Elevated Frequencies of SARS-CoV-2 Specific T Cells in Patients with Post Acute COVID19

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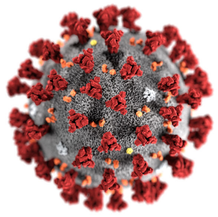

Abstract

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection rates have decreased globally since the start of 2021, in part due to novel vaccines, but as many as 50% - 80% of individuals with resolved infection report persistent symptoms lasting weeks to months. SARS-CoV-2 infection is typically controlled by the immune system in 2-3 weeks and detection of replication-competent virus via nasal pharyngeal swab is rare after this period. Symptoms lasting longer than 4 weeks post-symptom onset, or hospital discharge, qualify as post-acute COVID-19 (PAC), which is thought to have multifactorial etiology driven primarily by immune dysregulation and inflammatory cytokine production. Because it has been shown that individuals infected with SARS-CoV-2 mount a strong inflammatory T cell response against the virus, we examined SARS-CoV-2-specific T cells in 15 subjects with PAC and 15 controls (AC) that had didn't have any symptoms after clearing the virus to determine if virus-specific T cells play a role in driving persistent inflammation and PAC. Peripheral blood mononuclear cells were collected, isolated by gradient centrifugation, and stimulated with Spike (S), Nucleocapsid (N), Membrane (M) and Envelope (E) SARS-CoV-2 peptide pools to determine the frequency, cytokine producing capacity and maturation / exhaustion marker state of SARS-CoV-2 specific-T cells using flow cytometry. Individuals with PAC had decreased frequencies of total naïve T cells, and increased frequencies of total effector memory cells CD8+ T cells and increased frequencies of total central memory CD4+ T cells compared to AC subjects. The combined response to all peptide pools demonstrated that subjects with PAC had significantly elevated frequencies SARS-CoV-2 specific CD8+ T cells producing TNFα and IFNγ compared to controls (P=0.026 and P=0.0003). While CD4+ T cell frequencies to the combined peptide only trend higher in the PAC subject, frequencies of TNFα producing CD4+ T cells directed against N and S peptide pools were significantly elevated compared to controls (P=0.007 and P=0.004). Within the PAC group, 60% of individuals had a CD8$^+$ T cell response to 3 or more peptides while the same response was only seen in 22% of AC subjects. These results suggest that SARS-CoV-2-specific T cells persist in patients with PAC and could in part contribute to the development and maintenance of post-acute COVID syndrome.

Background

- SARS-CoV-2 was first seen in 2019 and as of 4/9/2021 has infected 133,146,550 people worldwide causing 2,888,530 deaths[1].
- While the majority of people recover for SARS-CoV-2, 50%[2] - 80%[3] with resolved infection have residual symptoms termed long covid or PAC which is defined by a broad class of symptoms ranging from chronic headaches, anosmia, dyspnea and fatigue to sequelae across multiple organ systems that persist 4 weeks or longer post infection.
- PAC more commonly occurs in females, those with increased age or BMI, or in individuals reporting 5+ symptoms within a week of onset of primary infection[4].
- Previous studies demonstrate 100% of individuals infected with SARS-CoV-2 had T cell responses to structural peptides, including the nucleocapsid peptide[5].
- A study of long-term immune responses found SARS-CoV-2 specific T cells have a half life of 3-5 months in those that clear the virus normally[6].
- Proposed mechanisms behind PAC include autonomic dysfunction, organ system damage during acute infection, and prolonged inflammation.

Hypothesis

Inflammatory cytokines produced by SARS-CoV-2 specific T cells contribute to the development and maintenance of PAC.

Patient Recruitment and Methods

- Participants were recruited from the University of Colorado Hospital and around the Aurora/Denver area under the approved COMIRB protocol # 20-1219. All participants were PCR positive. COVID-19 symptoms were self reported and individuals with two or more symptoms that persisted for at least four weeks after onset or hospitalization – depending on severity – were classified as post-acute COVID (PAC) and individuals reporting symptoms for less than four weeks were classified as acute COVID (AC). Clinical characteristics are summarized in Table 1.
- Blood was collected and PBMC (Peripheral Blood Mononuclear Cells) were isolated by gravity centrifugation.
- PBMC was stimulated with peptide pools of the spike (S), nucleocapsid (N), membrane (M) and envelope (E) proteins of the USA-WA1/2020 strain of SARS-CoV-2. T cell activation, memory markers and cytokine production were enumerated by flow cytometry.
- Statistical significance was determined using Mann-Whitney T tests.

Table 1: Clinical characteristics.

	PAC	AC
Number of participants	15	15
Participants with preexisting conditions*	10 - 75%	4 - 27%
Age (year)	53 (22-64)	34 (24-71)
Female	53%	40%
Male	47%	60%
Number of symptoms	9 (2-17)	6 (0-15)
Symptom duration (days)**	225 (72-317)	14 (0-42)
Time from symptom onset to Visit 1 (days)	196 (41-571)	29 (10-241)

Information provided by patients and obtained from the EMR including medical history, demographics and COVID symptoms and testing. *Participants who reported one or more of the following: hypertension, cancer, cardiac, pulmonary, kidney, immune system, metabolic or hepatic disease. **Duration was calculated from onset for mild cases and from hospital discharge for severe cases.

Results

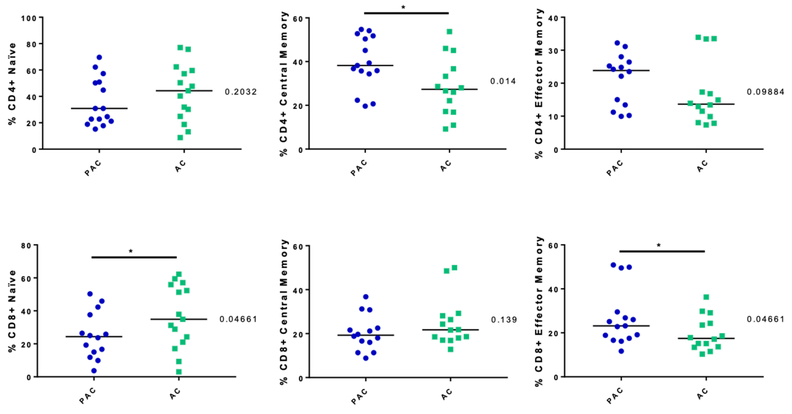

Fig 2. PAC subjects had decreased naïve and increased effector memory T cells compared with controls. PBMC were stained with monoclonal antibodies to determine the expression of CD45RA and CD27 to classify the maturation state. Naïve are defined as CD27$^+$CD45RA$^+$, central memory are CD27$^+$CD45RA$^-$, and effector memory CD27$^-$CD45RA$^-$. Terminally differentiated effector memory cells were also compared and not found to be significantly different between PAC and AC groups.

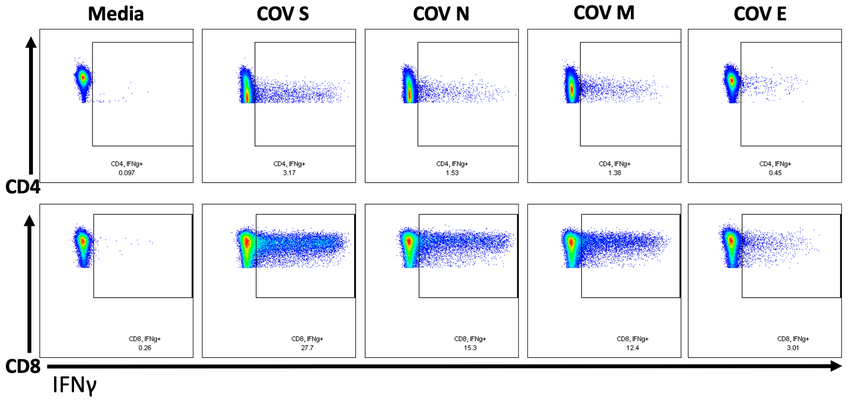

Fig 3. Representative flow cytometry dot plots of SARS-CoV-2-specific T cell responses. PBMC from subjects with PAC were stimulated with SARS-CoV-2 S, N, M and E 15mer peptide pools for 6 hours in the presence of GolgiPlug after 2 hours. The cells were stained with monoclonal antibodies against surface markers (CD3, CD4, CD8, PD-1, CD27, CD45RA), then they were fixed, permeabilized and intracellularly stained with IFNγ, TNFα, IL-2 and Ki67. The stained samples were acquired on a BD LSR II and analyzed using FlowJo. Cytokine positive populations were determined by expression in an unstimulated control.

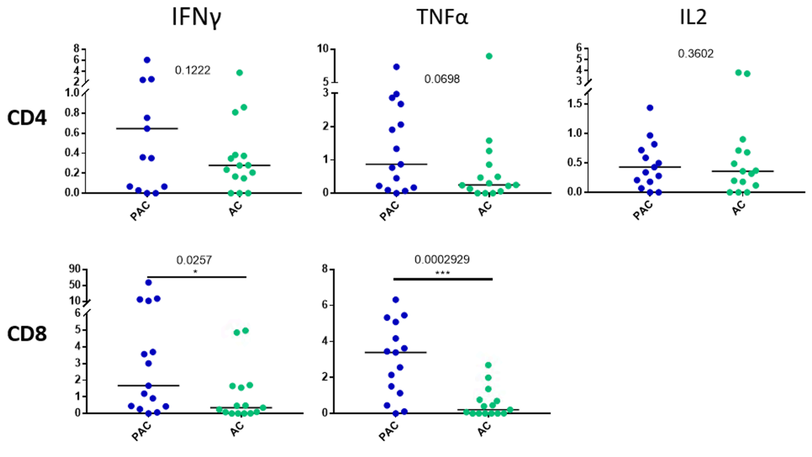

Fig 4. TNF-α and IFN-γ producing SARS-CoV-2 specific T cells against all peptide pools were significantly higher in PAC than controls. Each dot represents one patient's sum of percent IFNγ, IL-2 or TNFα on CD4+ and CD8+ T cells from PAC (Blue) or AC (teal) over background. Combined IFNγ or TNFα virus-specific CD8+ T cells against all peptide pools were significantly higher in PAC individuals compared to acute controls (AC). A similar trend was also seen in the virus-specific CD4+ T cell compartment. There was no significant difference in IL-2 producing virus-specific T cells between PAC and AC.

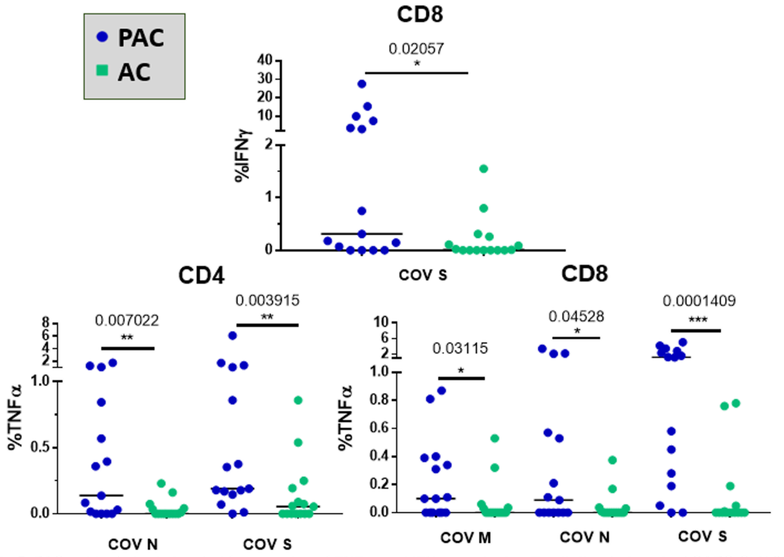

Fig 5. PAC subjects had elevated frequencies of TNF-α and IFN-γ producing T cell directed against SARS-CoV-2 S, N and M peptide pools compared with controls. While some of the strongest differences between those with PAC and AC are seen in response to the S peptide, in PAC specific T cells respond to the N and M peptide pools as well.

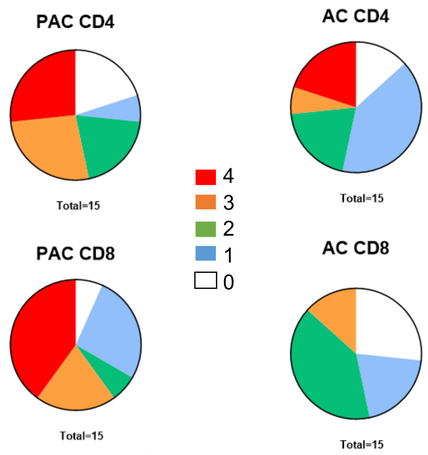

Fig 6. PAC specific T cells respond to more SARS-CoV-2 epitopes than AC patients. In these pie charts we compare the number of peptide pools each subject responds to by defining cytokine production of 0.1% or more over background as having a specific T cell response. In CD4 and CD8 specific T cells 53% and 60% respectively of those with PAC responded to three or more SARS-CoV-2 peptides and only 27% and 20% responded in those with AC.

Conclusions

- Total naïve T cells were decreased, while effector memory T cell were increased in PAC compared to AC subjects.
- Combined responses to all SARS-CoV-2 peptide pool were elevated in PAC compared to control subjects indicating the maintained T cell responses to the virus longer than those that cleared the virus normally.
- PAC subjects responded to a greater number of SARS-CoV-2 peptide pools that controls indicating that they maintain a broader virus-specific response than controls.

Future Directions

- Determine if elevated SARS-CoV-2 specific T cells in PAC subject are responding to persistent virus in distal organs (i.e. gut) and do they contribute to the development and maintenance of PAC.
- Examine inflammatory cytokine production by innate immune cell populations in the blood.
- Measure plasma CRP and D-Dimer to determine if systemic inflammation and SARS-CoV-2 specific T cells correlate.

References

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