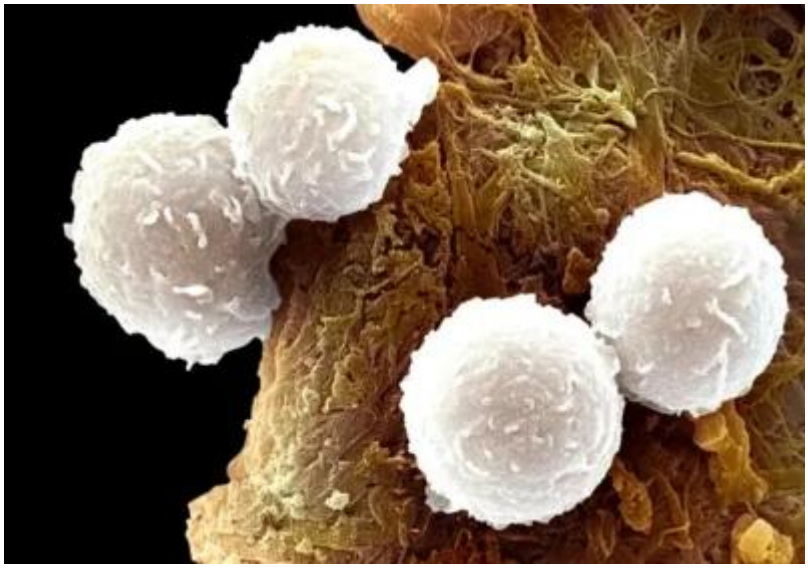
Post-acute COVID (PASC) or long-COVID syndrome is more common in hospitalization survivors, but even those who have experienced mild acute COVID-19 have a wide range of persistent and disabling symptoms. The most frequent symptoms of long COVID are neurological. Despite extensive mapping of the spectrum of neurological sequelae, no significant progress has been made in comprehending the underlying mechanisms. In this study, Australian authors investigated immunological signatures in patients diagnosed with neurological manifestations of post-acute COVID syndrome (neuro-PASC), especially the immune responses specific to the SARS-CoV-2 nucleocapsid (N) protein.

SARS-CoV-2 is an enveloped, positive-sense, single-stranded RNA virus. Its genome encodes four structural proteins, namely the spike (S), envelope (E), nucleocapsid (N), and membrane (M) proteins. Much attention has been focused on the S protein, which appears to be a major factor in the unique pathogenesis of SARS-CoV-2. However, many other SARS-CoV-2 proteins play equally critical roles in the viral life cycle. SARS-CoV-2 N protein is the basis of viral RNA genome packaging, but it is also the most abundant protein in virions and a highly immunogenic antigen.

Severe COVID-19 is associated with T-cell abnormalities and dysfunctions that range from excessive activation and exhaustion to defective activation and differentiation abnormalities. CD8+T cells appear preferentially dysregulated in severe COVID-19 compared to CD4+T cells. However, a recent study has discovered that SARS-CoV-2 infects human CD4+ T helper cells, but not CD8+ T cells, and that SARS-CoV-2  S protein binds to the CD4 molecule directly, which in turn mediates the entry of the virus into T-helper cells. According to researchers, CD4 stabilizes SARS-CoV-2 on the cell membrane until the virus encounters the host angiotensin-converting enzyme 2 (ACE2) to enter the cell.  The authors also argued that, from an evolutionary perspective, infection of CD4+ T helper cells by SARS-CoV-2 is an effective mechanism for viruses to evade the immune response. (Brunetti NS et al. SARS-CoV-2 uses CD4 to infect T helper lymphocytes. *eLife 2023;* 12:e84790.) https://elifesciences.org/articles/84790



***About the Study and Results***

The study included three groups of participants: 77 patients diagnosed with neuro-PASC, 44 healthy COVID convalescents, and 34 healthy individuals not infected with SARS-CoV-2. Patients with neuro-PASC had positive polymerase chain reaction (PCR) tests or seropositive IgG results for SARS-CoV-2. The authors focused on patients who developed neuro-PASC syndrome after a mild acute COVID-19 and were not hospitalized. They have experienced persistent neurological symptoms, such as headaches, fatigue, brain fog, and myalgia, for more than six weeks after the acute COVID-19. The group of healthy COVID convalescents had positive PCR tests or seropositive IgG results for SARS-CoV-2 before the vaccination. Still, they did not experience neurological symptoms for more than four weeks after acute COVID-19. Healthy controls had negative PCR tests or seronegative IgG results for SARS-CoV-2 before the vaccination.

30 subjects across all three groups were vaccinated with the Pfizer BNT162B2 or Moderna mRNA-1273 vaccines.

Cognitive deficits in patients diagnosed with neuro-PASC were evaluated by the second version of the National Institutes of Health Toolbox test, which measures processing speed, attention, executive functions, and working memory. The PROMIS-57 questionnaire was used to evaluate quality of life. The results of cognitive function assessment showed lower scores in the attention domain and higher scores in anxiety, depression, fatigue, sleep disturbance, and pain domains in patients with neurological manifestations of PASC

syndrome than in healthy COVID convalescents and healthy controls.

The results further demonstrated distinct immunological signatures in individuals diagnosed with neuro-PASC compared to the healthy convalescents and healthy controls. Patients diagnosed with neuro-PASC had a higher titer of IgG antibodies specific for SARS-CoV-2 N protein than healthy COVID convalescents and healthy controls.

Additionally, patients diagnosed with neuro-PASC had a higher percentage of CD8+ terminal effector memory (TEMRA) T cells than the control groups. However, the subsets of CD8+ T cells, such as CD8+ effector memory (TEM), and CD8+ TEMRA cells from neuro-PASC patients exhibited reduced functionality and were less activated by the SARS-CoV-2 N antigen than those from COVID convalescents. Although CD8+ memory T cells showed reduced activation in response to the SARS-CoV-2 N protein, they exhibited enhanced interleukin (IL)-6 production.

According to the authors, functionally anergic CD8+ TEMRA cells observed in patients diagnosed with neuro-PASC might contribute to the pathogenesis of this syndrome.

In contrast, CD4+T cells from neuro-PASC patients were more activated by the SARS-CoV-2 N antigen and produced more tumor necrosis factor-alpha than those from healthy COVID convalescents.

IL-6 production by CD8+T cells correlated with the severity of neurologic symptoms, including pain, whereas a reduced functionality of CD8+ TEM cells correlated with high depression scores. Increased levels of proteins associated with immunoregulatory pathways correlated with symptom severity and cognitive scores.

*Conclusion*

This study has shown different immunological signatures and immune responses specific to the SARS-CoV-2 N protein in patients with neurological manifestations of post-acute COVID syndrome (neuro-PASC) compared with healthy COVID convalescents and healthy controls. Although individuals with neuro-PASC had a higher percentage of CD8+ TEMRA cells than control groups, their CD8+ TEM and CD8+ TEMRA cells exhibited reduced functionality and were less activated by the SARS-CoV-2 N antigen than those from healthy COVID convalescents.

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