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New advances in CNS immunity against viral infection

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The central nervous system (CNS) is an immunologically specialized organ where restrictive barrier structures protect the parenchyma from inflammation and infection. This protection is important in preventing damage to non-renewable resident cell populations, such as neurons, responsible for functions ranging from executive to autonomic. Despite these barriers, the CNS can be infected through several entry portals, giving rise to meningitis and encephalitis. Following infection, resident cells recruit peripherally derived immune cells to sites of viral infection. In this review, we discuss recent advances in immune recruitment and entry at barrier structures as well as current immunotherapeutic strategies for the treatment of persistent viral infections.

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Introduction

CNS viral infections are a major cause of death and disability globally. Furthermore, the socio-economic burden of CNS infections is growing rapidly with the (re) emergence of highly pathogenic neurotropic viruses [1–3]. Despite this growing patient population, specific therapies for CNS infections are largely unavailable. The current standard-of-care for viral infection is antiviral therapy [4,5]. However, these drugs are often ineffective in depleting viral reservoirs because they are either non-specific or fail to cross the specialized CNS barrier structures into areas of viral invasion [2]. Consequently, there remains an outstanding and growing need for targeted treatment of these viral diseases.

Inextricably intertwined in our ability to treat CNS viral infections is our knowledge of brain anatomy, drainage, and immunity, as reviewed elsewhere [6–9]. Application of these principles can assist in creating targeted viral and immune-mediated therapeutics. Over the past decade, several instrumental findings have altered our perspective of CNS immunity. Once thought to be immune-privileged, the CNS is immune competent, dynamic, and in direct contact with the peripheral immune system [10,11]. Within this review, we discuss recent advances in immune recruitment and entry at barrier structures as well as current immunotherapeutic strategies for the treatment of persistent viral infection.

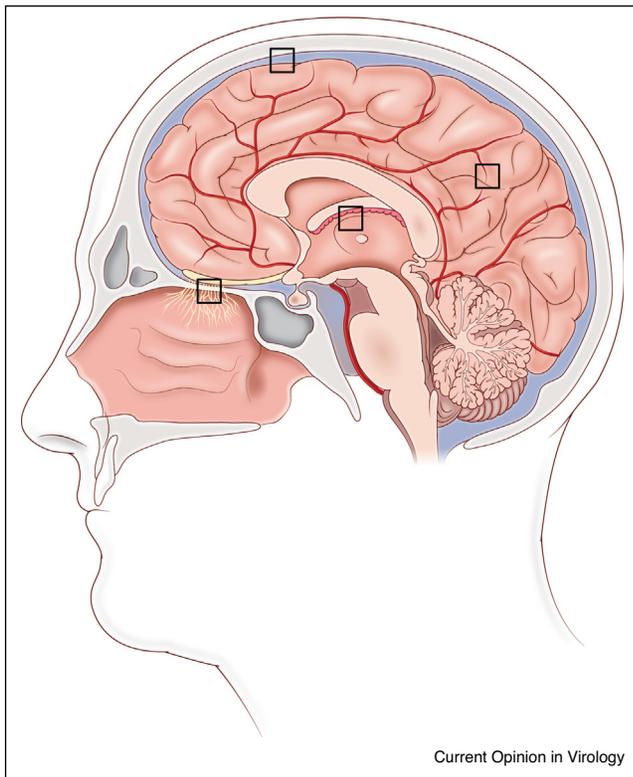
CNS anatomy and drainage

The brain parenchyma, enveloped by the meninges, is suspended in cerebrospinal fluid (CSF) within the cranial vault (Figure 1). The CSF, produced by the choroid plexus (Figure 2) within the ventricular spaces, and the meninges (Figure 3), comprised of the dura, pia and arachnoid mater, protect the CNS from injury and infection. Immediately beneath the skull lies the dura mater (Figure 3), a dense connective tissue layer consisting of fenestrated vessels (without tight junctions) derived from the carotid artery, sensory nerve fibers of the cranial nerves, and an abundant repertoire of immune cells, including meningeal macrophages [9,12*]. Inferior to the dural tissue lies the arachnoid mater — a trabecular network that creates a subarachnoid space for CSF flow. Within the arachnoid mater lie granulations responsible for CSF absorption into the superior sagittal sinus, the venous drainage system. Lastly, the pia mater above the parenchyma gives rise to perivascular (Virchow-Robin) spaces where penetrating arteries enter the parenchyma. These spaces are known to contain CSF and perivascular macrophages [9].

Between cells of the CNS parenchyma resides interstitial fluid that maintains neuronal and glial homeostasis through solvent exchange between the capillary vasculature and CSF [13]. Recently, Iliff *et al.* described a system known as the ‘glymphatics’, where the disparate systems of CSF influx and efflux drive clearance of interstitial fluid and its suspended solutes [14,15]. Specifically, CSF moves from the meningeal space along penetrating arteries and their periarterial spaces into the CNS parenchyma. Mediated in part by astrocytic endfeet, interstitial fluid is driven through the parenchyma into perivenous spaces where, ultimately, fluid residing in the perivascular spaces can be resorbed.

The CSF and interstitial fluid is drained in part by lymphatics into the deep cervical lymph nodes. The

Figure 1



Sagittal view of the central nervous system and its barrier structures. The CNS is protected by several anatomically restrictive barrier structures that include the choroid plexus (Figure 2), meninges (Figure 3), blood–brain barrier (Figure 4), and olfactory epithelium (Figure 5). These barrier structures protect the CNS infection but can also serve as an entry point for pathogens and/or immune cells.

CNS lymphatics consists of the nasal lymphatics, which travel from olfactory bulb (OB), through the cribriform plate and below the skull, and the dural lymphatics, which travel alongside the superior sagittal sinus and across the transverse sinus [16–18]. Although studies have shown the connection between glymphatics and lymphatics, the exact mechanism of CSF drainage into the lymphatics remains unknown [15].

In addition to the CNS clearance and drainage systems, the brain and spinal cord are well perfused by an extensive vascular network. Unlike choroid plexus or dural vasculature, blood vessels that traverse the CNS pia and parenchyma are tightly regulated (Figure 4) [19,20]. Endothelial cells comprising these vessels form tight junctions. In addition, parenchymal blood vessels are further fortified by pericytes, basement membrane, and astrocytes. These structures decrease permeability, making it difficult for small molecules and cells to enter the CNS parenchyma.

While the aforementioned anatomy protects the CNS, it can still be infected by viruses via the meninges or

blood–brain barrier (BBB), retrograde transport by peripheral nerves, or by circulating/infiltrating peripheral immune cells. Invasion of neural tissue can initiate serious neurological disorders like meningitis and encephalitis, as reviewed previously [21,22]. These infections can cause significant and irreversible damage to the CNS. Understanding how CNS resident innate immune cells respond to these infections as well as their role in guiding peripheral immune cell recruitment to sites of viral invasion should aid the development of interventions to treat CNS infections.

Innate antiviral immunity to CNS infection

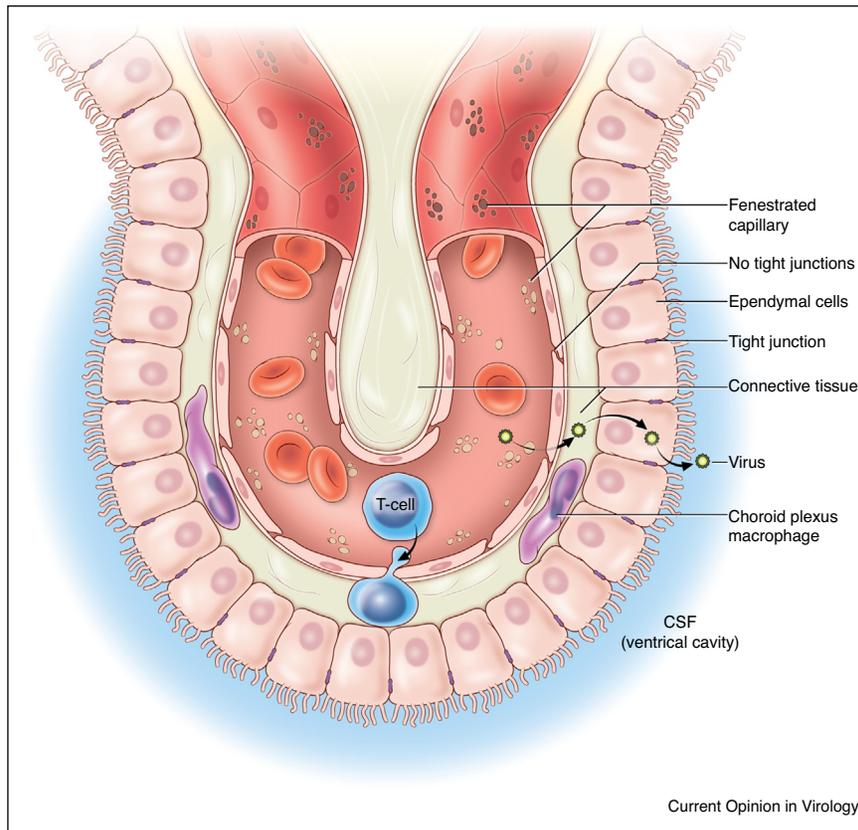
Upon viral entry into the body, an immune response is generated in secondary lymphoid organs. Primed immune cells are then recruited to the CNS by local inflammatory signaling. Activated by conserved pathogen-associated molecular patterns (PAMPs), pattern recognition receptors (PRRs), retinoic acid-inducible gene (RIG)-I-like receptors, and toll-like receptors (TLRs) initiate innate immune reactions [23,24]. Viral nucleic acids bind to these receptors expressed by innate immune sentinels (e.g. microglia, macrophages, dendritic cells, astrocytes) and cause release of type I interferon (IFN-I) as well as the production of interferon stimulated genes (ISGs). IFN-I limits viral spread by upregulating antiviral proteins, recruiting peripheral immune cells, and altering endothelial tight junction proteins to decrease BBB permeability [23,25,26*,27,28]. A recent intravital imaging study performed in the brains of lymphocytic choriomeningitis virus (LCMV) infected mice revealed that the absence of IFN-I signaling prevented microglia differentiation from a ramified state, decreased peripheral myeloid cell patrolling of cerebrovasculature, and significantly increased the number of infected brain-resident myeloid cells [28]. Within this viral model system, IFN-I signaling was responsible for the entire innate immune program within the CNS.

Following infection, IFN-I responses within the CNS can be global or region-specific. For example, long-distance IFN-I signaling can prevent viral spread from one brain region to another. This has been observed following vesicular stomatitis virus (VSV) infection, where microglial IFN-I production in the olfactory bulb is thought to limit the anterograde spread of virus into the hindbrain regions [29,30]. IFN-I responses can also tighten the BBB and limit the extent of viral entry from the blood. Astrocytic interferon- α receptor signaling was shown to decrease BBB permeability and protect the hindbrain from West Nile virus (WNV) infection [26*]. These data demonstrate that with certain infections IFN-I can provide a region-specific antiviral defense.

Antiviral immune responses within specific anatomical niches

While the local IFN-I response plays an important role in CNS protection, the activation and recruitment of the

Figure 2



Choroid plexus. The choroid plexus resides in ventricular spaces of the brain and is responsible for creating cerebral spinal fluid (CSF). The choroid plexus is a dynamic barrier structure home to many immune cells, including choroid plexus macrophages. Unlike the BBB, but similar to the dura mater, choroid plexus blood vessels are fenestrated and do not express tight junctions, which provides easier access to pathogens and immune cells into the stromal space. Choroid plexus epithelia (ependyma) serve as a barrier between fenestrated blood vessels and the CSF. Expression of tight junctional proteins between individual epithelial cells protects the CSF by restricting solute and cellular movement. During infection, the choroid plexus can serve as a gateway for immune recruitment and entry into the CSF. Immune cells must first enter the choroid plexus stroma before traversing the epithelial barrier.

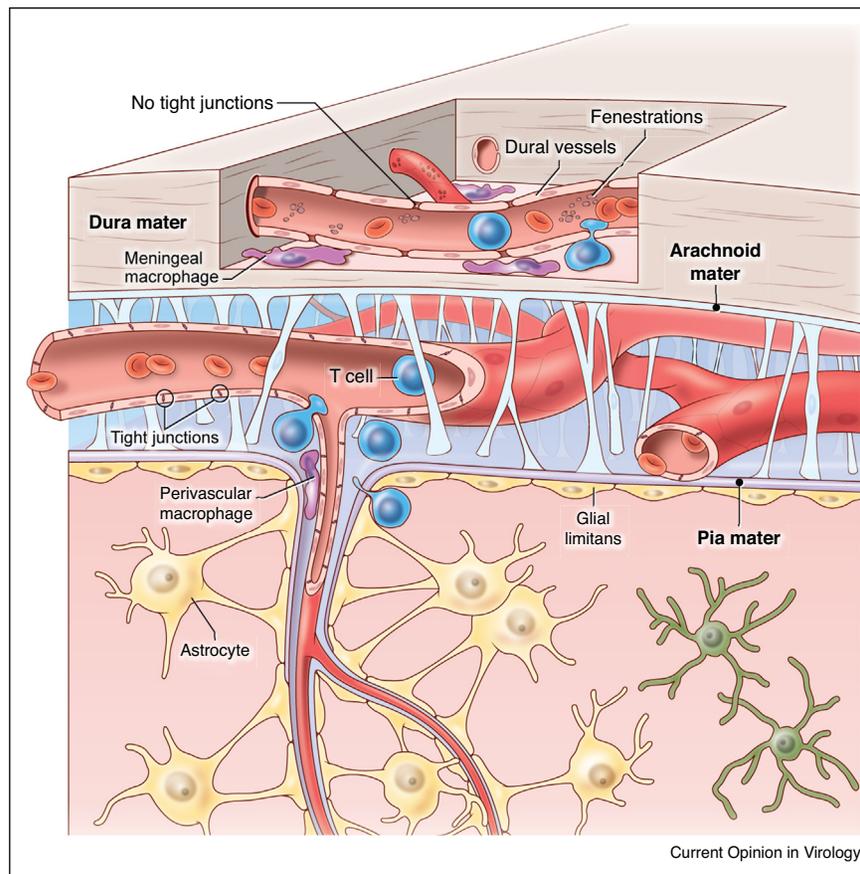
adaptive immune cells is usually necessary for viral clearance. Lymphoid cells can gain access to the CNS via multiple entry portals as shown in Figure 1. These areas are also sites of pathogen entry into the CNS, which is likely why the immune system invests so much into surveying these structures. The presence of adhesion and antigen presenting molecules facilitates extravasation and routine immune surveillance of these entry portals despite the presence of specialized barrier proteins [31]. Each barrier structure has its own anatomical specializations that result in a tailored immune defense against invading pathogens. Some of the recent insights into pathogen-specific immunity within these distinct anatomical regions are described in more detail below.

A. Meninges

Although viral meningitis has been studied extensively, little is known about how resident meningeal cells recruit and direct peripheral immune cell traffic. Peripheral

immune cells can enter the CNS through the blood meningeal barrier [12,32], as depicted in Figure 3. The meningeal stromal cell network consists of blood endothelial cells, pericytes, fibroblasts, and smooth muscle cells [19]. Blood vessels within the dura mater are fenestrated and do not express tight junctions [33], permitting easier access to peripheral immune cells that must still traverse the arachnoid mater before entering the subarachnoid space. By contrast, meningeal vessels beneath the arachnoid mater are non-fenestrated, express tight junctions, and are surrounded by a network of fibroblastic reticular cells that can modulate peripheral immune cells. Following coronavirus infection, meningeal endothelial cells and fibroblastic reticular cells release CCL19 and CCL21 to recruit/reactivate antiviral CCR7⁺ CD4⁺ and CD8⁺ T cells in response to resident myeloid and neural cell infection [34]. The entry of CCR7⁺ T cells is likely facilitated by stromal cell reorganization after infection [35]. Without CCR7⁺ lymphoid

Figure 3



Meninges. The meninges consist of three layers that envelope the brain and spinal cord. The dura mater, the outermost layer, is vascularized by fenestrated vessels and is home to a repertoire of immune cells, including meningeal macrophages that line the vessels. Interior to the dura lies the arachnoid mater. The arachnoid, its trabecula and the pia mater form the subarachnoid space, a compartment where cerebrospinal fluid (CSF) freely flows. The arachnoid mater also contains tight junctions that help keep materials in the dura mater separate from the subarachnoid space. Within the subarachnoid space resides pial vessels that dive into the CNS parenchyma. Pial vessels are non-fenestrated and express tight junctions. The spaces between these vessels and the parenchyma (referred to as perivascular spaces) are inhabited by perivascular macrophages. The final layer, which lies beneath pia mater, is referred to as the glial limitans — a layer of surface-associated astrocytes that protect the brain and prevent migration of solutes and cells from the CSF into the parenchyma. During states of infection, the meninges can serve as an entry point for extravasating immune cells. Dural vessels are especially susceptible to immune cell and pathogen entry because they are fenestrated and do not express tight junctions.

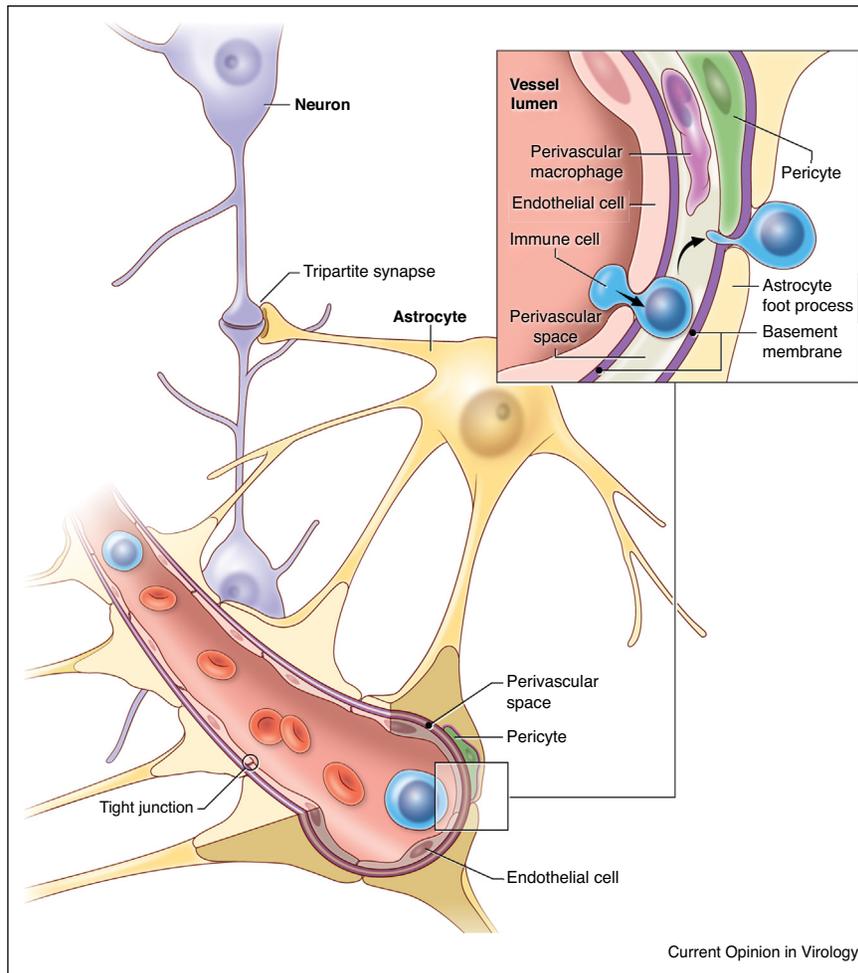
cell recruitment, animals cannot control viral replication and die of infection [34^{**}]. However, it is unclear exactly how these immune cells enter the virally infected CNS parenchyma from the meninges [36], although the process presumably involves migration across the glial limitans. Non-infectious animal models suggest that meningeal and/or perivascular macrophages ‘license’ T cells for CNS entry through chemokines (e.g. CCL5, CXCL9-11, CXCL12), adhesion molecules, and cognate antigen presentation [37^{*}]. Upon entering the meninges, activated T cells utilize these cues to interact with local macrophages, reactivate, and, ultimately, gain access to the CNS parenchyma. However, it is presently not known whether similar steps are required for antiviral T cells to enter the infected brain after migrating into the meninges. Understanding how the meninges regulate immune

recruitment and traffic into the infected brain should improve our ability to stimulate productive immune responses against parenchymal infections.

B. Choroid plexus

The choroid plexus consists of fenestrated vasculature without tight junctions that is surrounded by an epithelial network (sealed by tight junctions) capable of hosting a diverse immune repertoire, including T and B cells (Figure 2) [12^{*},19]. Although the choroid plexus is directly infected by some viruses (e.g. Coxsackie B3, Echovirus 30, LCMV), it is likely that this structure also plays a primary role in the antiviral defense against other CNS viruses because of its anatomical localization within the ventricular system, ability to globally activate the CNS through release of inflammatory mediators into

Figure 4



Blood–brain barrier (BBB). The blood brain barrier creates a selective interface between the blood and CNS parenchyma. It consists of endothelial cells and their tight junction proteins, basement membrane, pericytes and astrocytic end feet. In conjunction with barrier function, the BBB can also modulate cerebral blood flow through the neuro-vascular unit, a connection involving neurons, pericytes, astrocytes, and the blood–brain barrier. This allows neural and astrocytic activity to modulate blood vessel tone, resulting in an increase or decrease of regional perfusion. During infection, the BBB is permissive to immune extravasation from the blood into the perivascular spaces. Immune cells usually enter the perivascular spaces before gaining access to the parenchyma. These spaces are inhabited by perivascular macrophages.

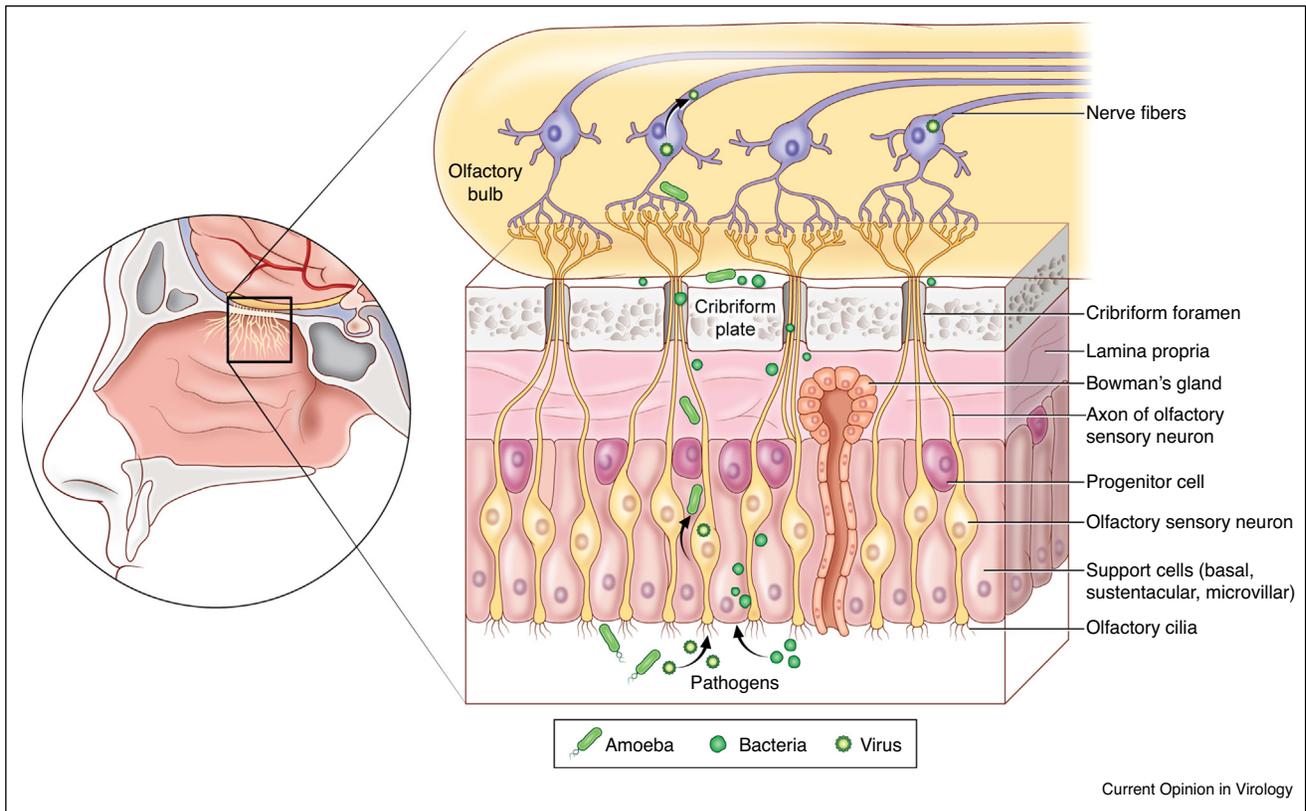
the CSF, and role as a gateway for immune cell entry [38,39]. Under steady state conditions, immune cells travel from the blood across fenestrated endothelium into stromal spaces (connective tissue) within the choroid plexus. More recently, it was observed that CD4⁺ T cells use IFN γ to help maintain immune populations within the choroid plexus during homeostasis by promoting expression of adhesion, chemokine and antigen presenting molecules on the epithelium. Interestingly, deletion of the IFN γ receptor was shown to reduce steady state immune cell numbers within the choroid plexus as well as immune trafficking into the CSF [40] — a process that depends in part on NF κ B/p65 signaling [41]. While immune cell traffic through the choroid plexus has been studied in the context of CNS injury and autoimmune disease [42,43], less is known *in vivo* about how this

structure functions immunologically following CNS viral infection [38]. However, data obtained in other models of CNS inflammation might provide clues into the role of the choroid plexus in antiviral immunity.

C. CNS parenchyma

Unlike the meninges, the CNS parenchyma is home to few immune cells [12*]. Resident microglia survey and respond rapidly to CNS infections [44]. After herpes simplex virus-1 (HSV-1) infection, microglia utilize the cGAS-STING cytosolic DNA sensing pathway to induce release IFN-I, which abrogates viral spread and lethality by activating a neuronal antiviral program [45–47]. Microglia-mediated protection was also observed following dengue virus (DENV) infection, where depletion of CNS myeloid cells with clodronate increased viral

Figure 5



Olfactory epithelium and bulb. The olfactory epithelium forms part of the nasal airway and serves as a direct interface between the peripheral nervous system (PNS) and CNS. Olfactory sensory neurons within the olfactory epithelium are surrounded by support cells including basal, sustentacular, and microvillar cells. These neurons extend axon fibers that cross the cribriform plate and synapse in the olfactory bulb of the CNS. Because the dendrites of olfactory sensory neurons extend directly into the airways, these cells can be infected by viruses. Viruses that infect olfactory sensory neurons often hijack axonal transport machinery to invade the CNS. Other pathogens, such as bacteria and amoeba, can also access the CNS by traversing the olfactory epithelium and entering holes in the cribriform plate.

replication and mortality [48]. Loss of CNS myeloid cells abolished antiviral CD8⁺ T cell recruitment to the DENV-infected brain, suggesting that microglia play a critical role in the antiviral defense against this pathogen.

Although innate activation and recruitment can be initiated by microglia, neurons can also contribute to protective immunity during infection. Following HSV-1 infection, deletion of STAT1 signaling specifically in neurons markedly increased viral titer in the brain and trigeminal ganglia as well as viral spread into non-neuronal tissues, resulting in increased mortality [47]. Neuronal antiviral protection also occurs following WNV infection. CD8⁺ T cells are required to clear WNV [49,50], and neurons can participate in this clearance by releasing T cell recruitment chemoattractants like CXCL10, which has also been observed following rabies virus infection [51,52]. Interestingly, it was recently demonstrated that WNV infection promotes release of CCL2 and CXCL10 from neurons that depends on activation of receptor-interacting protein-kinase 3

(RIPK3), which in turn promotes recruitment of leukocytes to the brain without causing neuronal cell death [53^{**}]. Use of this particular pathway in neurons reflects a nontraditional, neuroprotective role for RIPK3 that usually induces necroptotic cell death in other cell types [54]. Induction of necroptosis within virally infected neurons would have a profoundly negative effect on the CNS. It is very important after infection that the CNS parenchyma inhibits viral spread and attracts adaptive immune cells without injuring neurons, which are largely considered a non-replicative cell population.

D. Olfactory epithelium

Olfactory sensory neurons (OSN) are located in the olfactory epithelium of the nose and provide a bridge between the periphery and CNS. Hair-like cilia expressing olfactory receptors extend from OSN dendrites into the mucus layer of the epithelium, and their axons project through the cribriform plate onto mitral cells located in the olfactory bulb (Figure 5). This system is responsible for our sense of smell, but provides a direct conduit for

viruses and other pathogens to enter the CNS via the nose. There are many viruses (e.g. VSV, HSV-1, RABV, Nipah virus, Hendra virus, influenza A virus, etc.) that enter the CNS via this route [22,55]. Viral invasion of OSNs can rapidly lead to encephalitis and host death, as a virus travels from the olfactory bulb to the hindbrain [56]. Little is known about the immunological defense mounted by the olfactory epithelium following infection. Olfactory ensheathing cells (OECs) are glial cells that insulate OSNs, have phagocytic capabilities, and can clear neuronal debris during development [57]. These cells can respond to bacterial PAMPs via TLR2 and TLR4, inducing release of IFN-I [58–60]; however, it is unclear how OECs contribute to the immune response against neurotropic nasal viruses. Another strategy used by the olfactory epithelium to defend against viral infection is apoptosis. OSNs are a renewable neuronal population that will sometimes undergo apoptosis following infection to limit the degree of viral dissemination into the CNS [61]. Despite this ability to rebuild, infection and inflammation within the olfactory epithelium can be detrimental to olfactory function, giving rise to a temporary or permanent loss of smell [62,63].

Nasal associated lymphoid tissue (NALT) is also housed within the nose and is thought to play a role in antimicrobial immunity as well as the efficacy of vaccine delivery nasally [64]. NALT is home to a diverse population of APCs, including macrophages, B cells and dendritic cells. These APCs facilitate adaptive immune responses to infection [65]. The NALT can participate in the initiation of nasal immune responses; however, a recent study demonstrated that following influenza virus infection, T resident memory (Trm) cells localized primarily in the nasal tissue (e.g. olfactory epithelium) outside of the NALT [66**]. Interestingly, these Trm's contributed to the primary defense against re-challenge by a heterologous strain of influenza virus, which prevented spread of the pathogen into the lower respiratory tract [66**]. These data indicate that antiviral immunity within the olfactory epithelium can limit viral dissemination into the CNS as well as the lower respiratory tract. Additional studies are required in this important barrier tissue to determine all the cellular participants within the nasal cavity that coordinate such a formidable defense against continuous microbial challenges.

Advancements in treatment of persistent viral infections

Viruses can evade adaptive immune responses and remain in the CNS indefinitely. Some examples include human immunodeficiency virus (HIV), simian immunodeficiency virus (SIV), John Cunningham virus (JCV), cytomegalovirus (CMV), varicella zoster virus (VZV), LCMV, HSV and WNV, among others. By creating their own microenvironments, viral reservoirs can develop in a

region-specific and/or cell-specific manner, undergoing significant genomic evolution compared to the periphery. Reactivation of CNS viral reservoirs can result in profound neurological disorders, including epilepsy, cognitive impairment, and motor dysfunction despite antiviral treatment [67].

Over the past decade, immunotherapy has revolutionized medicine. By utilizing immune-specific therapies, refractory diseases have become responsive to treatment. The application of these techniques to persistent viral infection could aid in the clearance of CNS reservoirs. However, use of immunotherapy to treat persistent viral infections offers two unique challenges. First, neurotropic viruses often take residence in the CNS behind selective barriers to evade immune cell traffic and detection. To target these viruses, immunotherapies need to gain access to the anatomical niches despite low levels of neuroinflammation — a scenario often encountered during states of viral persistence. Second, immunotherapeutic regimens have the potential to cause a great deal of tissue pathology [68], which must be considered when attempting to purge a virus from a sensitive compartment like the CNS. Using adoptive T cell therapies, it is possible to eradicate a virus from the CNS parenchyma without causing immunopathology, although this might not be the case with every pathogen [69**].

Tailored immunotherapy for treatment of persistent viral infections can consist of immunomodulators or adoptive cell transfer. During peripheral viral infection, immunomodulatory therapy has focused primarily on cytokine or interferon-based treatments. However, these treatments alone often fail to clear viral reservoirs but instead reactivate or facilitate adaptive immune clearance [70]. For example, TNF α blockade paradoxically reactivates CD4⁺ and CD8⁺ T cell effector function to stimulate viral control during a persistent LCMV infection [71]. Accordingly, immunomodulatory treatment has immense potential to resolve infectious diseases where adaptive immune function remains intact and the nature of the infection is understood. For example, during WNV infection, CXCR4 antagonism increases CD8⁺ T cells entry into the brain from the perivascular spaces, thereby decreasing viral load and mortality [49]. Similarly, administration of CXCL9 into the CNS during HSV-1 infection increases the recruitment of antiviral CD8⁺ T cells and promotes survival by bypassing the role of the UL13 kinase — a viral protein that downregulates neuronal CXCL9 release during infection [72]. Alongside chemokine-based and cytokine-based treatments, checkpoint inhibitors, such as PD-1/PDL-1 blockade, have also been used to modulate immune responses during persistent viral infection [73,74*]; however, their efficacy in treating CNS infections is currently unknown. To address this question, a current clinical trial (NCT03239899) is underway to determine whether PD-1 blockade is safe in HIV-1

patients and can be used to improve antiviral immunity in the CNS, which is thought to be a reservoir for the pathogen.

Immunocytotherapy or adoptive immunotherapy is another potentially effective strategy to purge a persistent CNS viral infection when the host immune system is incapable of doing so [75,76]. During a persistent LCMV infection, adoptive transfer of virus-specific memory CD8⁺ and CD4⁺ T cells can eradicate virus from the CNS parenchyma, meninges, and choroid plexus without causing cytopathology or BBB breakdown [69^{••},77]. During this clearance process, antiviral T cells convert microglia into CD11c⁺ antigen presenting cells that they purge of virus non-cytopathically [69^{••}]. These data demonstrate that antiviral T cells are not inherently pathogenic to the CNS and have the potential to clear a virus without causing damage [78].

One of the suggested etiologies of multiple sclerosis (MS) includes Epstein-Barr virus (EBV) infection [79]. Because of incomplete EBV clearance and T cell exhaustion, infected B cells persist in the meninges as well as perivascular spaces and associate with MS lesions [80,81]. Therefore, removal of these inflammatory B cells may ameliorate some of the CNS dysfunction associated with MS [82]. A recent case report revealed that adoptive transfer of autologous EBV-specific CD8⁺ T cells improved cognition and motor function in a patient with secondary progressive MS [83]. Following treatment, the patient showed expansion of EBV-specific CD8⁺ T cells in the blood, decreased intrathecal immunoglobulin production, and reduced CNS lesions by MRI. These promising preliminary data suggest that adoptive immunotherapy directed against EBV might be efficacious in treating MS patients, and an expanded clinical trial will evaluate this possibility more definitively.

Another virus-induced CNS disease that might benefit from adoptive immunotherapy is progressive multifocal leukoencephalopathy (PML) — a progressive demyelinating disease caused by JC virus. JCV has a high seroprevalence within the general population; most carriers remain asymptomatic unless immunocompromised. In a recent study, a patient with JCV-induced PML was successfully treated by adoptively transferring JCV-specific CD8⁺ T cells directed against the VP1 and large T viral proteins [84]. This treatment cleared virus from the CSF and improved neurological function. A clinical trial (NCT02694783) is currently underway to test this approach in a larger cohort of PML patients. It will be interesting to find out whether JCV-specific CD8⁺ T cells alone can provide lasting viral control in all patients. Similar to adoptive immunotherapy against LCMV [85], the antiviral defense against JCV might require CD4⁺ T cell support of CD8⁺ T cells to provide durable control [86].

Future directions

Understanding how peripheral immune cells are recruited to the CNS and interact with resident cells to fight viral infections can greatly improve the development of immunotherapeutic regimens. The need for treatments has become more relevant than ever with the (re)emergence of pathogens like Zika virus, which can cause neonatal microcephaly, adult encephalitis, and neural precursor cell death [87,88], and Ebola virus, a pathogen that causes acute hemorrhagic fever and can reactivate up to nine months after an acute infection, leading to meningoencephalitis [89,90]. Thus far, research on these pathogens has focused on vaccine development [91,92]; however, this approach will not necessarily resolve infection in persistently infected hosts. Therefore, continued development of therapies to treat patients persistently infected with neurotropic viruses must remain a high priority.

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