Immunological Subpopulations Within Critically Ill COVID-19 Patients

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections cause coronavirus disease 2019 (COVID-19).[1] Most SARS-CoV-2 infections are self-limiting and pauci-symptomatic. However, a minority of SARS-CoV-2 infections develop pulmonary and extrapulmonary organ dysfunction (such as hypoxemic respiratory failure, acute kidney injury, and thrombotic complications[2]) that require organ support (COVID-19 critical illness or severe COVID-19, equivalent to World Health Organization Clinical Progression Scale of ≥6 points).[1]

Dysregulated immune responses are key to the pathogenesis of COVID-19.[3,4] Briefly, as an intracellular pathogen, the unique nucleic acid structures and viral replication intermediates of SARS-CoV-2 are sensed by endosomal Toll-like receptors in innate immune cells and the cytosolic retinoic acid-inducible gene-like receptors present in most cells. This sensing of danger signals results in the production of pro-inflammatory cytokines through the nuclear factor-kB transcriptional program and inhibition of viral replication through interferons (IFNs) activating the interferon-stimulated

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genes program.[5,6] The cell-mediated effector immune responses to SARS-CoV-2 infection consist of the transcription factor T-bet and IFNγ dependent type 1 effector immune responses by innate lymphoid cells, natural killer cells, helper cells, and cytotoxic T cells.[6] These immune responses result in viral clearance and illness resolution, especially in patients with self-limiting infections.

However, certain SARS-CoV-2 characteristics and host factors can adversely influence these responses to generate the complex dysregulated immune responses seen in severe COVID-19 illness.[5] SARS-CoV-2 encodes viral proteins capable of evading recognition by immune cells, reducing IFN production, impairing IFN signaling, and impairing IFN-stimulated genes effector function program, all of which impair SARS-CoV-2 clearance.[5] Host factors such as old age and genetic defects[7] can result in delayed IFN responses, leading to persistence of virus and exaggerated systemic inflammation, resulting in severe disease.[5] Severe COVID-19 illness is also associated with inborn errors in the IFN pathway, and antibodies to IFNs.[8,9] Furthermore, the effector immune responses associated with helminth infections[6] and with extracellular pathogens (bacterial and fungal infections)[6] appear activated, and they persist in patients with severe COVID-19 illness.[3]

In this context, let us consider the cohort study by Dupont and colleagues[10] in this issue of *CHEST*. The authors performed immunological assessments in 96 adults with severe COVID-19 illness. This cohort included 26 patients with *immune comorbidities* (history of malignancy or active malignancy or solid organ transplant), and approximately 60% (16/26) of these patients were receiving immunosuppressant medications. The authors performed Ward's Hierarchical Agglomerative Clustering, using the following variables: D-dimers, cytokines (IL-6, IL-1β, and tumor necrosis factor-alpha, complement proteins (C3, sC5b-9), gamma globulin levels, and counts of the cytotoxic T cells (CD8), helper T cells (CD4), B cells (CD19), and natural killer cells. The authors identify three phenotypes. First, *humoral response deficiency phenotype,* characterized by B cell lymphopenia and hypogammaglobulinemia, was most prevalent in patients with *immune comorbidities*. Second,

hyperinflammatory phenotype, characterized by pan T cell lymphopenia and highest cytokines levels, was most prevalent in patients receiving mechanical ventilation. Third, *complement-dependent phenotype,* characterized by the highest complement protein levels. The overall critical care mortality was 31%, with the highest mortality in the *hyperinflammatory phenotype* and the least in the *complement-dependent phenotype.* The authors conclude that these phenotypes should inform eligibility criteria for clinical trials testing immunomodulation.

When contextualizing this work, the key limitations to consider include are a single-center study, without an independent validation cohort and with immunological assessments only at a single time point—particularly because the average duration of symptoms at the time of sampling was 8 days. Additionally, the sensitivity analysis for testing cluster allocation excluded only 16 of the 26 patients with *immune comorbidities,* namely, those receiving immunosuppressive medication. The cytokine profile measured in this study appears limited, particularly when compared with the extended cytokine profile assessed longitudinally to identify COVID-19 phenotypes previously.[3] However, the cytokines measured include those being considered as potential treatment targets in COVID-19, such as IL-6 (with IL-6 receptor antagonists such as tocilizumab) and IL-1β (with IL-1 receptor antagonist such as anakinra), giving the study context relevance. Similarly, in patients with COVID-19, despite the overall lymphopenia, there is a brisk plasmablast response, profoundly altered T cell subsets, and differential changes in B and T cell subsets that contribute to COVID-19 immune phenotypes, none of which are measured in this study.[11-13] Although C-reactive protein, IL-8, ferritin, and human leukocyte antigen-DR isotype were measured and reported, these variables were not considered in the unsupervised clustering analyses. The relevance is that IL-6 levels are associated with high C-reactive protein and decreased human leukocyte antigen-DR isotype expression, and there is a strong positive correlation between ferritin and D-dimers in severe COVID-19.

COVID-19 immune phenotypes have been reported previously.[3,12] Lucas and colleagues[3] performed longitudinal immunophenotyping (using cell subsets, cytokine, chemokines, and other markers) in patients with moderate (n = 80) and severe (n = 33) COVID-19 illness, using SARS-CoV-2 negative health-care workers (n = 108) as control subjects. In this study, the four immune signatures identified correlated with three distinct immune trajectory clusters. Two clusters

represented severe COVID-19 illness, and one, moderate illness with better outcomes. The two severe COVID-19 illness clusters had increased levels of inflammasome-associated cytokines (IL-1α, IL-1β, IL-6, IL-18, and tumor necrosis factor), type 1 (IL-12, chemokines linked to monocyte recruitment, and IFNγ); type 2 (chemokines linked to eosinophil recruitment, IL-4, IL-5); and type 3 (IL-23, IL-17A) effector immune response markers. Mathew and colleagues[12] performed high dimensional phenotyping of lymphocyte subsets and integrated the immune and clinical features in 125 COVID-19 patients with different illness severity. The authors report three groups of patients referred to as *immunotypes.* Two of these clusters were identified using principal component analyses, and the third immunotype was identified using additional information on clinical characteristics. Immunotype 1 signature included CD4 T cell activation, brisk plasmablast response, and lower frequencies of proliferating effector/exhausted CD8 T cells. The immunotype 2 signature included conventional effector CD8 T cell subsets, less CD4 T cell activation, and less proliferating plasmablasts and memory B cells. The immunotype 3 signature was characterized by minimal lymphocyte activation. Given the differences in biological measurements between these studies, head-to-head comparisons between phenotypes are inappropriate.

Severe COVID-19 illness immunology is complex. Immunological subpopulations reported depend on immunological measurements used, timing of measurements, and the analytic methods. Given the wealth of open-source data, it is essential that the research community engages in deriving and validating immunological subpopulations of COVID-19. Until then, studies such as these are hypothesis generating and have limited direct impact on clinical care.

Acknowledgments

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