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The type I interferon antiviral response in the choroid plexus and the cognitive risk in COVID-19

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The type I interferon (IFN) response is the body's typical immune defense against viruses. Previous studies linked high expression of genes encoding type I IFNs in the brain's choroid plexus to cognitive decline under virus-free conditions in aging and neurodegeneration. Multiple reports have documented persisting cognitive symptoms following recovery from COVID-19. Cumulative evidence shows that the choroid plexus is one of the brain regions most vulnerable to infection with the coronavirus SARS-CoV-2, and manifests increased expression of genes encoding type I IFNs even in the absence of viral traces within the brain. In this Perspective, we propose that the type I IFN defensive immune response to SARS-CoV-2 infection in the choroid plexus poses a risk to cognitive function if not resolved in a timely manner.

The brain's choroid plexus (Box 1) serves as the interface within the brain's ventricles between the cerebrospinal fluid (CSF) bathing the brain and the circulation. This interface is responsible for allowing passage of nutrients and other molecules that the brain needs for its lifelong health, while preventing entry of microorganisms such as viruses and bacteria to the brain. Here, based on the understanding that this anatomical barrier can express an antiviral immunological signature under virus-free conditions[1–4], we propose that although the choroid plexus is endowed with an active protective role that can destroy viruses before they infect the brain, its defensive activities could also have negative consequences for the brain if not fully resolved in a timely manner. Multiple reports document cases of broad-spectrum neurological symptoms in individuals with COVID-19 after recovery, including persistent cognitive impairment[5,6]. In principle, cognitive decline following viral infection might result from a direct damage to the brain caused by the virus itself, or from the body's response to the virus, if not optimally controlled. Given the global scale of the COVID-19 pandemic, the elucidation of the mechanisms potentially driving cognitive deterioration and the identification of predisposing factors are a pressing medical need. In this Perspective, we propose a link between impaired cognitive ability after severe infection with SARS-CoV-2 and the type I interferon (IFN) antiviral response in the choroid plexus epithelium. While such a response is part of the body's natural immune defense against viruses, when persistent, it was shown to be detrimental to brain function[1,7].

SARS-CoV-2 neurotropism in the choroid plexus

The choroid plexus has been proposed as one of the potential sites through which SARS-CoV-2 virus might enter the brain[8,9]. However, direct SARS-CoV-2 neuroinvasion is debatable, as traces of SARS-CoV-2 RNA or proteins are not always detected in brain autopsy samples of individuals with COVID-19 disease[10]. In addition, in cases where traces of SARS-CoV-2 RNA or proteins were found within the brain, they did not correlate with neurological symptoms[10]. Moreover, in mice, SARS-CoV-2 spike protein accumulates in several regions of the body, including the choroid plexus, but is not detected in the brain parenchyma or CSF[11]. It is thus very unlikely that direct viral damage to the brain is the leading cause of cognitive deficits following COVID-19. Human brain organoids exposed to SARS-CoV-2 spike pseudovirus or SARS-CoV-2 viral isolates had little to no presence of viral proteins in neuronal and glial cells, but high infection of choroid plexus epithelial cells[12,13]. These observations in human brain organoids were linked to the higher expression of angiotensin-converting enzyme 2 (ACE2), one of the receptors for SARS-CoV-2, in the choroid plexus epithelial cells relative to neuronal

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BOX 1

Anatomy and functions of the choroid plexus

The choroid plexus is a complex brain structure protruding from the brain ventricles and suspended within the ventricular spaces, filled by the CSF. It consists of a highly folded monolayer of polarized epithelial cells, with their apical side characterized by villi facing the CSF, and their basal side lying on a stroma populated by mesenchymal and immune cells and vascularized by fenestrated blood capillaries (Fig. 1a). The epithelial cells of the choroid plexus are tightly connected (Fig. 1a), thereby providing an efficient physical barrier between the blood and the CSF. A large number of transporter proteins expressed by the choroid plexus epithelium regulate the exchange of compounds between the brain and the periphery, including the clearance of toxins from the brain into the blood (Fig. 1a). The choroid plexus epithelium is the main producer of CSF and a source of several neurotrophic factors (for example, insulin-like growth factor 1 and brain-derived neurotrophic factor; Fig. 1a). The choroid plexus epithelium receives and integrates immune signals coming from both the brain and the circulation, and in turn expresses leukocyte trafficking molecules and produces immune signals that can remotely affect cells within the parenchyma, including microglia and astrocytes (Fig. 1a). Perturbations of choroid plexus homeostasis, for example, during infections, inflammation and also in aging and neurodegeneration, can limit immune surveillance of the brain, as well as alter microglial homeostasis, leading to cognitive decline[27].

and glial cells[12] (Fig. 1a). Consistent with these results, analysis of publicly available human and mouse brain transcriptome databases showed that while *ACE2* gene expression is displayed by many neuronal and nonneuronal cell types, it is higher in some brain areas, including the choroid plexus[9]. Similarly, histological examination of postmortem human brains showed more intense ACE2 protein staining in the choroid plexus epithelium than in the brain parenchyma[14]. Conceivably, due to its relatively high expression of ACE2, the choroid plexus epithelium may be a site with high risk of attack by the SARS-CoV-2 virus (Fig. 1b) and, at the same time, as we propose here, the site that intercepts the virus and mounts an antiviral response that blocks viral entry into the brain parenchyma.

Studies with human brain organoids infected with SARS-CoV-2 (refs. [12,13]) and single-nucleus transcriptome analysis of postmortem choroid plexus samples from individuals with COVID-19 (refs. [15,16]) collectively show prominent choroid plexus alterations, including impairment of blood–CSF barrier properties, increased cell death, cell functional deficits, aberrant leukocyte infiltration and inflammation. In agreement, a retrospective analysis of clinical findings in the CSF of patients with COVID-19 and neurological symptoms revealed that the majority of individuals had a high CSF/serum albumin ratio, an indicator of blood–CSF barrier dysfunction[17]. Given the broad importance of choroid plexus functions for brain homeostasis (Box 1), their impairment as an outcome of SARS-CoV-2 infection likely plays a role in COVID-19 neuropathogenesis.

Type I IFN and cognitive impairment

Type I IFN (for example, IFN-α and IFN-β) signaling is a distinctive immunological defense response to viral infection. Type I IFN responses at the brain's barriers, including the choroid plexus epithelium, have been linked to cognitive impairment in a mouse model of infection with the vesicular stomatitis virus M2 (ref. [18]). Of note, however, bulk RNA-sequencing analyses of the choroid plexus transcriptome showed enhanced type I IFN signaling also under noninfectious conditions, such as normal aging[1] and neurodegenerative diseases of diverse etiology that are also associated with reduced cognitive ability in mice and humans, such as Alzheimer's disease[2,3] and Niemann–Pick's disease type C[4]. In particular, type I IFN signaling at the choroid plexus is associated with cognitive impairment and reduced hippocampal neurogenesis in aged mice[1]. It is also associated with altered expression of several genes involved in the maintenance of blood–CSF barrier integrity and CSF composition in the J20 mouse model of Alzheimer's disease[2]. Intracerebroventricular injection of an antibody blocking the type I IFN receptor (IFNAR) restored cognition in aged mice[1], while overexpression of IFN-β in the choroid plexus of young mice induced an aging-like microglial phenotype and cognitive impairment[7], suggesting a direct link between type I IFN signaling at the choroid plexus and cognitive dysfunction.

Single-nucleus transcriptome analysis of postmortem brain samples from individuals who died with COVID-19 showed strong expression of type I IFN signatures (for example, *IFITM3* and *STAT3*) in the choroid plexus in the absence of both RNA and protein traces of SARS-CoV-2 in the brain[15]. Analysis of the cortices of the same postmortem brains identified subpopulations of microglia and astrocytes that expressed genes previously linked with Alzheimer's disease (for example, *C1Q* and *CD14*) and inflammation or astrogliosis (for example, *IFITM3* or *GFAP*), respectively[15]. In addition, broad dysregulation of genes associated with synaptic transmission was found in upper-layer excitatory neurons (for example, *VAMP2*, *SNAP25* and *ATP6V0C*) as well as in proximal inhibitory neurons, potentially suggestive of cognitive deficits[15]. Furthermore, the in silico reconstruction of the choroid plexus-to-cortex signaling networks showed enhanced complement and CCL/CXCL chemokine pro-inflammatory pathways[15]. In support, the postmortem choroid plexus of individuals who were infected with SARS-CoV-2 expressed the *C3*, *C7* and *C1S* complement genes[15]. In a mouse model of mild respiratory COVID, chemokines including CCL11, CCL2 and CXCL10 were persistently elevated in the CSF up to 7 weeks after infection[19]. Increased expression of complement components and CCL/CXCL chemokines in the choroid plexus has been associated with brain diseases. The postmortem examination of the choroid plexus of patients with Alzheimer's disease revealed the presence of lipid deposits containing complement components (for example, C1q, C3 and C5) correlating with cognitive decline[20]. Similar deposits were also observed in the choroid plexus of *ApoE*$^{-/-}$ and ApoE4 knock-in (ApoE4-KI) mice fed a high-fat diet, concomitantly with high expression of genes encoding type I IFNs (also present in ApoeE4-KI mice fed normal chow)[20]. Of note, *Ccl11* chemokine gene expression in the choroid plexus was found to increase in mice during aging and was connected with type I IFN signaling[1]. Moreover, systemic administration of CCL11 in mice impairs hippocampal neurogenesis and induces cognitive deficits[19,21]. Finally, expression of the chemokines encoded by *Ccl2* and *Cxcl10* in the choroid plexus was linked to myeloid cell homing to the brain under neurodegenerative conditions, and their reduced expression at the choroid plexus is associated with disease progression in the 5xFAD mouse model of Alzheimer's disease[22]. Yet excess *Ccl2* and *Cxcl10* transcripts in the choroid plexus of mouse embryos are associated with disruption of the blood–CSF barrier following maternal immune activation during gestation, an experimental model for postnatal neurodevelopmental disorders[23].

Taken together, it is possible that, upon SARS-CoV-2 infection, the choroid plexus relays inflammatory signals to the brain in a manner that, at least in part, may involve local type I IFN responses, and that these mediators have the potential to cause cognitive impairment. As such, it is conceivable that, in patients infected with SARS-CoV-2, type I IFN signaling in the choroid plexus epithelium constitutes a defensive

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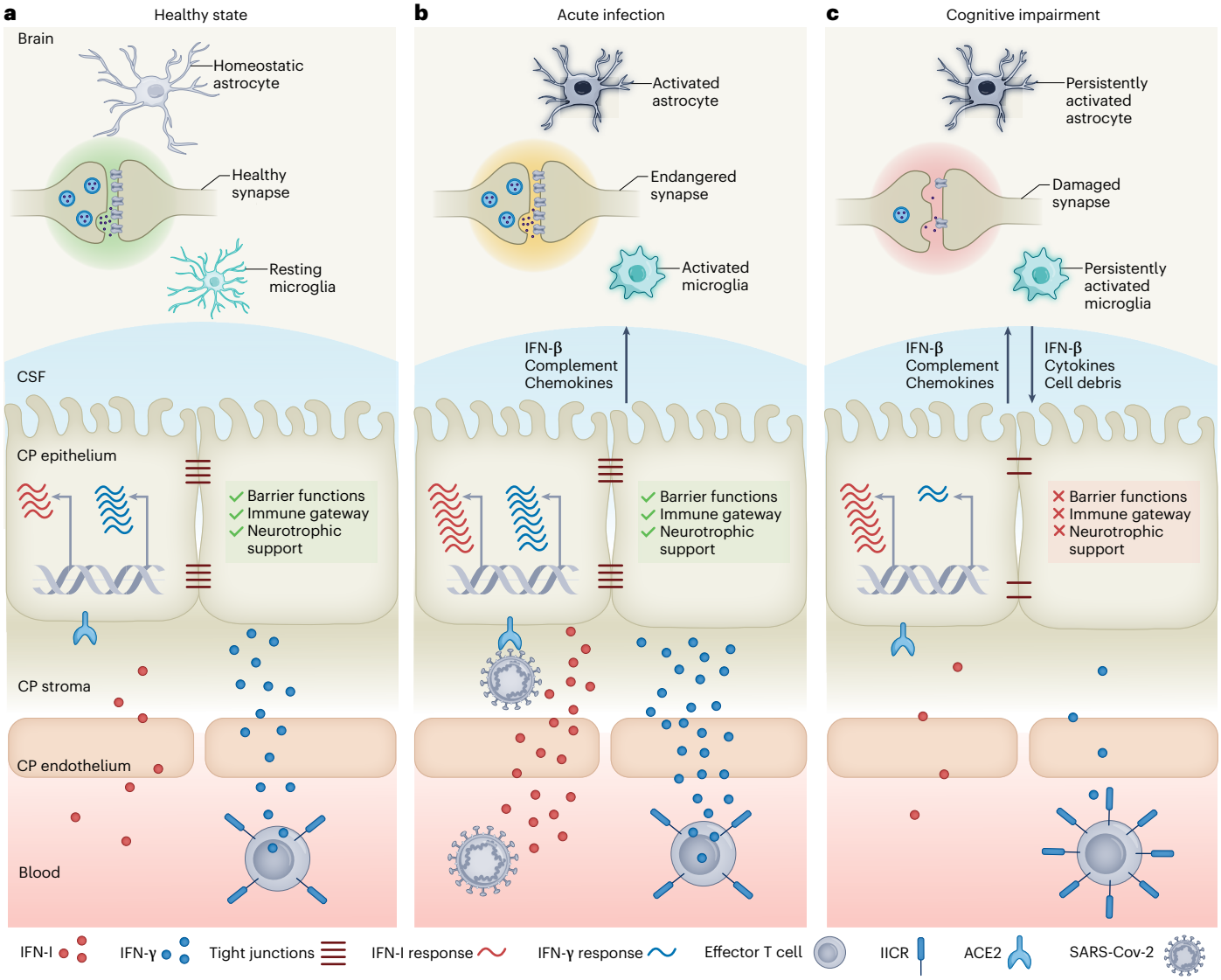

Fig. 1 | Type I IFN signaling in the choroid plexus epithelium after resolution of SARS-CoV-2 infection may trigger a cascade of events leading to cognitive decline. a, In the healthy state, the choroid plexus epithelium responds to type I IFN (IFN-I) signals and IFN-γ released by effector T cells in the circulation. The balance between type I IFN and IFN-γ responses in the choroid plexus (CP) epithelium regulates homeostatic choroid plexus functions such as barrier integrity, immune cell trafficking to the brain and release of neurotrophins, which are crucial for the maintenance of normal brain function[27]. **b**, During acute SARS-CoV-2 infection, type I IFN and IFN-γ responses in the choroid plexus epithelium increase as a result of elevated type I IFN and IFN-γ signals in the circulation, and possibly also due to a direct response to viral infection by choroid plexus epithelial cells, which express several molecules required for SARS-CoV-2 entry, notably ACE2. Consequently, the choroid plexus epithelium releases pro-inflammatory mediators such as IFN-β, complement (for example, C1S, C3 and C7)[15] and CCL/CXCL family chemokines (for example, CCL2, CCL11 and CXCL10)[19], which are in turn sensed by microglia and astrocytes, leading to their activation. **c**, Pro-inflammatory cytokines, including IFN-β, released by activated microglia and astrocytes, as well as debris from damaged cells, maintain the choroid plexus epithelium in an inflamed state. Following severe COVID-19, the host's systemic immune effector functions, including type I IFN and IFN-γ production, are reduced. T cell populations with an exhausted phenotype, characterized by high expression of inhibitory immune checkpoint receptors (IICRs) like PD-1, increase in frequency. Increased inflammation within the brain and reduced IFN-γ from the periphery sustain elevated activation of type I IFN signaling in the choroid plexus epithelium, leading to progressive loss of choroid plexus function, microglial and astrocyte dysfunction, deterioration of synapses, and cognitive impairment.

response to arrest viral propagation to the brain (Fig. 1b). This physiological defensive response, however, if persistent, may negatively affect the brain parenchyma, especially microglia and astrocytes[7,15,24,25] (Fig. 1b,c). Moreover, the type I IFN responses in microglia and astrocytes can further fuel the type I IFN responses in the choroid plexus epithelium through their production of pro-inflammatory cytokines such as IFN-β, tumor necrosis factor, interleukin (IL)-6 and IL-1β[1,24,25] (Fig. 1c). Thus, the protective type I IFN antiviral response in the choroid plexus epithelium might trigger an inflammatory reaction within the brain parenchyma that perpetuates the choroid plexus type I IFN response and endangers brain function.

IFN-γ and cognitive loss

Type I IFN responses in the choroid plexus epithelium may not only be the consequence of direct signals from within the brain parenchyma, but also be amplified by reduction of type II IFN (or IFN-γ) signals from the periphery[1,2]. IFN-γ is necessary for normal brain function, including cognitive performance and social behavior[1,26]. In addition, IFN-γ

is required for the expression by the choroid plexus epithelium of leukocyte trafficking molecules (for example, CCL2, CXCL10, ICAM-1 and VCAM-1) that recruit immune cells from the circulation, such as monocyte-derived macrophages, that could resolve brain damage[27] (Fig. 1a). Reduced IFN-γ signaling at the choroid plexus has been associated with aging[1] and chronic neurodegeneration[2,22], and decreased expression of IFN-γ in the plasma of patients with Alzheimer's disease correlates with cognitive decline[28]. Reduced production of IFN-γ in the periphery may result from decreased systemic immune effector functions[27]. Immunological features compatible with systemic immune exhaustion and suppression have been documented in patients with COVID-19, including impaired IFN-γ activity[29], increased expression of inhibitory immune checkpoints[30] (Fig. 1c), lymphopenia[31] and increased proportions of regulatory T (T_{reg}) cells with enhanced suppressive capabilities[32]. Of note, increased numbers of T_{reg} cells may be a direct consequence of increased expression of type I IFN in the periphery[33].

Because the periphery represents a more accessible compartment for intervention than the brain, the revitalization of systemic immunity after resolution of SARS-CoV-2 infection might be a potential strategy to prevent cognitive vulnerability. Such treatment should be administered after the acute phase of the infection to avoid any potential adverse synergy between immunotherapy-related and COVID-19-related immune effects. It was reported that enhanced IFN-γ signaling at the choroid plexus can be achieved by targeting inhibitory immune checkpoints, such as the 1PD-1–PD-L1 pathway, in the periphery as a strategy to fight neurodegeneration[34]. The possibility of deploying the same strategy to treat late sequelae of COVID-19 is currently being investigated in several clinical trials[31].

Concluding remarks

The mechanisms underlying the cognitive deficits observed in some individuals recovering from COVID-19 are a matter of intense investigation. While the uncontrolled antiviral defense response at the choroid plexus may not be the sole factor inducing cognitive dysfunction after severe SARS-CoV-2 infection[35], it is very likely an important component of this pathway. We base this contention on the well-established negative effects of chronic type I IFN signaling in the choroid plexus epithelium in aging and chronic neurodegeneration, in mice and humans, which impacts microglial and astrocytic activities that may impair cognitive function. While further studies are needed, we argue that the site, level and duration of the immune response to SARS-CoV-2, and perhaps to other viruses, critically affect the fate of the brain, and potentially other tissues, in the infected host.

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All the authors contributed to the writing of the manuscript and the design of the figure.

Competing interests

M.S. is an inventor of the intellectual property that forms the basis for development of PD-L1 immunotherapy for AD.

Additional information

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