UJEMI PERSPECTIVES

SARS-CoV-2 potential pathways of neuroinvasion and the role of astrocytes in neuroinvasion

Garshana Rajkumar

Department of Microbiology and Immunology, University of British Columbia, Vancouver, British Columbia, Canada

SUMMARY Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is well characterized by its respiratory infection and associated respiratory complications. However, increasing evidence has shown that the virus can affect other organ systems besides the lungs such as the brain. Disease manifestations in infected patients such as encephalitis, psychosis, cerebrovascular injury, fatigue, loss of taste, and loss of smell have been reported in over one-third of hospitalized COVID-19 patients. Moreover, MRI-based screenings have shown structural damage to the brain of patients recovering from COVID-19. Despite these reported complications, there have been few studies that have examined the neurotropism of SARS-CoV-2 and the cause of these neurological complications. One suggested cause is direct neural infection also known as neuroinvasion. Recent studies using human brain organoids and mouse models have shown the neuroinvasive capacity of SARS-CoV-2 and its ability to directly infect neurons. However, the specific mechanism by which the virus enters the central nervous system and causes damage to neurons is unknown. Furthermore, for the virus to invade the brain it must bypass the blood brain barrier. Astrocytes play a key role in the maintenance of the blood brain barrier but their involvement in SARS-CoV-2 neuroinvasion has not been fully explored. This article will examine the neurotropism of SARS-CoV-2, focusing on the potential pathways SARS-CoV-2 uses to enter neurons in the brain and what role astrocytes play in neuroinvasion. Answering these questions could provide novel insight into new potential pathways and mechanisms that may be critical for disease prevention, developing therapeutics, and understanding the long-term complications of COVID-19 also known as long COVID.

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the cause of the coronavirus disease (COVID-19), has infected millions of people globally and is well characterized by its clinical symptoms such as interstitial pneumonia, upper respiratory tract infection, and death (1). Although the virus is primarily known to target the respiratory system, increasing evidence has shown that SARS-CoV-2 has extrapulmonary effects including in the central nervous system (CNS) (2). Over one third of hospitalized COVID-19 patients develop neurological complications which range from encephalitis, psychosis, cerebrovascular injury, fatigue, loss of taste, and loss of smell (3). Despite the numerous reports of neurological complications, their direct cause is still unclear.

One proposed cause is direct neural infection and invasion of the CNS also known as neuroinvasion. In a recent study by Song *et al*, they were able to show the neuroinvasive capacity of SARS-CoV-2 through its ability to infect human brain organoid cells in culture with accompanying metabolic changes in infected and neighboring neurons (4). They were also able to demonstrate neuroinvasion *in vivo,* using mice overexpressing human angiotensin-converting enzyme 2 (ACE2) (4). Furthermore, viral RNA and proteins have been found in the brain and cerebrospinal fluid of COVID-19 patients (3). Despite this research, the mechanisms by which the virus enters the CNS and causes damage to neurons is still unknown.

Additionally, for the virus to invade the brain, it must be able to bypass the blood brain barrier (5). Astrocytes are key players in CNS infections and are essential for the maintenance

Published Online: September 2022

Citation: Rajkumar G. 2022. SARS-CoV-2 potential pathways of neuroinvasion and the role of astrocytes in neuroinvasion. UJEMI Perspectives 6:1-6

Editor: François Jean (Ph.D.), University of British Columbia

Address correspondence to Garshana Rajkumar

garshanar@gmail.com

of the blood brain barrier (BBB). As well, studies have found that the occurrence of SARS-CoV-2 in the brain is associated with significant astrogliosis, an increase in the size and number of astrocytes in response to stress (2). However, the involvement of astrocytes in SARS-CoV-2 neuroinvasion has not been fully explored.

PROPOSED RESEARCH QUESTIONS

One study revealed that more than half of hospitalized COVID-19 patients continue to have neurological complications three months after the acute stage of infection (6). Additionally, studies have confirmed impaired cognition in COVID-19 patients recovering from hospitalization (7). Therefore, it is crucial to understand the neuroinvasive capacity of SARS-CoV-2 for disease prevention and diagnosis, to find potential therapeutics and guide treatment options for patients with long-term neurological complications, and to understand the long-term complications of SARS-CoV-2 infection also known as long COVID. The mechanisms of neuroinvasion and the role that astrocytes play in response to SARS-CoV-2 neuroinvasion have yet to be fully explored. This article will build upon this knowledge gap by firstly, examining the potential pathways that SARS-CoV-2 uses to enter neurons in the brain and secondly, examining the role that astrocytes play in response to neuroinvasion.

PROPOSED PROJECT NARRATIVE

What potential pathways does SARS-CoV-2 use to enter neurons in the brain? Understanding the potential pathways of neuroinvasion is critical for interpreting the neurological manifestations of COVID-19 (8). A deeper knowledge of the pathways of neuroinvasion could provide potential avenues of therapeutics for patients with neurological symptoms and provide more insight into the long-term effects of SARS-CoV-2 infection. There have been many proposed pathways of entry into the brain, but this paper will focus on two categories: the Olfactory route and the Hematogenous route (Fig. 1).

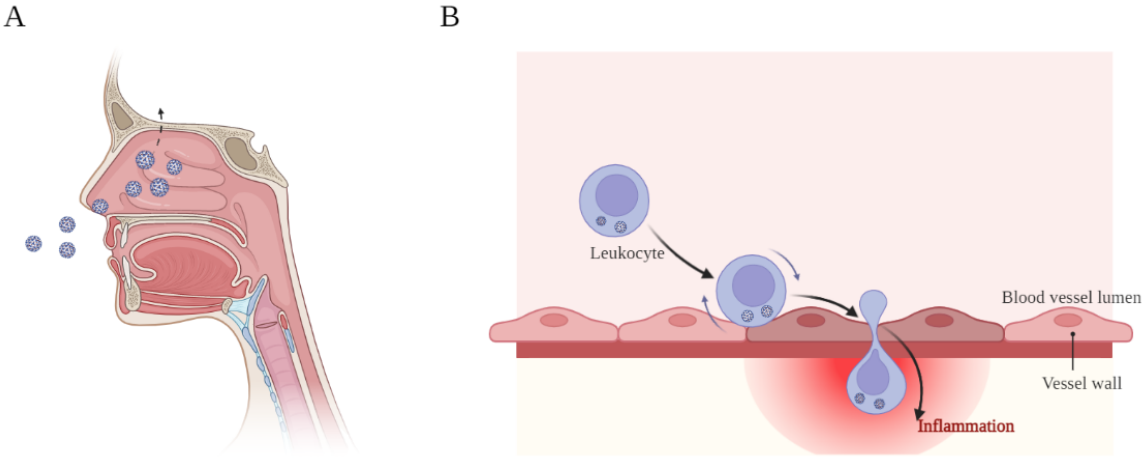

FIG. 1 Proposed olfactory and hematogenous routes of neuroinvasion. One proposed olfactory mechanism involves inhaled SARS-CoV-2 viruses infecting the olfactory mucosa to gain access to the CNS (A). One proposed hematogenous mechanism involves infected leukocytes crossing the bloodstream into the CNS. Figure created with BioRender.com.

One proposed olfactory mechanism is that the virus infects the nasal mucosa to gain access to the CNS. Recent studies have detected the presence of SARS-CoV-2 virions in the olfactory bulb and neuroepithelium of the nasal mucosa (9,10). Olfactory bulb neuroinvasion was further supported through animal models where it was shown that SARS-CoV-2 can enter the brain of mice expressing ACE2 through intranasal infection (10). In a study done by Meinhard *et al*, they were able to show that SARS-CoV-2 could access the CNS through the neural-olfactory mucosal interface and suggested from there it can enter primary respiratory and cardiovascular control centers in the medulla oblongata (10). This mechanism could

explain the cases of patients seen with damage to the lower brain stem since the medulla oblongata is housed there (10).

Another proposed olfactory mechanism is through axonal transport. It is possible that the virus infects olfactory nerves since they have been shown to express ACE2 which can interact with the SARS-CoV-2 spike protein (11). This infection is followed by trans-synaptic transmission which allows the virus to disseminate to other areas of the brain (11). Both olfactory pathways could also potentially explain the loss of smell and taste symptoms that some patients experience.

One proposed hematogenous mechanism is that SARS-CoV-2 could infect endothelial cells or leukocytes that transverse from the bloodstream into the CNS. It is thought that the virus could infect vascular endothelial cells since they express ACE2.From there, the virus has the potential to damage the BBB and create an entryway into the CNS (12, 8). In a recent study by Krasemann *et al*, their data provided strong support for SARS-CoV-2 brain entry across the BBB resulting in increased interferon signaling (3). They were able to show infection of brain endothelial cells by exposing human induced pluripotent stem cell derived brain capillary endothelial cells to SARS-CoV-2. Their results also suggested active replication and transcellular transport of the virus across the BBB (3). It has also been suggested that the virus could infect leukocytes to get past the BBB similar to what has been seen in SARS-CoV-1 and human immunodeficiency virus (13, 14, 15, 16). These proposed mechanisms might explain the endothelial damage and cerebral bleeding observed in some COVID-19 patients (13).

What role do astrocytes in the blood brain barrier play in response to SARS-CoV-2 neuroinvasion? Astrocytes are a type of glial cell that are prevalent in the CNS and contribute to its defense and homeostasis (5). They also serve as immune modulators and impact immune responses by releasing cytokines, chemokines, and growth factors (5). Astrocytes also regulate the permeability of the BBB through their influence on the expression of tight junctions in endothelial cells (5). Therefore, determining the role that astrocytes play in response to SARS-CoV-2 neuroinvasion could provide potential explanations to how the virus bypasses the BBB and explain the neurological pathophysiology of COVID-19. Furthermore, identifying the role that astrocytes play in neuroinvasion could potentially provide beneficial uses in developing diagnostics and disease prevention.

Infection with SARS-CoV-2 is known to trigger reactive astrogliosis which changes the gene expression, biochemistry, and morphology of astrocytes (17). This can shift astrocytes into a pro-inflammatory phenotype that promotes CNS damage through the release of cytokines, chemokines, and neurotoxic factors (5). These reactive astrocytes can also become facultative antigen presenting cells which attract immune cells and further contribute to immune cell infiltration and neuroinflammation (18).

In a study done by Crunfli *et al*, they found that neural stem cell-derived human astrocytes are susceptible to SARS-CoV-2 infection through a mechanism that involves the interaction between the virus's spike protein and neuropilin 1 (NRP1) (2). Their results showed that infected astrocytes underwent changes in energy metabolism and changes in key proteins and metabolites used to fuel neurons and fuel the biosynthesis of neurotransmitters (2). Furthermore, they found that SARS-CoV-2 infection elicits a secretory phenotype of astrocytes that reduces neuronal viability (2). Overall, these results suggest that SARS-CoV-2 reaches the brain, infects and alters astrocytes, then consequently leads to neuronal dysfunction (Fig 2). This deregulation of astrocyte function might also explain the structural alterations seen in the brains of COVID-19 patients and some of the observed neurological complications.

POTENTIAL IMPACT/CONCLUSIONS

As more evidence emerges, we are beginning to see increasing cases of people who contract COVID-19 and have persisting complications also known as long COVID (19). An increase in the number of patients with persisting neurological complications due to SARS-CoV-2 could potentially burden our drained health care system and might become a prevalent

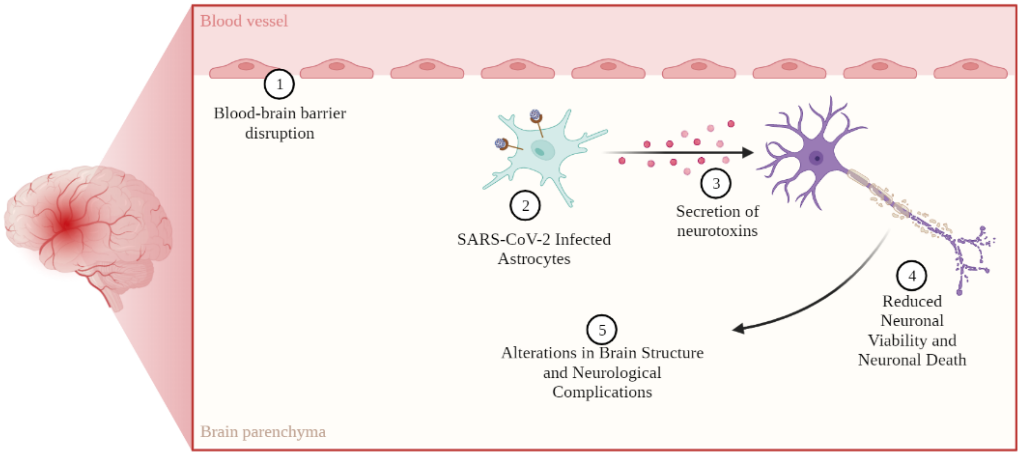

FIG. 2 Overview of the proposed mechanism to how SARS-CoV-2 causes astrocyte dysfunction. SARS-CoV-2 utilizes blood brain barrier disruption to enter the brain (1). The virus infects astrocytes using an interaction between the spike protein and neuropilin 1 receptor (2). This shifts astrocytes into a secretory phenotype that reduces neuronal viability (3,4). This process could potentially explain the structural alterations and neurological complications seen in some COVID-19 patients (5). Figure created with BioRender.com.

issue in the future. By exploring the possible pathways of neuroinvasion and the role of essential cells in the CNS like astrocytes, there is potential to alleviate this burden by developing targeted therapeutics and developing neuroprotective measures for patients with COVID-19 (20). Therefore, it might be worth considering finding ways to prevent SARS-CoV-2 neuroinvasion of the CNS and infection in astrocytes when treating COVID-19 patients.

However, with new variants of concern (VOCs) emerging, more research needs to be done to see how VOCs vary in terms of their neurological effects and complications. Clinically we see less patients experience loss of taste and smell when they are infected with Omicron when compared to Delta (21). This could potentially be due to Omicron having multiple mutations in the receptor binding motif that ACE2 and most monoclonal antibodies interact with (22). This altered interaction with ACE2 could potentially result in less infection of the olfactory mucosa and could explain the fewer cases of loss of smell seen in patients infected with this variant.

With the proposed neuroinvasion pathways using different receptors and variants having different affinities to these receptors, future research could build upon this review article by investigating this further and seeing if one variant can lead to more neurological complications compared to another. Additionally, since astrocyte infection is mediated through NRP1, future research could examine to see if VOCs with higher affinity to NRP1 lead to increased prevalence of astrocyte infection.

ACKNOWLEDGEMENTS

I would like to thank the MICB 406 teaching team, Dr. François Jean and Dr. Andrea Olmstead for their endless support and guidance throughout this paper. I would also like to thank my MICB 406 peers for providing feedback and for the great discussions during our brainstorming sessions.

REFERENCES

1. **Maiese A, Manetti AC, Bosetti C, Del Duca F, La Russa R, Frati P, Di Paolo M, Turillazzi E, Fineschi V.** 2021. SARS-Cov-2 and the brain: A review of the current knowledge on neuropathology in Covid-19. Brain Pathology **31**.
2. **Crunfli F, Carregari VC, Veras FP, Vendramini PH, Valença AGF, Antunes ASLM, Brandão-Teles C, Zuccoli Gda S, Reis-de-Oliveira G, Silva-Costa LC, Saia-Cereda VM, Smith BJ, Codo AC, Souza GFde, Muraro SP, Parise PL, Toledo-Teixeira DA, Castro ÍMSde, Melo BMS, Almeida GM, Firmino EMS, Paiva IM, Silva BMS, Guimarães RM, Mendes ND, Ludwig RG,**

Ruiz GP, Knittel TL, Davanzo GG, Gerhardt JA, Rodrigues PB, Forato J, Amorim MR, Silva NB, Martini MC, Benatti MN, Batah S, Siyuan L, João RB, Silva LS, Nogueira MH, Aventurato ÍK, Brito MRde, Alvim MKM, Júnior JRda S, Damião LL, Maria Ercilia de Paula Castilho Stefano, Sousa IMPde, Rocha EDda, Gonçalves SM, Silva LHLda, Bettini V, Campos BMde, Ludwig G, Tavares LA, Pontelli MC, Viana RMM, Martins R, Vieira AS, Alves-Filho JC, Arruda E, Podolski-Gondim G, Santos MV, Neder L, Cendes F, Louzada-Junior P, Oliveira RD, Cunha FQ, Damásio A, Vinolo MAR, Munhoz CD, Rehen SK, Nakaya HI, Mauad T, Duarte-Neto AN, Silva LFFda, Dolhnikoff M, Saldiva P, Farias AS, Moraes-Vieira PMM, Fabro AT, Sebollela AS, Módena JLP, Yasuda CL, Mori MA, Cunha TM, Martins-de-Souza D. 2021. SARS-COV-2 infects brain astrocytes of COVID-19 patients and impairs neuronal viability. medRxiv. Cold Spring Harbor Laboratory Press.

3. **Krasemann S, Haferkamp U, Pfefferle S, Woo MS, Heinrich F, Schweizer M, Appelt-Menzel A, Cubukova A, Barenberg J, Leu J, Hartmann K, Thies E, Littau JL, Sepulveda-Falla D, Zhang L, Ton K, Liang Y, Matschke J, Ricklefs F, Sauvigny T, Sperhake J, Fitzek A, Gerhartl A, Brachner A, Geiger N, König E-M, Bodem J, Franzenburg S, Franke A, Moese S, Müller F-J, Geisslinger G, Claussen C, Kannt A, Zaliani A, Gribbon P, Ondruschka B, Neuhaus W, Friese MA, Glatzel M, Pless O.** 2022. The blood-brain barrier is dysregulated in covid-19 and serves as a CNS entry route for SARS-COV-2. Stem cell reports. Elsevier.
4. **Song E, Zhang C, Israelow B, Lu-Culligan A, Prado AV, Skriabine S, Lu P, Weizman O-E, Liu F, Dai Y, Szigeti-Buck K, Yasumoto Y, Wang G, Castaldi C, Heltke J, Ng E, Wheeler J, Alfajaro MM, Levavasseur E, Fontes B, Ravindra NG, Van Dijk D, Mane S, Gunel M, Ring A, Kazmi SAJ, Zhang K, Wilen CB, Horvath TL, Plu I, Haik S, Thomas J-L, Louvi A, Farhadian SF, Huttner A, Seilhean D, Renier N, Bilguvar K, Iwasaki A**. 2021. Neuroinvasion of SARS-COV-2 in human and Mouse Brain. Journal of Experimental Medicine. The Rockefeller University Press.
5. **Tavčar P, Potokar M, Kolenc M, Korva M, Avšič-Županc T, Zorec R, Jorgačevski J**. 1AD. Neurotropic viruses, astrocytes, and COVID-19. Frontiers. Frontiers.
6. **Lu Y, Li X, Geng D, Mei N, Wu P-Y, Huang C-C, Jia T, Zhao Y, Wang D, Xiao A, Yin B**. 2020. Cerebral micro-structural changes in COVID-19 patients – an MRI-based 3-month follow-up study. EClinicalMedicine **25**:100484.
7. **Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, Xiang J, Wang Y, Song B, Gu X, Guan L, Wei Y, Li H, Wu X, Xu J, Tu S, Zhang Y, Chen H, Cao B**. 2020. Clinical course and risk factors for mortality of adult inpatients with covid-19 in Wuhan, China: A retrospective cohort study. Lancet (London, England). Elsevier Ltd.
8. **McQuaid C, Brady M, Deane R**. 2021. SARS-COV-2: Is there neuroinvasion? - fluids and barriers of the CNS. BioMed Central. BioMed Central.
9. **Morbini P, Benazzo M, Verga L, Pagella FGM, Mojoli F, Bruno R, Marena C**. 2020. Ultrastructural evidence of direct viral damage to the olfactory complex in patients testing positive for SARS-COV-2. JAMA Otolaryngology–Head & Neck Surgery **146**:972.
10. **Meinhardt J, Radke J, Dittmayer C, Franz J, Thomas C, Mothes R, Laue M, Schneider J, Brünink S, Greuel S, Lehmann M, Hassan O, Aschman T, Schumann E, Chua RL, Conrad C, Eils R, Stenzel W, Windgassen M, Rößler L, Goebel H-H, Gelderblom HR, Martin H, Nitsche A, Schulz-Schaeffer WJ, Hakroush S, Winkler MS, Tampe B, Scheibe F, Körtvélyessy P, Reinhold D, Siegmund B, Kühl AA, Elezkurtaj S, Horst D, Oesterhelweg L, Tsokos M, Ingold-Heppner B, Stadelmann C, Drosten C, Corman VM, Radbruch H, Heppner FL**. 2020. Olfactory transmucosal SARS-COV-2 invasion as a port of central nervous system entry in individuals with covid-19. Nature News. Nature Publishing Group.
11. **Yachou Y, El Idrissi A, Belapasov V, Ait Benali S**. 2020. Neuroinvasion, neurotropic, and neuroinflammatory events of SARS-COV-2: Understanding the neurological manifestations in COVID-19 patients. Neurological Sciences **41**:2657–2669.
12. **Zhang L, Zhou L, Bao L, Liu J, Zhu H, Lv Q, Liu R, Chen W, Tong W, Wei Q, Xu Y, Deng W, Gao H, Xue J, Song Z, Yu P, Han Y, Zhang Y, Sun X, Yu X, Qin C**. 2021. SARS-COV-2 crosses the blood–brain barrier accompanied with basement membrane disruption without tight junctions alteration. Nature News. Nature Publishing Group.
13. **Jha NK, Ojha S, Jha SK, Dureja H, Singh SK, Shukla SD, Chellappan DK, Gupta G, Bhardwaj S, Kumar N, Jeyaraman M, Jain R, Muthu S, Kar R, Kumar D, Goswami VK, Ruokolainen J, Kesari KK, Singh SK, Dua K**. 2021. Evidence of coronavirus (COV) pathogenesis and emerging pathogen SARS-COV-2 in the nervous system: A review on neurological impairments and Manifestations - Journal of Molecular Neuroscience. SpringerLink. Springer US.
14. **Spiegel M, Schneider K, Weber F, Weidmann M, Hufert FT**. 2006. Interaction of severe acute respiratory syndrome-associated coronavirus with dendritic cells. Journal of General Virology **87**:1953–1960.
15. **Nicholls JM, Butany J, Poon LL, Chan KH, Beh SL, Poutanen S, Peiris JS, Wong M**. 2006. Time course and cellular localization of SARS-COV nucleoprotein and RNA in lungs from fatal cases of SARS. PLoS Medicine 3.

16. **Trojanowicz B, Ulrich C, Kohler F, Bode V, Seibert E, Fiedler R, Girndt M**. 2016. Monocytic angiotensin-converting enzyme 2 relates to atherosclerosis in patients with chronic kidney disease. Nephrology Dialysis Transplantation.
17. **Lee M-H, Perl DP, Nair G, Li W, Maric D, Murray H, Dodd SJ, Koretsky AP, Watts JA, Cheung V, Masliah E, Horkayne-Szakaly I, Jones R, Stram MN, Moncur J, Hefti M, Folkerth RD, Nath A**. 2021. Microvascular injury in the brains of patients with covid-19. New England Journal of Medicine **384**:481–483.
18. **Colombo E, Farina C**. 2016. Astrocytes: Key regulators of neuroinflammation. Trends in Immunology **37**:608–620.
19. **Taquet M, Dercon Q, Luciano S, Geddes JR, Husain M, Harrison PJ**. Incidence, co-occurrence, and evolution of long-COVID features: A 6-month retrospective cohort study of 273,618 survivors of covid-19. PLOS Medicine. Public Library of Science.
20. **Newcombe VF, Dangayach NS, Sonneville R**. 2021. Neurological complications of covid-19. Intensive Care Medicine **47**:1021–1023.
21. **Boscolo-Rizzo P, Tirelli G, Meloni P, Hopkins C, Madeddu G, De Vito A, Gardenal N, Valentinotti R, Tofanelli M, Borsetto D, Lechien JR, Polesel J, De Riu G, Vaira LA**. 2022. Covid-19-related smell and taste impairment with widespread diffusion of SARS-COV-2 omicron variant.
22. **Kannan SR, Spratt AN, Sharma K, Chand HS, Byrareddy SN, Singh K**. 2021. Omicron Sars-COV-2 variant: Unique features and their impact on pre-existing antibodies. Journal of Autoimmunity. Academic Press.