SIGLEC-9 RESTRAINS ANTIBODY-DEPENDENT NATURAL KILLER CELL CYTOTOXICITY AGAINST SARS-COV-2

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BACKGROUND

- The host immune response to SARS-CoV-2 infection is critical in disease pathogenesis. Severe Coronavirus disease (COVID-19) has been associated with various alterations to the profiles of immune cells, including natural killer (NK) cells.
- NK cells play a central role in anti-viral immunity through direct cytotoxicity and/or antibody-dependent cell cytotoxicity (ADCC).
- We aimed to understand whether anti-SARS-CoV-2 NK functions are impaired during severe COVID-19 and what host factors modulate these functions.
- We focused on determining the role of Siglecs in modulating the anti-SARS-CoV-2 NK cytotoxicity.

METHODS

A. Peripheral blood mononuclear cells from COVID-19 infected and uninfected were co-cultured with Spike-expressing 293-T cells as a target cells to evaluate direct cytotoxicity.
B. Total IgG isolated from plasma from patients added to the co-culture assay to evaluate the ADCC
C. NK degranulation was evaluated by flow cytometry by expression of CD107a (NK degranulation marker) and cytokine production (IFN-γ, and TNF-α).

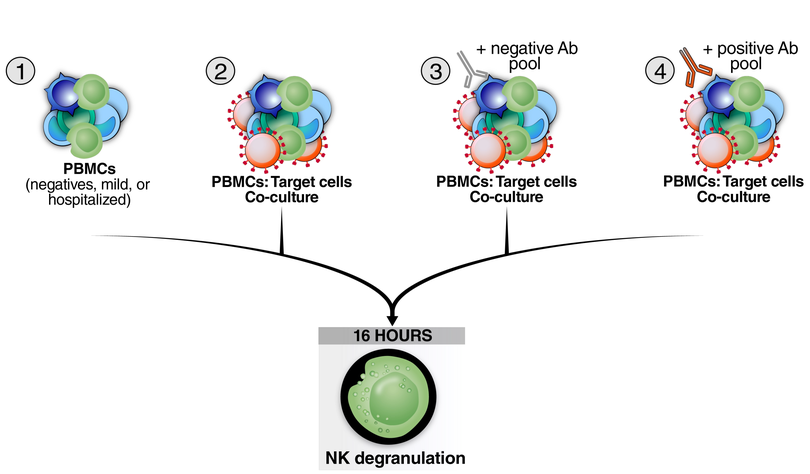


Figure 1 Schematic overview of the experiments to evaluate the direct cytotoxicity and ADCC mediated degranulation of CD56^{dim} NK cells during different severities of COVID-19 infection. To examine direct cytotoxicity-mediated degranulation, PBMCs from each donor, from three COVID-19 status groups (n=8 SARS-CoV-2 negative, n=12 mild COVID-19, and n=21 hospitalized COVID-19) were co-cultured at 10:1 effector-to-target ratio (2) or not (1) with SARS-CoV-2 Spike-expressing 293T target cells. To examine ADCC-mediated degranulation, identical co-cultures were performed in the presence of the negative (3) or positive (4) antibody pool. Direct cytotoxicity was assessed by subtracting (1) from (2). ADCC was assessed by subtracting the results of subtracting (1) from (3) from the results of subtracting (1) from (4).

RESULTS

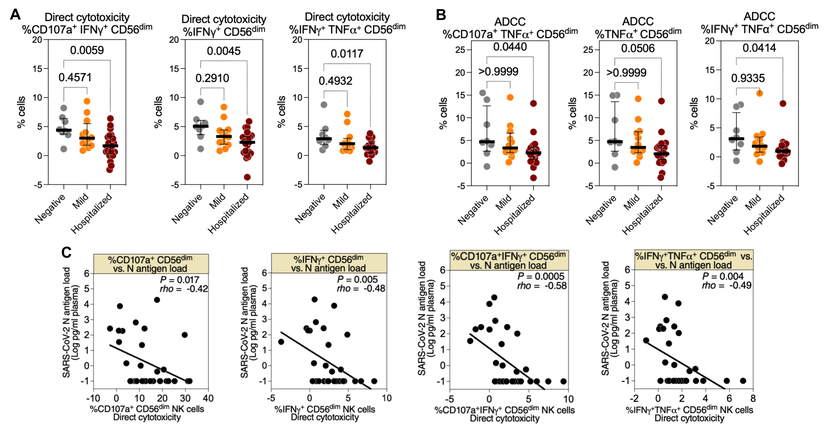


Figure 2. Hospitalized COVID-19 is associated with reduced CD56^{dim} NK cells degranulation against SARS-CoV-2 Spike-expressing target cells. Direct cytotoxicity-mediated degranulation and cytokine production of the CD56^{dim} NK population was assessed as the percentage of **(A)** CD107a$^+$ IFN-γ$^+$, IFN-γ$^+$, and IFN-γ$^+$ TNF-α$^+$ cells. **(B)** ADCC-mediated degranulation and cytokine production of the CD56^{dim} NK population was assessed as the percentage of CD107a$^+$ TNF-α$^+$, TNF-α$^+$, and IFN-γ$^+$ TNF-α$^+$ cells. **(C)** Spearman's rank-order correlations between plasma N-antigen load and the percentage of CD56^{dim} NK cells expressing CD107a$^+$, CD107a$^+$ IFN-γ$^+$, and IFN-γ$^+$ TNF-α$^+$ during direct cytotoxicity assays; only samples from COVID-19 positive donors were used (n=33).

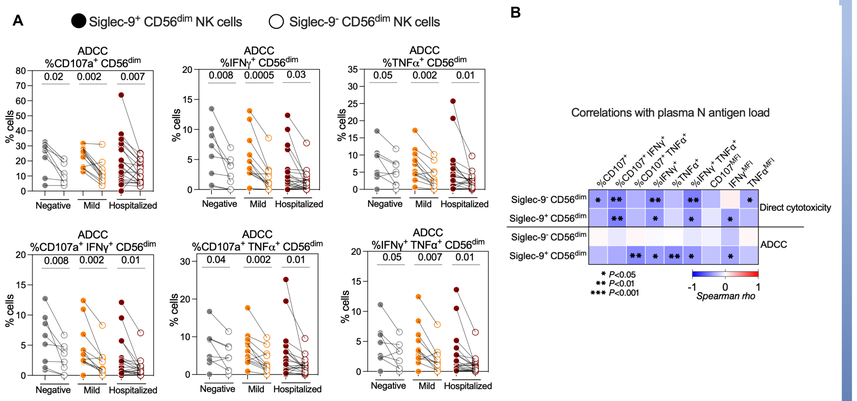


Figure 3 The Siglec-9$^+$ CD56^{dim} NK sub-population exhibits a higher SARS-CoV-2 specific ADCC than does the Siglec-9$^-$ CD56^{dim} NK sub-population. ADCC-mediated degranulation and\or cytokine production of the Siglec-9$^+$ and Siglec-9$^-$ CD56^{dim} NK cells within each COVID-19 status group as assessed by the percentage of cells expressing **(A)** CD107a, IFN-γ, TNF-α, CD107a and IFN-γ, CD107a and TNF-α, and IFN-γ and TNF-α. **(B)** Wilcoxon's signed-rank tests were used to compare the Siglec-9$^+$ and Siglec-9$^-$ CD56^{dim} NK cells within each COVID-19

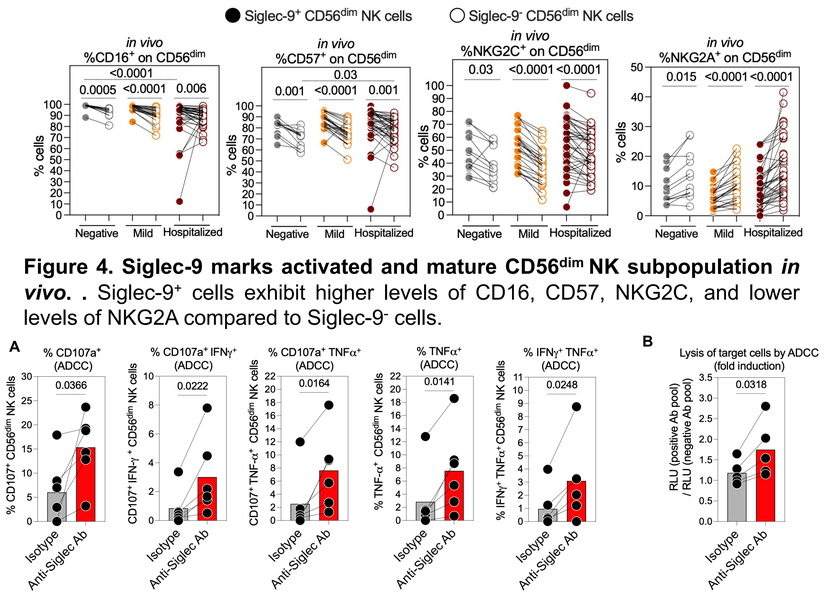
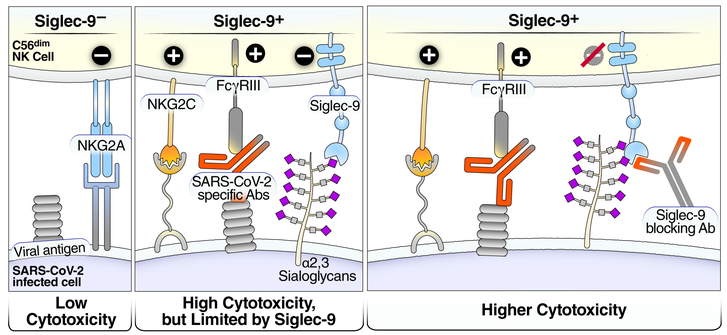


Figure 4. Siglec-9 marks activated and mature CD56^{dim} NK subpopulation *in vivo*. . Siglec-9$^+$ cells exhibit higher levels of CD16, CD57, NKG2C, and lower levels of NKG2A compared to Siglec-9$^-$ cells.

Figure 5. Blocking Siglec-9 interactions, using a Siglec-9 blocking antibody, enhances the anti-SARS-CoV-2 ADCC of CD56^{dim} NK cells. (A) The impact of the Siglec-9 blocking antibody, compared to an isotype control, on the ADCC-mediated NK degranulation and\or cytokine production against SARS-CoV-2. **(B)** The impact of the Siglec-9 blocking antibody, compared to an isotype control, on the ADCC-mediated lysis of SARS-CoV-2 target cells.

CONCLUSIONS



- Hospitalized COVID-19 is associated with dysfunctional NK cells with low expression of CD16
- Inhibitory Siglec-9 define NK subpopulations that are highly cytotoxic against SARS-CoV-2 target, blocking Siglec-9 by antibody elicits higher ADCC by this restrained population.

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