The authors from the United States conducted a cross-sectional study using multidimensional immunophenotyping and machine learning method to identify biological features associated with long COVID more than one year after the acute infection. The results suggest that persistence of the severe acute respiratory syndrome coronavirus type-2 (SARS-CoV-2) antigens, the reactivation of latent herpesviruses and chronic inflammation may contribute to this syndrome.

More than two years after the global COVID-19 pandemic, it is evident that infection with the SARS-CoV-2 can result in a novel disease known as long-COVID-19 or post-acute COVID-19 syndrome (PACS). Despite the fact that this disease is more prevalent among hospitalization survivors, even those who have experienced mild acute COVID-19 have a wide range of organ dysfunction and clinical symptoms. These symptoms and clinical signs include fatigue, muscle weakness, dyspnea, cough, headache, hypoxia, memory impairment, loss of taste and smell, anxiety, depression, and dysautonomia.

These sequelae significantly impair physical and cognitive functions, and reduce the quality of life. Prospective studies indicate that one out of eight individuals with COVID-19 have persistent somatic symptoms that are the result of SARS-CoV-2 infection. The underlying pathogenesis of long COVID remains unclear, however, current hypotheses include the persistence of virus or viral remnants in tissues, a development, or aggravation of autoimmunity, microbial dysbiosis, the reactivation of non-SARS-CoV-2 latent viral infection, and tissue damage resulting from chronic inflammation.

About the study

The present cross-sectional study included 268 participants, divided into five different groups: healthcare workers infected with SARS-CoV-2 before vaccination, patients with long COVID, group of patients with long COVID from an independent study, healthy, uninfected, vaccinated controls, and group of convalescent vaccinated controls without symptoms of long COVID. 76% of participants with long COVID were infected in 2020, when the majority of new cases were caused by parental SARS-CoV-2 strain (WA-1).

In the long COVID group, the most common symptoms were fatigue (87%), brain fog (78%), memory disorders (62%) and confusion (55%). The postural orthostatic tachycardia syndrome (POTS) was also frequently reported.

On average, samples were taken more than a year after the acute infection. The groups

underwent a systematic, multidimensional immunophenotyping and unbiased machine learning to identify potential biomarkers for long COVID. The anti-viral reactivity patterns in the cohorts were examined using three complementary approaches: rapid extracellular antigen profiling (REAP), serum epitope repertoire analysis (SERA) and enzyme-linked immunosorbent assay (ELISA). REAP, which measures antibody reactivity to 225 viral surface proteins, was used to assess global anti-viral responses. Non-SARS-CoV-2 antigens were analyzed using the SERA platform.

The results

The analysis of the circulating immune cells

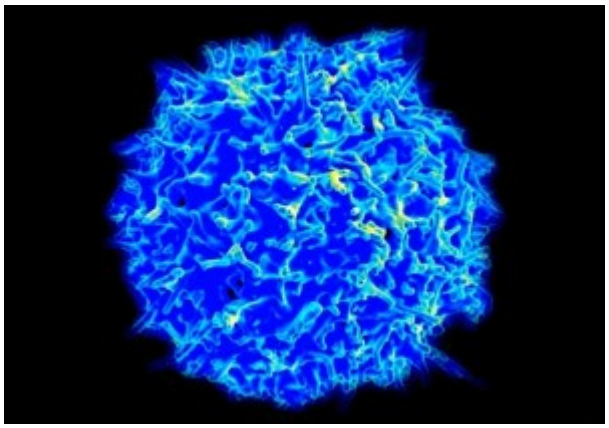
Long COVID cohorts had significantly altered populations of circulating immune cells. Compared with convalescent controls, the long COVID group displayed an increased expression of class II major histocompatibilty complex molecules (MHC class II). They also had an increased number of non-conventional monocytes (CD14lowCD16high), and decreased population of conventional type 1 dendritic (cDC1) cells, which are responsible for antigen presentation and cytotoxic T cell priming. The levels of neutrophils, eosinophils, conventional and intermediate monocytes, and cDC2 populations were not significantly different between the groups.

The median relative percentage of B lymphocytes in activated populations (CD86highHLA-DRhigh) and double-negative subsets (IgD–CD27–CD24–CD38-) was higher in long COVID group compared with convalescent controls. The absolute count of double-negative B cells was also increased in long COVID group compared with convalescent controls. The

circulating levels of other B cell subsets, including naïve B cells, were not significantly different among the groups.

An analysis of the circulating T lymphocyte populations revealed a significant reduction in the number of CD4+ T central memory cells (CD45RA–CD127+CCR7–) and an increase in the absolute number of exhausted CD4+T cells (PD-1+TIM3+) in the long COVID group. The naïve CD4+and CD8+T cells were not significantly different. Interestingly, the number of TIGIT+CD8+T cells in the cerebrospinal fluid of individuals with long COVID was increased, indicating possible immune exhaustion.

After stimulation with phorbol myristate acetate and ionomycin, cells from individuals with long COVID produced significantly more intracellular IL-2 (CD4+and CD8+T cells), IL-4 (CD4+T cells) and IL-6 (CD8+T cells) than vaccinated convalescent controls and unvaccinated healthy controls. The participants with long COVID also had polyfunctional CD4+T cells co-expressing IL-4 and IL-6.



Anti-SARS-CoV-2-specific antibody responses

An analysis of anti-SARS-CoV-2 antibody responses was performed using ELISA. The patients with long COVID, who had received two doses of vaccine, had significantly higher anti-S1 IgG levels than the convalescent vaccinated controls without symptoms of long COVID. There was no difference in the levels of total anti-S IgG and anti-receptor-binding domain (RBD) IgG between the long COVID group and the group of convalescent vaccinated controls without symptoms of long COVID.

Unvaccinated patients with long COVID had significantly higher anti-nucleocapsid (N) IgG

levels than unvaccinated convalescent controls without symptoms of long COVID.

Antibody responses to herpesviruses

Only participants who received two doses of the vaccine were analyzed. 98 patients with long COVID and 38 control participants had reactivities against 38 viral conformational epitopes. Vaccinated individuals with long COVID had elevated REAP scores for several herpes virus antigens, including the Epstein–Barr virus (EBV) minor viral capsid antigen gp23, the EBV fusion-receptor component gp42 and the varicella zoster virus (VZV) glycoprotein E. They also had lower REAP scores for the herpes simplex virus (HSV)-1 glycoprotein gL.

Polyfunctional CD4+T cells co-expressing IL-4/IL-6, found in participants with long COVID, correlated with reactivity against EBV lytic antigens, but not against SARS-CoV-2 antigens. According to the authors, these results may suggest T-helper-2-cell-shifted CD4+T cell activation in response to EBV in individuals with long COVID, as previously proposed for myalgic encephalomyelitis/chronic fatigue syndrome.

Since anti-EBV IgM levels were not elevated in individuals with long COVID and there was no evidence of EBV viremia, the researchers hypothesized that the higher reactivity to EBV lytic antigens may be due to recent EBV reactivation, rather than acute infection.

In summary, the REAP and SERA results showed increased IgG reactivity to EBV and VZV surface antigens without evidence of primary EBV infection or acute viremia in individuals with long COVID.

The analysis of hormones and soluble immune mediators

A parallel analysis of circulating hormones and immune mediators revealed that median levels of cortisol, complement component C4b, galectin-1, chemokines CCL4, CCL19, CCL20, a proliferation-inducing ligand (APRIL), luteinizing hormone (LH) and IL-5 were significantly different in individuals with long COVID.

Participants with long COVID had a significant increase in the levels of complement component C4b, chemokines CCL4, CCL19, and CCL20, galectin-1, APRIL and LH, and marginal but significant decrease in the level of IL-5.

Circulating cortisol levels were significantly reduced in both groups with long COVID

(participants with long COVID, and participants with long COVID from an independent study). The decrease in cortisol did not correlate with a compensatory increase in adrenocorticotropic hormone (ACTH) levels, suggesting that the response of hypothalamic–pituitary axis to regulate cortisol may be inappropriately attenuated. Importantly, ACTH has an extremely short half-life in the plasma, which may impair the accuracy of detecting changes.

The subsequent statistical modelling revealed that long COVID status was significantly associated with lower cortisol levels, taking into account individual variations in age, gender, body mass index, sample-collection time and cohort. The serum cortisol level was the most significant predictor of long COVID status in the model.

In conclusion, the analysis of immune profiles and hormone levels in individuals with long COVID more than one year after acute infection indicates that persistence of SARS-CoV-2 viral antigens, the reactivation of latent herpesviruses and chronic inflammation may contribute to this syndrome. As cortisol plays a central role in a variety of homeostatic and stress responses, the authors suggested that the finding of persistently lower cortisol levels in individuals with long COVID more than a year after acute infection warrants further investigation.

The article was published in Nature.

Journal Reference

Klein J, Wood J, Jaycox JR. *et al*. Distinguishing features of long COVID identified through immune profiling. Nature 623, 139–148 (2023). (Open Access)
https://doi.org/10.1038/s41586-023-06651-y