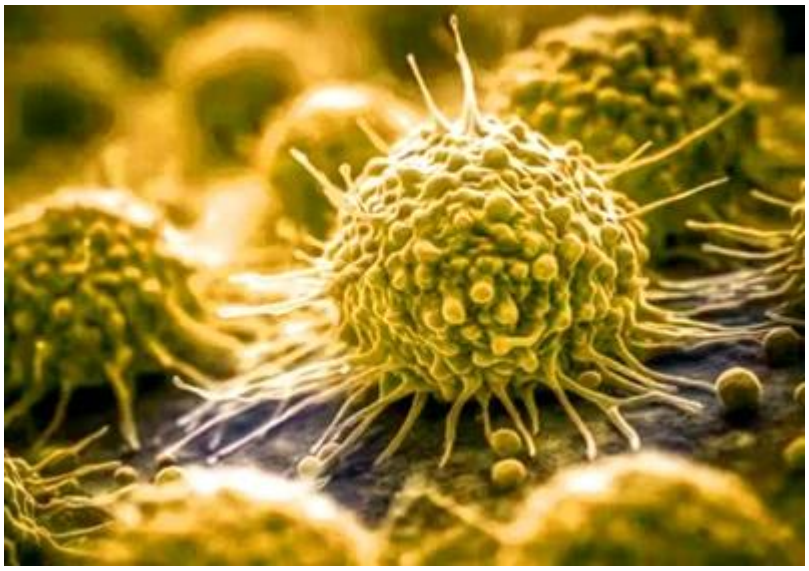
Infection with severe acute respiratory syndrome coronavirus type-2 (SARS-CoV-2) can lead to a new disease called long COVID-19 or post-acute COVID-19 syndrome (PACS). This syndrome can occur in various populations, including children and young adults, and those who have had only mild COVID-19. As viral infections have a lasting effect on the immune system and can result in organ-specific, disease-typical post-infectious consequences, the Austrian authors in this study investigated parameters of humoral and cellular immunity in unvaccinated COVID-19 convalescents ten weeks and ten months after initial infection with the Wuhan Hu-1 strain of SARS-CoV-2.

In an earlier study, the same research team reported that COVID-19 has long-lasting effects on the immune system 10 weeks after infection, even in those who had mild COVID-19. COVID-19 convalescents had a sustained reduction in neutrophil count, associated with T cell activation, as evidenced by increased expression of HLA-DR+ (CD8+ T cells) and CD38+ (CD4+ and CD8+ T cells) and significantly higher numbers of CD3+CD4+CD127+CD45RA– and CD3+CD8+CD45RO+CCR7– effector memory T cells (Tem), CD19+IgM+CD38+ transitional B cells, and plasmablasts (CD19+IgM−CD38+). In the present study, in the same group of previously studied COVID-19 convalescents, the researchers investigated the long-term effects of COVID-19 10 months after infection.



About the study

The authors compared the parameters of humoral and cellular immunity in unvaccinated COVID-19 convalescents 10 weeks and 10 months after acute infection. The age- and size-matched, uninfected control group negative for SARS-CoV-2 at the time of venipuncture and asymptomatic 10 weeks before blood donation served as a reference. For the period between the two visits, none of the COVID-19 convalescents had reported symptoms of COVID-19. As licensed vaccines became available later, all COVID-19 convalescents included in this study were unvaccinated.

The frequencies of reported comorbidities were comparable in COVID-19 convalescents (66%) and the uninfected control group (65%). The highest prevalences in both groups were reported for allergic diseases, followed by cardiovascular, metabolic, and chronic lung diseases. All other comorbidities (diabetes mellitus, hematopoietic diseases, immunosuppressive conditions, liver, neurological, or renal diseases) were reported only by a few individuals (<10%).

The authors assessed the concentrations of IgG antibodies specific for the SARS-CoV-2 spike (S) protein, receptor-binding domain (RBD), and nucleocapsid protein (N protein) over 10 months. Whole blood leukocyte subsets, with a special focus on T and B lymphocyte subpopulations, were assessed by multiparametric flow cytometry. Levels of serum cytokines and T-cellular and natural killer (NK)-cellular interferon (IFN)-γ were also measured.

Results

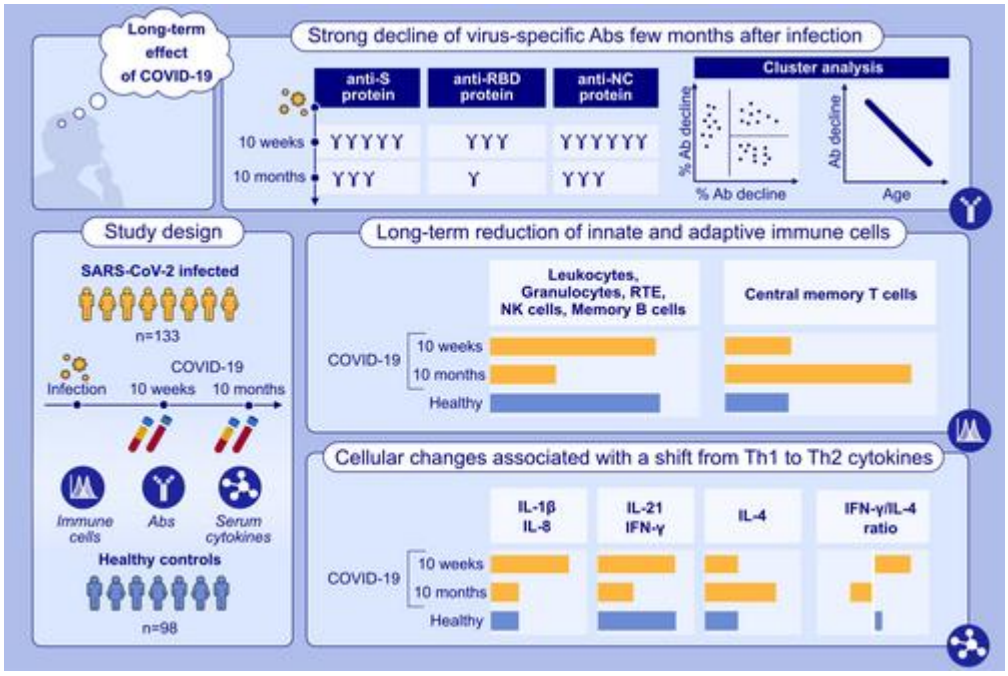
132 patients diagnosed with COVID-19 between May and August 2020, when SARS-CoV-2 Wuhan Hu-1 was the only circulating virus strain, and 98 uninfected healthy controls were enrolled in this case-control study. COVID-19 was confirmed by RT-PCR and/or anti-SARS-CoV-2 antibody assay. The cohort was analyzed 10 weeks (77.8 ± 24.6 days) and 10 months (9.5 ± 0.8 months) after the acute SARS-CoV-2 infection.

The IgG antibodies specific for the SARS-CoV-2 S protein, RBD, and N protein were stable or even elevated in 26 subjects. This group was excluded from further analysis because of a possible clinically silent infection.

In the remaining 106 COVID-19 convalescents, IgG antibodies specific for the SARS-CoV-2 proteins significantly decreased between 10 weeks and 10 months after the infection. At 10 weeks after the infection, the concentration of IgG antibodies specific for the S protein

was below the cutoff value in only 3 of 106 (2.8%) COVID-19 convalescents, but after 10 months, this number increased to 19 (17.9%) convalescents. Anti-RBD IgG levels were below the cutoff value in 26 of 106 (24.5%) COVID-19 convalescents at 10 weeks, but after 10 months, this number increased to 86 (81.1%) convalescents. None of the 106 subjects was negative for nucleocapsid protein, neither at 10 weeks nor at 10 months after the infection. Strong declines of antibodies specific for the N and S proteins were associated with younger age and lower numbers of CD3–CD56+ NK cells and CD19+CD27+ memory B cells.

According to the waning pattern of anti-SARS-CoV-2 antibodies, the authors divided all COVID-19 convalescents into three groups. Group 1 was characterized by a small to moderate (≤50%) decline of antibodies specific for the N protein and a variable decline of antibodies specific for the S protein. Group 2 was characterized by a strong decline of antibodies specific for the N protein (>50%), but a small decline of antibodies specific for the S protein (≤40%), and group 3 showed a strong decline of antibodies specific for both N (>50%) and the S proteins (>40%). There was a significant difference in the mean age between groups 1 and 3 (56.2 ± 11.5 *vs.* 45.9 ± 12.3).



Abbreviations: CD, cluster of differentiation; COVID-19, coronavirus disease 2019; IL, interleukin; IFN, interferon; NC, nucleocapsid protein; NK, natural killer; RBD, receptor binding domain; RTE, recent thymic emigrants; S, spike protein; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

After 10 weeks, COVID-19 convalescents had lower numbers of circulating neutrophils than uninfected healthy controls. After 10 months, COVID-19 convalescents had lower leukocyte numbers than those at 10 weeks, including neutrophils, monocytes, and lymphocytes. The changes in total leukocyte, lymphocyte, and granulocyte counts were similar for all groups. Monocyte count was reduced in groups 2 and 3 but not in group 1.

T cells

At 10 weeks after COVID-19, convalescents had a sustained reduction of neutrophil counts, accompanied by T cell activation, as evidenced by increased expression of HLA-DR+ (CD8+ T cells) and CD38+ (CD4+ and CD8+ T cells). According to the authors, these results may be a consequence of the SARS-CoV-2 particle shedding, the extensive tissue damage in severe COVID-19 cases, increased IL-4 serum levels found in most patients, or the moderately elevated IL-17A levels typically seen in severe COVID-19 cases.

After 10 months, T cell activation observed after 10 weeks disappeared, except in 10 patients who were hospitalized due to severe acute COVID-19, in whom HLA-DR+ and HLA-DR+CD38+ T cells remained elevated. These changes in T cell types were similar in all groups.

At 10 weeks after infection, the numbers of most naïve T cells, defined as CD3+CD45RA+CD62L+CD31+ recent thymic emigrants, were similar in COVID-19 convalescents and healthy controls. However, after 10 months, these numbers were almost cut in half in COVID-19 convalescents. This affected the CD3+CD4+CD45RA+CD62L+CD31+ helper T cell subset more than the CD3+CD8+CD45RA+CD62L+CD31+ cytotoxic T cell subset. Notably, younger patients had higher numbers of circulating recent thymic emigrants than older patients.

At 10 months after COVID-19, a decrease in circulating recent thymic emigrants was accompanied by an increase in absolute numbers of CD3+CD45RO+CCR7+ central memory CD4+ and CD8+ T cell subsets. Absolute numbers of overall CD45RO+ memory T cells were not increased, but CD3+CD4+CD45RO+CCR7+ and CD3+CD8+CD45RO+CCR7+ central

memory T cell subsets were increased in 55% and 68.4%, respectively. These changes in T cell subsets were similar in all groups of COVID-19 convalescents with different antibody waning patterns.

At 10 weeks after COVID-19, convalescents had an increase in CD3+CD4+CD127+ effector memory T cells and a decrease in Foxp3+ CD3+CD4+CD127−CD25+ T regulatory cells, with the tendency to return to almost normal levels after 10 months. However, CD3+CD4+CD45RA+CD127+ cells remained elevated throughout the observation period.

These findings show that 10 months after COVID-19, the pool of recent thymic emigrants was mainly depleted and not central memory T cells. The changes of T cell subsets, such as normalization of effector and Treg numbers, decline in recent thymic emigrants, and increase in central memory T cell numbers, were independent of the antibody waning pattern.

NK cells

CD56+CD3- NK cell numbers were significantly lower at 10 months than those at 10 weeks, and this drop was mainly attributable to groups 2 and 3.

B cells

At 10 months after the infection, the overall numbers of B cells declined in the entire COVID-19 study group, with a significant drop in groups 2 and 3 and a plateau in group 1. A similar decline in overall circulating CD19+CD21+CD27+ memory B cells was due to a significant decrease in non-class-switched CD19+IgD+CD27+ memory B cells. These reductions were accompanied by the normalization of CD19+IgM+CD38+ transitional B cells and CD19+IgM-CD38+ plasmablasts, which were similar in all groups. Groups 2 and 3 had reduced non-class-switched B cells, whereas a significant reduction of class-switched memory B cells was exclusively seen in group 3. In contrast, a drop of CD5+ B cells was observed across all three groups.

These findings show a decrease in non-class-switched CD19+IgD+CD27+ memory B cells in COVID-19 convalescents 10 months after the infection compared to healthy controls.

Serum cytokine levels

At 10 weeks after COVID-19, convalescents had higher levels of certain cytokines, which may be indicative of Th1-dominated inflammation (e.g., interleukin (IL)-1β, IL-8, and IL-12) than the uninfected control group. At 10 months after COVID-19, these alterations resolved, paralleled by a Th2-dominated serum cytokine pattern, as evidenced by increased IL-4 and IL-10 levels compared to those at 10 weeks after the infection. These findings suggest that cellular changes 10 months after COVID-19 were associated with a shift from Th1 to Th2 cytokine pattern.

IFN-γ levels were similar in COVID-19 convalescents and uninfected healthy controls 10 weeks after COVID-19, but they decreased significantly after 10 months. This was accompanied by a decrease in IL-21 levels, while the overall low IL-17 levels did not change significantly.

Interestingly, the extent of the Th1 to Th2 cytokine pattern shift was dependent on the antibody waning pattern. After 10 weeks, group 1 showed considerable IFN-γ serum levels and low IL-4 levels. However, IL-4 levels increased during the observation period, leading to a moderate change in the IFN-γ/IL-4 ratio, accompanied by an increase in IL-10 levels. In contrast, groups 2 and 3 had IFN-γ-dominated responses after 10 weeks and considerable IL-4 levels, which reversed during the observation period. This led to more dramatic changes in IFN-γ/IL-4 ratios, accompanied by decreased numbers of CD19+CD27+ B memory cells and CD3–CD56+ NK cells.

Interestingly, almost all COVID-19 convalescents suffering from a post-acute COVID-19 syndrome (93%) had an inverted IFN-γ/IL-4 ratio and were more frequently stratified in group 3 than in groups 2 and 1. The authors speculated that such inversion of the IFN-γ/IL-4 ratio could be a biomarker for post-COVID-19 syndrome.

Conclusion

This follow-up study of immune parameters in unvaccinated COVID-19 convalescents 10 weeks and 10 months after a first and mostly mild infection with the Wuhan Hu-1 strain of SARS-CoV-2 showed a long-term reduction in innate and adaptive immune cells, associated with a shift from Th1 to Th2 cytokine pattern.

The authors claimed that these findings were unexpected. Instead of an activation and expansion of CD3+CD4+ and CD3+CD8+ effector memory cells and transitional B cells,

and plasmablasts, the results showed significantly reduced numbers of adaptive immune cells, including T cells (particularly CD3+CD45RA+CD62L+CD31+ recent thymic emigrants) and B cells (non-class-switched CD19+IgD+CD27+ memory B cells). These findings could explain an immunological mechanism of post-acute COVID-19 syndrome.

Since all leukocyte lineages originate from pluripotent CD34+ hematopoietic stem cells in the bone marrow, the authors speculated that SARS-CoV-2 could infect these cells, which could explain a multi-lineage reduction observed after 10 months. Alternatively, SARS-CoV-2 may affect the bone marrow stromal microenvironment (mesenchymal progenitors, osteoblasts, fibroblasts, and endothelial cells).

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Kratzer B, Gattinger P, Trapin D et al. Differential decline of SARS-CoV-2-specific antibody levels, innate and adaptive immune cells, and shift of Th1/inflammatory to Th2 serum cytokine levels long after first COVID-19. Allergy. 2024;79:2482–2501. https://onlinelibrary.wiley.com/doi/10.1111/all.16210