Monocytes and macrophages in severe COVID-19 – friend, foe or both?

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While most COVID-19 patients have mild symptoms, about 15% of patients develop severe COVID-19 often with fatal outcomes, and scientists have been on the hunt to understand the underlying immune mechanisms driving disease severity. In a recent study in Immunity, Szabo et al.1 provide comprehensive longitudinal insights into the phenotypical and functional shifts of T cells, monocytes and macrophages driving inflammation in the lungs of patients with severe COVID-19.

COVID-19, a disease caused by the coronavirus SARS-CoV-2, has continuously been putting a toll on life worldwide since the first cases started to spread at the end of 2019. SARS-CoV-2 is highly contagious, can be transmitted via various routes including via aerosols and predominantly infects the respiratory system. Thus, the disease often manifests with symptoms similar to a classic cold or flu, such as coughing, a runny nose and fever. However, in some cases patients suffer from severe disease characterized by major dysregulation of immune responses, cytokine storm syndrome, massive lung tissue damage resulting in difficulties or inability to breathe on their own and the need for intubation and ICU care.2 Over the past year, multiple studies have profiled immune cell frequencies, phenotypes and responses in the blood of patients with COVID-19 trying to correlate these to the risk of developing mild vs. severe COVID-19, providing comprehensive insights into the potential mechanisms responsible for the massive lung damage observed in severe COVID-19. They performed longitudinal high-dimensional profiling of blood and airway monocyte and macrophage populations (as well as T cells) describing their phenotypes, transcriptomes and corresponding cytokine secretion profiles. Overall Szabo et al. found myeloid cells to be hyperinflammatory, with aberrant CD163+HLA-DR+CCR2+ monocytes increased in the blood and a reduction of CD16+ monocytes in the lungs of patients with severe COVID-19 (Figure 1), matching previous reports by Silvin et al. and Schulte-Schrepping et al.3,4

In order to be able to correlate immune profiles during severe COVID-19 across tissues and over time, Szabo et al. collected blood samples and saline washes of the endotracheal tube which, as they had shown previously, contain most of the respiratory immune cell populations, from intubated ICU patients with severe COVID-19 for up to 10 consecutive days. By
multiparameter profiling of these samples by flow cytometry, Szabo et al. detected lowered T cell frequencies but increased frequencies of aberrant monocytes in the blood of patients with severe COVID-19, in line with a few other publications.3,4,5 Yet another recent report showed that the overall monocyte numbers were barely altered, or were even decreased for certain subsets, while dendritic cell (DC) numbers were generally decreased in severe patients.6 Disease driven changes in the immune cell compartment generally appeared more manifested in the blood than within the airways. The authors found that within the airways myeloid cell frequencies generally increased with age, while T cell frequencies were inversely correlated. Further, these frequency distributions were not only pronounced in patients with severe COVID-19 who eventually succumbed to the disease, but also were predictive of disease outcome for at least 75% of cases -- an observation that could be useful for adjusting the therapy strategy for individual patients.1

Figure 1. Changes in myeloid cell frequencies and phenotypes during severe COVID-19. In patients with severe COVID-19 lymphocyte frequencies within the blood decrease with time and severity of disease, while inflammatory monocyte frequencies increase, resulting in aberrant CD163\(^{\text{hi}}\)HLA-DR\(^{\text{lo}}\) monocytes dominating within the blood stream. These aberrant monocytes subsequently infiltrate the airways following a CCL2 gradient, with CCL2 highly produced by activated resident airway T cells. CD163\(^{+}\) myeloid cells can be found aggregated in areas of alveolar damage, suggesting a contribution of these cells to the pathology of severe COVID-19.
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Given the diversity of myeloid cell types, from DCs to neutrophils, monocytes and macrophages to many others, the obvious question was which myeloid subsets contributed most to these changes? To address this, Szabo et al. clustered myeloid populations based on surface marker expression and identified nine main clusters that were profoundly altered within the airway and blood samples from healthy donors vs. patients with severe COVID-19. Besides a drastic reduction of CD16+ intermediate monocytes in the airways of COVID-19 patients, the frequencies were highly increased for CD163+HLA-DR+ and CD86+HLA-DR+ cells in the airways and for CD163+HLA-DR+ monocytes in the blood. These monocytes were not present in healthy blood controls where the airways contained mostly CD163hi macrophages and only a few CD163+ intermediate monocytes, suggestive of increased monocyte infiltration from the blood stream into the airways during severe COVID-19. This dramatic shift between a high expression of HLA-DR in the airways but downregulation of HLA-DR on blood monocytes is in line with other recent publications, suggesting an immature or dysregulated phenotype of monocytes and macrophages as a driver for pathogenesis during SARS-CoV-2 infection. In general, few publications over the past year have addressed the dysregulation of monocytes and macrophages in COVID-19. Liao et al. had found inflammatory monocyte-derived FCN1+ macrophages in the bronchoalveolar fluid of patients with COVID-19, while Zhou et al. observed increased frequencies of CD14+CD16+ inflammatory monocytes secreting MCP1, IP-10 and macrophage inflammatory protein-1α (MIP-1α) contributing to the cytokine storm observed in patients with severe COVID-19. These hyperinflammatory responses by myeloid cells, including a macrophage activation syndrome, during infection with SARS-CoV-2 have been attributed as a major driver of disease severity. However, the data presented here by Szabo et al. also appear to show a dramatic shift in DC frequencies, and perhaps functions, between healthy donors and severe COVID-19 blood and airway samples, the relevance of which needs to be studied further. Looking at the overall findings of this publication and other publications showing, for example, dysregulated type I interferon responses during COVID-19, it would be interesting to also address the impact of DCs on COVID-19 outcome in future studies.

Another question should be how exactly do aberrant monocytes or lung macrophage populations contribute to the lung damage (or other pathologies) observed in patients with severe COVID-19? Do differently localized subsets or subsets of embryonic vs. bone marrow-derived origin contribute in different ways? In an attempt to address some of these questions, Szabo et al. performed scRNA-seq on paired blood and airway samples from patients with severe COVID-19 and showed that pro-inflammatory gene signatures were highly upregulated in airway monocytes and macrophages compared with blood monocytes, as indicated by the expression of CD206 or ITGAV. Meanwhile, blood monocytes showed increased expression of genes for “homing” receptors such as CCR2 or CX3CR1. Airway myeloid cells had increased transcript levels for CCL2, which was also upregulated in airway T cells (which are mostly composed of tissue resident memory T cells becoming highly activated during severe COVID-19), which, Szabo et al. hypothesized, was facilitating enhanced recruitment of monocytes from the blood to the lungs. Similarly, Zhou et al. and others had previously shown elevated levels of CCL2 and CCL7 in the BALF from severe patients, thus increasing monocyte recruitment into the lung. Matrix metalloproteinases (e.g. MMP9), which had been implicated previously in driving tissue damage, were also highly upregulated in airway myeloid cells of patients with severe COVID-19 and may contribute to pathogenesis.

In addition, CD163+ myeloid cells were aggregated in the alveolar spaces of lung autopsy samples from COVID-19 patients with diffuse alveolar damage, leading Szabo et al. to conclude that myeloid cell recruitment into the alveoli is perpetuating inflammation and pathogenesis. Although diffuse alveolar damage is one key characteristic of the pulmonary pathology exhibited by COVID-19, which massively impedes alveolar gas exchange, simply the presence of CD163+ myeloid cells in the alveolar spaces does not tell us much about why these cells aggregate there. Their functions could be pro-inflammatory or also aiding tissue regeneration but also anti-inflammatory, as CD163+ macrophages often have been described as immunosuppressive in the context of tumor microenvironment. It is intriguing to speculate that this immunosuppressive function could be the case in COVID-19, while pro-inflammatory monocytes keep “firing” cytokines resulting in a pro-anti-inflammatory loop that cannot be properly resolved in severe COVID-19. Szabo et al. identified that cytokine and chemokine gradients from the blood to the airways changed over time, with the higher levels of the monocyte chemoattractant MCP-1/CCL2, MIP-1α/CCL3 or MIP-1β/CCL4 in the airways facilitating monocyte infiltration and recruitment, which may explain the aggregation of CD163+ myeloid cells in alveolar spaces.

In conclusion, Szabo et al. provide a comprehensive data set of paired blood and airway monocytes and macrophages from patients with severe COVID-19 employing multiparameter flow cytometry and
scRNA-seq, confirming their aberrant and hyperinflammatory profiles, and an increased influx of aberrant monocytes into the lung following a CCR2-CCL2 gradient, which likely give rise to alveolar damage promoting CD163+ myeloid cells. More functional data are needed now to confirm the hypothesis built by Szabo et al., and other authors, providing more explicit mechanisms of tissue damage, specialized functions and contributions of each of the identified aberrant monocyte or macrophage subsets. This will be crucial to fully understand pathogenesis during severe COVID-19, and thus, how to prevent and/or treat it to improve disease outcome for patients, perhaps in a more personalized or cell-targeted way than the current “standard” therapies such as anti-IL-6 treatment, remdesivir or targeting of GM-CSF.

**AUTHOR CONTRIBUTION**

Regine J Dress: Conceptualization; Writing-original draft; Writing-review & editing. Florent Ginhoux: Conceptualization; Writing-original draft; Writing-review & editing.

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