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Profiling natural killers in COVID-19

Emily M. Mace, PhD *New York, NY*

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Natural killer (NK) cells play a crucial role in the control of viral infections and their critical function has especially been underscored by studies describing severe viral disease in individuals with missing or dysfunctional NK cells. As innate lymphoid effector cells, NK cells are rapid responders to infection through cytokine production and direct lysis of virally infected cells. They are conventionally defined in human peripheral blood as $CD56^{bright}$ NK cells or $CD56^{dim}$ NK cells, which are thought to represent primarily cytokine-producing and cytolytic subsets, respectively. Despite their important role in human health, the role of NK cells in the control of and response to SARS-CoV-2 infection has remained enigmatic. In some contexts, NK cells can direct lytic activity against SARS-CoV-2–infected cells.[1] However, the potent cytokine-producing potential of NK cells can also contribute to lung injury in viral respiratory infections, suggesting that NK cell cytokine production can similarly contribute to the lung pathology that is a hallmark of severe COVID-19.

Many confounding factors have made it difficult to map the trajectory of NK cell responses and define the role they play in the outcome of SARS-CoV-2 infection. These factors include the effects of therapeutic interventions such as high-dose steroids on innate immune cell function, the natural phenotypic and functional heterogeneity of human NK cell subsets and their dynamic response to infection, and the limited functional and in-depth longitudinal analyses of previous studies. In their study, published in *Immunity*, Krämer et al aimed to address each of these factors by performing comprehensive longitudinal functional and phenotypic analyses of NK cells from 4 cohorts of patients from distinct treatment centers and excluding those being treated with high-dose steroids.[2] This has generated a truly extraordinary data set from 205 COVID-19–affected individuals and 81 controls that was collected from days 2 to 41 after onset of COVID-19 symptoms and provides novel insight into the association between NK cells and COVID-19 disease severity.

Krämer et al combined single-cell RNA sequencing (scRNA-Seq) with multicolor flow cytometry or mass cytometry, ultrasensitive bead-based analyses of plasma soluble factors such as cytokines, and cell biologic assays to measure NK cell function and antiviral activity. Disease severity was described by using World Health Organization classifiers; patients treated with dexamethasone were excluded from the study. As has been described by previous studies, COVID-19 disease severity was correlated with a decrease in NK cell frequency.[3,4] In their study, Krämer et al[2] further showed that loss of NK cells occurred at weeks 1 and 2 in both moderate and severe disease; however, NK cell frequencies normalized after week 2 in moderate COVID-19 disease but continued to decline in severe disease. scRNA-Seq analysis identified 6 distinct NK cell subsets aligning with previously defined NK cell peripheral blood subsets[5] that were universally identified in all 4 cohorts from all treatment centers. These subsets included "inflamed" $CD56^{dim}$ cells, which were marked by interferon-related genes; "proliferating" $CD56^{dim}$ cells; cytokine-producing" $CD56^{dim}$ cells, which were marked by *IFNG*, *CCL4* and *CCL3*; and HLA^{hi} $CD56^{dim}$, $CD56^{dim}$, and $CD56^{bright}$ subsets. Strikingly, there was a strong association between COVID-19 disease severity and the expansion of NK cell subsets that were found at minor frequencies in healthy donors; that is, the inflamed and proliferating $CD56^{dim}$ NK cell subsets were associated with severe disease, whereas the cytokine-producing $CD56^{dim}$ NK cells were associated with moderate disease. This is consistent with previous reports showing that NK cells in severe COVID-19 are activated and proliferating,[3,4,6] and it suggests that this phenotype could also be linked to the depletion of NK cells observed at later time points in severe COVID-19. Krämer et al[2] further validated their transcriptomic findings with flow cytometry and, in 1 cohort, cellular indexing of transcriptomes and epitopes by sequencing, thus linking COVID-19 disease severity with the presence of these conserved NK cell subsets that were selectively expanded in infection. Further analysis of transcriptomic data identified IFN-α and TNF gene modules associated with NK cell subsets. Specifically, an early IFN-α signature was present in week 1 in individuals with both moderate and severe COVID-19 disease; however, it was strongest in week 1 and persisted until week 3 in individuals with severe but not moderate disease. In contrast, those with moderate COVID-19 disease had a strong TNF signature in week 1 that was sustained throughout the course of disease (summarized in Fig 1). This observation was functionally validated by measuring the effect of the addition of exogenous cytokines and validating that the addition of IFN-α repressed TNF genes, whereas mimicking severe disease by the addition of TNF in the presence of a high IFN-α level led to higher expression of IFN-α–associated genes. Together, the phenotypic, transcriptomic, and biologic evidence demonstrated distinct NK cell signatures associated with moderate and severe COVID-19 disease and assigned these to dominant TNF and IFN-α signatures, respectively.

Krämer et al[2] further demonstrated functional and mechanistic properties of these NK cell signatures. NK cells were functionally impaired in patients with severe COVID-19 disease, and whereas NK cells from healthy donors reduced viral spike protein levels on SARS-CoV-2–infected cells, the NK cells from patients with COVID-19 had impaired cytotoxicity and antiviral function that

From the Department of Pediatrics, Vagelos College of Physicians and Surgeons, Columbia University Irving Medical Center, New York.

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Corresponding author: Emily M. Mace, PhD, Columbia University Irving Medical Center, 630 West 168th St, New York, NY 10032. E-mail: em3375@cumc.columbia.edu.

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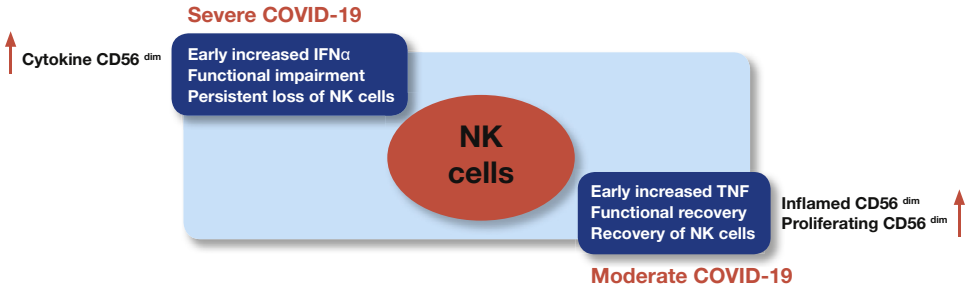

FIG 1. Graphical representation of the functional and phenotypic features of circulating NK cells identified by Krämer et al.[2]

was due at least in part to decreased production of IFN-γ and TNF. This functional phenotype could also be generated by incubating NK cells from healthy donors with plasma from patients with COVID-19, thus demonstrating that the source of the transcriptomic phenotype was not cell-intrinsic but could be generated by these soluble factors. Finally, Krämer et al[2] showed that COVID-19 NK cells from patients with severe late-stage disease were impaired in their ability to limit antifibrotic activity against primary human lung fibroblasts, thus linking NK cells to lung pathology.

This study is remarkable in several respects, not least because it represents a concerted and unparalleled effort in longitudinal sample collection, single-cell analyses, data analysis, and analysis of NK cell function. It satisfyingly links subsets identified by previous scRNA-Seq studies of NK cells to subsets that are expanded in COVID-19 and uses these as a foundation to dissect the interplay between IFN-α and TNF signaling pathways associated with moderate or severe disease. This study also uses a large cohort in multiple centers to further validate and build on previous findings that NK cells can have an activated or proliferative phenotype in COVID-19 and certain subsets of $CD56^{dim}$ cells expand in severe disease.[3,4,6,7] This longitudinal analysis delineates the path that COVID-19 infection can take, including how NK cells can contribute to the antiviral response and also be shaped by the course of infection. Given the link between previously defined NK cell subsets and the NK cell subsets described by Krämer et al,[2] it is likely that this interplay between IFN-α and TNF signaling represents a conserved mechanism of NK cell–associated function and dysfunction in disease and provides interesting insight into the functional role of NK cell subsets that are expanded or contracted in disease. Further studies will be necessary to determine which cell types initiate the soluble factors to which NK cells respond in COVID-19 infection and further explore how therapeutic intervention can help restore the antiviral properties of these and other innate effectors. In addition, questions remain about the following issues: the role of other cytokines, including the anti-inflammatory molecule TGF-β, which is also found at higher concentrations in plasma of patients with severe COVID-19[8]; the role of IL-15 in the generation and function of NK cells in COVID-19 disease, given its potential role in fatty acid metabolism and dysfunction of $CD56^{dim}$ $CD16^{high}$ NK cells[9]; and the role of anti–type I interferon-neutralizing antibodies in the disease response.[10] However, this study represents an important step forward in our understanding of NK cell biology and, importantly, our understanding of the immunopathology of the disease that has led to an extraordinary health crisis in our time.

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