillo and Landete et al. investigated DCs and monocytes from blood and bronchial secretions of patients with varying COVID-19 severity and with healthy controls. The authors conclude that circulating monocytes and DCs migrate from the blood into the inflamed lungs. While sampling differences in sex, collection timing, bacteria/fungal infection, and corticosteroid treatment limit interpretation, we believe that reprogramming monocyte or macrophages by targeting immunometabolism, epigenetics, or the cytokine milieu holds promise in resolving lung inflammation associated with COVID-19.

Introduction

COVID-19 disease morbidity and mortality have swept the globe since early 2020. The role of the immune response in SARS-CoV-2 pathogenesis and its determinants for the clinical course and outcomes remain elusive. An effective and coordinated immune response controls viral replication and limits immune pathology for a favorable clinical outcome (e.g., asymptomatic or a self-limiting respiratory viral infection). In contrast, an ineffective and dysregulated immune response could eventually control the virus, but sustained and excessive inflammation results in organ injury and an unfavorable outcome (e.g., respiratory failure, acute respiratory distress syndrome [ARDS], and multiorgan failure).

Approximately 14% of hospitalized patients with symptomatic COVID-19 infection develop severe disease with respiratory failure requiring intensive care unit support (1, 2). Clinical severity involves multiple systems, including respiratory, neurological, cardiovascular, hematological, renal, and even dermatological. Although other respiratory viruses, such as influenza, respiratory syncytial virus, other coronavirus, metapneumovirus, etc., can cause severe pneumonia and ARDS, SARS-CoV-2 differs in severity and duration, suggestive of hyperinflammation and/or impaired repair or resolution of inflammation.

As the scientific community elucidates the immunopathology of COVID-19, elevated proinflammatory cytokines and chemokines have led to the term *cytokine storm*. We and others feel this term is a misnomer, as other clinical syndromes, such as non-COVID ARDS, sepsis, and other cytokine release syndromes after CART-T therapy, are among clinical entities with similar cytokine profiles (3), yet these entities do not usually display a protracted ARDS course. Further, cytokine release syndrome secondary to CART-T therapy responds therapeutically to anti–IL-6. In addition to cytokine abnormalities and antibodies specific to COVID-19, dysregulated cellular immune responses include CD4$^+$ T cell activation, hyperactivated or exhausted CD8$^+$ T cells, decreased follicular helper cells, variable plasmablast responses (4), decreased regulatory T cells (5), decreased γδ T cells, increased NK cell activity, and increased neutrophil extracellular traps (6). Emerging literature also implies that myeloid cells play a role in COVID-19 severity (7). In this issue of the *JCI*, Sánchez-Cerrillo, Landete, and colleagues explore the role of myeloid cells in COVID-19 (8). The authors reveal the need for extensive profiling and evaluation of myeloid cell function and fate at different stages and severities of the disease.

The role of myeloid cells in ARDS

Myeloid cells including monocytes and macrophages initiate and maintain lung inflammatory responses to a variety of local or systemic insults (9); they also orchestrate resolution of lung inflammation (10). Research in mouse models of ARDS underscores the importance of monocytes that are recruited from blood. These blood-recruited monocytes mature into monocyte-derived macrophages that are involved with lung repair and lung fibrosis. Similarly, resident lung macrophage populations also contribute at different phases of the repair/fibrosis processes. Notably, the literature regarding the role of monocytes and macrophages in human ARDS pathogenesis is mainly descriptive and speculative. Previous studies in ARDS patients show increases in total alveolar macrophages (AMs); specifically, surface and maturation markers implied that AMs are of monocytic origin and are proinflammatory (11,
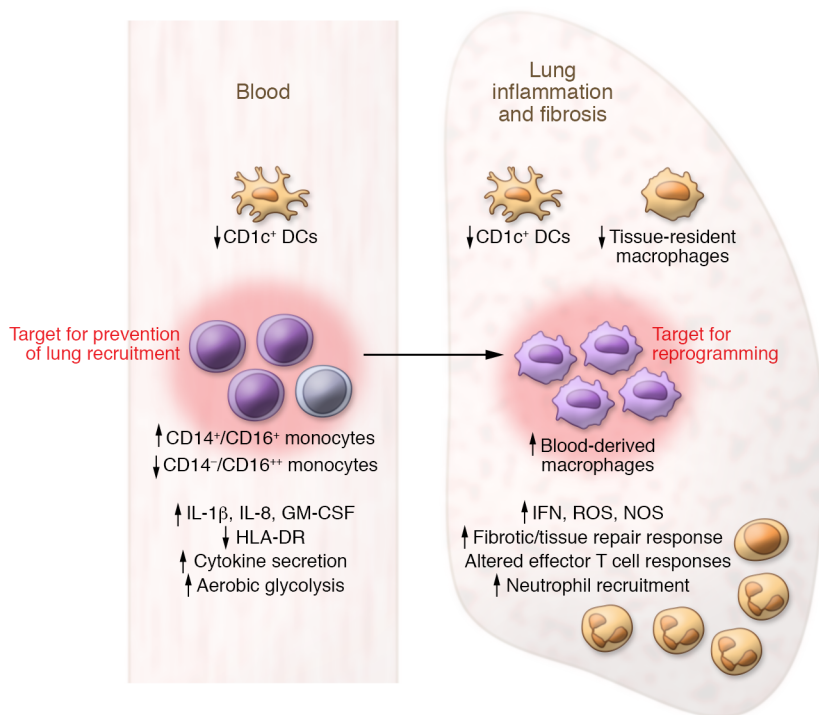
Figure 1. Model of changes in myeloid subsets during severe COVID-19. Sánchez-Cerrillo and Landete et al. (8) showed that in patients with severe COVID-19, circulating intermediate monocytes CD14⁺CD16⁺ increased and nonclassical CD14⁻CD16⁺⁺ monocytes decreased. Corresponding surface markers CD11b, CD18, CD80⁺ (M1), CD206, CD69, and tissue factor CD142 all increased; the MHC class II cell surface receptor HLA-DR decreased. DCs decreased both in the blood and the lung. Cytokine secretion also increased, and phagocytosis was impaired. Within the lung, tissue-resident macrophages decreased, while mononuclear phagocytes derived from blood increased (8). Targeting circulating monocytes or lung-residing macrophages therapeutically may resolve inflammation, mitigate fibrosis, and repair lung damage.

12). AMs from ARDS patients also secrete more IL-1β and IL-8, which correlate with increased mortality (13, 14). Transcriptionally, AMs and peripheral blood monocytes upregulate inflammatory genes, which correlates with worse outcomes (15). These data highlight the importance of immune cell tissue origin and suggest the need for comprehensive analysis of both blood and lung. One such mass cytometry analysis has revealed three unique AM populations in non-COVID ARDS that are defined by CD169 and PD-L1 expression. Notably, increased abundance of these three populations correlated with more ventilator-free days (16). Furthermore, a persistently activated AM transcriptional profile and lack of transition into a prorepair signature was associated with poor prognosis (15). However, clinical trials targeting macrophage function (GM-CSF [ref. 17] and IFN-β [ref. 18]) in ARDS patients have failed. Blocking one target in an intricate milieu in ARDS is oversimplistic, and timing of intervention is pivotal. With the advent of high-throughput, high-dimensional flow cytometric, proteomic, and transcriptomic technologies, a better understanding of human monocyte/macrophage subsets, origins, functions, and correlations with clinical outcomes in both COVID-19-induced and non-COVID-19-induced ARDS is possible and critical.

Monocytes and macrophages in COVID-19 disease

Monocytes and lung macrophages are substantially altered, both in number and function, during COVID-19 disease. While disease severity affects the number and proportion of monocyte subsets in the blood and lung, some patterns emerge. Generally, in blood, the nonclassical monocyte subset (CD14⁻CD16⁺⁺) is depleted, yet the intermediate subset (CD14⁺CD16⁺) is expanded (19-22). The alveolar space is enriched for monocytes and monocyte-derived macrophages (19, 23), suggesting the lungs increase recruitment from the blood. Phenotypically, blood monocytes show diminished HLA-DR surface expression, possibly due to excess IL-6 exposure, suggesting reduced antigen-presenting capacity. Elevated complement receptor 3 (CD11b/CD18) expression (24) activates the cells to release cytokines. It is unclear whether blood monocytes and lung macrophages are directly infected by the virus or whether cellular changes are due to excessive inflammatory mediators. Functionally, monocytes from COVID-19 patients secrete the cytokine GM-CSF (25), although spontaneous IL-6 secretion varies by study (25, 26). The combination of these two cytokines switches monocyte differentiation from a DC fate to an inflammatory macrophage phenotype, consistent with the phenotyping of cells collected from the lower respiratory airway of COVID-19 patients (8, 23). Monocytes from COVID-19 patients also spontaneously secrete IL-1β and IL-8 (26). Excessive cytokine secretion is supported energetically by a switch to aerobic glycolysis (27). Transcriptional profiling has revealed a type I IFN gene signature, overexpression of genes that promote reactive oxygen and nitrogen species formation, inflammation-related chemokines, and a tissue-repair phenotype (28). This gene signature is consistent with a robust antiviral response and fibrotic response, which likely contribute to the collateral tissue damage and fibrosis seen in later stages of this disease. Although direct evidence that human monocyte-macrophages participate in COVID-19 pathogenesis is lacking, the findings described above and others (29) strongly implicate these cells as key players in the lung inflammation and damage seen in the disease.

Pulmonary redistribution of myeloid subpopulations and COVID-19 severity

Sánchez-Cerrillo and Landete et al. (8) profiled immune cells from the blood and respiratory secretions of a cohort of

patients with critical disease and compared them with those of patients having either mild or severe disease and also with those of healthy controls. With results similar to those of previous studies, the authors noted that patients' blood was depleted of classical and intermediate monocyte subsets and DCs, and that inflammatory intermediate and nonclassical monocytes, granulocytes, and CD1c+ cDCs appeared in their lungs. Diminished blood monocytes correlated with increased inflammatory markers, procalcitonin, and C reactive protein, levels, but not with IL-6 and type I IFN (Figure 1). Exhausted effector CD8+ T cells were enriched in the lungs of patients with COVID-19 compared with their blood and the blood of healthy controls. The dominance of inflammatory monocytes over CD1c+ DCs may explain the abundance of exhausted lung CD8+ T cells. By surveying monocyte and DC populations in both the blood and the lungs, the authors conclude that the depletion of circulating monocytes and DCs is a result of migration to the inflamed lungs. Expression of myeloid CD40, an activation marker, was increased on classical and nonclassical blood monocytes in mild, but reduced in critical, COVID-19 patients, although expression was not different from that of healthy controls (8).

There are important limitations of Sánchez-Cerrillo and Landete et al. (8) that need considering: (a) the subjects in the control arm were predominantly females. Sex and sex hormones influence macrophage phenotype and function, as we and others have previously demonstrated (30). (b) The timing of sample collection, and consequently immune profiling, after infection was delayed in the critical group. Myeloid cell activation and plasticity dynamically change over the course of the infection, and consistent collection intervals would address variability. (c) The presence of bacterial or fungal superinfection modulates myeloid responses and likely modifies antiviral immune function in the lungs. A control group with active bacterial pneumonia without COVID-19 infection would add specificity. (d) A large proportion of critically ill patients received combinations of biologicals (anti–IL-6 blockade) and corticosteroids, which alter macrophage

subpopulations and their activation. The use of immunomodulators and corticosteroids influences immune response and should be omitted if possible. (e) Superior flow cytometric phenotype and analysis would have allowed accurate quantification of lung myeloid subsets. (f) Comparison with non-COVID ARDS would determine specificity of their findings.

Outstanding biology and future directions

Immune profiling and sequencing of myeloid subpopulations in blood and alveolar compartments will uncover how these immune cells participate in response to SARS-CoV-2 infections and their interplay with other immune cells, such as T cells and neutrophils, and with nonimmune cells, such as endothelial cells and alveolar epithelial cells. Direct infection of SARS-CoV-2 in myeloid cells or phagocytosis of infected epithelial cells will need to be elucidated. Understanding the signaling pathways in infected macrophages could lead to therapeutic targets, including JAK and inflammasome inhibitors, which are currently being tested in clinical trials. A full understanding of how the cytokine milieu diminishes effective monocyte-macrophage responses to the virus is needed. Moreover, determining how risk factors that associate with severe disease, such as age, sex, obesity, and diabetes, affect monocyte-macrophage dynamics in COVID-19 disease, as well as how therapeutic trials modulate myeloid responses longitudinally, will be informative.

It is imperative when considering therapies that target myeloid responses, to account for factors including timing and stage of the infection (early vs. late) and nature of the pathway to be modulated (single cytokine or receptor vs. upstream signaling pathway). For instance, abrogating myeloid proinflammatory responses to COVID-19 could limit disease and progression to organ injury, but could also lead to super infection with bacterial and fungal pathogens.

The potential of monocyte-macrophage reprogramming (immunometabolically, epigenetically, or through cytokine modulation) to resolve lung inflammation and repair the damaged lung is an unexplored cellular approach that could treat patients with COVID-19 while the world

and the scientific community wait for a protective and safe vaccine (Figure 1).

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