Abstract: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) is identified as the cause of coronavirus disease 2019 (COVID-19), and is often linked to extreme inflammatory responses by over activation of neutrophil extracellular traps (NETs), cytokine storm, and sepsis. These are robust causes for multi-organ damage. In particular, potential routes of SARS-CoV2 entry, such as angiotensin-converting enzyme 2 (ACE2), have been linked to central nervous system (CNS) involvement. CNS has been recognized as one of the most susceptible compartments to cytokine storm, which can be affected by neuropilin-1 (NRP-1). ACE2 is widely-recognized as a SARS-CoV2 entry pathway; However, NRP-1 has been recently introduced as a novel path of viral entry. Apoptosis of cells invaded by this virus involves Fas receptor–Fas ligand (FasL) signaling; moreover, Fas receptor may function as a controller of inflammation. Furthermore, NRP-1 may influence FasL and modulate cytokine profile. The neuro-immunological insult by SARS-CoV2 infection may be inhibited by therapeutic approaches targeting soluble Fas ligand (sFasL), cytokine storm elements, or related viral entry pathways. In the current review, we explain pivotal players behind the activation of cytokine storm that are associated with vast CNS injury. We also hypothesize that sFasL may affect neuroinflammatory processes and trigger the cytokine storm in COVID-19.

Keywords: central nervous system; coronavirus; COVID-19; cytokine storm; hyperinflammation; neuropilin-1.

Introduction

The ongoing coronavirus disease 2019 (COVID-19) pandemic originated from Wuhan, China, and is one of the most important causes of mortality in the world (Petit et al. 2020; Woolf et al. 2020). Speculations regarding viral elements responsible for prevalent neurological illnesses, including Parkinson’s disease (PD) (Fazzini et al. 1992), acute disseminated encephalomyelitis (ADEM) (Reichard et al. 2020; Yeh et al. 2004), and multiple sclerosis (MS) have been explored with rising attention (Louapre et al. 2020). In particular, findings indicating that numerous variants of coronaviruses (CoVs), such as HCoV-229E and HCoV-OC43 can be detected within PD, ADEM, and MS cases’ cerebri, show the presence of a bridge between neurodegenerative and viral pathologies (Arbour et al. 2000; Saleki et al. 2020; Yeh et al. 2004).

Many therapeutic interventions target viral infection and immunoregulation for clinical management of COVID-19, including Tocilizumab, Sarilumab, Siltuximab, Baricitinib, Ruxolitinib, Imatinib, and convalescent plasma (Bernal-Bello et al. 2020; Cheng et al. 2005; Didangelos 2020; López et al. 2020; Maes et al. 2020; Richardson et al. 2020; Shen et al. 2020; Xu et al. 2020a). The definition of the term “cytokine storm” has remained without a clear definition. Broadly-speaking the term refers to the hyper-inflammatory state characterized by harmful levels of cytokines, containing tumor necrosis factors (TNFs), interferons (IFNs), and interleukins (ILs) (Mehta et al. 2020; Sinha et al. 2020). Importantly, researchers have suggested several pathways and initiator components for the start of the storm mechanism, such as the angiotensin-converting enzyme (ACE)/ Angiotensin II (Ang II)/Angiotensin type-1 receptor (AT-1R) axis. Moreover, severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) has been known to take an ACE2-mediated
Cytokine storm

Definition and role of cytokine storm in COVID-19

The term “cytokine storm” has not been clearly defined. Generally, it refers to an overactive immune response that consists of the secretion of IFNs, ILs, TNFs, chemokines, or other inflammatory components. These compounds are involved in an intact innate immune reaction essential for successful clear up of pathogens. But, during cytokine storm, these mediators reach harmful levels, causing damage to self cells (Fajgenbaum and June 2020). However, differentiating a proper from an overactive inflammatory reaction in the pathophysiology of severe disease is a difficult task (Sinha et al. 2020).

Mechanisms of cytokine storm elements involvement in CNS damage of COVID-19

Renin angiotensin system (RAS) is an endocrine system recognized, mainly for its regulatory functions, including electrolyte homeostasis, body liquid volume modulation, and cardiovascular regulation of the peripheral circulation. However, brain RAS is an independent form of RAS expressed locally in the brain, which is known to be involved in brain functions and disorders. Recent evidence
points to the substantial involvement of excessive brain ACE/Angiotensin II/AT1R axis in increased activation of oxidative stress, apoptosis, and neuroinflammation that leads to neurodegeneration in several neurological diseases (Turner 2015).

Upon the binding of SARS-CoV2 S protein to ACE2, its intracellular attaching region dampens ACE2. Next, Ang II quantity is amplified in the serum, augmenting the Ang II/AT1R axis induction, which is continued through trans-signaling of the IL-6-sIL-6Ra complex, in which the gp130-regulated induction of STAT3 takes place in the epithelium of the lung. In addition to a role in the entry by ACE2, S protein has been frequently utilized in vaccine design (Rahmani et al. 2021). Although SARS-CoV2 activates nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) by PRRs, the simultaneous induction of NF-κB and STAT3 is recognized as the precise enhancer of the NF-κB activation system (the IL-6 inducer). This overactivation of NF-κB by the IL-6 amplification in the lungs leads to cytokine storm, which had been observed in critically ill COVID-19 patients (Cuervo and Grandvaux 2020; Hirano andMurakami 2020). It may be justifiable to speculate that being emptied of ACE2 and induction of ACE/Angiotensin II/AT1R axis could have a key role in the clinical status of COVID-19. Indeed, prominent circulatory amounts of Ang II were found to be significantly different in COVID-19 patients compared to the control cases, and these plasma levels of Ang II showed a linear correlation with lung damage (Saponaro et al. 2020; Turner 2015; Wu et al. 2020b). Hence, in contrast to prior clinical perspectives, RAAS inhibitors should not be discontinued in stabilized COVID-19 patients, as the abandoning of ACE inhibitors and ARBs may have damaging effects on such cases (Guo et al. 2020; Liu et al. 2020b). In a recent study, the first clinical evidence has shown that ACE inhibitors or ARB therapy in COVID-19 patients with hypertension were associated with a lower rate of disease severity and a shift towards lower IL-6 quantities (Meng et al. 2020). SARS-CoV2-related ACE2 decrease causes a series of complex and entwined molecular reactions by four axes consisting of dysregulation of the ACE2/angiotensin II/AT1R axis, attenuation of ACE2/MasR axis, increased activation of ACE2/bradykinin B1R/DABK axis, and activation of the complement cascades (Ghode et al. 2017). Also, the therapeutic effect of ACE2 in critical acute lung failure has been demonstrated in vivo (Imai et al. 2005). Moreover, the role of inflammatory cytokines storm in neurodegenerative disease and exacerbation has been well-established, indicating such mechanisms may contribute to alterations in COVID-19 patients’ status of neurological disease (Isacson 2020). The role of immune receptors involved in CNS disease in cytokine storm has been provided in Table 1.

## Major cytokine storm elements cause multi-organ damage in light of SARS-CoV2 entry pathways

### Soluble Fas ligand may interact with MMPs to induce lung damage

Studies showed that the lung infiltration and overactivation of neutrophil extracellular traps (NETs) via thrombosis, mucous secretions in the lung airways, and excessive inflammatory mediators production might contribute to organ damage, lethal blood clots, and mortality in the COVID-19 cases (Barnes et al. 2020; Middleton et al. 2020; Yao et al. 2020). Previous research has indicated that neutrophils are needed for an effective immune response against pulmonary rat CoV infection; however, these cells might as well contribute to lung injury via excessive inflammatory responses (Haick et al. 2014). Moreover, researchers have demonstrated that neutrophils induce apoptosis in lung epithelial cells by soluble Fas ligand (sFasl) (Serrao et al. 2001). The Fas system, including both membrane-bound (mFas and mFasl) and soluble (sFas and sFasl) forms, plays essential roles in apoptosis and immune regulatory responses. mFasl can be cleaved by several matrix metalloproteinases (MMPs), releasing sFasl into the extracellular environment (Nagata and Golstein 1995). sFasl inhibits the interaction between Fas and Fasl on cell surface and blocks programmed cell death (Felderhoff-Mueser et al. 2001).

On the other hand, in 2018, Margaryan et al. showed that sFasl could exhibit pro-inflammatory effects and increased neutrophil activation in patients with type 2 diabetes mellitus (T2DM) (Margaryan et al. 2018). Also, following stimulation with sFasl, the ability of neutrophils for reactive oxygen species (ROS), IL-1β, and IL-8 production is increased. Indeed, such changes introduce deregulation in the redox system in favor of pro-inflammatory activity in neutrophils (Peng et al. 2020; Wang et al. 2008; Zhang et al. 2018); Moreover, increased IL-8 production by neutrophils promotes chemotaxis of macrophages and other immune cells, and enhances metalloproteinases activity, which may further contribute to the tissue damage (Mogi et al. 2001; Ottonello et al. 1999). Corroborating the above, explorations indicated that the lung cells infected with SARS-CoV2 overexpressed neutrophil-attracting chemokines and complements, resulting in systemic inflammation and lung neutrophilia in ARDS (Blanco-Melo et al. 2020; Gralinski et al. 2018). Therefore, sFasl may be a dysfunctional agent among immunoregulatory responses against COVID-19 and can contribute to the maintenance of harmful inflammatory
processes that are involved in activation and survival of neutrophils by downregulation of Fas. Determining the role of sFasL and MMPs as therapeutic targets in combat against the excessive inflammatory immune response in severe COVID-19 requires further investigation of neutrophils, sFasL, and MMPs in COVID-19. The role of sFasL in storm initiation is shown in Figure 1.

**Multi-faceted role of neuropilin-1**

NRP-1 is a cell surface glycoprotein that is related to axonal rearrangement in the embryonic nervous system. It is also a receptor for collapsin/semaphorin family of proteins (Soker et al. 1998). This receptor is also expressed on oligodendrocyte precursor cells (OPCs) and the microglia (Sherafat et al. 2021). Additionally, NRP-1 is expressed on the endothelial cells acting as a receptor for VEGF and furin-cleaved substrates (Cantuti-Castelvetri et al. 2020; Moin et al. 2021). This receptor is also implicated in metastasis; for example, it is expressed in metastatic clear cell Renal Cell Carcinoma (mccRCC), which is an incurable type of kidney cancer. In these cells, NRP-1 is expressed as a co-receptor of VEGFs, enhancing the metastatic capacity of such cells, and thus blocking such receptors by NRP-1 receptor blockers (e.g., NRPa-308) could provide a promising therapeutic option for such malignancies (Dumond et al. 2021). The extracellular regions of NRP-1 comprise two coagulation factor regions (b1 and b2), two CUB regions (a1 and a2), and one MAM region (Daly et al. 2020). For SARS-CoV2, NRP-1 could promote the internalization of CendR peptides/ligands through an endocytic mechanism that acts similar to macropinocytosis. NRP-1 could also enhance host cell infection through other viruses like the Epstein–Barr virus (EBV) (Kyrrou et al. 2021). Together, change in expression of NRP-1 plays an important role in

**Table 1: The role of major cytokine storm receptors in neurodegenerative disease.**

<table>
<thead>
<tr>
<th>Sub-family</th>
<th>Signaling pathway</th>
<th>Cytokine storm (TNF or IL-1)</th>
<th>Neurodegenerative disease</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toll-like receptors (TLRs)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>TLR3</td>
<td>IRF3 and NF-κB</td>
<td>IFN-1, IL-1, IL-6, TNF-α</td>
<td>PD, stroke, MS, MG</td>
<td>Wang et al. (2020), Deng et al. (2017), Saresella et al. (2014), and Cufi et al. (2013)</td>
</tr>
<tr>
<td>TLR7</td>
<td>IRF7 and NF-κB</td>
<td>IFN-1, IL-1, IL-6, TNF-α</td>
<td>stroke, MS, MG, AD</td>
<td>Brea et al. (2011), Derkow et al. (2013), Cavalcante et al. (2016), and Lehmann et al. (2012)</td>
</tr>
<tr>
<td>TLR8</td>
<td>IRF7 and NF-κB</td>
<td>IFN-1, IL-1, IL-6, TNF-α</td>
<td>stroke, MS, MG</td>
<td>Brea et al. (2011), Johnson et al. (2013), and Wang et al. (2013b)</td>
</tr>
<tr>
<td>TLR9</td>
<td>IRF7 and NF-κB</td>
<td>TNF, IFN-1, IL-6, IL-12</td>
<td>PD, GBS, MS, MG, AD</td>
<td>Zhu et al. (2016), Wang et al. (2012), Zhou et al. (2017), Cavalcante et al. (2016), and Wang et al. (2013a)</td>
</tr>
<tr>
<td>Tumor necrosis factor (TNF) superfamily</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphotoxin alpha (TNF-β)</td>
<td>TRAFs with classical or non-classical pathways</td>
<td>TNF-β, TNF-α, IL-1, IL-6, IL-8, IL-12, IFN-γ, IL-18</td>
<td>PD, MS, AD, stroke, AD</td>
<td>Zelano et al. (1998), Moussa et al. (2017), Raine et al. (1998), Csencsits-Smith et al. (2016), Rahmani et al. (2020), and Mattson (1997)</td>
</tr>
<tr>
<td>OX40 ligand (CD252)</td>
<td></td>
<td>MG, AD, PD, stroke</td>
<td></td>
<td>AD Desideri et al. (2008)</td>
</tr>
<tr>
<td>CD40 ligand (CD154)</td>
<td></td>
<td>MG, MS, stroke</td>
<td></td>
<td>Xiaoyan et al. (2006), Carboni et al. (2003), and Mao et al. (2020b)</td>
</tr>
<tr>
<td>CD178 ligand</td>
<td></td>
<td>PD, GBS, MS, MG, AD</td>
<td></td>
<td>Landau et al. (2005), Geleijns et al. (2005), Volpe et al. (2016), Tüzün et al. (2003), and Su et al. (2003)</td>
</tr>
<tr>
<td>CD27 ligand (CD70)</td>
<td></td>
<td>PD, GBS, MS, MG, AD</td>
<td></td>
<td>Saunders et al. (2012), Che et al. (2016), Hintzen et al. (1999), Yilmaz et al. (2015), and Chen et al. (2016)</td>
</tr>
<tr>
<td>RIG-I-like receptors (RLRs)</td>
<td>RIG-I</td>
<td>IFN-1</td>
<td>PD, MS, AD, stroke, GBS</td>
<td>Li et al. (2019), Varzari et al. (2014), de Rivero Vaccari et al. (2014), Suzuki et al. (2013), and Ma et al. (2018)</td>
</tr>
<tr>
<td>MDA5</td>
<td>IRF3, IRF3, NF-κB</td>
<td></td>
<td></td>
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</table>

AD, Alzheimer’s disease; CD, cluster of differentiation; GBS, Guillain–Barré Syndrome; IFN, interferon; IL, interleukin; IRF, interferon regulatory factor; MDA5, melanoma differentiation-associated protein 5; MG, myasthenia gravis; MS, multiple sclerosis; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; PD, Parkinson’s disease; RIG-I, retinoic acid-inducible gene-I; TNF, tumor necrosis factor; TRAF, TNF-receptor associated factor.
the normal physiology of the human body and the pathological situations.

**SARS-COV2-specific viral entry pathways contribute to CNS alteration**

ACE – II receptors exist chiefly on pulmonary alveolar cells. However, they can be found throughout the body in vascular endothelial cells of the gastrointestinal (GI) tract, heart, and cerebrum (Saleki et al. 2020). Increasing evidence such as trials that explored on rodents infected with SARS-CoV34 or MERS-CoV13, highlight the CNS invasiveness of SARS-CoV2 for earlier CoVs. These studies demonstrated potential penetration of these CoVs by the olfactory route through the cribriform plate and ethmoid bone, and finally their dissemination to the CNS. In the CNS, these CoVs may localize to areas, including the brainstem and thalamus (Arbour et al. 2000; Netland et al. 2008; Yeh et al. 2004; Žanin et al. 2020). SARS-CoV2, similar to its older variant SARS-CoV1, may be capable of acting through ACE2 receptors. Notably, this indicates the potential of SARS-CoV2 to infect the brain stem, through a completely non-hematogenous retrosynaptic neural pathway, as well as exacerbate respiratory conditions through hematogenous routes (Saleki et al. 2020). Also, angiotensin II has been linked to severity of lung injury in COVID-19 (Wu et al. 2020b). Although ACE2-mediated entry may be shared between some SARS-CoV variants, increasing research has discovered specific pathways. More intriguingly, novel perspectives by De Virgiliis and Di Giovanni have suggested the concept of “the neuroimmune unit” as a main influencer of lung functions in COVID-19, inflammation, and senescence, revolving in particular around the utilization of the vagus nerve route and the ACE2 receptor. Also, methods like neural stimulation and drug-based neuromodulation to decrease inflammation with the purpose of avoiding respiratory failure have been suggested as means of fighting COVID-19 cytokine storm (De Virgiliis and Di Giovanni 2020).

NRP-1, which is known to strongly react with furin-cleaved substances, has recently emerged as a possible pathway for viral entry and infection (Cantuti-Castelvetri et al. 2020; Coutard et al. 2020; Longping et al. 2014). Indeed, co-expression of NRP-1 with ACE2 and the transmembrane protease serine 2 (TMPRSS2) has shown significant infectivity potentiating influences in Human embryonic kidney 293 T (HEK-293T) cells. This study also explored the influence of the NRP-1-inhibiting monoclonal antibody, mAb3, on infection of Caco-2 cells through wild-type and mutant SARS-CoV2, and showed that preincubating with NRP-1-inhibiting antibody decreased the wild-type infection by...
40% (Teesalu et al. 2009; Walls et al. 2020). Contrary to low expression of ACE2 on the lung tissue, NRP-1 and NRP-2 are significantly expressed on most of the lung tissue (Durante et al. 2020; Mao et al. 2020a).

Olfactory dysfunction may be a sign of infectious CNS damage, and is clinically linked to anosmia or hyposmia. In addition, ACE2 pathway has been linked to upregulation of NRP-1 in olfactory bulb in COVID-19 cases. NRP-1 regulates the entrance of nanoparticles laced with SARS-CoV2 S – originated CendR peptides into cultured cells, olfactory epithelial cells, and the CNS in mice (Cantuti-Castelvetri et al. 2020; Yazdanpanah et al. 2020). NRP-1 is a major player in directing and pruning of axons, mainly via its interplay with Semaphorin-3A (SEMA3A), a protein found profoundly in both the nervous system and the vascular system (Nakanishi et al. 2019). When compensatory neuronal processes are formed following spinal cord damage, plexins function as signal transducers accompanied by NRP-1, which is overexpressed in the motor cortex after the spinal cord damage. Inhibiting NRP-1 results in the blockade of axonal pruning of compensatory neural pathways in the CNS further highlighting the critical role of NRP-1 in cellular signaling and its role in restoring motor performance after spinal cord injuries. Moreover, this pruning is essential for sensory function. When NRP-1 production was restricted in the cochlea of mice embryos, postnatal rodents showed disarranged nerve cells and continuously deteriorating hearing dysfunction (Nakanishi et al. 2019; Salehi et al. 2017). Regarding smelling, studies have shown that NRP-1 is involved in Kallman syndrome, a congenital illness detected through hypogonadism and anosmia. Deregulation in axonal guidance, produced as a result of a defect in NRP-1 and SEMA3A interaction, contributes to the pathogenesis of this syndrome (Cariboni et al. 2011; Guo and Vander Kooi 2015; Hanchate et al. 2012). These results are significant during the present pandemic of SARS-CoV2, considering that COVID-19 has been widely linked to olfactory dysfunction.

BBB disruption is a major consequence of cytokine storm. Currently, it is evident that several cytokines like TNF-α may cross the BBB efficiently, possibly within 30 min of administration (Gutierrez et al. 1993). These actions may be achieved by a saturable influx transport (SIT) or retrograde axonal transport system (e.g., IL-1α, IL-6, and TNF-α) and circumventricular organs–brain regions where the BBB is not complete and cytokines can penetrate via simple diffusion (e.g., GDNF, glial cell-derived neurotrophic factor). As mentioned, the retroaxonal path may be taken up by SARS-CoV2 via ACE2 (Meschkat et al. 2020). Cytokines potentially insult the BBB and increase its permeability without penetrating the brain, by mechanisms such as induction and break down of tight junctions of microvascular endothelial cells in the BBB (Pan et al. 2006; Spray et al. 2006). Also, inhibition of VEGF signaling via an NRP-1-blocking peptide in vivo had a restorative effect after activation of CD8 T cell-activated BBB disintegration that can be activated by CD8 T cells, suggesting NRP-1 as a potential therapeutic aim in neuroinflammatory illnesses involving BBB disruption (Suidan et al. 2012). Therefore, both cytokine storm and NRP-1 deregulation may exert similar actions in COVID-19.

State-of-the-art evidence has found that viral entry pathways may explain some neurological behaviors in COVID-19 cases. Studies have discovered parallels among the pro-nociceptive influences of VEGF-A in animals (Beazley-Long et al. 2013) and humans (Hulse 2017), and clinical findings indicate elevated VEGF-A quantities in bronchial alveolar lavage fluid from COVID-19 cases (Ray et al. 2020) along with significantly decreased levels in the sera of asymptomatic cases compared with symptomatic cases (Long et al. 2020). Moreover, a study has depicted that SARS-CoV2 S protein co-opts VEGF-A/NRP-1 receptor pathway to trigger analgesia (Moutal et al. 2021). Multi-system damage by SARS-CoV2 and its link with the role of NRP-1 and ACE2 entry pathways is depicted in Figure 2.

**Combination therapy against viral entry pathways and cytokine storm elements: role of NRP-1 and FasL**

Recent research has focused on blocking entry elements (e.g., ACE2, NRP-1). The cardiovascular disease treatments, ACE inhibitors and angiotensin II receptor blockers (ARBs), were shown to promote ACE2 production in vivo (Kriszta et al. 2021). Researchers speculated elevated ACE2 expression may accelerate viral penetration into the host cells during the infection for its replication (Diaz 2020). However, after conducting human studies, it has been found that ACE2 alteration may not change susceptibility to COVID-19. When compared to ARBs, ACE inhibitors showed mild improvement in COVID-19 cases as combination therapy, but did not show benefits as monotherapy (Morales et al. 2021). These results may be because ACE2 presents a dual role; both as a viral entry pathway and a means of protection against oxidative stress in lung injury (Li et al. 2016). The clinical value of ACE2 inhibition is to be further explored in future studies.

Interestingly, more recent research has shown promising preclinical results for NRP-1. The furin cleavage result of SARS-CoV-2 Spike protein uses the VEGF-A-attaching area on NRP-1 that provides a polybasic extension finishing in a C-terminal arginine. This area has long been a focus of
After finding that VEGF-A/NRP-1 signaling pathway improves neuropathic pain, researchers tested their speculation that disrupting of this process via SARS-CoV-2 Spike protein hinders the pain signaling. In this study, verified hits from a small molecule and natural product examination of nearly 500,000...

**Figure 2:** Role of cytokine storm and entry pathways in respiratory and CNS injury. The triangle of viral entry pathways, cytokine storm, and multi-system damage has been depicted. (white, Ia) NRP-1 is linked to FasL and thereby mediates cellular survival. (Ib) Co-expression of NRP-1 with ACE2 potentiates SARS-CoV2 infectivity. (II) Followed by attachment of the virus to ACE2 via its spike, break down of spike is initiated. (III) Aktivated S2 region starts to function. (IV) Following the activation of S2, viral fusion with membrane and (V–VIII) viral replication steps take place. (magenta, I) Also, mFasL is cleaved via MMPs, resulting in sFasL. (II–III) Apoptosis is initiated. (IV) Immune-mediated macrophage autophagy inhibition results in apoptosis failure and (V) increase in DAMPs. (VI–VIII) Finally, activation of innate immune system (e.g., inflammasomes [Rasoulinejad et al. 2020]) as well as immune cell recruitment leads to cytokine storm, damaging CNS, lungs, and other organs. Of note, respiratory damage itself is connected to CNS damage, because parasympathetic nerves may help the virus spread in the nervous system retrosynaptically. Collectively, the link among tightly connected neuroimmunopathology of COVID-19 and multi-organ damage may lie among SARS-CoV2 entry pathways. Created with BioRender.com. ACE2, angiotensin-converting enzyme 2; C terminus; CASP, cysteine–aspartic acid protease; CCLs, chemokine (C–C motif) ligand; CNS, central nervous system; DAMPs, damage-associated molecular patterns; ER, endoplasmic reticulum; ERGIC, endoplasmic-reticulum–Golgi intermediate compartment; FADD, Fas-associated protein with death domain; FOXO, forkhead box O; GIPC, GAIP-interacting protein; ILs, interleukins; mFasL, membrane-bound FAS ligand; MMPs, matrix metalloproteinases; NRP-1, neuropilin-1; PARP, poly(ADP-ribose) polymerase; PSR, phosphatidyl serine receptor; RNA, ribonucleic acid; S, spike; SARS-CoV, severe acute respiratory syndrome coronavirus; serine 2; TMPRSS2, transmembrane protease, and VEGFR; vascular endothelial growth factor receptor.
candidates attaching the VEGF-A binding site on NRP-1 were found. Precisely, nine chemical series with lead- or drug-resembling physicochemical features were detected. Researchers showed via enzyme-linked immunosorbert assay (ELISA) that six candidates hindered VEGF-A-NRP-1 attachment more efficiently than EG00229 (Jarvis et al. 2010), a well-established NRP-1 blocker. Further validation in vitro showed that all chosen candidates inhibited VEGF-A triggered VEGFR2 phosphorylation. Additionally, two candidates presented robust suppression of a novel vesicular stomatitis virus protein which uses the SARS-CoV-2 Spike for penetration and fusion. These candidates define an initial step in the long journey to discover small molecule inhibitors of the VEGF-A/NRP-1 pathway for the therapy of neuropathic pain and cancer with the additional capability of suppressing the SARS-CoV2 cellular penetration (Perez-Miller et al. 2021).

Recent research has proposed suppression of cytokine storm elements as a means of COVID-19 treatment (Cabler et al. 2020). A recent perspective suggested that a potential immunotherapy for the severe COVID-19 could be achieved by the suppression of transforming growth factor-beta (TGF-β); it is in line with the clinical and laboratory characteristics, and lung abnormalities found in COVID-19 or the earlier SARS cases (Chen 2020). Moreover, small molecule NRP-1 antagonists integrate angiogenesis- and tumor-blocking functions with immunoregulation via decreasing TGF-β biosynthesis in T<sub>reg</sub> cells (Powell et al. 2018). Together with the fact that, unlike ACE2, NRP-1 is expressed on some cells of the nervous system, it should be considered that inhibiting NRP-1 may block our proposed pathological triangle; viral entry and cytokine storm (e.g., by dampening TGF-β), and CNS damage (e.g., anosmia). ACE2 is located in cholesterol rafts (Lu et al. 2008). Cilastatin is a specific competitive blocker of renal dehydropeptidase I (DHP-I) enzyme, which is attached to cholesterol rafts of renal tubular cells. Interestingly enough, Cilastatin has been shown to effectively block viral replication through DHP-1 inhibition and cytokine storm by mediating Fas/FasL reactions in a recent preclinical study. Cilastatin is a possible therapy that could avoid SARS-CoV2 internalization and duplication and safeguard from cell injury via specific inhibition of DHP-1 enzyme located in cholesterol lipid rafts of the apical membrane, avoiding Fas/FasL trimerization and decreasing ROS, hyperinflammation, cell death, and cytokine storm (González-Nicolás et al. 2020). Finally, in light of the discussed evidence, further exploration of combinational therapies that target multiple elements in the proposed triangle may pave the path for next-generation COVID-19 treatments.

Difficulties in identifying the link between cytokine storm and multi-faceted cell injury

Corticosteroids, colchicine, hydroxychloroquine, azithromycin, and aspirin have been utilized for COVID-19 treatment in clinical trial studies. However, to date, their benefits have not been proven (Lamontagne et al. 2021; Lopes et al. 2021; Salah and Mehta 2021; Siemieniuk et al. 2020a,b,c, 2021a,b; Skipper et al. 2020). The anti-neutrophil effects of these drugs are well-studied (Colotta et al. 1992; Cronstein et al. 1995; Ćulić et al. 2002; Merzon et al. 2021; Yaqinuddin and Kashir 2020). However, a main difficulty of linking drug targeting is that, blocking the pro-inflammatory state does not translate to enhancement of important outcomes, such as mortality. This suggests large trials and cohorts are needed to confirm the therapeutic value of newly uncovered and versatile infection pathways. As of now, new in silico and in vitro studies regarding viral entry pathways and cytokine storm elements are completing the missing pieces of the neuroimmunological puzzle of COVID-19 reasonably. NRP-1 influences membrane expression of FasL and MMPs (Ghode et al. 2017). Among the remaining research questions, the presence of such effects in COVID-19 is to be explored. These results indicate that drug repurposing or development efforts should be directed towards agents that mediate primary elements of viral entry and cytokine storm activation, including ACE2, NRP-1, FasL, and MMPs.

Conclusion

The COVID-19 pandemic is ongoing. Understanding the neuroimmunological alterations in COVID-19 by studying interconnected mechanisms is a robust approach that may open new prospects for COVID-19 therapy and management. The uncontrolled cytokine storm in COVID-19 may be accompanied by detrimental CNS effects, which may be due to or be exacerbated by neuroinflammation. COVID-19-derived cytokine storm may be calmed by targeting initiators of the storm as well as associated viral entry pathways. Such data highlight the need for pharmacological and molecular research investigating the entwined triangle of cytokine storm, CNS involvement, and viral entry.

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References


