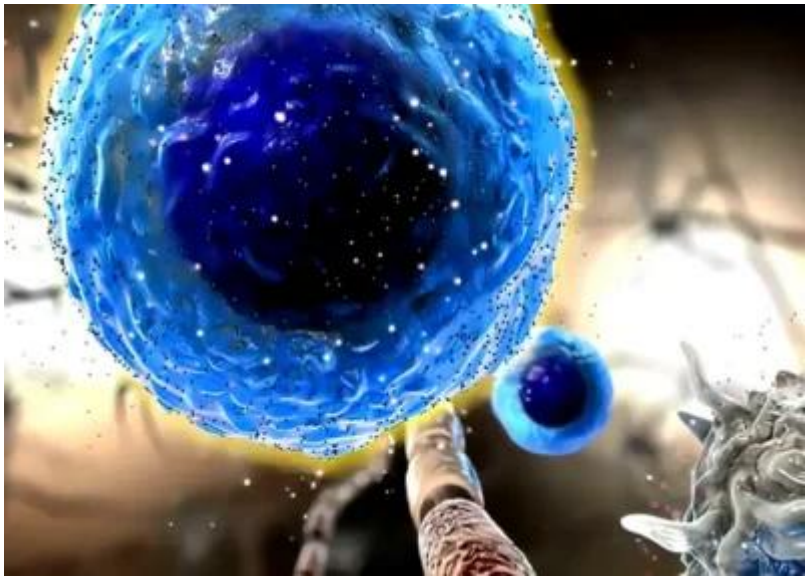
More than three years after the global COVID-19 pandemic, it is clear that infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can lead to a new disease called long-COVID-19 or post-acute COVID-19 syndrome. Long/post-COVID, evidently represents a heterogeneous nosological entity, despite the existence of similar or overlapping symptoms between patients, and clear diagnostic criteria are yet to be established. The mechanisms underlying long COVID and the differences in its manifestation are poorly understood. In this study, the consortium of researchers, led by authors from Switzerland, evaluated the presence of anti-chemokine autoantibodies in COVID-19 convalescents.

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, which appears to be a major pathogenic factor of the SARS-CoV-2 is a glycosylated homotrimer with each monomer composed of subunits S1 and S2, separated by host cell proteases. The S1 domain comprises the N-terminal domain (NTD), the receptor binding domain (RBD) with a receptor binding motif (RBM), and two C-terminal domains. The RBD recognizes human host cell angiotensin-converting enzyme 2 receptor (ACE2).

Chemokines are small proteins, a class of chemotactic cytokines that attract different cytokines, cells, and substances to specific sites. They are involved in biological processes, such as homeostasis, angiogenesis, immune response, inflammation, chemotaxis, and metastases. Depending on the number of amino acids between the first two cysteine residues, the chemokines are classified into four subfamilies, CXC, CC, CX3C, and XC.

Acute COVID-19 is characterized by high expression of certain chemokines, which recruit neutrophils and monocytes to infection sites. These chemokines are significant for the pathophysiological processes of COVID-19 by sustaining inflammation and causing collateral tissue damage. The variety of anti-chemokine autoantibodies, induced upon SARS-CoV-2 infection, may modulate the inflammatory response, disease manifestation, and the persistence of COVID symptoms.



About the study

The researchers investigated the presence of anti-chemokine autoantibodies in plasma samples collected from 43 COVID-19 convalescents from three independent cohorts (Lugano, Milano, or Zurich) 6 and 12 months after the onset of acute SARS-CoV-2 infection. The study also included 23 healthy uninfected controls, confirmed by a negative serologic test.

Twelve months after the infection with SARS-CoV-2, the Lugano cohort was asked to answer a questionnaire about self-reported symptoms of long-COVID. The average number of long-term symptoms was 3.3, and 65% of participants reported at least one persistent symptom. Previously hospitalized COVID-19 convalescents more frequently (73%) reported long COVID symptoms than outpatient (48%) convalescents.

The enzyme-linked immunosorbent assay and pseudovirus-based neutralization assay evaluated the binding and neutralizing capacity of anti-SARS-CoV-2 antibodies in plasma samples. The results showed significantly higher levels of antibodies against RBD of S1 protein and values of half-maximal SARS-CoV-2 neutralizing titers (NT50) in previously hospitalized convalescents than in outpatient convalescents. The same results have been found in men compared to women.

The concentrations of anti-RBD IgG antibodies and NT50 values did not correlate with concentrations of autoantibodies against “COVID-19 signature” chemokines, which include

CCL19, CCL22, and CXCL17. These chemokines are believed to distinguish COVID-19 convalescents from healthy individuals.

Temporal dynamics of chemokines and anti-chemokine autoantibodies

In the Milan cohort, several chemokines significantly increased during COVID-19 (CCL2, CCL3, CCL4, CCL19, CCL21, CCL22, CCL25, CXCL2, CXCL8, CXCL9, CXCL10, CXCL13, and CXCL16) and after 7 months (CCL19, CCL21, CCL22, CXCL2, CXCL8, CXCL10, CXCL13, and CXCL16). There was no correlation between concentrations of chemokines and their corresponding autoantibodies in acute COVID-19 and after 7 months.

In the Lugano cohort, some chemokines rapidly increased and persisted in plasma for at least 6 months after the acute SARS-CoV-2 infection. Twelve individuals had elevated concentrations of CCL3 and CCL4 chemokines six months after COVID-19, but, there was no correlation between chemokine levels and their corresponding autoantibodies.

At 6 and 12 months after the onset of COVID-19, temporal dynamics demonstrated a significant increase in concentrations of autoantibodies against CCL8, CCL13, CCL16, CCL19, CXCL7, and CX3CL1, regardless of the vaccination status. Autoantibodies to CXCL17 remained generally stable.

The concentrations of autoantibodies against “COVID-19 signature” chemokines (CCL19, CCL22, and CXCL17) differed from those against “COVID-19 severity signature” chemokines (CXCL5, CXCL8, and CCL25). In the Milan cohort, concentrations of autoantibodies against “COVID-19 signature” chemokines during COVID-19 were higher than in healthy controls. In the Lugano cohort, concentrations of autoantibodies against CCL19 (but not CCL22 or CXCL17) during COVID-19 were higher than in healthy controls and continued to rise until 12 months.

Correlation of anti-chemokine autoantibodies with severity of COVID-19

Six months after the onset of COVID-19, outpatient convalescents had higher concentrations of autoantibodies against 8 chemokines (CXCL7, CXCL8, CXCL16, CCL19, CCL22, CCL27, CCL20, and CX3CL1) and significantly higher cumulative IgG reactivity against chemokines than healthy controls.

Six months after the infection, previously hospitalized convalescents had higher

concentrations of anti-CCL19 autoantibodies than healthy controls. In this group of participants, anti-CXCL5 and CXCL8 autoantibodies correlated negatively with anti-RBD IgG. Similar results were found in the Milan and Zurich cohorts.

Importantly, the outpatient convalescents from the Lugano cohort had a broader pattern and higher overall concentrations of anti-chemokine autoantibodies than hospitalized convalescents. They had higher levels of autoantibodies against the "COVID-19 severity" chemokines (CXCL5, CXCL8, and CCL25) than hospitalized convalescents. This difference was not related to therapy given during hospitalization. These findings suggest that higher levels of autoantibodies directed against specific chemokines are associated with favorable disease outcomes.

The link between anti-chemokine autoantibodies and long-COVID

Researchers also investigated whether specific patterns of anti-chemokine antibodies registered 6 months after the infection could predict long COVID syndrome. The results showed that convalescents without long COVID symptoms had significantly higher cumulative levels and levels of specific patterns of anti-chemokine autoantibodies than COVID-19 convalescents who developed long COVID syndrome, especially outpatients and women.

Convalescents without long COVID had higher concentrations of autoantibodies to CCL21, CXCL13, and CXCL16 than convalescents with long COVID syndrome. The authors emphasized that autoantibodies against CCL21, CXCL13, and CXCL16 distinguished the individuals with long COVID from those without long COVID with high significance, and therefore these autoantibodies were called "long COVID signature".

The chemokines CCL21, CXCL13, and CXCL16 are important for tissue trafficking and activation of T and B lymphocytes, so, the authors speculated that autoantibodies to these chemokines might positively influence the long-term outcome by antagonizing or modulating the activation, recruitment and retention of T and B lymphocytes.

Anti-chemokine autoantibodies in other infectious or autoimmune diseases

The authors also evaluated anti-chemokine autoantibodies in patients with other infectious or autoimmune diseases, such as chronic HIV-1 infection ($n = 24$), the infection with *Borrelia burgdorferi*-Lyme disease ($n = 27$), ankylosing spondylitis ($n = 13$), rheumatoid arthritis ($n = 13$) and Sjögren syndrome ($n = 13$).

Individuals with chronic HIV-1-infection had significantly higher levels of autoantibodies against 14 chemokines (CCL2, CCL3, CCL4, CCL5, CCL20, CCL21, CCL22, CCL23, CCL27, CCL28, CXCL7, CXCL8, CXCL9 and CXCL12) than healthy controls. Plasma from *Borrelia*-infected individuals did not differ from the healthy controls, except for elevated anti-CXCL14 autoantibodies during the acute infection. Individuals with ankylosing spondylitis, rheumatoid arthritis, or Sjögren syndrome had higher levels of autoantibodies against four chemokines (CCL4, CCL19, CCL25, and CXCL9) than healthy controls.

Conclusion

This study showed the presence of autoantibodies against certain chemokines in COVID-19 and long-COVID syndrome. A higher level of anti-chemokine autoantibodies observed in outpatient convalescents compared to convalescents hospitalized during acute COVID-19 indicates their association with favorable disease outcomes. Also, convalescents who developed long COVID syndrome displayed lower cumulative levels and specific anti-chemokine autoantibody patterns compared to convalescents without long COVID.

Additionally, different patterns of anti-chemokine autoantibodies distinguished not just different COVID-19 trajectories, but also other infectious and autoimmune diseases.

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