The 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, young adults, and those who have recovered from mild COVID-19. Long COVID/post-COVID syndrome represents a heterogeneous entity, despite the existence of similar or overlapping symptoms between patients. In this study, the authors from the Netherlands investigated a passive transfer of total human immunoglobulin (Ig)G (hIgG) from patients with long COVID to mice. They tested the hypothesis that autoantibodies may play a crucial role in the pathogenesis of long COVID, in, at least, some patients.

Autoantibodies that target diverse self-antigens, including chemokines, G protein-coupled receptors, neurotransmitters, and various immunomodulating proteins were detected in patients diagnosed with long COVID syndrome. A recent study that investigated the presence of autoantibodies against certain chemokines in long-COVID syndrome, found higher levels of anti-chemokine autoantibodies in outpatient convalescents than in convalescents hospitalized during acute COVID-19. Convalescents who developed long COVID syndrome displayed lower cumulative levels and specific anti-chemokine autoantibody patterns compared to convalescents without long COVID, indicating their association with favorable disease outcomes. In addition, different patterns of anti-chemokine autoantibodies distinguished different COVID-19 trajectories.
https://discovermednews.com/autoantibodies-against-chemokines-have-favorable-outcome-in-covid-19-convalescents/

About the study

The study included 34 individuals diagnosed with long COVID according to the WHO criteria, as well as two distinct control groups: healthy control subjects who were sampled before the SARS-CoV-2 pandemic, and 15 healthy control COVID-19 convalescents who recovered from mild infection and lacked any residual symptoms. None of the participants were hospitalized for COVID-19, and venous blood was taken at least 90 days after the initial SARS-CoV-2 infection.

Human cytokine assays were used to detect interleukin (IL)-1β, IL-6, IL-10, tumor necrosis factor (TNF), interferon (IFN)-α2a, IFN-β, IFN-γ, glial fibrillary acidic protein (GFAP), neurofilament light chain (NfL), and total Tau. The protein profiling analysis was performed using Olink Proteomics technology. Long COVID patients were stratified based on GFAP and

IFN levels in three subgroups, LC1, LC2, and LC3.

For *in vivo* experiment, the authors used adult male and female C57BL/6 mice. The pooled purified hIgG from each of the three long COVID subgroups and the healthy control group sampled before the SARS-CoV-2 pandemic, were injected intraperitoneally into eight mice per group (four females and four males) at a single dose of 260 mg/kg, which represents about 1/3 of the total circulating IgG per mouse. On day 15 after injection, the presence of hIgG was examined in various mouse tissues, including the heart, skeletal muscle, dorsal root ganglia, and dorsal horns of the spinal cord where afferent nerves of sensory neurons form synapses with interneurons to process sensory information, including pain perception. The intensity and area of human IgG staining in murine lumbar spinal cord and lumbar dorsal root ganglia (L3-L5) were evaluated by immunohistochemistry.

Behavioral tests were performed before the administration of hIgG injection. Mechanical sensory thresholds were measured with the Von Frey test and thermal sensitivity was assessed with the Hargreaves test. The general locomotor activity was assessed with an open field test, and motor strength, coordination, and balance were measured using two rotarod paradigms: one with a fixed speed and another with an accelerating speed over time to detect subtle impairments in motor coordination.

Results

Plasma levels of proinflammatory cytokines IL-1β, IL-6, TNF, and GM-CSF were comparable between long COVID patients and healthy COVID-19 convalescent controls.

However, long COVID patients had dysregulated IFN signatures at the time of sampling (over six months after infection) even though they had mild acute COVID-19. Compared to healthy controls, long COVID patients had elevated IFN-β, a type-I IFN produced by most nucleated cells upon viral infection, and lower IFN-γ, the type-II IFN produced mainly by T cells and NK cells. The other type-I IFN, IFN-α2a, produced predominantly by plasmacytoid dendritic cells, was unaffected.

The astroglial activation marker GFAP was detectable in 10 of 34 long COVID patients, while it was undetectable in all healthy COVID convalescents. There were no differences in the levels of neurodegenerative markers TAU and NfL between the long COVID patients and healthy COVID convalescents.

Proteomics analysis of 2865 proteins and clustering of the top 200 components revealed

multiple protein clusters that accumulated differentially within three subgroups. The LC1 subgroup consisted of 12 patients and was characterized by elevated levels of astroglia activation markers GFAP and neurodegenerative markers TAU and NfL compared to other subgroups. This subgroup was also characterized by higher levels of cytoskeleton, nervous system, and Golgi-related proteins, and reduced lysosome proteins and bacterial response.

The LC2 subgroup, which consisted of 10 patients, had higher IFN-α2a and IFN-β levels than the LC3 subgroup. The LC2 subgroup also had higher levels of muscle-related proteins and lower levels of neurotransmitter proteins than the LC1 and LC3 subgroups.

The LC3 subgroup consisted of 12 patients. Compared to the LC2 subgroup, the LC3 subgroup had elevated leukocyte activation proteins and lower type-I IFNs, TAU, acute-phase proinflammatory cytokines IL-1β and IL-6, and muscle and neural proteins. According to the authors, elevated plasma levels of muscle and neural proteins could indicate the release of these proteins from local tissue damage and their entry into the circulation.

There was no difference in IFN-γ levels between subgroups.

In vivo results

The pooled purified human IgG from three long COVID subgroups and the healthy control group sampled before the SARS-CoV-2 pandemic were injected intraperitoneally into mice.

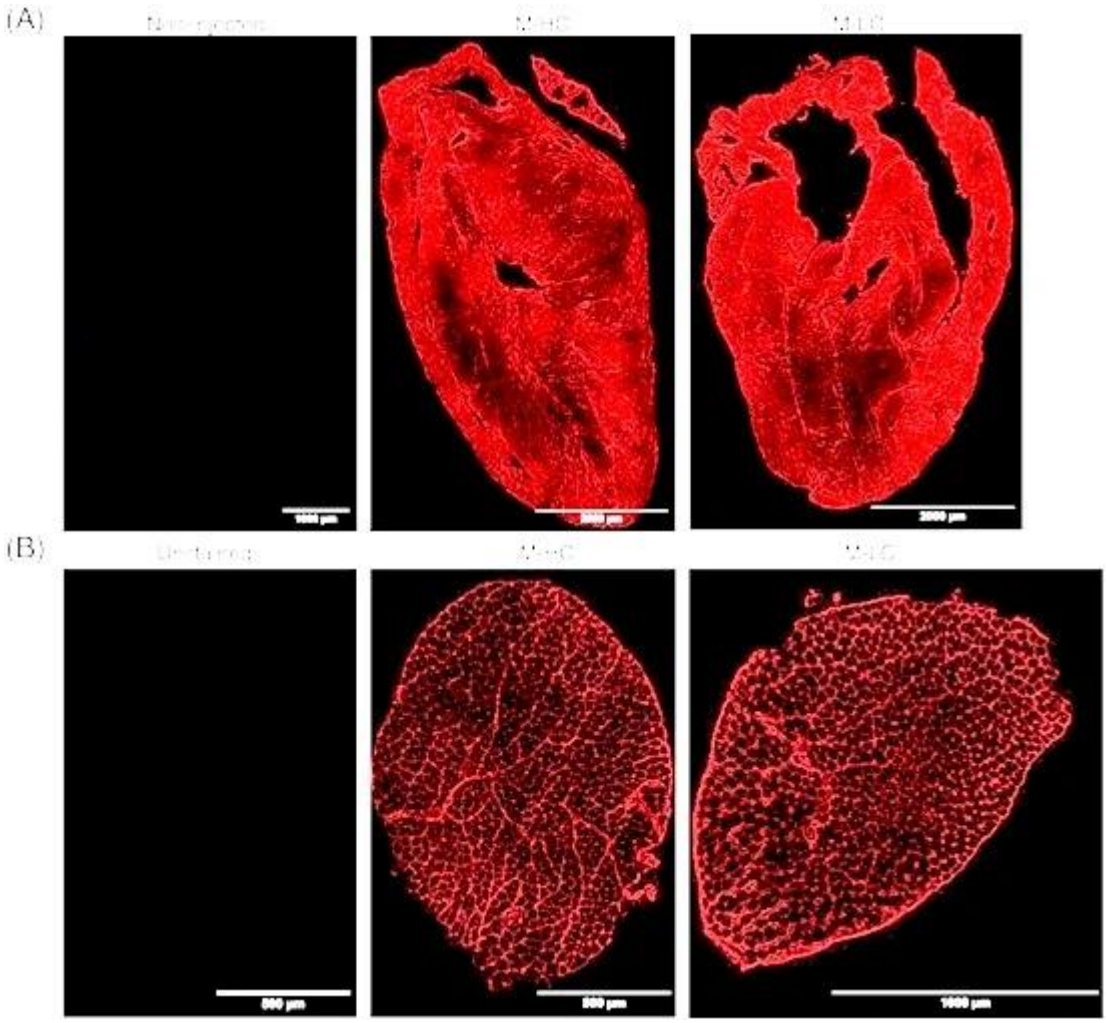

Figure from the original article of Chen H-J et al.: Injected human IgG of Long COVID (LC) patients and healthy donors (HC) in murine heart and skeletal muscles. (A) Representative staining of hIgG (red) in the heart of M-HC and M-LC. As a negative control, the hearts of non-injected mice were included and stained for the presence of hIgG (left panel). (B) Representative staining of hIgG in skeletal muscle of M-HC and M-LC. As a negative control, unstained skeletal muscle (left panel).

Behavioral tests demonstrated thermal hypersensitivity in both mice groups, in the first group injected with human IgG from long COVID patients and in the second group injected with hIgG from healthy controls. In mice injected with hIgG from healthy controls, thermal hypersensitivity normalized within 2-3 days, but in mice injected with hIgG from long COVID patients thermal hypersensitivity persisted until the last measurement 15 days post-injection.

Further analysis showed that IgG transfer from different subgroups of long COVID patients

to mice resulted in distinct subgroup-specific sensory symptoms and pain-associated behavior. Remarkably, IgG transfer from the two subgroups LC1 and LC3, characterized by higher plasma levels of neuronal proteins and leukocyte activation markers, induced sensory hypersensitivity with distinct kinetics. Mice injected with hIgG from the LC1 subgroup exhibited a significant increase in mechanical and thermal sensitivity from 3 to 15 days after intraperitoneal hIgG injection. In mice receiving hIgG from the LC3 subgroup, mechanical sensitivity started more rapidly, as early as 24 hours after intraperitoneal hIgG injection.

However, mice receiving hIgG from the LC2 subgroup did not develop mechanical and heat stimulation sensitivity. Interestingly, patients in the LC2 subgroup had lower plasma levels of neuronal proteins involved in neurotransmitter transport and secretion, but higher plasma levels of proteins involved in skeletal and cardiac muscle pathways than patients in the LC1 and LC3 subgroups.

There was no difference in motor strength, coordination, and balance between mice injected with human IgG from long COVID patients and those injected with hIgG from healthy controls. However, the results of general locomotor activity were subgroup-specific. Only mice injected with hIgG from the LC2 subgroup, characterized by enriched skeletal and cardiac muscle proteome profiles, showed reduced walking distance one day after injection, about 40% less than mice injected with hIgG from healthy controls.

Human IgGs from either long COVID patients or healthy controls were detectable in all mice tissues tested, including the heart, skeletal muscles, dorsal horns of the spinal cord, and satellite cells in the dorsal root ganglia. The intensities of hIgG from healthy donors were not different from those of long COVID patients (Figure).

Conclusion

This study identified three distinct long COVID subgroups using quantitative plasma biomarker analysis, with proteome signatures enriched in distinct pathways. According to the authors, various underlying mechanisms trigger different local/systemic tissue damages, leading to the corresponding biomarker release into circulation. Alternatively, these markers are not direct pathogenic factors that drive disease progression but rather consequences of long COVID development based on different pathophysiology.

Passive transfer of IgG from these subgroups to mice caused subgroup-specific mechanical and thermal sensitivity and impaired locomotor activity. These findings demonstrate that

the transfer of IgG from long COVID patients to mice replicates disease symptoms, underscoring IgG's causative and heterogeneous role in the pathogenesis of long COVID.

The authors concluded that establishing a mouse model that mimics the pathology of long COVID may be a promising way to improve the understanding of the pathophysiology of long COVID and promote the development of targeted and personalized therapeutic interventions.

The results of the study have been published on a preprint server and are currently being peer-reviewed.

Journal Reference

Chen H-J, Appelman B, Willemen H et al. Transfer of IgG from Long COVID patients induces symptomology in mice. bioRxiv preprint posted May 31, 2024. https://doi.org/10.1101/2024.05.30.596590