Identification and Characterization of an Unconventional NK Subset in COVID-19

Andrew Platt[1,2], Izabella Lach[1], Jeffrey R. Strich[1], Gustaf Wigerblad[3], Ryan Curto[4], Shreya Singireddy[4], Jocelyn Wu[4], Katherine Raja[4], Kapil K. Saharia[5], Mariana Kaplan[3], Daniel S. Chertow[1,2]

1 Critical Care Medicine Department, Clinical Center, National Institutes of Health, Bethesda, MD, USA. **2** Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA. **3** Systemic Autoimmunity Branch, National Institute of Arthritis and Musculoskeletal and Skin Disease, Bethesda, MD, USA. **4** University of Maryland School of Medicine, Baltimore, MD, USA. **5** Institute of Human Virology, University of Maryland, Baltimore, MD, USA.

Introduction

The dysregulation of the **innate immune system** in **Coronavirus Disease 2019 (COVID-19)** results in a wide variability in illness severity and outcome[3].

Natural Killer (NK) cells are a subset of innate immune cells that play a critical role in combating viral infection. In COVID-19, dysregulation in this cell type in has been correlated to greater disease severity[2].

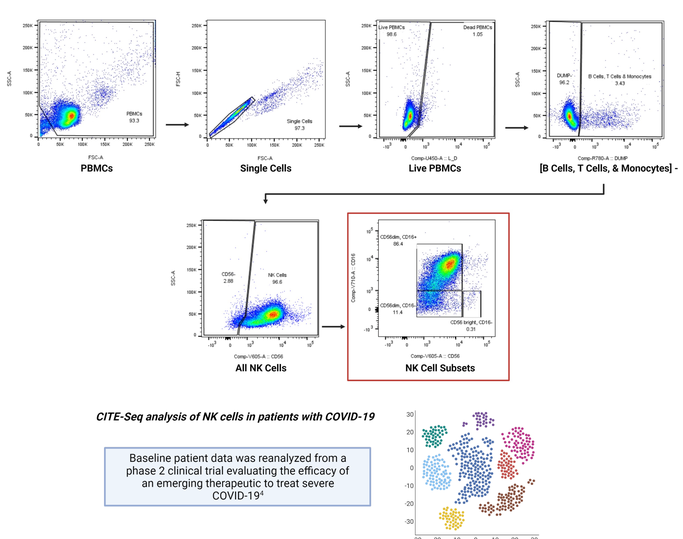
NK cells are divided into two main subsets based on their surface expression of **CD56** and **CD16**: $CD56^{dim}$ $CD16^{+}$ and $CD56^{bright}CD16^{-}$. A third unconventional subset, $CD56^{dim}$ $CD16^{-}$, is expanded in patients with COVID-19[1].

OUR GOALS: Characterize phenotypic changes in the NK cells of patients with severe COVID-19 and describe the functionality of the expanded atypical NK cell subset.

Methods

- PBMCs and plasma collected from patients with COVID-19 on Extra-Corporeal Membrane Oxygenation (ECMO).
- Healthy donor NK cells stimulated with 10% healthy vs COVID-19 plasma *ex vivo*.
  - Cells analyzed by flow cytometry.
- CITE-Seq single cell RNA sequencing data used from previously published dataset of hospitalized patients.

Representative gating strategy for identification of NK cell subsets



CITE-Seq analysis of NK cells in patients with COVID-19

Baseline patient data was reanalyzed from a phase 2 clinical trial evaluating the efficacy of an emerging therapeutic to treat severe COVID-19[4]

Results

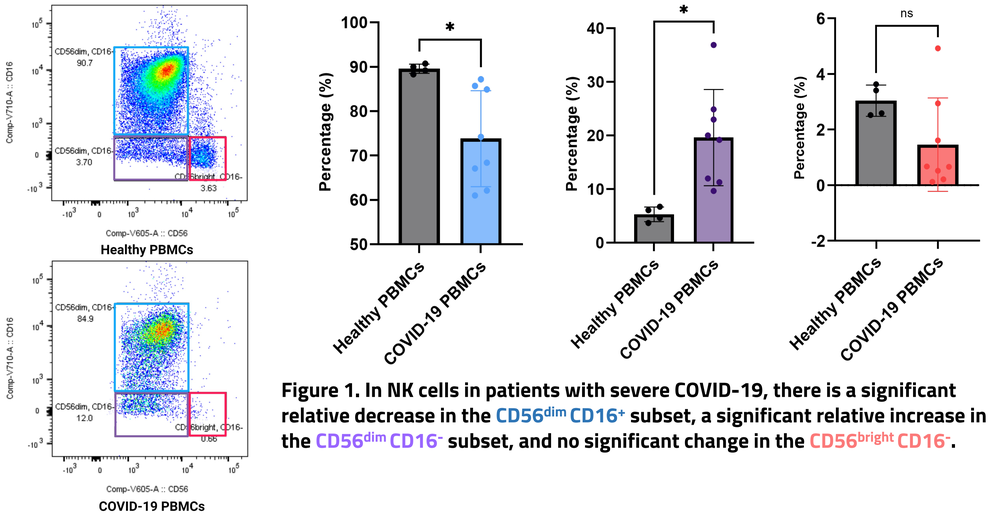


Figure 1. In NK cells in patients with severe COVID-19, there is a significant relative decrease in the $CD56^{dim}$ $CD16^{+}$ subset, a significant relative increase in the $CD56^{dim}$ $CD16^{-}$ subset, and no significant change in the $CD56^{bright}$ $CD16^{-}$.

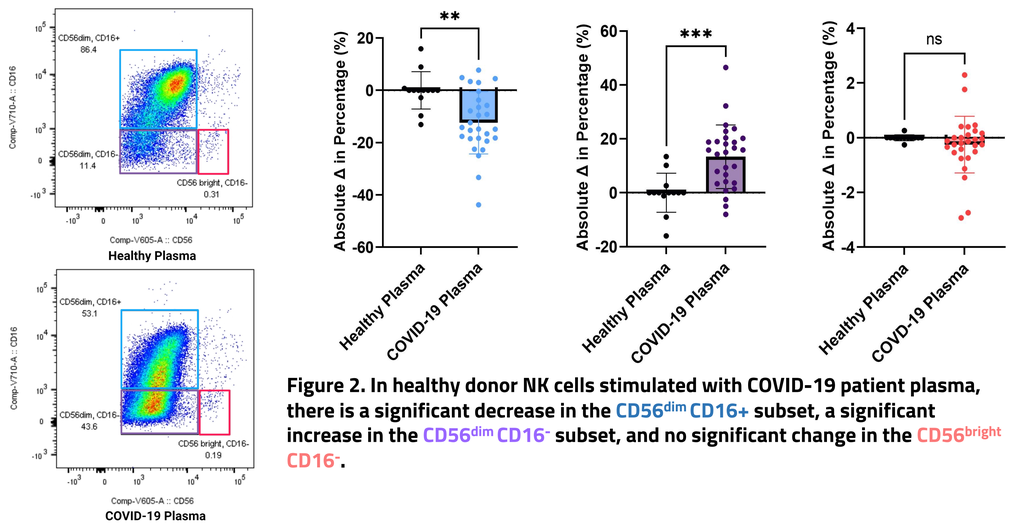


Figure 2. In healthy donor NK cells stimulated with COVID-19 patient plasma, there is a significant decrease in the $CD56^{dim}$ CD16+ subset, a significant increase in the $CD56^{dim}$ $CD16^{-}$ subset, and no significant change in the $CD56^{bright}$ $CD16^{-}$.

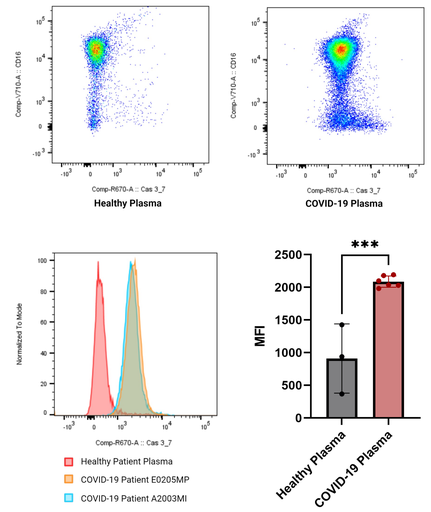


Figure 3. NK cells stimulated with COVID-19 patient plasma have a significantly higher level of Caspase-3/7 activity.

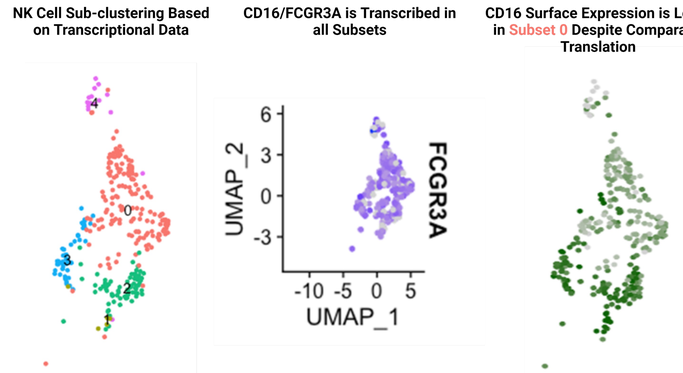


Figure 4. In patients with COVID-19, a subset of NK cells lose surface expression of CD16 despite expression of CD16 at the transcriptional level.

Discussion

- The $CD56^{dim}$ $CD16^{-}$ subset is expanded in patients with severe COVID-19.
  - The phenotype is seen across multiple different patient cohorts using several approaches.
  - This phenotype can be replicated *in vitro.*
- Given the findings of loss of CD16 and increased Caspase-3/7 activation, this suggests a common pathway through phosphatidylserine.

Conclusions

- A more robust characterization of the $CD56^{dim}$ $CD16^{-}$ subset by RNA sequencing is needed.
- Functional assays will be required to define the role of these NK cells
- The kinetics of $CD56^{dim}$ $CD16^{-}$ cells and their role in mild disease remains open.

References

1. Leem, G. et al. Abnormality in the NK-cell population is prolonged in severe COVID-19 patients. *J Allergy Clin Immunol.* 2021. **148**(4): 996-1006.
2. Paludan SR. et al. Innate immunological pathways in COVID-19 pathogenesis. *Sci Immunol.* 2022. **7**(67): eabm5505.
3. Schultze, J. and Aschenbrenner, A. COVID-19 and the human innate immune system. *Cell.* 2021. **184**(7):11671-1692.
4. Strich J et al. Fostamatnib for the Treatment of Hospitalized Adults With Coronavirus Disease 2019: A Randomized Trial. 2021. *Clinical Infectious Diseases.* Ciab732.