Persisting Neuroinflammation after 18-weeks in SARS-CoV-2 Infected African Green Monkeys

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Introduction: Many individuals report ongoing neurological symptoms, including impaired concentration and cognition, months to years after infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). We hypothesize that these neurological complaints are due to persistent neuropathological changes/injury after acute infection. **Methods**: To begin to explore our hypothesis, brain and cerebrospinal fluid (CSF) were acquired from ten African green monkeys (AGMs) after 18-weeks post-SARS-CoV-2 infection with the 2019-nCoV/USA-WA1/2020 strain. Formalin-fixed, paraffin-embedded brain tissues underwent a histopathological investigation for the presence of virus/viral proteins and overall pathology, including microhemorrhages, neuronal apoptosis, and neuroinflammation. Our findings from 18-week infected AGMs were compared to the findings from our previously published 4-week infection AGM study, using the same viral strain. Metabolomic analysis of CSF acquired at baseline and necropsy was performed by ultra-HPLC/MS. **Results**: Consistent with most post-mortem human studies, we did not detect viral spike protein in brain tissue. Although we previously reported an increased frequency of microhemorrhages and neuronal cell death, confirmed by active caspase 3 IHC, in 4-week infected AGMs, this appeared to be largely resolved at 18-weeks. Notably, microhemorrhages seen at 18-weeks post-infection (p.i.) had a similar appearance to those seen at 4-weeks p.i., with densely packed red blood cells on the parenchymal side of the blood vessel. Most striking was the presence of significant wide-spread neuroinflammation and nodular lesion formation persisting at 18-weeks p.i., which appeared worse than that seen at 4-weeks p.i. Microglial lesions, a pathological consequence of chronic neuroinflammation, were found at a higher frequency in the brainstem and cerebellum of 18-week p.i., as compared to 4-weeks p.i. and control animals. To gain further insight into the CNS environment, we performed untargeted metabolomics on baseline and necropsy CSF collections. This revealed significant changes in 22 metabolites, including decreased cortisol and malate and increased citrate and hippurate, which suggest inflammation and alterations of the citric acid cycle within the CNS compartment. **Conclusions**: Our findings suggest significant neuroinflammation and possible altered and/or impaired energy metabolism underly the neurological symptoms that continue well after infection.