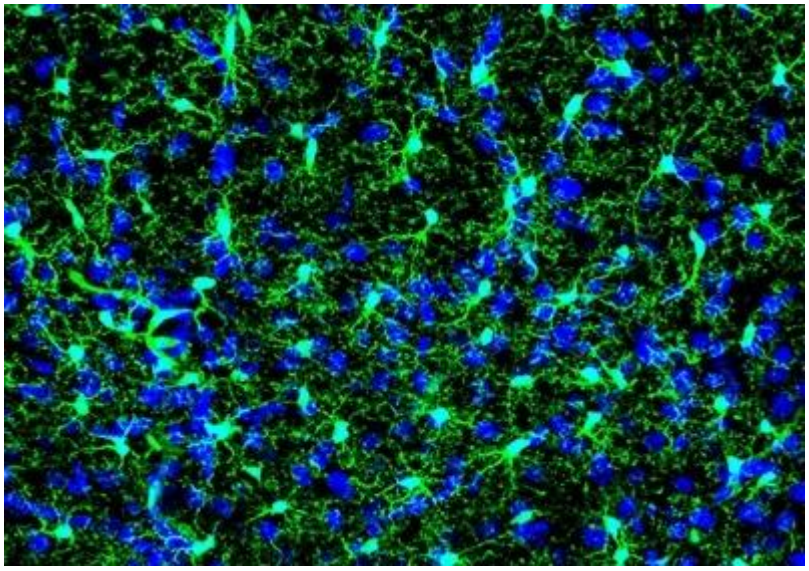
After recovering from COVID-19, a large proportion of patients suffer from long-lasting post-COVID-19 symptoms, called long-COVID or post-acute COVID syndrome (PACS). Post-COVID symptoms often include neuropsychiatric complications that last for months after the initial infection. In this *in vitro* study, researchers from the United States investigated whether the full-length recombinant severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike (S) protein and its receptor-binding domain (RBD) stimulate human microglia to release proinflammatory mediators.

SARS-CoV-2 is an enveloped, positive-sense, single-stranded RNA virus. Its genome encodes four structural proteins: the spike (S), envelope (E), nucleocapsid (N), and membrane (M) proteins. The S protein, which appears to be a major pathogenic factor, is composed of subunits S1 and S2, separated by host cell proteases. The S1 domain comprises the N-terminal domain (NTD), the RBD with a receptor binding motif (RBM), and two C-terminal domains. The RBD is a short immunogenic fragment that facilitates the binding of the S protein to the host cell angiotensin-converting enzyme 2 (ACE2) receptor.

Microglia are the resident mononuclear phagocytes of the central nervous system (CNS) and account for 5 to 10 percent of total adult brain cells. These cells are highly heterogeneous in the healthy CNS, acting as the first line of defense in the brain. Activated microglia have important functions in the CNS, especially in neuroinflammation and neurodegenerative diseases.

The *postmortem* examination of the brain samples from individuals who died of COVID-19 revealed extensive microglial activation and neuroinflammation associated with brain pathology. Additionally, neuroradiological studies provided evidence of glial dysfunction, neuronal injury, and persistent neuroinflammation in the brains of individuals diagnosed with post-COVID neuropsychiatric symptoms.
https://discovermednews.com/brain-proton-mr-spectroscopy-changes-in-neurometabolites-in-post-covid-patients/



About the Study and Results

This *in vitro* study evaluated the influence of the full-length recombinant SARS-CoV-2 S protein and its RBD on the secretion of proinflammatory mediators such as interleukin (IL)-1β, IL-6, IL-18, the chemokine CXCL8, tumor necrosis factor-alpha (TNF-α), matrix metallopeptidase 9 (MMP-9), and S100B protein in the immortalized human microglia-SV40 cell line.

The results showed that recombinant SARS-CoV-2 S protein stimulated the release of proinflammatory mediators from the immortalized human microglia-SV40 cell line in a dose-dependent manner. Various concentrations (1, 5, or 10 ng/ml) of the full-length recombinant S protein significantly increased concentrations of IL-1β, TNF-α, S100B, CXCL8, IL-6, and MMP9 after 24 hours of stimulation, compared to controls.

The RBD alone significantly increased concentrations of TNF-α, IL-18, and S100B. These findings were confirmed with the S protein and RBD from two different sources.

Researchers also investigated whether the proinflammatory responses in human microglia, triggered by the full-length recombinant S protein or the RBD, were mediated by Toll-like receptor (TLR2 and TLR4) signaling or ACE2. They incubated human microglia cell line with anti-TLR2, anti-TLR4, and anti-ACE2 antibodies and stimulated for 24 hours with the full-length S protein.

The results showed that only pretreatment with anti-TLR4 antibodies completely suppressed the increase in IL-1β, CXCL8, IL-6, S100B, and MMP9 induced by the full-length SARS-

CoV-2 S protein. On the other hand, an increase in TNF-α, IL-18, and S100B induced by RBD alone was completely suppressed only by pretreatment with anti-ACE2 antibodies.

Conclusion

This study showed that recombinant full-length SARS-CoV-2 S protein and RBD alone stimulated human microglia *via* different receptors to secrete various proinflammatory mediators. According to the authors, these findings confirmed that the SARS-CoV-2 S protein contributes to neuroinflammation *via* several mechanisms involved in CNS pathologies.

This study has been published on a preprint server and is currently being peer-reviewed.

Journal Reference

Tsilioni I, Theoharides TC. Recombinant SARS-CoV-2 Spike Protein and its Receptor Binding Domain stimulate release of different pro-inflammatory mediators via activation of distinct receptors on human microglia cells. Research Square preprint. (Open Access). https://doi.org/10.21203/rs.3.rs-2394904/v1