Does SARS-Cov-2 invade the brain? Translational lessons from animal models

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The current coronavirus disease (COVID-19) outbreak, caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has raised the possibility of potential neurotropic properties of this virus. Indeed, neurological sequelae of SARS-CoV-2 infection have already been reported and highlight the relevance of considering the neurological impact of coronavirus (CoV) from a translational perspective. Animal models of SARS and Middle East respiratory syndrome, caused by structurally similar CoVs during the 2002 and 2012 epidemics, have provided valuable data on nervous system involvement by CoVs and the potential for central nervous system spread of SARS-CoV-2. One key finding that may unify these pathogens is that all require angiotensin-converting enzyme 2 as a cell entry receptor. The CoV spike glycoprotein, by which SARS-CoV-2 binds to cell membranes, binds angiotensin-converting enzyme 2 with a higher affinity compared with SARS-CoV. The expression of this receptor in neurons and endothelial cells hints that SARS-CoV-2 may have higher neuroinvasive potential compared with previous CoVs. However, it remains to be determined how such invasiveness might contribute to respiratory failure or cause direct neurological damage. Both direct and indirect mechanisms may be of relevance. Clinical heterogeneity potentially driven by differential host immune-mediated responses will require extensive investigation. Development of disease models to anticipate emerging neurological complications and to explore mechanisms of direct or immune-mediated pathogenicity in the short and medium term is therefore of great importance. In this brief review, we describe the current knowledge from models of previous CoV infections and discuss their potential relevance to COVID-19.

Introduction

Highly pathogenic coronavirus (CoV) infections are well-established sources of previous epidemics in humans, i.e. severe acute respiratory syndrome CoV (SARS-CoV) and Middle East respiratory syndrome CoV (MERS-CoV). The novel CoV named SARS-CoV-2, which shares a highly homological sequence with SARS-CoV, is responsible for the current COVID-19 outbreak with more than 2 million patients diagnosed and over 146 000 deaths, which exceeds by far the total of SARS and MERS in 2002 and 2012, respectively [1-3].

Despite the short duration of the current pandemic outbreak, several neurological and neuroradiological phenotypes have been reported [4,5], requiring urgent investigation into the mechanisms and etiology.
underlying the interplay between SARS-CoV-2 and the central nervous system (CNS).

A translational neuroscience approach is mandatory to explore the possible CNS involvement in CoV infections, accelerate scientific knowledge transfer to the clinical frontline and test new disease-oriented treatments. Indeed, both clinical features of the previous CoV epidemics (SARS and MERS) and lessons from animal models used in the study of SARS and MERS constitute valuable tools to understand the viral pathogenesis in the host and to characterize mechanisms of viral access and dissemination in the CNS. Meanwhile, several laboratories are rushing to study SARS-CoV-2 in a number of different animals, including primates, mice, rats, hamsters and ferrets [6]. Here, we will provide a neurological perspective by analysing the main features of these models and point out relevant similarities and specificities in comparison to SARS-CoV-2.

A comprehensive systematic search of Medline, Scopus, Web of Science and https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports/ was performed.

Severe acute respiratory syndrome

Clinical and neuropathological features in human patients

In 2002, the outbreak of SARS in Guangdong Province, China led to the discovery of SARS-CoV, a highly pathogenic CoV, as the causative pathogen of the epidemic [7]. Although the virus is primarily a respiratory pathogen, there are reports of neurological manifestations, such as epileptic seizures and encephalitis, that may suggest a CNS involvement of the infection [8,9] (Table 1). Complementing these reports, post-mortem neuropathological studies have detected the SARS-CoV N protein and RNA polymerase gene fragment in neurons of infected patients and pathological changes such as brain tissue edema and vasculitis of cerebral veins [10].

Organ tropism and neuroinfective routes

Compelling evidence demonstrates that SARS-CoV attaches to the cell membrane by binding to human angiotensin-converting enzyme 2 (hACE2), now also known to be the SARS-CoV-2 functional receptor [11]. Human tissue studies have shown an abundant presence of these receptors not only in the epithelia of the lung and small intestine, but also in arterial and venous endothelial cells and arterial smooth muscle cells in all organs studied, including the brain [12]. Based on a transgenic mouse expressing hACE2, it was possible to show that angiotensin-converting enzyme 2 (ACE2) is also expressed at neuronal level, namely in the cytoplasm of cell bodies [13].

Distinctive properties in the structure of mouse ACE2 (as compared with hACE2 proteins) significantly reduce the virus tropism for mouse tissues. Hence, in order to overcome this species-related difference, a transgenic model has been generated in which a vector carrying a hACE2-coding sequence was introduced in wild-type mice under control of the human cytokeratin 18 (K18) promoter [14]. Notably, when K18-hACE2 transgenic mice were infected with SARS-CoV, the infection would start in the respiratory epithelium and rapidly spread to the alveoli. More importantly, neuroinvasive routes were later explored using the same model, by monitoring the kinetic profile of viral antigen [15]. Strikingly, the authors showed that the viral spread started in the olfactory bulb and progressively invaded subcortical and cortical regions. Such a trans-neuronal hypothesis could not apply for other infected regions, such as those brainstem nuclei that are not directly connected to the olfactory bulb. The authors raise the possibility that, once the virus is established in the brain, it might spread along specific neurotransmitter pathways or via non-neuronal routes (blood or Virchow–Robin spaces) [15]. Overall, their results showed that, in this model, SARS-CoV primarily entered the brain via the olfactory nerve.

Alternatively, other authors theorize that CoV can primarily use a hematogenous route to penetrate the CNS using dendritic or white blood cells as reservoirs [16]. This presumption is based on pathological studies that have shown that monocytes and macrophages can be infected by SARS-CoV [17] and on cell-line studies that revealed that dendritic cells (regulators of immune responses) can be infected and impaired by this virus [18] (Table 1).

Clinical and pathological lessons from animal models

Multiple animal models have been explored in the context of SARS, including non-human primates, hamsters, ferrets and mice (Table 2). A comprehensive descriptive review of all suitable models is beyond the scope of this review and we refer the reader to a number of excellent reviews [19-21]. It can be inferred that there is no single ideal animal model for SARS, although the evidence collected so far has significantly contributed to advancing the field. In particular, it is well established that models range from those in which only virus replication is observed (young BALB/c, B6 mice) to those in which replication is...
accompanied by minimal signs and histopathology (such as non-human primates, ferrets and hamsters) [19]. Curiously, old immunodeficient BALB/c mice exhibit a clinical syndrome, supporting age as a risk factor for more severe clinical phenotypes [19].

Transgenic K18-hACE2 mice infected with SARS-CoV develop a severe pulmonary phenotype, starting in the respiratory epithelium with rapid alveolar dysfunction [14]. In this model there is a massive infiltration of macrophages and lymphocytes in the lungs, promoting a release of pro-inflammatory cytokines not only at pulmonary level, but also in the brain. In a relatively short time frame (within 5 days), K18-hACE2 mice develop a severe phenotype, that includes a lethargic-like state, suggesting CNS involvement.

In follow-up studies in the same mouse strain, K18-hACE2 [15], the authors demonstrated an extensive involvement of the transgenic mouse brain. SARS-CoV produced a widespread infection involving vital brainstem nuclei, such as dorsal motor nucleus of the vagus, nucleus tractus solitarii and area postrema. This model also raised questions as to the cause of neuronal destruction and death in these animals [15]. As there was no pathological evidence of inflammation, the authors considered the possibility of apoptosis as the cause of neuronal death, although this was not confirmed [Terminal deoxynucleotidyl transferase (TdT) dUTP Nick-End Labeling (TUNEL)-positive cells were not detected]. It was proposed that a dysregulated cytokine response could be the cause of death in these animals. At day 4 post-infection, infected K18-hACE2 mice had an upregulation of the proinflammatory cytokines interleukin (IL)-1, tumor necrosis factor alpha and IL-6. The authors also propose a possible direct involvement of the dorsal vagal complex, a vital region of the brain that plays an important role in orchestrating cardiorespiratory function. In fact, animals intracranially inoculated with low-dose virus exhibited limited viral spreading but succumbed rapidly [15].

Overall, these data show the relevance of the transgenic approach in converting the mouse response to infection from mild to severe leading to CNS involvement.

Table 1 Clinical characteristics and pathology of severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV) and SARS-CoV-2 in humans

|                     | SARS-CoV | MERS-CoV | SARS-CoV-2 |
|---------------------|----------|----------|------------|
| **Systemic**
| manifestations      |          |          |            |
| Mild to severe      |          |          |            |
| Fever and lower respiratory illness |          |          |            |
| ICU care required in ~30% patients |          |          |            |
| ARDS in ~20% patients |          |          |            |
| Gastrointestinal infection |          |          |            |
| **Pulmonary**
| pathology           |          |          |            |
| Consistent with pneumonia and acute lung injury | Samples not available for investigation | Consistent with pneumonia and acute lung injury |
| **Human ligand**    | Protein S1 binds to ACE2 protein of the host cell surface | DPP4 (also known as CD26) | Protein S1 binds to ACE2 protein (10-20-fold higher affinity compared with SARS-CoV) |
| **Neurological**    | Sporadic case reports | Sporadic case reports | 34% of hospitalized patients and sporadic case reports |
| **CNS involvement** | Human neurons are infectible [53] and ACE2 neuronal expression has been identified in human CNS [54] | Capable of infecting human neuronal cells in in-vitro cell lines [53]. DPP4 has a low expression in the brain [56] | – |
| **Neuropathology**  | SARS genome sequences detected in the brain in autopsies; also, edema and scattered red degeneration of neurons [17] | Samples not available for investigation | – |
| **Mortality**       | 9.6%     | 34.4%    | 5.3%a      |

ACE2, angiotensin-converting enzyme 2; ARDS, acute respiratory distress syndrome; CNS, central nervous system; DPP4, dipeptidyl peptidase-4; ICU, intensive care unit. See Ref. [17,53-56]. aAs of 3 April 2020.
Additional key findings emerging from these different SARS models include: (i) the fundamental role of innate immunity in the response to SARS-CoV infection; (ii) the different severity observed in young versus old BALB/c mice underlies the age dependency in the clinical manifestations; and (iii) different kinetics of viral infection (faster clearance in animals) [22,23].
Middle East respiratory syndrome

Clinical and neuropathological features in human patients

Currently, MERS-CoV is still a relevant threat for populations in the Middle East, with a high lethality (close to 35%) [2]. Patients exhibit predominantly pulmonary clinical involvement in contrast to fewer patients presenting neurological manifestations such as coma, ataxia, focal motor deficits and peripheral nerve symptoms [24,25]. Unfortunately, there are no published data regarding human neuropathological findings (Table 1).

Organ tropism and neuroinfective routes

The MERS-CoV ex-vivo models supported the clinical tropism for the pulmonary tract by showing that the virus can replicate in human lung cultures (in bronchial, bronchiolar and alveolar epithelial cells) [26]. This cell line susceptibility study also revealed that, although presenting a lower viral expression and no cytopathic effects, MERS can infect human neuronal lines. Dipeptidyl peptidase-4 (DPP4), also known as CD26, was identified as a functional receptor for MERS-CoV. DPP4 is generally expressed in human bronchiolar epithelial cells and bronchial lung tissue [27]. It can also be found in the intravascular portion of vascular endothelial cells and in the cerebrospinal fluid [28]. After identification of DPP4 as a functional receptor, which is expressed in the airway epithelia of rodents, it was expected that rodents would have been vulnerable to infection. This turned out to be wrong, as the human binding domain differs from that of rodents [29]. This limitation was overcome by developing mice expressing human DPP4 that exhibited high susceptibility to infection and displayed the features of human disease [30], including a lethargic state, and showing high mortality and extrapulmonary involvement (Table 2). The authors detected a severe lung infection, but brain invasion was not seen until day 4 of infection, suggesting substantially different kinetics of MERS-CoV infection in the lung and brain [30].

A different animal model using human DPP4 transgenic mice studied the differences in viral replication in animals infected by a clinical aerosol transmission simulator compared with intranasal instillation-inoculated mice [31]. They found that the disease onset, lung lesion and viral replication progression were slower in the MERS-CoV aerosol-infected mice than in the MERS-CoV instillation-inoculated mice. Furthermore, after aerosol infection, they detected high viral loads after 3–9 days in the lungs versus 7–9 days in the brain. Again, although both lungs and brain are infected, the timing is different, with a later infection of the brain [31]. Such different kinetics could suggest a hematogenous route of infection. Indeed, neuroinvasive routes were not explored in either of these models. In addition, similarly to SARS-CoV, MERS-CoV has been shown to replicate in human dendritic cells and macrophages, which would support the hematogenous hypothesis [32].

Clinical and pathological lessons from animal models

A number of models have been developed and discussed in detailed review articles [33,34] (Table 2). In a non-human primate model of MERS, de Wit and colleagues inoculated rhesus macaques with MERS-CoV, which primarily affected the epithelium of the lower respiratory tract, giving rise to a mild-to-moderate interstitial pneumonia [35]. This model was able to replicate virus shedding and replication in tissues, as well as gene expression and cytokine and chemokine profiles. However, despite the mild clinical syndrome, no neurological signs and symptoms were reported. Thus, the self-limiting nature of MERS-CoV infection, as transient patterns at various levels of the model, suggests that this model does not fully resemble the lethal infection observed in humans [35]. It is of note that, when macaques were immunocompromised by immunosuppressive agents, the MERS-CoV replicated to significantly higher titers and disseminated in other organs (CNS not examined). Surprisingly, histopathological alterations were reduced in the immunosuppressed animals [36]. Together, these data suggest a prominent role of the host response in the manifestation of the disease.

The macaque model allowed the testing of a number of potential drugs as novel therapeutics. Remdesivir, an antiviral agent used also for COVID-19, was able to prevent/treat the histological and radiological signatures of the disease [37].

In studies using transgenic mice expressing human DPP4, it was possible to induce features of human disease in the animals [30]. From the studied cells, pneumocytes, brain microglia, astrocytes and neuronal cells all presented high titers of virus. With regard to pathology, whereas infected mice presented an extensive pulmonary inflammatory infiltrate, the only findings in the brain were a mild perivascular cuffing [30]. However, in a different study using human DPP4 transgenic mice, a few days after the appearance of pulmonary lesions, pathological changes were documented in the brain, with dilatation and congestion of the cerebral vessels and few areas of cellular necrosis...
in the cerebral cortex, hippocampus and thalamus [31].

As in SARS-CoV-2, MERS-CoV infection was also shown to induce a profound acute inflammatory response within the lungs and brain of hCD26 Tg mice, with upregulation of multiple genes related to the inflammatory response [30].

COVID-19

Clinical and neuropathological features in human patients

COVID-19 is the most recent and dramatic pandemic, caused by SARS-CoV-2. Registered lethality varies between European countries ranging from 1.5% in Germany to over 10% in Italy [1]. As in SARS and MERS, pulmonary clinical involvement is most prominent. However, more recently, neurological phenotypes involving central and peripheral nervous system have emerged and are being increasingly recognized, i.e. anosmia, ageusia, necro-hemorrhagic encephalitis and Guillain–Barré syndrome [4–5, 38]. So far there are no published human neuropathological findings (Table 1).

Organ tropism and neuroinfective routes

The SARS-CoV-2 ultrastructure was recently characterized by high-resolution cryo-electron microscopy [39]. Remarkably CoV spike glycoprotein, by which the virus binds the cell membrane, binds ACE2 with a higher affinity compared with SARS-CoV. In addition, most of the available antibodies to SARS-CoV targeting ACE-binding domain were unable to bind the SARS-CoV-2 spike protein, indicating that binding sites differ between SARS-CoV and SARS-CoV-2. Such a finding indicates the urgent need for generating specific antibodies for SARS-CoV-2 binding domain, but might also explain the distinct pathogenic properties of SARS-CoV-2 [40]. In addition to ACE2 receptor, SARS-CoV-2 uses the serine protease type II transmembrane serine protease (TMPRSS2) for spike protein priming [41, 42]. Very recently, in a preliminary report, Brann et al. took advantage of bulk mouse whole olfactory mucosa (WOM) RNA sequence data derived from macaque, marmoset and human and found in both mouse and human datasets that olfactory sensory neurons do not express two key genes involved in CoV-2 entry, i.e. ACE2 and TMPRSS2 [43]. In contrast, olfactory epithelial support cells and stem cells express both of these genes, as do cells in the nasal respiratory epithelium. Taken together, these findings suggest possible mechanisms through which CoV-2 infection could lead to anosmia or other forms of olfactory dysfunction. Moreover, these findings may question the olfactory bulb as an entry route for CoVs into the CNS [43].

To our knowledge, no study has evaluated, so far, any type of pathway targeted at the CNS or peripheral structures.

Clinical and pathological lessons from animal models

Several laboratories worldwide are accelerating attempts to develop a suitable animal model for COVID-19. Experimental infection with SARS-CoV-2 in these models provides basic information to address a number of fundamental questions regarding its pathogenicity, the interaction with the different hosts and, hopefully, establishing the criteria for prevention and care. In line with observations in SARS models, non-human primates and wild-type mice infected with SARS-CoV-2 exhibit a relatively mild clinical disease, in spite of the evidence that Quantitative reverse transcription polymerase chain reaction (RT-qPCR) revealed a massive infection of the respiratory tract [44, 45] (Table 2). Rhesus macaques infected with bronchoalveolar lavage fluid obtained from an affected patient developed a histopathologically confirmed interstitial pneumonia, associated with a widespread presence of SARS-CoV-2 in the respiratory tract. Clinical signs were mild and no viral RNA was detectable by means of RT-qPCR in the blood of the primates during the whole course of infection (14 days) [44]. These findings demonstrate the causal relationship between SARS-CoV-2 and interstitial pneumonia, reminiscent of COVID-19. Moreover, and consistent with observations in SARS models, Bao et al. [45] used the hACE2 transgenic mice and infected them with SARS-CoV-2 inducing interstitial pneumonia, with typical histopathological elements and, accordingly, viral antigens were found in airway epithelia. These lines of experimental evidence are relevant as they demonstrate the causal relationship between SARS-CoV-2 and pulmonary involvement, but, unlike in SARS models, nervous system involvement was not documented in these experiments. However, it is unclear if brain tissue was systematically assessed and the most susceptible brain regions explored for direct or indirect viral presence. Overall, the pathogenicity of SARS-CoV-2 is lower as compared with SARS-CoV in mice. Indeed, as discussed above, hACE2 transgenic mice infected with SARS-CoV exhibited widespread organ damage, whereas SARS-CoV-2, at least in this model, was confined to lungs, indicating a differential pathogenicity [45]. These studies reveal important commonalities between SARS-CoV-2 and SARS-CoV infection and identify a potential target.
for antiviral intervention. In fact, very recently, a TMPRSS2 inhibitor approved for clinical use (camostat mesylate) was tested and blocked SARS-CoV-2 entry into lung cells [42]. Finally, the same authors were able to show that the sera from convalescent patients with SARS cross-neutralized SARS-2-spike-driven entry [42]. If the same effect occurred in pre-clinical models, then we would be closer to both a preventive and a disease-oriented treatment.

Unanswered questions
- Major routes of CNS infection: (i) spread via olfactory bulb and/or (ii) synapse-connected route to the medullary cardiorespiratory center from the mechanoreceptors and chemoreceptors in the lung and lower respiratory airways; and/or (iii) hematogen via brain endothelial ACE2 receptors.
- Major pathogenic pathways for CNS involvement: (i) direct viral pathogenicity; and/or (ii) immune-mediated pathogenicity targeting brain tissue; and/or (iii) inflammatory involvement of brain blood vessels; and/or (iv) intravascular coagulation secondary to the systemic inflammatory response as a major cause of thrombosis, hemorrhage and stroke.
- Host individual susceptibility factors that underlie the variable severity of the disease in human patients. However, a relevant issue is also represented by gender. The clinical observation of a specific involvement of males might suggest a specific protective estrogenic effect.

Unravelling these points could clarify if CNS contributes to respiratory failure in patients with COVID-19 [15,46] and may provide a rationale to preventive and therapeutic strategies for major neurological events such as stroke, encephalitis or other reported complications.

Advantages and limitations

Established ex-vivo and animal models of CoV infection may help to dissect pathogenicity, infective routes and nervous system targets of CoVs. If we manage to mimic the pathological hallmarks of COVID-19 we may have the tools to test treatment efficacy and evaluate the efficacy of vaccines and therapeutics.

Among limitations, the following emerge as of primary importance.
- Neurological subtle clinical phenotypes are not easily reproducible in animal models.
- Neurological severe phenotypes are dependent on using specific transgenic approaches to enhance virulence, which limit direct translation to humans.
- Severity of the clinical features does not always parallel either the viral replication level or the histopathological findings, hinting at indirect disease mechanisms (such as inflammation and prothrombotic states) that have not been attained in the present models.
- Innate animal characteristics seem to influence viral infection kinetics leading to faster virus clearance.

Conclusions

The ongoing outbreak of SARS-CoV-2 confirms that human CoVs are primarily respiratory pathogens and Koch postulates have already been fulfilled in this regard [45].

Previous reports suggest that SARS-CoV and MERS-CoV can occasionally cause clinically relevant CNS infections. In fact, animal models suggest invasiveness of these viruses through the CNS, either via the olfactory bulb or through blood dissemination of infected and activated monocytes passing through a permeable blood–brain barrier as a consequence of the systemic inflammatory response.

With regard to the pathogenesis of immune-mediated CNS pathology, data derive broadly from mice infected with murine hepatitis virus strains, a beta-CoV genetically related to human CoV-OC43 [47]. Briefly, three mechanisms of immune-mediated CNS lesions can be recognized. (i) An excessive host response to the infection can occur resulting in a systemic inflammatory response syndrome that causes a multiple organ dysfunction (including CNS). The main pathogenic mechanism in this case includes tissue ‘dyoxia’ due to intravascular coagulation and dysfunction of the microcirculation homeostasis. (ii) Direct viral infection of immune cells, including macrophages, microglia and astrocytes in the CNS, may activate glial cells that locally produce pro-inflammatory cytokines, including IL-6, tumor necrosis factor alpha, IL-1β and IL-12 [48]. Moreover, activated immune cells may contribute to tissue damage by producing toxic agents, recruiting and activating further immune cells and inducing apoptosis. Immune-mediated events, either through T-cells or by means of other cytokine and chemokine pathways, may also eventually lead to demyelination. (iii) An autoimmune reaction is generated by an adaptive immune response directed against host epitopes or proteins either misrecognized by pathogen-directed antibodies or expressed by damaged tissues (and previously cryptic to the adaptive immune system) [49,50].

In order to speed up clinically useful discoveries, it would be desirable to follow some indications such as:
(i) to build a systematic, consecutive, prospective registry including epidemiological data in patients with COVID-19 with attention to neurological manifestation to fully understand if SARS-CoV-2 infections can cause CNS involvement and to what extent; (ii) to measure SARS-CoV-2 RNA in the cerebrospinal fluid of symptomatic vs. asymptomatic patients; and (iii) to perform autoptic investigations of patients with COVID-19 in order to find and characterize virus distribution across tissues (cerebral blood vessels, endothelia, glia and neurons) and neuropathological consequences such as antibody-based neuroinflammatory responses in gray and white matter, vasculitis, neuroglial death or apoptosis and ischaemic or hemorrhagic events. Taking into account the fact that other CoVs are prone to infecting neurons in animal models as well as in humans [16,50] we must keep an open mind regarding medium- to long-term sequelae and consequences of the acute infection. Therefore, despite immune-mediated control of acute infection being attained, host-mediated immune regulatory mechanisms may fail to clear the virus potentially leading to ‘chronic infections’ and hence impact chronic neurological diseases, such as Parkinson’s disease and multiple sclerosis [51] as well as acute disseminated encephalomyelitis [52]. This calls for long-term patient follow-up in the clinics and also exploring the effect of SARS-CoV-2 in mouse models of neurodegenerative disorders to anticipate the occurrence of chronic SARS-CoV-2 CNS infection.

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Data availability statement
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