The microglial NLRP3 inflammasome is involved in human SARS-CoV-2 cerebral pathogenicity: A report of three post-mortem cases

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Abstract

We herein report, by using confocal immunofluorescence, the localization of the SARS-CoV-2 nucleocapsid within neurons, astrocytes, oligodendrocytes and microglia in three deceased COVID-19 cases, of between 78 and 85 years of age at death. The viral nucleocapsid was detected together with its ACE2 cell entry receptor, as well as the NLRP3 inflammasome in cerebral cortical tissues. It is noteworthy that NLRP3 was colocalized with CD68 + macrophages in the brain and lung of the deceased, suggesting the critical role of this type of inflammasome in SARS-CoV-2 lesions of the nervous system/lungs and supporting its potential role as a therapeutic target. It is widely recognized that patients with coronavirus disease 2019 (COVID-19) present diverse neurological injuries leading to long-term sequels, but the pathogenic mechanisms involved are still largely unknown (Azizi and Azizi, 2020). Using confocal immunofluorescence analysis (Supplementary Materials and Methods), we report the potential role of NLRP3 inflammasome in brain pathogenicity of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) in three deceased patients with COVID-19. Importantly, the viral nucleocapsid (NC) protein was localized in a variety of typical cells of the central nervous system (CNS), that were identified using antibodies against NeuN (neurons), GFAP (astrocytes), CNPase (oligodendrocytes) and Iba-1 (microglia);In contrast, immunostaining was not detected with only secondary fluorescent probes-conjugated antibodies without primary antibodies in postmortem brain samples from COVID-19 deceased (Supp 1).Next, potential mediators of the pathogenicity of this virus were identified in the human CNS. Interestingly, NC colocalized with the cell entry receptor of SARS-CoV-2, Angiotensin-Converting Enzyme 2 (ACE2), in the brain tissue .Notably, co-immunostaining of NC with a key player of the neuroinflammatory axis: the inflammasome NLRP3 was also observed. Furthermore, NLRP3 was co-detected with CD68, a monocyte/macrophage and microglia marker. To determine whether similar viral pathogenic features were observed in lungs and brain, these viral and immune markers were studied in lungs.Remarkably,NC protein colocalized with NLRP3 and CD68 in Postmortem lung samples. In contrast, no immunostaining was detected for NC in a lung sample of a person who died from a cause non-related to COVID-19 (Supp 12). Moreover, NLRP3 localized with CD68+ cells suggesting that SARS-CoV-2 may regulate the functions of NLRP3 in monocytes/macrophages from lungs and brain. The pathogenesis of virus-related damage to lungs was evidenced by microscopic histochemical examination, showing features of predominant interstitial fibrosis. It is noteworthy that colocalization of NC with markers of CNS's tropism and immune responses, was observed in all deceased COVID-19 patients, evidencing the high cerebral virulence of the SARS-CoV-2. On the other hand, in a series of 41 postmortem cases, microglial activation was found in 80.5% of them (34/41), confirming the important role of this CNS cell type, as well as the infiltration of T cells (38/41 cases) in cerebral SARS-CoV-2 injuries (Thakur et al., 2021). Moreover, brain samples (28/41 cases) had either low viral RNA titers or non-detectable levels of viral structural proteins (Thakur et al., 2021).This fact may reflect the extensive cerebral injuries secondary to viral neuroinvasion. Under these conditions, the capacity of the virus to replicate in brain host cells will be limited, given the CNS heterogeneous expression of genes relevant to viral entry (Matschke et al., 2020), and due to virus clearance by the neuroinflammatory response subsequent to the acute CNS attack (Dogra et al., 2021). In this sense, in a patient who died from COVID-19 with a severe neuropathological condition (large acute cerebral infarction) indisputably related to this disease, the viral RNA was not detected in any of the 16 brain regions studied (Serrano et al., 2021). Therefore, these studies suggest that SARS-CoV-2 CNS infection, replication and clearance mechanisms precede the worsening of COVID-19 neurological complications such as infarctions, hemorrhages and neurodegenerative processes; indicating that appropriate neurotherapeutic interventions should start as early as possible once the virus is detected.

NLRP3 inflammasome activation and the subsequent production of IL-1β in dysregulated reactive microglia, has been associated with CNS pathology (Barclay and

Shinohara, 2017). Accordingly, our findings shed light on the plausible pathogenic role, as well as the possible pharmacological targeting, of this intracellular multiprotein complex on SARS-CoV-2 brain damage. We co-detected NLRP3 expression along with CD68 in brain samples, a marker previously related to the ramified state of activated microglia, possibly indicating its phagocytic activity (Hendrickx et al., 2017). Previously, higher NLRP3 levels were also observed in the lungs, in agreement with our observations, indicating that the activation of this inflammasome type may be a crucial SARS-CoV-2 pathogenicity mediator in the lungs and brain (Rodrigues et al., 2020). In the lungs, CD68+ macrophages have been observed within the alveolar space in 10/12 biopsies of COVID-19 patients, taken within 20 days of symptoms onset (Doglioni et al., 2021). These pieces of evidence support the hypothesis of early NLRP3 inflammasome activation in SARS-CoV-2 infected macrophages (lungs) and microglia (brain), promoting the progression to complications in the respiratory and nervous systems, respectively (Brodin, 2021); possibly in a cumulative way, along with its well-known cytokine release syndrome (Moore and June, 2020). Interestingly, an added factor to this puzzle is the bidirectional communication between these organs, as NLRP3 inflammasome components may reach the brain through extracellular vesicles, also possibly carrying SARS-CoV-2 viral particles, coming from infected lungs, and vice versa (Kerr et al., 2018). Considering this, the specific inhibition of NLRP3 inflammasomes could be a rational approach for improving the COVID-19 severity conditions and its CNS-associated injuries. Indeed, several biotechnological and synthetic candidates targeting NLRP3 are already under development (Freeman and Swartz, 2020); while alternative strategies for inhibiting NLRP3 have also been proposed, such as the natural-occurring tetrapyrrolic compound Phycocyanobilin (McCarty et al., 2021). In this sense, cumulative experimental evidence strongly supports the safe application of this compound for COVID-19-induced damage to the nervous system (Pentón-Rol et al., 2021). Moreover, pharmacological synergy may be achieved by combining such NLRP3 inhibition approach with therapies that can restrict the cytokine release syndrome in severe COVID-19 patients, such as the recently described CIGB-258 peptide.

In a broad perspective, our evidence raises the question of whether SARS-CoV-2 may also activate other inflammasome types that are present in neurons or astrocytes, such as NLRP1 and AIM2, or NLRP2, respectively (deRiveroVaccari et al., 2014). These are relevant topics worth investigating.Further studies with more cases and age/sex-matching controls are also needed for the generalization of our findings. Nonetheless, thispaper gives novel clues for dissecting the complex neuroimmunological mechanisms of SARS-CoV-2-induced injury of the human CNS.

BIOGRAPHY

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