Age-Dichotomous Associations of Long COVID With Frequency of Public SARS-CoV-2 Memory TCRs

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BACKGROUND

- Approximately 18% of adults in the United States have experienced long COVID (LC).
- While the etiology of long COVID is likely multifactorial, we hypothesize that persistent SARS-CoV-2 or EBV viral antigen may drive increased immune activation and may serve as key underlying mechanisms.

OBJECTIVES

- To determine whether people with LC have increased levels of activated T cells and increased percentages of circulating memory CD8+ and CD4+ T cells specific for SARS-CoV-2 and EBV within the first six months post-SARS-CoV-2 infection compared to people who experienced rapid and complete recovery.

METHODS

- Cryopreserved PBMCs from 1 and 3-6 months post-first SARS-CoV-2 infection were obtained from people who went on to develop LC (n=18) and people who experienced full and rapid recovery (n=17, FR) from two cohorts.
- Flow cytometry of PBMCs was performed with a panel focused on T cells and monocytes.
- Memory CD4+ and memory CD8+ T cells were flow sorted. DNA was isolated and TCRβ chain repertoires were sequenced (n=138 repertoires, AmpliSeq for Illumina TCRβ-SR). TCRs with 5 or fewer templates were excluded as likely PCR or sequencing errors, and all repertoires were resampled to the same total template number.
- Public human SARS-CoV-2, EBV, CMV, and Influenza A-specific CD8+ and CD4+ TCRβ amino acid sequences were extracted from VDJdb (all pathogens, confidence scores 2-3) and from ImmuneCODE-MIRA-Release002.1 (SARS-CoV-2 only) without regard for HLA type matching. TCRβ sequences that appeared in more than one pathogen's dataset were removed.

- People over age 50 with long COVID had fewer public SARS-CoV-2-specific TCRs in their memory T cell repertoires.
- People with full recovery had higher frequencies of public SARS-CoV-2-specific TCRs in their memory CD4+ T cell repertoires at 4-6-months post-infection.

Table 1. Participant characteristics.

	Full Recovery n=17	Long COVID n=18
Age (y), median (IQR)	51 (43-57)	47 (36-57)
Male sex at birth, %	47.1	38.9
Hispanic ethnicity, %	5.9	5.6
Black/African American race, %	11.8	27.8
White race, %	70.6	55.6
Asian race, %	5.9	11.1
American Indian or Alaska Native, %	5.9	0
Multiracial or Not Reported, %	5.9	5.6
BMI, median (IQR)	30.4 (25-34)	28.6 (21.4-35.7)
Hypertension, %	29.4	22.2
COPD/emphysema, %	0	0
Diabetes, %	11.8	11.1
1+ vaccination prior to COVID, %	41.2	22.2

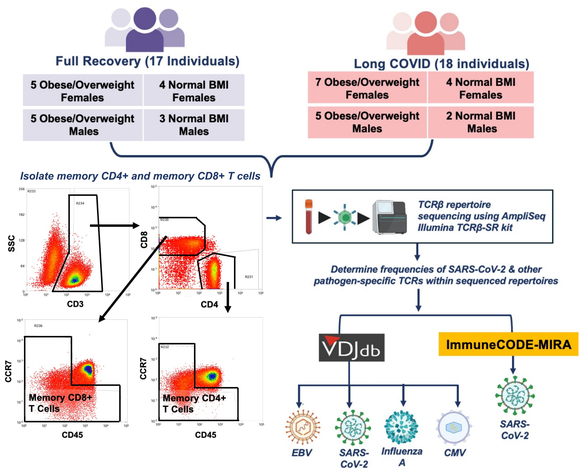


Fig. 1. Experimental outline.

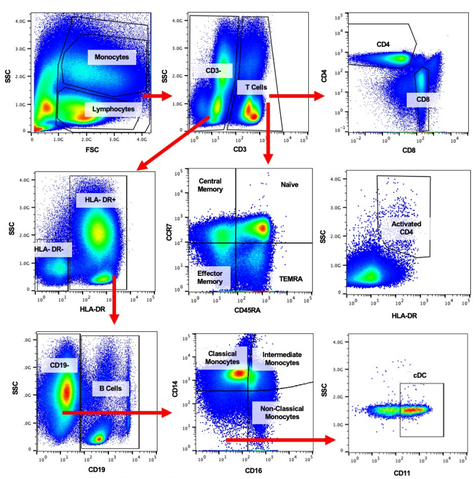


Fig. 2. Flow cytometry gating strategy.

RESULTS

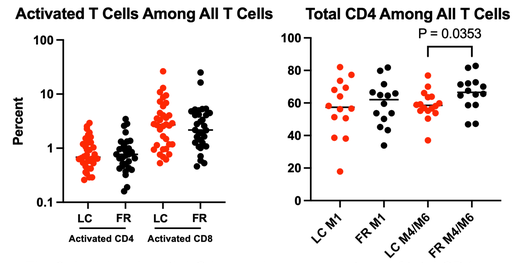


Fig. 3. Individuals with LC have a lower percentage of total CD4s among all T cells compared to fully recovered (FR) individuals at 4-6 months post-infection. The percentage of activated, naïve, TCM, TEM/TTM, TEMRAs, and PD-1 positive memory CD4+ and CD8+ T cells, B cells, dendritic cells, and classical, intermediate, and non-classical monocytes do not differ.

ImmuneCODE-MIRA-Release002.1

Proportion of public SARS-CoV-2-specific TCRβ sequences from ImmuneCODE-MIRA-Release002.1 that are present in the memory TCRβ repertoires

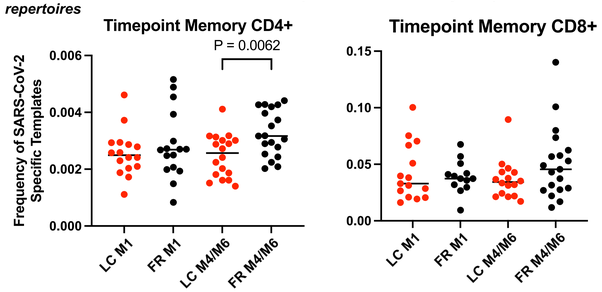


Fig. 4. Individuals at 4-6 months post-infection with LC demonstrated lower frequencies of public SARS-CoV-2-specifc TCRs in memory CD4+ TCRβ repertoires compared to fully recovered individuals.

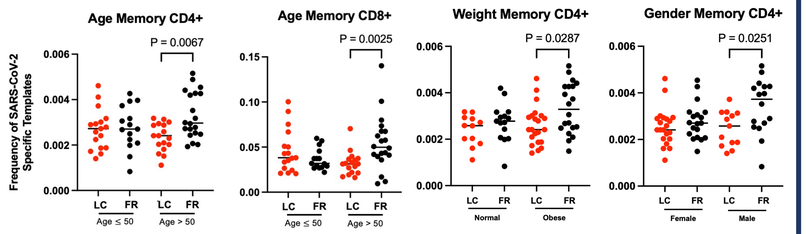


Fig. 5. Older (> 50) individuals with LC have lower frequencies of public SARS-CoV-2-specific TCRs in both memory CD4+ and memory CD8+ TCRβ repertoires compared to older individuals that recovered, with no differences observed between LC and fully recovered young (≤ 50) individuals. Overweight/obese individuals and males with LC demonstrated lower frequencies of public SARS-CoV-2-specific TCRs in memory CD4+ TCRβ repertoires compared to fully recovered individuals.

VDJdb

Proportion of public SARS-CoV-2 and EBV specific TCRβ sequences from VDJdb that are present in the memory TCRβ repertoires

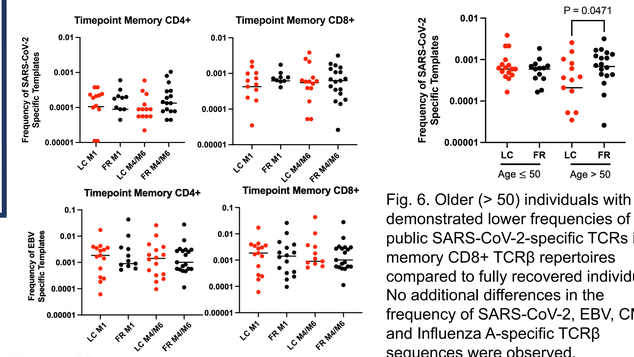
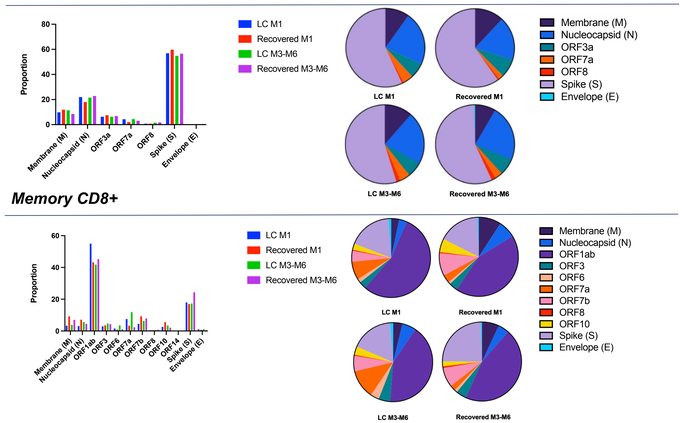


Fig. 6. Older (> 50) individuals with LC demonstrated lower frequencies of public SARS-CoV-2-specific TCRs in memory CD8+ TCRβ repertoires compared to fully recovered individuals. No additional differences in the frequency of SARS-CoV-2, EBV, CMV and Influenza A-specific TCRβ sequences were observed.

Memory CD4+



Memory CD8+

Fig. 7. The number of ORF/protein responding templates was summed for each repertoire to compare the distribution of cognate antigen location within the SARS-CoV-2 genome of public SARS-CoV-2-specific TCRs (ImmuneCODE-MIRA and VDJdb) identified within respective repertoires. (Left) The proportion of responding templates for each ORF/protein. The percentage of TCRs responding to spike did not differ between individuals with LC and full recovery. (Right) The overall distribution of cognate antigen location.

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