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Neurotropism and blood-brain barrier involvement in COVID-19

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The global pandemic of coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) persists despite the progress of vaccination and increased natural immunity. SARS-CoV-2 is associated not only with pneumonia and acute respiratory distress, but also with many symptoms related to the central nervous system (CNS), including loss of the sense of taste and smell, headache, convulsions, visual disturbances, and impaired consciousness. In addition, the virus has been implicated in CNS diseases such as cerebral hemorrhage, cerebral infarction, and encephalitis. SARS-CoV-2 binds to the receptor angiotensin-converting enzyme 2 (ACE2), which is used by the virus as a cell entry receptor. Although the mechanism by which SARS-CoV-2 enters the brain is still unclear, the possibility of direct entry through the olfactory nerve tract and entry into the brain through the blood-brain barrier (BBB) and blood-cerebrospinal fluid barrier (BCSFB) *via* blood circulation is indicated. The BBB likely serves as a site of entry for SARS-CoV-2 into the brain, and possibly contributes to the CNS symptoms of COVID-19 due to its dysfunction as a result of SARS-CoV-2 infection. The present review will focus on the effects of COVID-19 on the CNS, particularly on the BBB related cells involved.

KEYWORDS

COVID-19, SARS-CoV-2, blood-brain barrier, olfactory nerve, CNS

1 Introduction

Coronavirus disease 2019 (COVID-19) is a widespread pandemic caused by the highly contagious severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that has not been eradicated despite much time and vaccination progress. Many variants have been reported, and a variety of symptoms observed, often focusing on respiratory dysfunction, but it has also been shown to cause disorder in many other organs. Most patients with COVID-19 present mild to moderate symptoms, with the most common symptoms occurring as fever, upper respiratory tract symptoms such as dry cough and sore throat and headache, although some who are infected can remain asymptomatic. However, only a small percentage of cases progress to severe stages, leading to severe pneumonia, multiorgan dysfunction, and in the worst cases, death (Huang et al., 2020; Pascarella et al., 2020; Shi et al., 2020). An early review of data from the pandemic showed

that approximately 5% of COVID-19 patients had severe symptoms, such as acute respiratory distress syndrome (ARDS) and multiorgan-dysfunction (Wiersinga et al., 2020; Rahman et al., 2021), and the mortality was estimated to be around 0.15%–1.63%, with a median of 0.27% ((Ioannidis 2020; AuthorAnonymous, 2021).

Neurological symptoms in COVID-19 patients have been noted since the early days of the disease. Common neurological symptoms include headache, vomiting and dizziness, in addition to a characteristic loss of the senses of taste and smell (Menni et al., 2020; Wang et al., 2020). Interestingly, even in the absence of typical symptoms of COVID-19, SARS-CoV-2 can cause neurological disorders such as encephalitis, Guillain-Barré syndrome and seizures (Karadaş, Öztürk, and Sonkaya 2020; Nalleballe et al., 2020; Johansson et al., 2021). In severe cases, acute cerebrovascular disorders and impaired consciousness have been reported (Mao et al., 2020). The severity of COVID-19 has been implicated in the increased risk of subsequent neurological and psychiatric outcomes, involving mechanisms such as direct brain damage associated with viral entry into the CNS and induction of BBB dysfunction, as well as the neural effects of the immune response, inflammation, and hypercoagulability (Shehata et al., 2021; Taquet et al., 2021). This review summarizes the understanding of CNS dysfunction from the viewpoint of the blood-brain barrier (BBB) with SARS-CoV-2 infection and COVID-19.

2 Characteristics of SARS-COV-2

Many human coronaviruses cause respiratory diseases; some are endemic and among the causes of the common cold, whereas others, such as SARS-CoV and MERS-CoV, have caused serious epidemics with high mortality rates. SARS-CoV-2, like SARS-CoV and MERS-CoV, was likely first transmitted to humans *via* exposure to an infected animal, although all three can pass from human-to-human. The main structure of SARS-CoV-2 is composed of four proteins, Spike (S), Envelop (E), Nucleocapsid (N), and Membrane (M). The S protein plays the major role in cell binding and invasion (Kumar and Al Khodor 2020; Wu et al., 2020). The S protein is a transmembrane protein with spike-like projections and is characterized by two extracellular subunits S1 and S2; the S1 subunit facilitates receptor-binding, and the S2 subunit mediates membrane fusion and internalization of the virion (Du et al., 2009). The S protein promotes viral attachment to angiotensin-converting enzyme 2 (ACE2), thus fusing with the membrane and allowing the virus to enter the cell. SARS-CoV-2 closely resembles SARS-CoV virus that also causes severe acute respiratory syndrome (SARS), but several mutations in the receptor-binding region (RBD) of the S1 protein have greatly enhanced the binding affinity of the SARS-CoV-2 virus to ACE2. Such differences can be the basis for the high infectivity of COVID-19 (Angeletti et al., 2020; Tai et al., 2020). The S protein entry into target cells requires protein priming with cellular proteases, which has been shown to be mediated by the cellular transmembrane serine protease 2 (TMPRSS2) (Glowacka et al., 2011; Hoffmann et al., 2020). SARS-CoV-2 mainly infects cells in the respiratory tract, but infection of the CNS has also been observed and the mechanism has become clarified (3). Quantification of the virus in patients who died from COVID-19 indicated the neurotropic potential of the virus (Matschke et al., 2020; Puelles et al., 2020; Solomon et al., 2020; Serrano et al., 2021), and other evidence has been presented from cells, organoid models, and animals. Primary human brain endothelial cells were shown to be productively infected with SARS-CoV-2 by expressing ACE2 *via* lentivirus transduction (Nascimento Conde et al., 2020). Experiments on 3D human brain organoids similarly show that SARS-CoV-2 can enter the cells and have the potential neurotoxic effect (Ramani et al., 2020; Song et al., 2021). Several experiments using animal models, mainly mice, also indicate evidence for viral entry into the brain. Infection models using transgenic mice such as hACE and Hfh4-ACE2 mice have shown evidence for the presence of viral RNA in the brain (Menachery et al., 2016; Bao et al., 2020; Dinnon et al., 2020; Dong et al., 2022). Studies of non-human primate infection models in Rhesus macaques and African green monkeys similarly detected viral RNA in the brain, while pathological findings of neuroinflammation, microhemorrhage, and neuronal damage were obtained, suggesting that SARS-CoV-2 infection of the CNS may cause some of its neurological symptoms (Rutkai et al., 2022).

2.1 SARS-CoV-2 entry into the central nervous system *via* the olfactory pathway

Clinical symptoms, such as olfactory disorders, and experimental data suggest that SARS-CoV-2 may invade the CNS *via* the olfactory sensory neurons, although it is also likely that the virus crosses the BBB and enters the CNS. As for SARS-CoV-2 entry into the CNS *via* olfactory neurons, it has been posited that the virus can travel along their axons into the olfactory bulbs and beyond to other parts of the brain (Bougakov, Podell, and Goldberg 2021; Burks et al., 2021). The pathway of axonal transport through the olfactory nerve begins with infection of the olfactory epithelial cells *via* ACE2 receptors and spreads to the CNS by traversing synapses shared between olfactory neurons and neurons in the olfactory bulb (OB). Data from RNA mapping of the olfactory nerve tract to the CNS region of SARS-CoV-2 infection from human autopsy material confirms viral amplification along the olfactory nerve tract (Meinhardt et al., 2021). Neuropilin-1 (NRP-1) is a multifunctional transmembrane receptor that affects development of axons and is suggested to contribute to the

entry of SARS-CoV-2 into the brain *via* the olfactory epithelium (Perez-Miller et al., 2021). NRP-1 increases the infectivity of viruses in olfactory epithelial cells, and the high expression of NRP-1 in the olfactory epithelium of COVID-19 patients suggests that NRP-1 plays an important role in the entry of viruses through the olfactory pathway (Cantuti-Castelvetri et al., 2020). However, several studies have been presented that provide limited support for this pathway of viral entry. A kinetics study of S1 uptake by brain found that the olfactory rate was much lower than the rate of transport across the BBB(Rhea et al., 2021). Also, when SARS-CoV-2 is detected in olfactory neurons, it is mostly present in immature neurons, which lack the axonal projections necessary to transport the virus to the brain. Therefore, it is controversial whether the olfactory route is the main pathway by which SARS-CoV-2 enters the brain (Butowt et al., 2021; Zhang, Lee, et al., 2021).

2.2 SARS-CoV-2 and the blood-brain barrier/blood-cerebrospinal fluid barrier

Another promising route of SARS-CoV-2 entry into the brain is *via* the hematogenous route, crossing the BBB and/or blood-cerebrospinal fluid barrier (BCSFB). The interactions between SARS-CoV-2 and the BBB are multifactorial in that the virus may cross the BBB, infect brain endothelial cells, or alter functions of the BBB, with data suggesting that several receptors and signaling cascades are involved (Torices et al., 2021; Haidar et al., 2022; Krasemann et al., 2022). The BBB is primarily composed of brain endothelial cells, which are closely associated with other cells such as the pericytes and astrocytes that support the BBB endothelial phenotype. Brain endothelial cells express tight junction proteins that inhibit the diffusion of substances between cells, and a low number of pinocytic vesicles that prevents transcellular diffusion. Thus, viruses that can enter the CNS *via* the BBB must do so either by being transported *via* processes such as adsorptive transcytosis, or by altering BBB properties which then permits their direct entry (Erickson et al., 2021). The BCSFB, formed from epithelial cells of the choroid plexus, is also a barrier separating the central and peripheral systems and is considered a potential entry route of SARS-CoV-2. Experiments evaluating the neurotropism of the virus using an organoid model of choroid plexus epithelial cells showed that SARS-CoV-2 selectively infects choroid plexus epithelial cells and also disrupts BCSFB (Pellegrini et al., 2020). However, SARS-CoV-2 is variably detected by PCR in CSF samples from COVID-19 patients, including those with neurological symptoms (Jarius et al., 2022), indicating that most of the virus may be sequestered in the brain tissue. Since CNS entry *via* the BCSFB would first require SARS-CoV-2 to enter the CSF, it is controversial to consider the BCSFB as the main entry route.

Several studies have focused on the S protein, the main component of the virus, and its interactions with the BBB. In early experiments using 2D static and 3D microfluidic *in vitro* BBB models to verify the relation between the BBB and the proteins that compose SARS-CoV-2, including S protein, showed that those proteins disrupt the integrity of the barrier function of the BBB (Buzhdygan et al., 2020). However, the dosage of S protein used in the study are variable, with some higher than S protein concentrations typically detected in blood (Ogata et al., 2020), they range from 0 to 25 ng/ml and in most cases less than 1 ng/ml, so careful consideration is needed. Experiments that injected intravenously radioiodinated S1 protein showed that S1 is taken up into the brain much better in comparison to the intranasal route. This shows that S1 readily crosses the BBB, and additional studies suggest the mechanism involved is adsorptive transcytosis (Rhea et al., 2021). The same study also found that inflammation induced by bacterial lipopolysaccharide (LPS) caused some minor leakage of S1 across the BBB, but did not alter the transport mechanism which accounted for most of the S1 entry into brain. In mouse and hamster models that are permissive to SARS-CoV-2 infection, the virus caused no obvious ultrastructural or expression change in BBB tight junction proteins but altered the basement membrane and caused Evans blue dye leakage into the brain, indicating that there was BBB disruption. The virus infected brain endothelial cells, upregulated inflammatory cytokine expression, and crossed the BBB *in vitro*, although it caused only a moderate amount of paracellular disruption in hamster endothelial cells, suggesting that the virus crossed the BBB *via* transcellular mechanism s and without causing paracellular leakage (Zhang, Zhou, et al., 2021). Therefore, SARS-CoV-2 likely exerts effects on the BBB *via* its direct interactions with brain endothelial cells and indirectly through the immune system. However, it should be carefully considered that SARS-CoV-2 have low replication capacity in brain endothelial cells without inflammatory conditions or overexpression of ACE2 (Constant et al., 2021; Schimmel et al., 2021). Evidence supports that both the virus and its spike proteins can cross the BBB and enter the brain. Effects of S1 and SARS-CoV-2 on BBB as mediated through other types of brain cells are ongoing.

2.2.1 Interaction of SARS-CoV-2 and brain endothelial cells

Brain endothelial cells are thought to play an important role in SARS-CoV-2 invasion because they face circulatory dynamics and express receptors associated with viral invasion, such as ACE2 and TMPRSS2 (Baig et al., 2020). SARS-CoV-2 infects endothelial cells *via* ACE2 receptors without apparent the BBB disruption and enters the brain, simultaneously, it is suggested that SARS-CoV-2 infection causes endotheliitis in several organs, which leads to the BBB dysfunction and enables the virus to enter the brain directly (Varga et al., 2020). That human brain endothelial cells express ACE2 is supported by experiments using primary human brain endothelial cells and the hCMEC/D3 cell line, which also showed that SARS-CoV-2 can infect and

proliferate in brain endothelial cells (Buzhdygan et al., 2020; Zhang, Zhou, et al., 2021). Interestingly, the results of these studies showed that SARS-CoV-2 infection of brain endothelial cells did not cause any gross phenotypic alterations and did not affect tight junction protein integrity. The host protein nuclear factor (NF)-κB essential modulator (NEMO), which is involved in signalling cascades that regulate the transcription of numerous genes, regulates cell viability, and SARS-CoV-2 caused NEMO cleavage and cell apoptosis in brain endothelial cells, leading to possible vulnerability of the BBB (Wenzel et al., 2021).

Several studies suggest that the interactions of brain endothelial cells and SARS-CoV-2 could be increased with certain comorbid conditions. Cerebral ischemia, smoking, and diabetes have been shown to enhance ACE2 receptor expression in brain endothelial cells, supporting that these factors could potentially increase the ability of SARS-CoV-2 to infect or cross brain endothelial cells and thus increase the risk of COVID-19-associated neurological sequalae (Choi et al., 2020). Hypoxia was also shown to modulate expression of ACE2 and TMPRSS2 in human brain endothelial cells, suggesting involvement of endothelial damage caused by SARS-CoV-2 (Imperio et al., 2021). The results suggest that COVID-19 could be implicated in the induction of inflammation and apoptosis of brain endothelial cells involved in enhanced thrombus formation in patients with severe COVID-19 (Pons et al., 2020), which may cause cerebrovascular disease. Beta-secretase 1 (BACE1), an aspartic acid protease, is a transmembrane protein that can contribute to the loss of integrity of the BBB (Cheng et al., 2014). SARS-CoV-2 increased BACE1 in brain endothelial cells, resulting in decreased expression of tight junction proteins and accumulation of senescence-associatedβ-gal and p21, which are involved in cellular senescence. These results support the possibility that SARS-CoV-2 causes BBB vulnerability and senescence of brain endothelial vascular cells resulting in cerebrovascular disease (Choi et al., 2022).

2.2.2 Interaction of SARS-CoV-2 and the bloodbrain barrier related cells

Astrocytes have an important role in the induction and maintenance of the BBB phenotype and are anatomically close to brain endothelial cells. Several studies indicate that astrocytes can be infected by SARS-CoV-2. Of the samples that showed pathological signs of brain damage in autopsy patients with COVID-19, particularly in astrocytes, lesions from viral replication were observed (Crunfli et al., 2022). The same study also found that the susceptibility to infection *via* NRP-1 was shown in human astrocytes derived from neural stem cells, emphasizing the possibility that astrocytes may contribute to CNS infection Plasma biomarker testing of patients with COVID-19 showed increased glial fibrillary acidic protein (GFAP), a marker of astrocyte activity and damage (Kanberg et al., 2020). This finding is significant in severe COVID-19 patients and could be involved in the development of brain disorders in these patients. In the data investigating gene expression in astrocytes after SARS-CoV-2 infection, subpopulations of astrocytes were identified in which the expression of genes involved in inflammation and neurotoxicity, such as IFITM3, GFAP, and CHI3L1, were upregulated, and dysregulation of genes supporting neurotransmission was also observed (Yang et al., 2021). Additionally, infection and viral replication induced in human astrocytes from SARS-CoV-2 exposure was confirmed, indicating extensive inflammation and cytokine secretion. Interestingly, the ACE2 receptor was not identified in these astrocytes, suggesting that other invasion factors, DPP4 and CD147, are closely involved (Andrews et al., 2022). Brain pericytes surround blood vessels and play an important role in maintaining the BBB, including vessel maturation and stabilization, neuroprotection and repair in the event of damage. ACE2 has been shown to be abundantly expressed in platelet-derived growth factor receptor (PDGFR) β perivascular cells, which consist mainly of pericytes, and has been implicated in possible SARS-CoV-2 infection. Infection of pericytes in COVID-19 patients was associated with perivascular inflammation and fibrinogen leakage, suggesting that the integrity of the BBB is compromised (Bocci et al., 2021). S protein exposure increased ACE2 expression and triggered an inflammatory response in pericytes Additional evidence indicates that hypoxia enhances S protein related effects on brain pericytes, and that SARS-CoV-2 can cause vascular-mediated brain damage (Khaddaj-Mallat et al., 2021). Microglia are immune cells that respond to infection and have a wide range of functions, including activation of astrocytes and regulation of neurons. Microglia clustered prominently around blood vessels in COVID-19 patients, suggesting that they induce neuroinflammation (Schwabenland et al., 2021). Microglia, as well as astrocytes, showed subpopulations associated with COVID-19, showing increased levels of several genes involved in neuroinflammation (Yang et al., 2021).

3 Discussion

Since the early stages of the COVID-19 pandemic, neurological symptoms such as loss of sense of smell and taste have been noted, and many studies have focused on that aspect. Neurological complications are particularly common in severely ill COVID-19 patients, and residual effects are observed even after symptoms have abated (Helms et al., 2020). Serious brain disorders such as cerebral infarction, encephalitis, acute toxic encephalopathy, and epileptic seizures have also been reported in many cases (Roy et al., 2021). Recent studies have reported Long-COVID syndrome and identified several neurological signs, notably brain fog, suggesting neuroinflammation is involved (Theoharides et al., 2021). This could be related to damage to

brain endothelial cells caused by S protein and to the induction of inflammation and autoimmune reactions (Theoharides 2022).

These many accumulated reports suggest that the CNS is affected by COVID-19, and there have been various discussions regarding the mechanism, but no clear evidence has been presented. This review focused on the neurotropism of SARS-CoV-2, particularly with respect to its ability to cross the BBB and infect brain endothelial cells, astrocytes, and pericytes. Numerous papers indicate that the BBB is involved in COVID-19 infection of the CNS and that SARS-CoV-2 alters the homeostatic functions of the BBB. Therefore, the BBB is an ideal target for drug development aimed at preventing or facilitating recovery from neurological damage and disease caused by SARS-CoV-2.

Author contributions

TF performed the literature review, interpreted the data, and prepared the manuscript. ME and WB wrote or contributed to the writing of the manuscript.

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Conflict of interest

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