#0475

Long COVID is Associated with Lower Percentages of Mature, Cytotoxic NK Cell Phenotypes

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Introduction

Long COVID (LC), defined as ongoing symptoms present at least 3 months after infection that interfere with everyday functions, develops in approximately 5% of individuals after SARS-CoV-2 infection. There is growing evidence that tissue persistence of SARS-CoV-2 is associated with LC and that people with LC have dysregulated adaptive immune function that may originate from or potentiate viral persistence.[1] Natural killer (NK) cells play a critical role in clearing viral infections but can exhibit dysfunctional phenotypes during acute COVID-19 that may hinder viral clearance.[2] Acute COVID-19 infection can also induce adaptive NK cell responses, but there is a critical lack of knowledge about NK cell phenotypes in the post-acute phase and their relationship to LC.

Methods

We performed high-dimensional spectral flow cytometry NK cell phenotyping on PBMCs collected 4 months post SARS-CoV-2 infection from people with LC (n = 21) and those who had prior COVID-19 but fully recovered (n = 14) in the UCSF Long-term Impact of Infection with Novel Coronavirus (LIINC) cohort. Samples were collected in the first year of the pandemic prior to vaccination, antiviral treatment, or frequent reinfection. LC was defined as the presence of COVID-attributed symptoms for at least 3 months following initial infection. Severe LC was defined as the presence of more than 5 symptoms. All participants were confirmed to have consistent presence or absence of LC symptoms for up to 8 months.

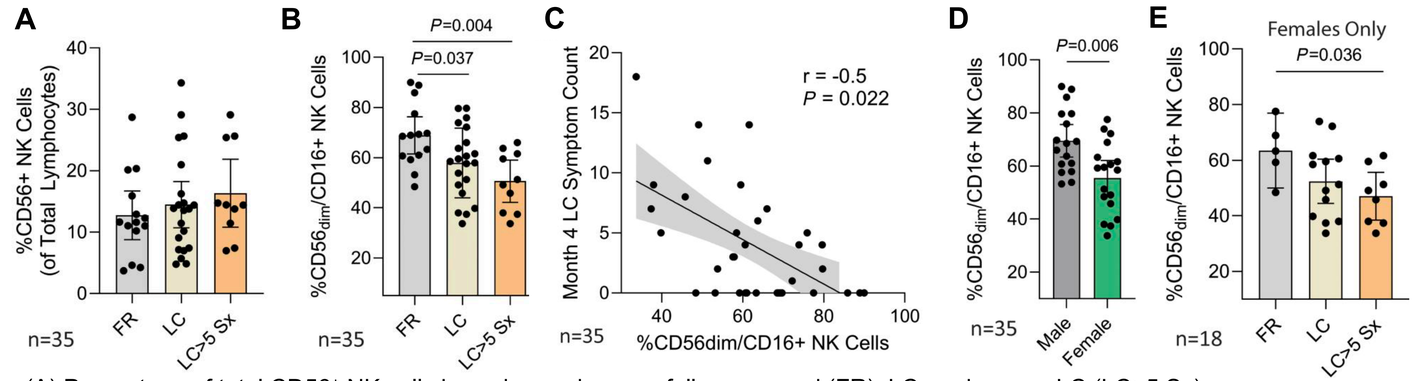
We examined associations between NK cell phenotypes, demographic and LC clinical factors, and SARS-CoV-2 antigen presence.

Cohort Demographic and Clinical Data

	Long COVID	Recovered
Sample Size	N = 21	N = 14
Age in Years [median (range)]	46 (19-71)	49 (31-71)
Female birth sex [n (%)]	13 (61.9%)	5 (35.7%)
Race/Ethnicity		
White, Not Hispanic/Latino	10/20 (50%)	9 (64.3%)
Hispanic/Latino	8/20 (40%)	2 (14.3%)
Black/African American	1/20 (5%)	1 (7.1%)
Asian	1/20 (5%)	2 (14.3%)
Hospitalized During Acute COVID-19	7 (33.3%)	2 (14.3%)
CMV IgG+	10 (47.6%)	8 (57.1%)
EBV EAD IgG+	7 (33.3%)	6 (42.9%)
Detectable SARS-CoV-2 S or N Antigen[1]	8 (38.1%)	4/13 (30.8%)
Median Long COVID Symptom Count at Month 4 (IQR)	5 (4-9)	N/A
Median Days from Acute COVID-19 to Month 4 Sample Collection (IQR)	129 (122-135)	118 (112-124)

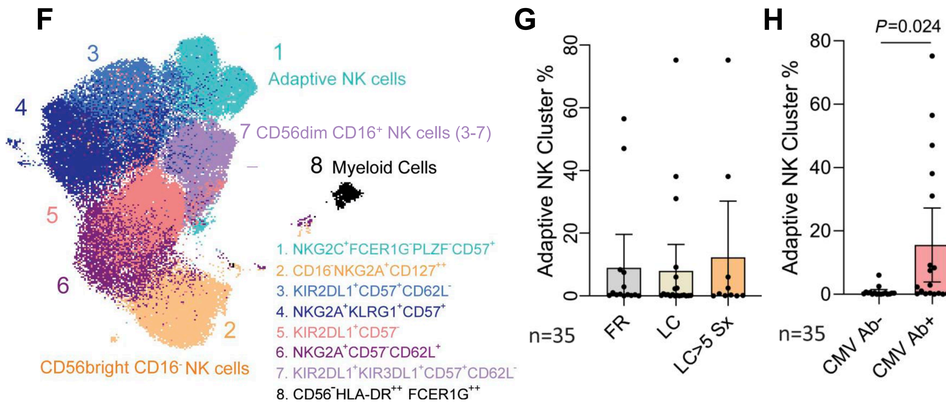
IQR=interquartile range, CMV=Cytomegalovirus, EBV EAD=Epstein Barr Virus Early Antigen D
[1] Detected anytime following acute COVID-10 (S=spike, N=nucleocapsid)

Mature cytotoxic NK cells are lower in periphery of people with long COVID



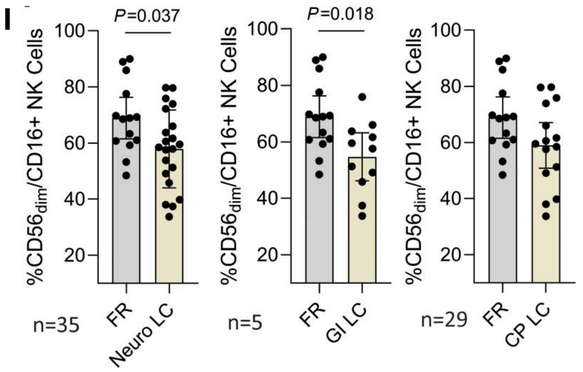
(A) Percentage of total CD56⁺ NK cells is unchanged across fully recovered (FR), LC, and severe LC (LC>5 Sx) groups.
(B) Percentage of mature cytotoxic NKs (CD56^{dim}CD16⁺) are lower in LC compared to FR, and even lower in severe LC.
(C) There is a negative correlation between number of LC symptoms at month 4 and percentage of cytotoxic NK cells.
(D) Female individuals in the cohort express lower frequencies of cytotoxic NK cells.
(E) The lower percentage of cytotoxic NK cells in LC compared to FR trend stays the same when looking at only female individuals.

No association between adaptive NK cells and LC phenotypes



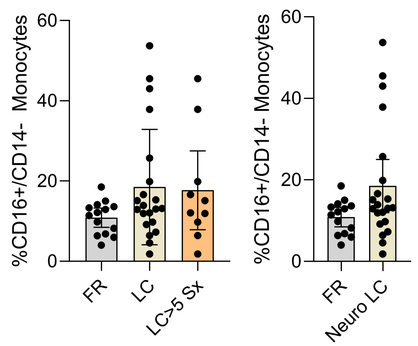
(F) Unsupervised clustering of NK cells based on differentially expressed markers
(G) No difference in adaptive NK cell frequency between conditions.
(H) Higher frequency of adaptive NK cells in individuals that are CMV seropositive.

Cytotoxic NK cells in individuals with tissue-based LC symptoms



(I) Lower mature cytotoxic NK cells in individuals experiencing neurological and gastrointestinal (GI) LC symptoms, but not cardiopulmonary (CP)

Trend towards increased viral-sensing monocytes in LC



Conclusions

- Cytotoxic NKs are reduced in periphery of people with LC.
- No association between adaptive NK cell frequency and LC.
- Cytotoxic NKs reduced in people experiencing neurological and GI LC symptoms.
- Viral-sensing monocytes trend towards an increase in people with LC.

NK cells mediate cytotoxicity through multiple methods, including antibody-dependent cellular cytotoxicity (ADCC) induced by CD16 binding to antibodies. It is possible that reduced cytotoxicity during initial tissue infection or late clearance of persistently infected cells may be related to the development of LC. Alternatively, these data could suggest redistribution of mature, cytotoxic NK cell subsets to tissues where antigen persists.

References:
1. Maucourant C, et al. Natural killer cell immunotypes related to COVID-19 disease severity. *Sci Immunol*. 2020;5(50):eabd6832.
2. Yin K, et al. Long COVID manifests with T cell dysregulation, inflammation and an uncoordinated adaptive immune response to SARS-CoV-2. *Nat Immunol*. 2024;25(2):218–225.

Funding: PolyBio Foundation, K24AI174971 and R01AI141003 (TJH), K23AI157875 and 1R01NS136197 (MJP), T32GM136547 and DGE2445150 (TT).