Prospective studies suggest that one in eight people infected with the severe acute respiratory syndrome coronavirus type-2 (SARS-CoV-2) have persistent symptoms. This novel disease, known as long-COVID or post-acute COVID-19 syndrome (PACS), is more prevalent among hospitalization survivors, but even people who have experienced mild acute COVID-19 have a wide range of organ dysfunction and clinical symptoms. The underlying pathogenesis of post-COVID syndrome remains unclear. Current hypotheses discuss the persistence of virus or viral remnants in various tissues, the development or aggravation of autoimmunity, micro-clotting and platelet hyperactivation, immune dysregulation, the reactivation of non-SARS-CoV-2 latent viral infections, and widespread organ damage resulting from chronic inflammation. The authors from the United States conducted this study to identify biological (immunological, virological, and hormonal) features that persist in participants diagnosed with post-COVID syndrome more than one year after acute infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

Severe acute respiratory syndrome coronavirus 2 (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) protein. The S protein is a glycosylated homotrimer with each monomer composed of subunits S1 and S2, separated by host cell proteases.

About the study

The study enrolled 268 participants, categorized into five different groups: healthcare workers infected with SARS-CoV-2 before vaccination, patients diagnosed with post-COVID syndrome, post-COVID patients from an independent study, healthy uninfected vaccinated controls, and vaccinated COVID-19 convalescent controls without post-COVID symptoms.

The COVID-19 vaccinated convalescents and post-COVID patients had mild acute SARS-CoV-2 infections that did not require hospitalization. Most (76%) patients diagnosed with post-COVID syndrome were infected in 2020 when the majority of cases were caused by the parental SARS-CoV-2 strain (WA-1).

On average, samples were collected more than a year after the acute COVID-19. Antiviral reactivity was investigated using three complementary methodologies, namely rapid extracellular antigen profiling (REAP), serum epitope repertoire analysis (SERA), and

enzyme-linked immunosorbent assay (ELISA).

Results

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

The analysis of the circulating immune cells

Post-COVID patients had significantly changed populations of circulating immune cells compared to vaccinated COVID-19 convalescent controls without post-COVID symptoms. Post-COVID patients had an increased number of non-conventional monocytes (CD14lowCD16high) and a decreased number of conventional type 1 dendritic (cDC1) cells, responsible for antigen presentation and cytotoxic T-cell priming. The numbers of neutrophils, eosinophils, conventional and intermediate monocytes, plasmacytoid dendritic, and cDC2 cells did not differ significantly between the groups.

Post-COVID patients also had a significantly higher median relative percentage of B

lymphocytes in activated populations (CD86highHLA-DRhigh) and double-negative subsets (IgD–CD27–CD24–CD38–), as well as the absolute count of double-negative B cells in comparison to vaccinated COVID-19 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 showed a reduced number of CD4+ T central memory cells (CD45RA–CD127+CCR7–) and an increased absolute number of exhausted CD4+ T-cells (PD-1+TIM3+) in post-COVID patients compared to vaccinated COVID-19 convalescent controls. The naïve CD4+ and CD8+ T-cells did not differ significantly between the groups.

The cerebrospinal fluid of post-COVID patients had an increased number of $TIGIT^{+}$CD8+ T-cells, consistent with possible immune exhaustion.

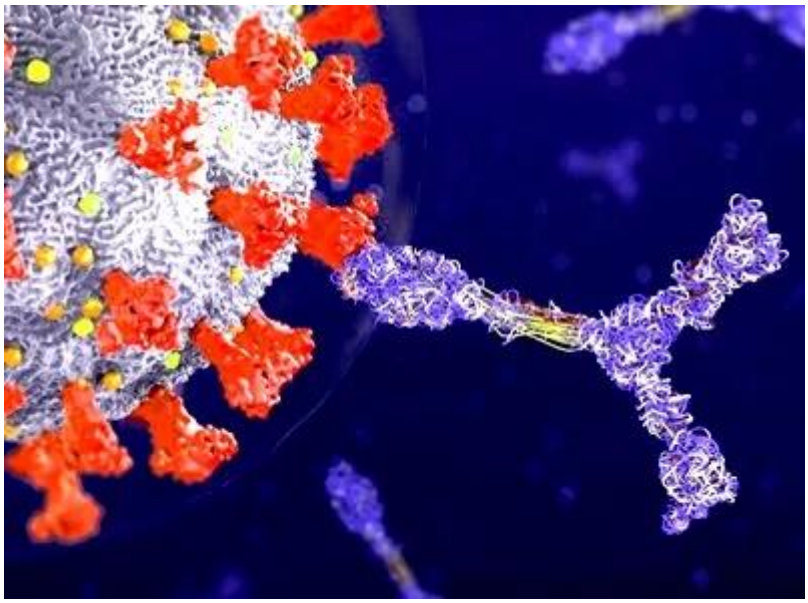
After being stimulated with phorbol myristate acetate and ionomycin, CD4+ cells from individuals with post-COVID syndrome produced significantly higher median levels of intracellular interleukin (IL)-2 and IL-4, while CD8+ T cells produced significantly higher median levels of IL-2 and IL-6 compared to vaccinated convalescents and unvaccinated healthy controls.

Post-COVID patients also had uniquely elevated median levels of IL-4/IL-6 double-positive CD4+ T cells and IL-4/IL-6 double-positive CD8+ T cells. The levels of interferon (IFN)-γ and IL-17 (in CD4+ cells) and tumor necrosis factor and granzyme B (in CD8+ cells) did not significantly differ across groups.

The antibody responses to SARS-CoV-2-specific antigens

An initial analysis of antibody responses to SARS-CoV-2-specific antigens, performed only in post-COVID participants vaccinated with two COVID vaccines, showed increased anti-S1 IgG levels compared to the vaccinated COVID convalescent controls. The levels of total anti-S IgG and anti-RBD IgG did not significantly differ between the two groups.

Importantly, unvaccinated post-COVID patients had higher levels of anti-N IgG than unvaccinated COVID convalescent controls.



The antibody responses to other viruses

The antibody responses to other viruses were only analyzed in participants vaccinated with two doses of COVID vaccines. Reactivities against 38 viral epitopes were detected in 98 post-COVID patients and 38 controls.

Post-COVID patients had higher 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.

The number of polyfunctional CD4+ T cells co-expressing IL-4/IL-6 detected in post-COVID patients correlated with reactivity to EBV lytic antigens, but not with reactivity to SARS-CoV-2-specific antigens. According to the authors, the CD4+ T-cell activation may shift in response to EBV, as previously speculated in individuals with myalgic encephalomyelitis/chronic fatigue syndrome.

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

To summarize, the results of the REAP and SERA revealed an increase in IgG reactivity to

EBV and VZV surface antigens, without evidence of primary EBV infection or acute viremia among patients diagnosed with post-COVID syndrome.

The analysis of hormones and soluble immune mediators

Patients diagnosed with post-COVID syndrome had higher median levels of complement C4b, chemokines CCL4, CCL19, CCL20, galectin-1, a proliferation-inducing ligand (APRIL), and luteinizing hormone (LH), and lower median levels of IL-5. The role of a cytokine APRIL, a member of the tumor necrosis factor family, following SARS-CoV-2 infection is still not fully investigated, although it is well-known that APRIL is expressed after viral infections playing an important role in B cell response and antibody production.

Importantly, the circulating cortisol levels decreased markedly in both the post-COVID groups, post-COVID patients, and post-COVID patients from an independent study. This result remained significant even after variations in demographics and sample-collection time adjustment.

A decrease in cortisol levels did not correlate with a compensatory increase in adrenocorticotropic hormone (ACTH) levels, suggesting an inappropriately blunted hypothalamic-pituitary axis response. The authors pointed out to extremely short half-life of ACTH in plasma, potentially compromising these results.

Subsequent statistical modeling showed an association between reduced cortisol levels and post-COVID syndrome, taking into account differences in age, sex, body mass index, time of sample collection, and cohort. Additionally, serum cortisol levels were the most significant predictor of post-COVID status in this model.

Conclusion

This study showed that several mechanisms, such as persistent SARS-CoV-2 antigens, reactivation of latent herpesviruses, chronic inflammation, and decreased cortisol levels may contribute to pathophysiological mechanisms of the post-COVID syndrome. The authors emphasized that the finding of persistently lower cortisol levels in post-COVID participants more than a year after the acute infection warranted further investigation.

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