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Illuminating viral infections in the nervous system

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Abstract

Viral infections are a major cause of human disease. Although most viruses replicate in peripheral tissues, some have developed unique strategies to move into the nervous system, where they establish acute or persistent infections. Viral infections in the central nervous system (CNS) can alter homeostasis, induce neurological dysfunction and result in serious, potentially life-threatening inflammatory diseases. This Review focuses on the strategies used by neurotropic viruses to cross the barrier systems of the CNS and on how the immune system detects and responds to viral infections in the CNS. A special emphasis is placed on immune surveillance of persistent and latent viral infections and on recent insights gained from imaging both protective and pathogenic antiviral immune responses.

Despite its immune-privileged status, the central nervous system (CNS) can respond vigorously to viral challenges. However, it is now abundantly clear that the CNS accomplishes this in a different manner to peripheral tissues. The CNS is protected by an elaborate barrier system that is the first line of defence against pathogen invasion. Crossing the blood–brain barrier or the blood–cerebral spinal fluid (CSF) barrier requires specialized viral adaptations. Nevertheless, many viruses have acquired strategies to quickly enter the nervous system following infection (TABLE 1). One particularly important strategy used by viruses is to enter the peripheral nervous system and travel via axon fibres to the CNS. The peripheral nervous system consists of nerve fibres and ganglia that connect the CNS to peripheral tissues. Because these peripheral nerves extend outside the protective barriers of the CNS, they represent a potential chink in the protective armour of the CNS that can be exploited by opportunistic infections. However, these nerves are protected from infection by peripheral innate and adaptive immune cells.

In addition to accessing the peripheral nervous system, neurotropic viruses use many other approaches to bypass the barrier systems and directly enter the CNS. These infections rarely go unnoticed, as the CNS is equipped with an elaborate network of innate immune sentinels (see Supplementary information S1 (movie)) that respond immediately to disturbances such

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SUPPLEMENTARY INFORMATION

See online article: S1 (movie) | S2 (movie) | S3 (movie) | S4 (movie) | S5 (movie)

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as those caused by viral replication. For example, the brain parenchyma is continuously scanned by microglial cells, which are derivatives of primitive myeloid precursors[1]. Highly dynamic microglial processes scan the entire extracellular space every few hours and are likely to detect most viruses that access the CNS parenchyma[2]. Specialized innate immune sentinels, such as macrophages and dendritic cells (DCs), are also found in the CNS structures that produce and transport CSF. The brain and spinal cord 'float' in CSF, which is synthesized by specialized epithelial cells in the choroid plexus and then flows through the ventricles and meninges (among other structures) (FIG. 1). The choroid plexus, meninges and perivascular spaces are not inhabited by microglial cells, but rather by macrophages (some of which resemble DCs) that are derived from the bone marrow[3,4]. These fluid-rich spaces are an excellent location for immune sentinels to undertake surveillance of pathogens that access the CNS by crossing the blood–CSF barrier. Having specialized sentinels continually scanning the CSF ensures the immediate detection of infectious agents and the initiation of a robust immune response.

It is well recognized that many cytopathic and non-cytopathic viruses can infect the human nervous system (TABLE 1), causing inflammatory diseases such as aseptic meningitis, encephalitis and meningoencephalitis. These diseases can severely affect human health and can be associated with long-term sequelae. In addition, new neurotropic viruses are continually emerging and can challenge the nervous system in new ways[5,6] (BOX 1). Emerging viruses are particularly problematic because they are often less equilibrated with their new host, and they can spread rapidly and induce severe pathology. This contrasts with viruses that cause persistent or latent infections, as these viruses have evolved strategies to replicate in the nervous system over prolonged time periods. Some persistent infections can be held in check by the immune system, and only re-emerge and cause disease during periods of immunosuppression, whereas other neurotropic viruses initiate chronic diseases owing to continual disruption of CNS homeostasis.

Box 1

Emerging viral infections

Emerging viral infections are those that have recently emerged or re-emerged and are likely to cause significant disease in humans or animals. Common causes of emerging infections include (but are not limited to): genetic mutation of existing viruses, the emergence of new viruses, expansion of viral host reservoirs, movement of viruses from one species to another and suppression of host immune surveillance.

Influenza virus is an excellent example of an existing virus that can quickly become an emerging infection owing to genetic mutation and variation. The RNA polymerase of influenza virus is error prone and generates mutations and antigenic drift over time[111]. In addition, the genome of influenza virus consists of eight separate RNA segments, which can be exchanged between different influenza virus strains when they infect the same cell. Both mutation and swapping of RNA segments enables influenza virus to change rapidly and generate new strains. These can become emerging infections if they replicate in new hosts, spread quickly and are able to cause serious disease.

Expansion of viral infections into new host reservoirs can also result in dramatic viral spread and disease. Recently, West Nile virus (WNV) spread into humans living in North America, resulting in the development of encephalitis, meningitis and acute flaccid paralysis in a portion of the infected population[6]. Although the exact route by which WNV first entered humans is unknown, it is considered likely that migratory birds (which serve as the normal host reservoir) and mosquito vectors participated in its dissemination.

Newly identified viruses can also fall into the category of emerging infections. The discovery of Hendra virus and Nipah virus in the 1990s led to the creation of a new genus (Henipavirus) within the <italic>Paramyxoviridae</italic> family[112]. Henipaviruses are normally found in bats, but can cause serious life-threatening disease following spread into domestic animals and humans. In humans, henipaviruses cause widespread multisystemic vasculitis and severe pathology in many tissues, including the brain. Patients infected with these viruses present with fever, headache, drowsiness, confusion and seizures, and disease progression can lead to loss of consciousness and death.

Finally, viruses that are normally benign (or controlled) can become emerging infections when they gain access to new environmental niches. This is exemplified by the emergence of JC virus-induced disease caused by therapeutic immunosuppression[110,113] or immunodeficiency associated with HIV infection[5,114]. JC virus infection is a common latent virus in the general population but in immunocompromised individuals JC virus can reactivate, target oligodendrocytes and cause disease (see Box 2).

After describing the predominant strategies used by viruses to enter and spread within the nervous system, this Review focuses on the unique dialogue established between the nervous and immune systems during states of viral persistence and latency. We then discuss how two-photon laser scanning microscopy (TPLSM) has been used to gain novel insights into CNS innate immune sentinels and the immune mechanisms that protect and harm the nervous system following acute viral infection.

Viral entry and spread in the nervous system

CNS barriers and anatomy

Maintaining homeostasis within the CNS is crucial for the protection of post-mitotic neurons and for optimal brain function. The blood–brain and blood–CSF barriers help to shield the CNS from free diffusion of vascular and cellular components (FIGS 1,2a). Most blood vessels that enter the CNS are composed of non-fenestrated endothelial cells that use tight junctions to limit the movement of cells and molecules through inter-endothelial gaps[7]. Endothelial cells also provide a physical acellular barrier to the perivascular space by producing a basement membrane consisting of laminin α4 and laminin α5. Smooth muscle cells and pericytes located along the vessels regulate vascular tone[7,8], and pericytes can also help to maintain and integrate blood–brain barrier components during development[9,10] (FIG. 2a).

Within the CNS parenchyma, juxtavascular microglial cells[11] and perivascular macrophages are closely apposed to the endothelium[12,13] and have the potential to interact with

infiltrating leukocytes[14] (FIG. 2a). Astrocytes also contribute to the blood–brain barrier system by forming the glial limitans, a dense network of basal lamina and astrocytic endfeet (FIGS 1,2a; Supplementary information S2 (movie)) that forms the outermost layer of the CNS parenchyma, just beneath the meningeal pia mater. Astrocytic endfeet also envelop brain parenchyma capillaries, providing an extension of the glial limitans through additional structural and barrier support[15]. Because astrocytes reside exclusively in the parenchyma, they do not contribute to meningeal or choroid plexus barriers, which are part of the blood–CSF barrier system. Meningeal blood vessels instead rely on non-fenestrated endothelium only, whereas choroid plexus blood vessels have normal fenestrated endothelium but are encased by a second layer of epithelial cells that provides some barrier protection. Although the blood–brain barrier and blood–CSF barrier are typically sufficient to shield the CNS from various insults (both biological and chemical), neurotropic viruses have evolved strategies to breach these barrier systems and enter the CNS.

Crossing the vascular endothelium

One means for viral entry into the CNS is through the CNS endothelium. Several viruses — including JC virus[16], poliovirus[17], Epstein–Barr virus (EBV)[18], mouse adenovirus 1 (MAV-1)[19], human T-lymphotropic virus type 1 (HTLV1)[20] and West Nile virus (WNV)[21] — have been shown to directly infect human brain microvascular endothelial cell lines *in vitro*. In many cases, infection alters endothelial cell behaviour by promoting increased production of chemokines (such as CC-chemokine ligand 2 (CCL2) and CCL5 (REFS 18,19)), altered expression of tight junction proteins[19–21], increased expression of vascular cell adhesion molecule 1 (VCAM1)[21] and decreased transendothelial electrical resistance[19]. These alterations have the potential to increase vascular permeability and permit viruses to bypass the first layer of the CNS barrier system (namely, the endothelium).

In vivo, viruses can also use endothelial-expressed proteins to bind and enter these cells. For example, the junctional protein junctional adhesion molecule A (JAM-A), which normally helps to form tight inter-endothelial barriers, can also serve as a reovirus receptor[22]. In humans, the retrovirus HTLV1 binds to various receptors, including glucose transporter type 1 (GLUT1; also known as SLC2A1), heparin sulphate proteoglycans and neuropilin 1, which were shown to be expressed by endothelial cells in spinal cord sections. In addition, HTLV1 viral transcripts were associated with the vas-culature of infected patients[20], suggesting that HTLV1 directly infects the blood–brain barrier to access the human CNS.

Trojan horse' entry

Another way that viruses can enter the CNS is via a 'Trojan horse' mechanism, in which infected leukocytes carry pathogens from the blood across the blood–brain barrier (FIG. 2a). Infection of monocytes and/or macrophages is considered a major mechanism used by lentiviruses — including simian immunodeficiency virus (SIV) and HIV — to migrate across CNS vascular barriers[23,24]. During SIV and HIV infections, a population of $CD16^+$ monocytes expands in the periphery. A study that tracked fluorescein-labelled monocytes during SIV infection revealed rapid localization (within 12–14 days) of these infected cells to brain perivascular spaces and the choroid plexus, indicating successful migration across the CNS barrier systems. SIV detection in both the brain and CSF coincided with monocyte

entry into the CNS[24], supporting the idea of monocyte involvement in viral neuroinvasion. In humans, peripherally derived $CD16^+$ monocytes were shown to be more susceptible to infection than other populations of blood monocytes, and in brain tissue $CD16^+$ monocytes were present in HIV-infected regions[23]. Initial monocyte entry could be due to normal turnover and repopulation of CNS perivascular macrophages and/or due to altered vascular permeability resulting from the production of CCL2, matrix metalloproteinases (MMPs) and pro-inflammatory cytokines by HIV-infected monocytes[23,25,26]. Sustained CCL2 production by monocytes and glial cells, together with viral protein-induced alterations in endothelial adhesion molecules and junctional protein expression[26], can then amplify blood–brain barrier breakdown and viral entry.

Other viruses, such as JC virus, also infect leukocyte populations that could facilitate CNS access. JC virus was detected in peripheral blood B cells during infection, and *in vitro* it can move from B cells to glial cells, which are a major target cell population *in vivo*[27,28]. Whether B cells serve as a 'Trojan horse' for viral entry and dissemination *in vivo* remains unclear. In general, the blood–brain and blood–CSF barriers carefully regulate the passage of immune cells into the CNS. However, viruses have learnt strategies to exploit normal haematological routes of immune surveillance and repopulation in order to replicate within the brain.

Accessing peripheral nerves

Another critical point of viral entry is through sensory and motor neurons that extend beyond the CNS barriers into the periphery (FIG. 2b). The differential expression of viral receptors on either sensory or motor neurons can dictate the type of peripheral nerve ending a particular neurotropic virus will target. Poliovirus, adenoviruses and rabies virus can bind to neurons at the neuromuscular junction owing to the neuronal expression of specific receptors, such as poliovirus receptor (PVR; also known as CD155), coxsackievirus and adenovirus receptor (CAR; which is bound by adenoviruses) and acetylcholine receptors and neural cell adhesion molecule (NCAM), to which rabies virus binds[29]. In addition, herpesviruses[30,31], including pseudorabies virus, use nectin 1 (also known as PVRL1) and nectin 2 (also known as PVRL2) to infect sensory neurons[29,32]. Peripheral viral entry is not limited to sensory and motor neurons, as olfactory nerves also act as a point of viral entry into the CNS[33].

Following entry, numerous viruses hijack the axonal transport system for intracellular movement (FIG. 2c,d). Such viruses include poliovirus, alphaherpes-viruses[29,30], WNV[34], Theiler's murine encephalomyelitis virus (TMEV)[35], rabies virus[36], measles virus[37] and Borna disease virus (BDV)[38,39]. Normally, axons use anterograde and retrograde transport systems to move cellular cargo between the cell body and synaptic boutons[40]. Anterograde transport uses the kinesin motor system, whereas retrograde transport relies on a dynein-based motor[40]. Herpes simplex virus 1 (HSV-1), pseudorabies virus, adenoviruses, poliovirus and rabies virus can interact either directly or indirectly with dynein or dynactin components of the retrograde transport system[30,40] (FIG. 2c). Use of the retrograde transport pathway allows for viral translocation to neuronal cell bodies and thus rapid CNS entry[29]. Therefore, it is vital for the peripheral immune system to locally control viral infection and

prevent initial access to these exposed neuronal processes whenever possible[41]. Overall, the axonal transport system is undoubtedly an effective route into the CNS, as many viruses have evolved strategies to exploit this machinery in their natural hosts.

Viral dissemination within the CNS

After arriving in the CNS, viruses use many different mechanisms to promote cell-to-cell dissemination. Viral spread can occur through release into synaptic clefts or via fusion events with neighbouring neurons[29]. Alphaherpesviruses (namely, HSV and pseudorabies virus) use the neuronal anterograde transport system to move from the cell body to the axon terminal, where the virus is released by exocytosis from the presynaptic terminal into the synaptic cleft[30,32,42] (FIG. 2d). During anterograde transport, viruses can also exit through axonal varicosities, before reaching the termini, and infect neighbouring cells[30,42] (FIG. 2d). Rabies virus disseminates along synaptically connected neurons in a retrograde (rather than anterograde) manner and thus emerges from neuronal dendrites rather than axons[36]. Like rabies virus, measles virus moves in a unidirectional, retrograde manner; however, it is thought that the virus spreads through ‘microfusions’ that form between neurons[29,37] (FIG. 2c). Therefore, viruses can use both standard synaptic release machinery and cellular fusion events to disseminate in the CNS and even reinfect peripheral tissues. Certain herpes simplex viruses, for example, are known to emerge from neurons and infect cells in the periphery (see below).

Persistent and latent viral infections

Once a virus gains access to the nervous system, there are several potential outcomes, including acute replication, persistence and latency. During all three scenarios, local and peripherally derived immune cells will mount a defence response and attempt to eradicate the pathogen and, in some cases, this immune defence results in severe disease. The ideal scenario is for the host to eliminate the invading virus quickly without causing disease or death; however, some viruses can evade this stage and establish persistence or latency. Persistent viral infections are defined by continual viral replication, whereas latency is a ‘dormant’ state during which the production of infectious virions is minimized or ceases entirely but the pathogen is not eliminated.

During latency, viruses usually integrate into the host genome and produce some antigenic material that the immune system can detect. This enables immune cells to maintain pressure on the pathogen. However, if the immune system becomes suppressed, latent viruses can reactivate, form productive virions and initiate serious disorders. In general, immune responses to persistent and latent viral infections can be highly disruptive to nervous system function. In the following sections, we discuss a few well-studied examples of how the immune system deals with persistent and latent viral infections of the nervous system.

Herpes simplex virus

One of the best-studied examples of immune viral control is observed following HSV-1 infection of sensory neurons, which are part of the peripheral nervous system and therefore extend beyond the protection of the CNS blood–brain barrier. Although HSV-1 latency is

thought to be regulated by the assembly of chromatin on HSV-1 DNA[43], both innate and adaptive immune responses have a crucial role in shaping viral latency in some infected neurons. HSV-1 initially infects peripheral epithelial cells before accessing neuronal cell bodies via retrograde axonal transport[43]. On reaching the nucleus, HSV-1 establishes a latent state where it remains quiescent until reactivation occurs. HSV-1 gene expression occurs in a consecutive order during lytic cycles, with initial expression of immediate early genes followed by early genes and late genes.

Owing to the limitations of studying HSV-1 in humans, many insights into the immune response to this virus have been described using mouse model systems. The first line of immune defence probably comes from Toll-like receptor (TLR) recognition of pathogen-associated molecular patterns (PAMPs) expressed by HSV-1. Stimulation of TLR2 or TLR9 on glial cells can result in the production of type I interferons (IFNs), interleukin-15 (IL-15), tumour necrosis factor (TNF) and the chemokine CCL2 (REF. 44), which recruits macrophages. TLR3, which is expressed by neurons and glial cells, was shown to be important for increased type I IFN responses and neuronal resistance to HSV-1 infection[44–46]. Generally, type I IFNs can generate an antiviral response through the induction of proteins such as RNase L and IFN-induced dsRNA-activated protein kinase (PKR), resulting in mRNA degradation and cessation of translation, respectively[47]. Following HSV-1 infection, IFNα decreases the expression of both immediate early viral genes (such as transacting transcriptional protein ICP4) and late viral genes (such as envelope glycoprotein D), and this leads to an enhanced quiescent state of the virus in latently infected neurons *in vitro*[48]. The importance of the type I IFN response in controlling HSV-1 is also clearly reflected by the fact that the virus encodes several proteins, such as the E3 ubiquitin ligase ICP0, that inhibit this cytokine pathway[49,50].

In addition to the innate immune responses that occur following HSV-1 infection, virus-specific T cells must be generated to control and maintain HSV-1 latency[51]. Macrophages and γδ T cells migrate into HSV-1-infected trigeminal ganglia 2–3 days after corneal infection. This coincides with HSV-1 replication in neurons and is associated with local production of TNF, nitric oxide (NO) and IFNγ [51], which controls viral replication. $CD4^+$ and $CD8^+$ T cells also localize near latently infected neurons, and $CD8^+$ T cells are crucially important in preventing HSV-1 reactivation[51–53] through MHC class I-dependent interactions[54]. Following infection, $CD8^+$ T cells recognize an immunodominant HSV-1 epitope — residues 498–505 of envelope glycoprotein B (gB498–505) — as well as undefined subdominant epitopes[53,55]. HSV-1-specific $CD8^+$ T cells were shown in mouse models to infiltrate trigeminal ganglia and polarize their T cell receptors on the cell surface towards infected neurons, and this is suggestive of direct neuronal engagement[53]. Studies using transplanted latently infected sensory ganglia have also shown local antigen-specific proliferation of $CD8^+$ memory T cells and enhanced local immunity[56,57]. In humans, $CD8^+$ T cells have been shown to be in close proximity to HSV-1-infected neurons[58–60]. It is thought that $CD8^+$ T cells control HSV-1 in part by blocking the induction of viral late genes and the production of virions during reactivation[61,62]. By contrast, the importance of $CD4^+$ T cells in controlling HSV-1 is less clear. $CD4^+$ T cell help during priming was shown to transiently affect HSV-1-specific $CD8^+$ T cell function; however, at later time points $CD4^+$ T cell help had no impact on the maintenance of viral latency[63]. In another study,

transplantation of latently infected dorsal root ganglia depleted of memory $CD4^+$ T cells resulted in decreased expansion of memory $CD8^+$ T cell populations, suggesting that $CD4^+$ T cells may have a role in local $CD8^+$ T cell activation[64].

During HSV-1 latency, low-level expression of non-latent genes can continually stimulate antiviral $CD8^+$ T cells in the trigeminal ganglia[65]. Evidence in support of this includes the expression of the early activation marker CD69 by $CD8^+$ T cells isolated from both mouse and human latently infected trigeminal ganglia[51,60] and the presence of immediate early viral transcripts (such as those encoding ICP0 and ICP4) in latently infected human trigeminal ganglia[66]. The maintenance of latency by $CD8^+$ T cells is mediated through IFNγ-dependent and -independent pathways. Release of IFNγ was shown to inhibit HSV-1 reactivation by decreasing the levels of HSV-1 transcripts encoding ICP0 and by promoting expression of p21 (also known as CIP1) and p27 (also known as KIP1), which inhibit the activity of cyclin-dependent kinase 2 (CDK2) and CDK4 (REFS 51,67). Because ICP0 is an HSV-1 transactivator required for efficient HSV-1 reactivation, and CDK2 is necessary for the expression of immediate early and early HSV-1 genes, IFNγ can efficiently shut down progression of HSV-1 gene expression and maintain viral latency[51,62,68].

In addition to the role of IFNγ, the release of granzyme B by $CD8^+$ T cells was shown to contribute to the control of HSV-1 reactivation. Surprisingly, granzyme B-expressing $CD8^+$ T cells were observed clustering around latently infected neurons in the absence of neuronal apoptosis[60], and further studies revealed that granzyme B promotes latency by cleaving the HSV-1 immediate early protein ICP4 (REF. 69). Recently, the HSV-1 latency-associated transcript (LAT) gene was shown to protect neurons *in vitro* from granzyme B-associated caspase 3 activation and killing[70]. This suggests that the absence of neuronal cytopathology following the release of granzyme B by $CD8^+$ T cells is due to anti-apoptotic mechanisms contributed by the viral genome. Overall, both the innate and adaptive immune systems coordinate to limit HSV-1 dissemination by inhibiting translation, promoting mRNA degradation, and using IFNγ-dependent and -independent pathways to block HSV-1 gene expression.

HIV and SIV

Viral persistence is not linked exclusively to neuronal populations in the CNS. For example, a general state of immunosuppression following HIV and SIV infection results in viral infection of perivascular macrophages and parenchymal microglial cells — two potential antigen-presenting cell (APC) types in the CNS[71]. During HIV-1 infection, circulating infected $CD14^+CD16^+$ monocytes enter the CNS[72]. The peripheral development and activation of these cells may be further perpetuated by systemic lipopolysaccha-ride (LPS), which results from bacterial translocation from the intestines following HIV infection[73]. $CD16^+$ monocytes are thought to be more susceptible to HIV infection, and the continued generation of these cells increases the pool of targets that can be infected and then act as 'Trojan horses' to carry additional virus into the CNS. Following peripheral monocyte infiltration, CNS perivascular macrophages and parenchymal microglial cells become infected with the virus. Furthermore, in an animal model of SIV infection, antiviral innate immune mechanisms in the CNS (such as the expression of mRNA encoding IFNβ and IFN-

induced GTP-binding protein MXA (also known as MX1)) were noted just 4 days after infection[74]. In macrophage cell lines, IFNβ can induce the production of a dominant-negative form of CCAAT/enhancer-binding protein-β (C/EBPβ) that has been referred to as LIP[75,76]. LIP blocks the acetylation of histones bound to SIV DNA and reduces viral replication[75,77]. Interestingly, an inverse correlation between SIV viral loads and the ratio of C/EBPβ to LIP was observed in macaque brains[76], suggesting a role for IFNβ-induced genes in CNS antiviral responses following SIV infection. However, *in vitro* studies in primary peripheral and bone marrow-derived macrophages have demonstrated that IFN responses eventually wane during viral persistence owing to microRNA-mediated regulation of IFNβ expression[78] and/or induction of suppressor of cytokine signalling 3 (SOCS3) expression by the HIV Tat protein[79]. If these observations extend to CNS macrophages, then it is likely that the innate immune response to HIV declines over time, resulting in renewed viral replication within the nervous system.

Similarly, reductions in $CD4^+$ or $CD8^+$ T cell numbers were shown to accelerate disease progression and increase viraemia following HIV or SIV infection[80–83]. The presence of $CD8^+$ T cells in the CNS during SIV infection[84,85] suggests local control of viral replication. In patients with presymptomatic HIV infection, HIV-specific $CD8^+$ T cells were detected at higher frequencies in the CSF than in the blood. These cells had a memory phenotype ($CD45RO^+CCR7^-$) and produced IFNγ in response to stimulation, indicating that they were still functional in these individuals[86]. In addition, analysis of post-mortem brains from presymptomatic HIV patients revealed that the number of $CD8^+$ T cells in the brain was inversely correlated with HIV viral loads[87]. This suggests that functional $CD8^+$ T cells can promote viral control within the CNS. Nevertheless, despite the early presence of functional virus-specific $CD8^+$ T cells in the CNS, both the innate and adaptive immune responses ultimately fail to control viral replication in this tissue. Indeed, HIV disseminates widely in perivascular macrophages as the disease progresses and can infect up to two-thirds of parenchymal microglial cells[88]. Infection of astrocytes by HIV-1 has also been noted, but to a lesser extent than that of macrophages and microglia[71].

HSV, HIV and JC virus (BOX 2) represent three excellent examples of viruses that can establish persistence or latency in the nervous system. The resulting chronic activation of the innate and adaptive immune systems can lead to alterations in CNS homeostasis. Viral elimination requires physical deletion of all cellular reservoirs, and this is challenging for the immune system to accomplish. It is nevertheless important to mechanistically understand and attempt to induce healthy states of immune control in which a potentially pathogenic virus (for example, JC virus or HSV) is held in check by constant immune pressure. This will require a more detailed knowledge of how the immune system operates dynamically in living, virally infected tissues. One way to gain these insights is through real-time imaging of immune surveillance in the virally infected nervous system.

Box 2

Progressive multifocal leukoencephalopathy

JC virus is a human virus that, similarly to HIV-1, thrives in non-neuronal cells in the central nervous system (CNS). It infects oligodendrocytes and astrocytes and can cause

progressive multifocal leukoencephalopathy (PML)[109,114,115]. Mutations in the JC virus caspid protein have been associated with PML and may be a crucial aspect of disease development[116]. JC virus normally remains latent in the kidneys, bone marrow, lymphoid tissues and brain[117,118]; however, viral reactivation can occur during states of immunosuppression, which can be induced by HIV infection or immunomodulatory drugs, such as the α4 integrin-targeting drug natalizumab (Tysabri; Biogen Idec/Elan)[110]. $CD8^+$ T cells are thought to be important in controlling JC virus, and the failure of these cells to persist has been associated with the death of patients with PML[118]. During HIV infection, enhanced blood–brain barrier breakdown and global immunosuppression may facilitate JC virus entry and replication within the CNS. Furthermore, because JC virus is cytopathic, it can cause a primary demyelinating disorder by destroying myelin-producing oligodendrocytes[115,119,120]. In general, this human virus represents an excellent example of a pathogen that is normally controlled by an intact immune system, but has the potential to cause a severe, potentially fatal neurological disorder if CNS immune surveillance is disturbed.

Real-time insights into CNS immunity

Brain tissue preparations

Since its inception[89], TPLSM has been used to examine the dynamics of CNS cellular residents and peripheral immune infiltrates[90–92]. These studies have educated us about the inner workings of the nervous system during states of health and disease. An important consideration when imaging the CNS by TPLSM is tissue preparation. Three approaches are now commonly used to image living brain tissue (FIG. 3): skull thinning[93], craniotomy[94] and acute brain slices[95].

For a TPLSM experiment to be considered ‘intravital’, skull thinning or a craniotomy (skull removal) must be performed, and most neuroscience studies so far have relied on craniotomies[94]. For this procedure, a small circular region of the skull is removed and replaced with a glass coverslip. An upright two-photon microscope is then used to image through the glass window into the underlying meninges and brain parenchyma. By contrast, skull thinning creates a translucent viewing window because the skull is surgically shaved down to a thickness of 20–30 μm. A direct comparison of the two surgical techniques revealed that craniotomies but not skull thinning procedures induce profound proliferation of astrocytes, which is indicative of a brain injury response[94]. Therefore, these data indicate that surgical preparations can substantially influence the results of TPLSM experiments and, in some cases, introduce artefacts. For example, craniotomies have been shown to induce a significantly higher level of neuronal den-dritic spine turnover in the barrel cortex than the less injurious approach of skull thinning[94]. This result can be attributed to the substantial glial response observed following craniotomy. Innate injury responses resulting from tissue preparation must be considered when studying the CNS by TPLSM, particularly when the aim is to examine innate and adaptive immunity to an infectious agent.

Although thinned and open skull preparations provide viewing windows for TPLSM experiments, both approaches have restricted imaging depth. For example, it is only possible

to image 200–400μm beneath the surface of a thinned skull; this region includes the meninges and neocortex but not deeper brain structures. To image deeper regions by TPLSM, the brain must be extracted and cut using a vibratome[95] (FIG. 3b). Equilibrated brain slices are then placed in an imaging chamber containing a flow of warm, oxygenated artificial CSF. These samples are often referred to as acute brain slices because they are used shortly after extraction. Acute brain slices are not an intravital preparation, but have been used to image cellular dynamics in the brain during states of health and disease. For example, brain slices were recently used to study innate and adaptive immune responses to infection with the parasite *Toxoplasma gondii*[96,97] — a pathogen that can replicate in humans and cause toxoplasmic encephalitis in immunocompromised individuals (such as patients with HIV). One study showed that stromal elements (that is, reticular fibres; FIG. 1) which are usually only found in the meninges of the brain were recruited into the brain parenchyma during *T. gondii*-induced encephalitis[96]. Interestingly, these structures appeared to guide $CD8^+$ T cell migration in a manner reminiscent of T cell movement on stromal networks in draining lymph nodes[98]. It remains to be determined whether the same stromal elements invade the brain parenchyma following viral infection.

An important caveat of using acute brain slices is that the procedure induces a severe tissue injury response along the cutting surface that can extend 50–100μm into the tissue. This tissue injury response is characterized by cellular necrosis and microglial cell activation[99], resembling in some ways what is observed following a craniotomy[94]. Tissue injury responses must be carefully considered when interpreting TPLSM-derived data obtained using brain slices or craniotomies. At present, the most physiological method of tissue preparation is skull thinning[93,94], and whenever possible this approach should be used to validate data obtained with the other two procedures.

Microglial cells

The CNS was originally thought to be devoid of professional APCs. However, following closer examination, it is now clear that this compartment is equipped with an elaborate (albeit specialized) sentinel system capable of rapidly detecting disturbances such as injury and infection. There is still much to be learnt about the unique ways in which APCs in the CNS influence immune responses to infections. At present, one challenge is deciding how best to study these APCs without perturbing their unique microenvironments. TPLSM through a thinned skull offers a solution to the issue of how to preserve tissue architecture. Using this approach, investigators have begun to study the dynamics of CNS sentinels in the normal brain and following injury. A recent TPLSM study demonstrated that under steady state conditions microglial cell processes are highly dynamic (see Supplementary information S1 (movie)). These cellular processes were found to extend and retract at a rate of approximately 1.5μm min^{-1}, which would allow for surveillance of the entire brain parenchyma once every few hours[2]. Thus, microglial cells are not static CNS residents with processes stably integrated into the neuropil; they are instead highly dynamic sentinels with mobile processes. This mobility enables them to remove debris and dead cells and quickly respond to CNS insults. For example, it was revealed that microglial cells can respond to injury within minutes by projecting their processes to the site of damage[100,101]. This rapid response depends, in part, on extracellular ADP and ATP released by the damaged cells,

which is detected by purinergic receptors (such as P2Y12) expressed by the microglial cells[100]. TPLSM studies have also revealed that following this early response microglial cell bodies physically converge on the injury site to participate in the clean-up[102]. This occurs over the course of several days[102] and does not appear to depend on P2Y12 (REF. 100).

Visualizing viral infections

TPLSM is rapidly advancing our understanding of immunity to infections. Watching innate and adaptive immune cells operate in real time provides insights that could not otherwise be obtained using traditional approaches, such as static imaging and flow cytometry. So far, most TPLSM studies involving infectious agents have focused on peripheral tissues. In the nervous system, investigators have focused their two-photon microscopes mainly on immune responses during autoimmunity[14], glial injury[2,100–102] and parasite infections[96,97], but a few studies have visualized immune responses to neurotropic viral infections[41,103,104].

Given the prominence of neurotropic viruses, it is important to understand mechanistically how the nervous system protects itself from these diverse invaders. Many human neurotropic viruses have relevant animal models that can be used to study how the viruses access the brain, trigger local immune responses and cause pathology (TABLE 1). Because viruses in humans rarely gain direct access to the CNS, the first immunological defence against a neurotropic virus usually occurs in peripheral tissues. A recent study using vesicular stomatitis virus (VSV) demonstrated that macrophages residing in the subcapsular sinus of draining lymph nodes prevent the virus from gaining access to the CNS and causing fatal disease[41]. VSV is a cytolytic negative-stranded RNA virus that is transmitted to mammals by insects. It is a member of the same family as rabies virus (namely, *Rhabdoviridae*). Following subcutaneous inoculation, VSV localizes to subcapsular sinus macrophages in the draining lymph node. However, if these macrophages are depleted before inoculation, the virus gains access to peripheral nerves in the lymph node, travels to the CNS and causes a fatal paralytic disorder in 7–10 days. These data demonstrate that subcapsular sinus macrophages prevent VSV from accessing the CNS through peripheral nerves by secreting type I IFNs and recruiting plasmacytoid DCs (which also secrete type I IFNs) to the subcapsular sinus. Thus, innate immune cells can have an important role in protecting peripheral nerves, and ultimately the CNS, from infection by a neurotropic virus.

The symptoms and severity of the disorders caused by neurotropic viruses depend on many factors, which include viral cytopathogenicity and tropism as well as the host immune response. A case in point is the fatal meningitis induced by lymphocytic choriomeningitis virus (LCMV). Intracerebral inoculation of mice with LCMV induces fatal convulsive seizures within 6–7 days and has served for decades as a model of $CD8^+$ T cell-mediated viral meningitis[105]. To gain novel insights into the pathogenesis of LCMV meningitis, a combination of techniques, including intravital TPLSM imaging (FIG. 4; Supplementary information S3,S4,S5 (movies)), was used to monitor the relative contribution of innate and adaptive immune cells to meningeal disease following infection[104]. During LCMV meningitis, the virus localizes to three specific cell types: fibroblast-like cells in the meningeal stroma (see Supplementary information S3 (movie)), epithelial cells lining the ventricles and epithelial cells in the choroid plexus. This tropism triggers the disorder by

recruiting immune cells to the meninges. Cytotoxic T lymphocytes (CTLs) localize precisely to these meningeal structures 5–6 days post infection[106], and their arrival coincides with the onset of fatal convulsive seizures. Interestingly, although CTLs are required for rapid-onset disease[107], their effector mechanisms are not[104]. Convulsive seizures were still observed in mice with single deficiencies in all major CTL effector pathways (for example, in granzymes, perforin, IFNγ, TNF, FAS (also known as CD95) and degranulation)[104].

Intravital TPLSM has been used to monitor the pathogenic activities of green fluorescent protein (GFP)-tagged LCMV-specific CTLs through a thinned skull window[104,108] (FIGS 3,4; Supplementary information S4,S5 (movies)). In symptomatic mice (at day 6 post infection), an abundance of virus-specific CTLs were found actively scanning the infected meninges; however, no visual evidence directly linked these CTLs to vascular pathology. At this same time point, intravenously injected quantum dots were observed leaking from meningeal blood vessels into the subarachnoid space (FIG. 4; Supplementary information S5 (movie)), and this leakage was ultimately attributed to the effects of CTL-recruited monocytes and neutrophils. The synchronous extravasation of neutrophils from the blood vessels into the subarachnoid space was shown to result in severe vascular leakage, whereas monocyte-derived macrophages appeared to participate in vessel damage from an extravascular position[104]. Depletion of both neutrophils and macrophages eliminated the rapid-onset convulsive seizures at day 6 post infection and extended survival by several days. These data demonstrate that CTLs can mediate a fatal CNS inflammatory disease in part by recruiting pathogenic innate immune cells that compromise the integrity of meningeal blood vessels. Furthermore, CTLs can directly contribute to the recruitment of innate immune cells by producing chemokines such as CCL3, CCL4 and CCL5 (REF. 104).

Future perspectives

We are at the cusp of an exciting new era in our understanding of viral infections of the nervous system. With new tools in hand, we can now watch neurotropic viral infections and the immune responses induced in real time. Viruses usually enter the nervous system through the periphery, and it is now clear that in draining lymph nodes innate immune cells (such as subcapsular sinus macrophages) prevent neurotropic viruses (including VSV) from accessing peripheral nerves and causing fatal CNS disease[41]. However, there is still much to be learnt regarding the dynamics of this process and whether innate immune cells also prevent viral access to peripheral nerves in non-lymphoid tissues (for example, rabies virus entry at neuromuscular junctions).

Because neurotropic viruses must first encounter peripheral immune defence mechanisms before accessing the CNS, careful consideration should be given to whether the immune system can be therapeutically modulated to keep viruses localized in the periphery. Vaccination is one obvious approach to prevent peripheral infections; however, when a vaccine is not available (or has not been administered), it is necessary to consider other therapeutic strategies. Neurotropic viruses usually attempt to bypass peripheral immune surveillance and directly access the CNS. Viral infection of the CNS can induce severe disorders, such as meningitis or encephalitis, which are usually treated with supportive care or, when applicable, antiviral drugs (for example, during HSV infection). However, it would

be preferable to keep viruses sequestered in peripheral tissues, which can usually tolerate more pathology than the CNS.

With viruses such as VSV, it might be possible to amplify peripheral immune responses before a virus has time to enter the CNS. This could be accomplished by boosting innate production of cytokines (such as type I IFNs) at viral entry points to the nervous system. In lymph nodes, subcapsular sinus macrophages and plasmacytoid DCs produce type I IFNs that can prevent viral replication in neurons, and it is likely that innate immune sentinels have similar roles at other viral entry points. It is therefore necessary to understand the relevant immune mechanisms that become operational as viruses attempt to enter the nervous system. As discussed, viruses use more than one strategy to access the CNS, so it is unlikely that one immune mechanism will apply to all viruses. In addition, viruses that enter the CNS through a haematological route by hijacking immune cells (for example, HIV and JC virus) might present a bigger challenge than those that simply enter through peripheral nerves.

Once a virus enters the CNS, it is necessary to consider mechanisms that underlie pathogenesis, and real-time imaging can again help in this regard. The innate immune sentinels in the CNS are usually among the first responders to CNS viral infections and can shape the subsequent adaptive immune response. Currently, we know very little about how innate immune sentinels respond in their distinct anatomical niches to CNS viral infections. These cells are typically extracted from their microenvironments and analysed *ex vivo*. Importantly, TPLSM now permits intravital investigation of innate immune sentinels responding to diverse viral challenges in a physiological microenvironment. As an example of a non-cytopathic viral infection, we have relied on LCMV because the virus reproducibly induces meningitis. In this model, it will be important to determine how innate immune sentinels predispose the meninges to fatal disease and whether these immune cells can be therapeutically modulated to change disease outcome. Thus far, TPLSM has been instrumental in revising our understanding of LCMV meningitis[92,104], and it is expected that similar advances will come from studying LCMV and other neurotropic viruses in real time.

Persistent and latent viral infections of the nervous system represent another frontier that has yet to be explored by TPLSM. Persistent viral infections can disrupt CNS homeostasis and cause neurological dysfunction, but little is known about the exact mechanisms that give rise to this dysfunction. It is also unclear how the nervous system is surveyed by the innate and adaptive immune systems during states of viral persistence. HSV infection of sensory ganglia is the prototypical example of a viral infection that can be kept in check by constant immune surveillance. As long as the host–pathogen equilibrium is maintained, the consequences to the host are minimal. A classic example of a breakdown in this equilibrium is the increased incidence of JC virus-induced progressive multifocal leukoencephalopathy (PML) in immunocompromised patients[109,110]. Reduced immune surveillance allows a pathogen that can normally be controlled to reactivate from latent reservoirs and cause severe pathology in the CNS. Although we are not likely to purge viruses such as HIV, HSV and JC virus once they establish latency in the nervous system, it is important to support the adaptive immune system as it attempts to establish a relatively benign equilibrium. At present, this is best achieved by not impeding established antiviral immune responses with

immunosuppressive regimens. However, it may be possible to induce such equilibria with therapeutic vaccines that are specifically designed to elicit responses that hold a virus in check. The development of effective therapeutic vaccines will require a more detailed knowledge of the exact immune parameters that maintain viruses in a latent state. Our understanding of immune responses to persistent and latent viral infections has come a long way, but there are still many mechanistic details that await discovery.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Glossary

Immune-privileged
A term used to describe areas of the body with a decreased inflammatory response to foreign antigens, including tissue grafts. These sites include the brain, eye, testis and placenta.

Blood–brain barrier
A barrier formed by tight junctions between endothelial cells that markedly limits entry to the CNS by leukocytes and all large molecules, including to some extent immunoglobulins, cytokines and complement proteins.

Meninges
The membranes surrounding the brain and spinal cord. There are three layers of meninges: the dura mater (outer), the arachnoid mater (middle) and the pia mater (inner).

Aseptic meningitis
Infection and inflammation of the meninges that is not caused by bacteria. Enteroviruses such as echovirus and coxsackie virus are the most common cause of viral meningitis, but cytomegalovirus, HSV, HIV, JEV, LCMV, mumps virus, rabies virus, VZV and WNV can also cause the disease.

Encephalitis
Infection and inflammation of the brain parenchyma. This can be caused by adenovirus, cytomegalovirus, coxsackievirus, EBV, echovirus, HSV, measles virus, poliovirus, mumps virus, rabies virus, rubella virus, VZV and WNV.

Meningoencephalitis
A disease that resembles both meningitis and encephalitis and is characterized by infection and inflammation of both the meninges and brain parenchyma.

Two-photon laser scanning microscopy (TPLSM)

Laser scanning microscopy that uses pulsed infrared laser light for the excitation of conventional fluorophores or fluorescent proteins. This technique greatly reduces photodamage to living specimens and improves the depth of tissue penetration, owing to the low level of light scattering within the tissue.

Tight junctions
A belt-like region of adhesion between adjacent epithelial or endothelial cells that regulates paracellular flux. Tight-junction proteins include the integral membrane proteins occludin and claudin, in association with cytoplasmic zonula occludins proteins.

Pericytes
Cells embedded in the vascular basement membrane of microvessels that are thought to be derived from the vascular smooth muscle lineage. They make close cellular contact with endothelial cells and this interaction is essential for the maintenance of vessel function, as well as for the regulation of angiogenesis and vascular remodelling.

Anterograde and retrograde transport systems
Cargo is moved between the cell body (soma) and the synapse of neurons using two transport mechanisms. The anterograde transport system uses kinesin motors to move cargo from the cell body to the synapse, whereas the retrograde system moves material from the synapse back to the cell body using dynein.

Antigenic drift
A process by which circulating influenza viruses are constantly changing, which allows the virus to cause annual epidemics of illness. Antigenic drift occurs when mutations accumulate in the haemagglutinin and neuraminidase genes and alter the antigenicity of these proteins such that the ‘drifted’ strains are no longer neutralized by antibodies that were specific for previously circulating strains.

Pathogen-associated molecular patterns (PAMPs)
Molecular patterns that are found in pathogens but not in mammalian cells. Examples include terminally mannosylated and polymannosylated compounds (which bind the mannose receptor) and various microbial components, such as bacterial lipopolysaccharide, hypomethylated DNA, flagellin and double-stranded RNA (all of which bind Toll-like receptors).

γδ T cells
T cells that express the γδ T cell receptor. These T cells are present in the skin, vagina and intestinal epithelium as intraepithelial lymphocytes.

MicroRNAs (miRNAs)
Small RNA molecules that regulate the expression of genes by binding to the 3′-untranslated regions z (3′-UTRs) of specific mRNAs.

Quantum dot
A nanocrystalline semiconductor of extremely small size (5–50 nm in diameter) that absorbs incident photons and then emits light of a slightly longer wavelength. Because of a

phenomenon called the quantum confinement effect, the colour (wavelength) of the emitted light is determined by the size of the nanocrystal.

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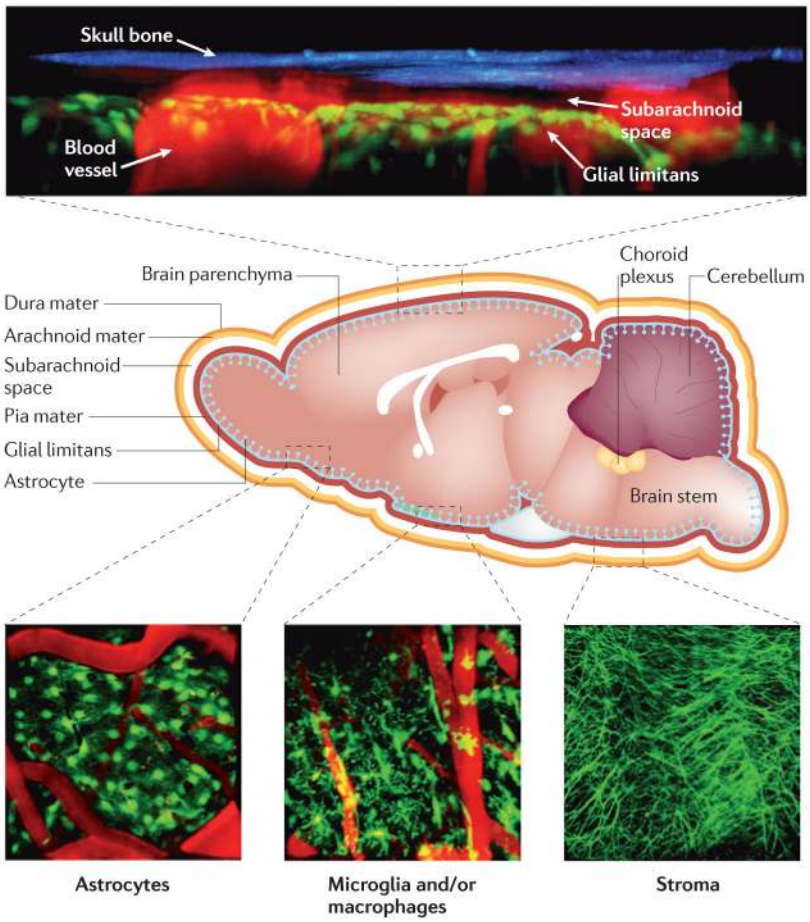

Figure 1. Anatomy of the brain

The outer lining of the brain sits beneath the skull bone and is collectively referred to as the meninges. The meninges are composed of the dura mater, arachnoid mater and pia mater. Cerebral spinal fluid (CSF) flows between the arachnoid and the pia mater through the subarachnoid space. This space also contains meningeal macrophages, stromal cells, trabeculae that physically connect the arachnoid to the pia mater and blood vessels that traverse the meninges and penetrate down into the brain parenchyma (not shown). The meninges are considered part of the blood–CSF barrier. The glial limitans lies beneath the pia mater, and is comprised of basal lamina and astrocytic endfeet. This layer keeps the meninges separate from the underlying brain parenchyma, which contains neurons, astrocytes, oligodendrocytes and microglial cells (see Supplementary information S2 (movie)). Although not depicted, the glial limitans can also be found around post-capillary venules within the brain parenchyma. Microglial cells continually scan the brain parenchyma for damage and foreign materials such as infectious agents (see Supplementary information S1 (movie)). The anatomical features presented in this figure are illustrated with three-dimensional projections captured in a living mouse brain by intravital two-photon laser scanning microscopy (TPLSM).

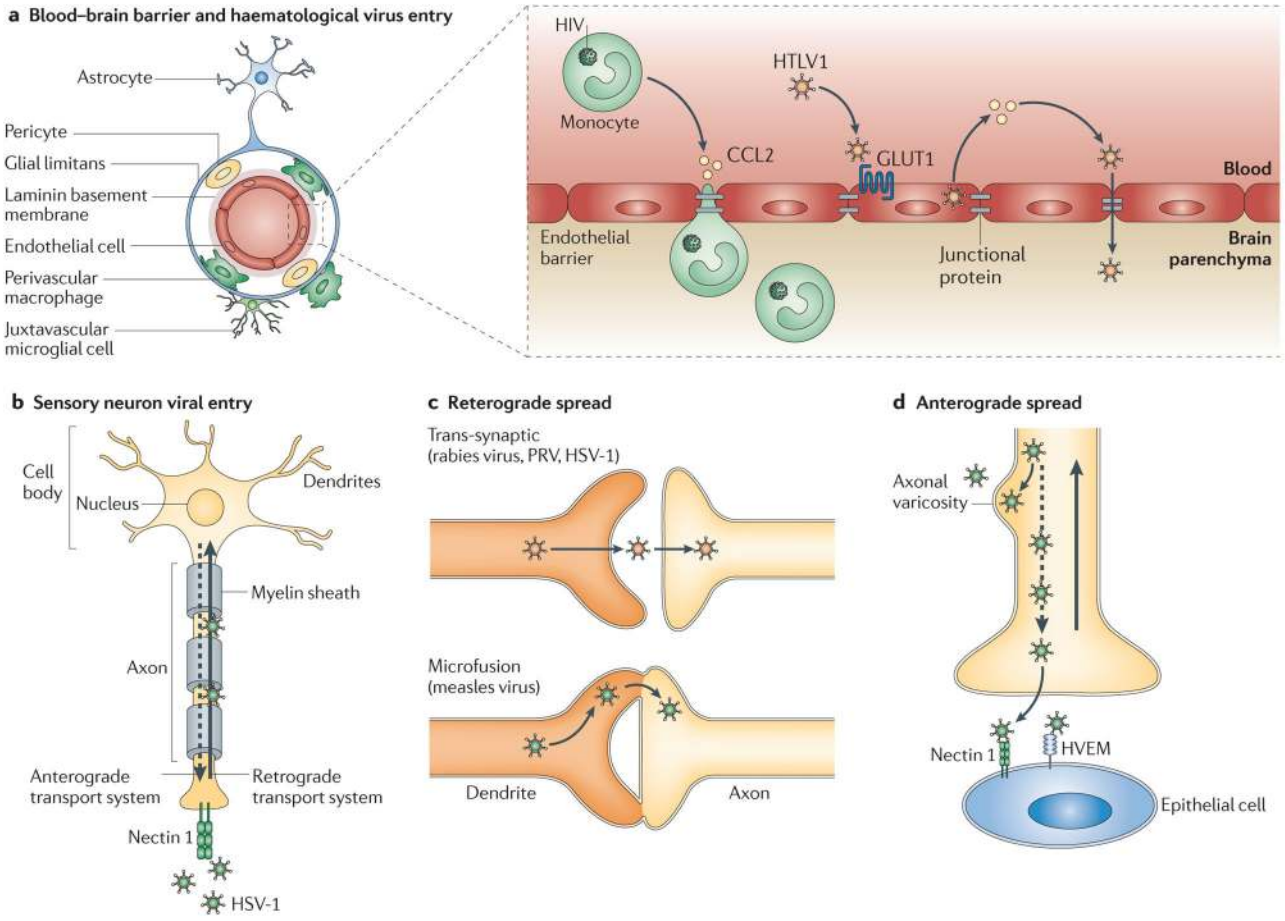

Figure 2. CNS viral entry and spread

a | The blood–brain barrier is a multi-layered barrier to free diffusion of vascular components into the brain parenchyma. Specialized endothelial cells (red) have junctional proteins that restrict movement through inter-endothelial gaps and secrete a laminin basement membrane (light pink). Both perivascular macrophages and pericytes lie in close apposition to the vessel wall. Astrocytes form the glial limitans that is comprised of a laminin barrier and astrocytic endfeet. In addition, parenchymal juxtavascular microglial cells have processes that extend along blood vessels and even down into the perivascular space towards the basal lamina, allowing for sampling of this space (not shown). Although the blood–brain barrier normally protects the central nervous system (CNS) from pathogens, viruses have adapted strategies to enter through both haematological and axonal routes. Some viruses, such as HIV, use a 'Trojan horse' method of entry by travelling in monocytes. Infected monocytes pass through the blood–brain barrier during normal turnover of perivascular macrophages or as a result of the production of pro-inflammatory mediators, such as CC-chemokine ligand 2 (CCL2), which compromise the barrier. Other viruses, including human T-lymphotropic virus type 1 (HTLV1), bind to endothelial receptors such as glucose transporter type 1 (GLUT1), allowing for infection of endothelial cells and release of pro-inflammatory mediators (for example, CCL2). **b |** Viral CNS entry also occurs through peripheral neurons. Herpes simplex virus 1 (HSV 1) entry into sensory neurons is

facilitated by nectin 1 that is expressed on axons. Viral spread to the neuronal cell body is then expedited by hijacking of the fast axonal retrograde transport system (solid black arrow). **c** | Cell-to-cell transport of viruses can be conducted using various strategies. Rabies virus, pseudorabies virus (PRV) and HSV-1 are released at a synapse and use a retrograde trans-synaptic pathway to infect neighbouring neurons. Measles virus dissemination between neurons is thought to occur through microfusions between neighbouring cells. **d** | In the case of HSV-1, anterograde transport (from cell body to axon) can lead to infection of neighbouring cells when the virus exits via axonal varicosities before reaching the axon termini. During HSV-1 reactivation, the virus uses the anterograde system (dashed black arrow) to reach axon termini and reinfect epithelial cells by binding to nectin 1 or herpesvirus entry mediator (HVEM) receptors .

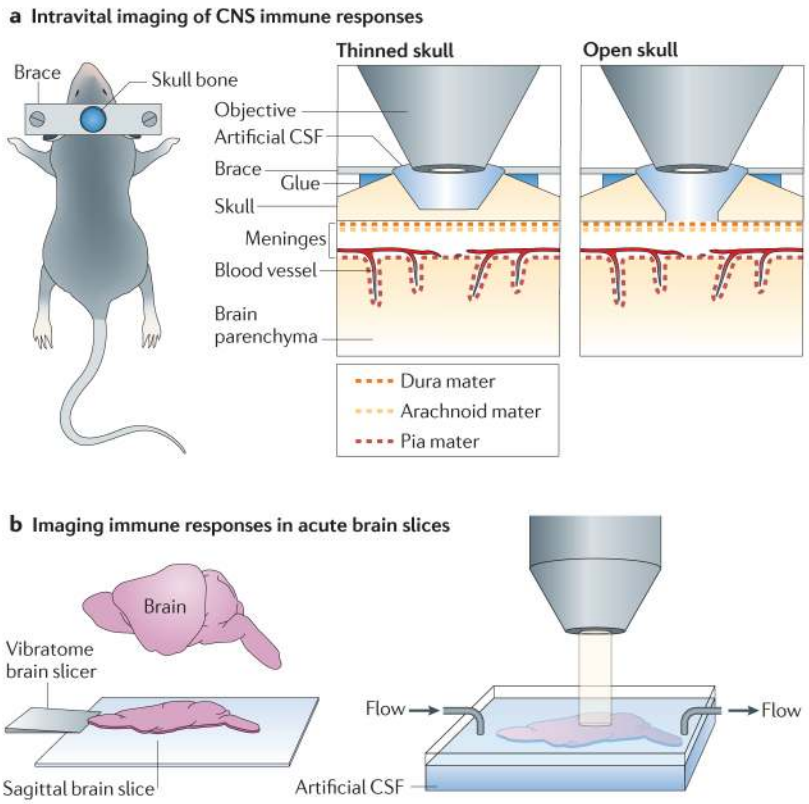

Figure 3. Imaging antiviral immune responses in the brain

Three approaches are commonly used to image immune cells and resident cells of the central nervous system (CNS) in living brain tissue. **a** | To perform intravital imaging, mice are anaesthetized and immobilized with a metal brace to prevent movement artefacts. A section of the skull bone is then surgically thinned to a thickness of 20–30 μm or removed entirely. Skull thinning is the most physiological preparation method because the underlying meninges and brain parenchyma are not disturbed. Following a craniotomy (skull removal procedure), a glass viewing window is usually inserted. This surgical procedure disrupts the meninges and causes a severe brain injury response[94]. After skull thinning or removal, four-dimensional time-lapse imaging can be performed using an upright two-photon microscope. The microscope objective is dipped into artificial cerebral spinal fluid (CSF), which is added to ensure that the normal CSF composition is maintained. Depending on the exact preparation and microscope used, imaging depths of up to 200–400 μm beneath the skull can be achieved. **b** | Another approach used to image deeper structures relies on acute brain slices. For this preparation, the brain is removed and sliced in ice-cold artificial CSF using a vibratome. The slice is then equilibrated in artificial CSF and later placed in an imaging chamber through which warm, oxygenated artificial CSF flows.

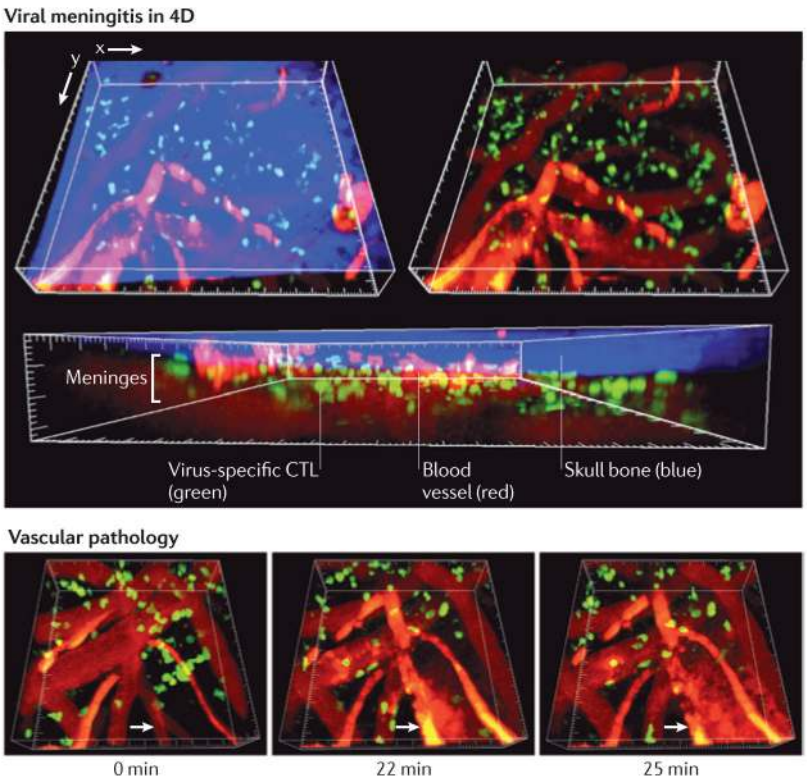

Figure 4. Viral meningitis in real time

Immune responses to central nervous system (CNS) viral infections can be visualized in real time using intravital two-photon laser scanning microscopy (TPLSM). A representative maximal projection of a three-dimensional (3D) *z* stack is shown for a symptomatic mouse 6 days following infection with lymphocytic choriomeningitis virus (LCMV; upper panels) (see Supplementary information S4 (movie)). The skull (blue) was surgically thinned to maintain a physiological setting for the intravital imaging experiment. Quantum dots (red) were injected intravenously just before imaging to visualize blood vessels, and naive LCMV-specific $CD8^+$ T cells tagged with green fluorescent protein (GFP; green) were injected 1 day before infection to provide a traceable representative of the pathogenic cytotoxic T lymphocyte (CTL) response. GFP^+ LCMV-specific CTLs invade and begin patrolling the meningeal space 5–6 days post infection. This is associated with reduced vascular flow and integrity. The lower panels show representative 3D projections from a time-lapse experiment in which the contents of a blood vessel (red) first slow and then begin leaking into the subarachnoid space (white arrowhead). This leakage represents a breach in the blood–cerebral spinal fluid barrier (see Supplementary information S5 (movie)).

Table 1

Neurotropic human viruses that have relevant animal models

Human virus	Genome	Family	Viruses used in animal models	Animal host
HCMV	dsDNA	*Herpesviridae*	MCMV, RCMV, GPCMV	Mice, rats, guinea pigs
HSV-1	dsDNA	*Herpesviridae*	HSV	Mice
Coxsackievirus	(+)ssRNA	*Picornaviridae*	Coxsackievirus B3	Newborn mice
Poliovirus	(+)ssRNA	*Picornaviridae*	Poliovirus	Primates, PVR-
WNV	(+)ssRNA	*Flaviviridae*	WNV	Mice
JEV	(+)ssRNA	*Flaviviridae*	JEV	Primates, mice
Chikungunya virus	(+)ssRNA	*Togaviridae*	Chikungunya virus	Primates, mice
Bornavirus	(−)ssRNA	*Bornaviridae*	Bornavirus	Rats, mice
LCMV	(−)ssRNA	*Arenaviridae*	LCMV	Primates, rats, mice, guinea pigs
Measles virus	(−)ssRNA	*Paramyxoviridae*	Measles virus	Primates, CD46 and CD150 transgenic mice
Rabies virus	(−)ssRNA	*Rhabdoviridae*	Rabies virus	Dogs, mice
HIV	ssRNA (RT)	*Retroviridae*	HIV, SIV, SHIV	Primates, humanized mice

dsDNA, double-stranded DNA; GPCMV, guinea pig cytomegalovirus; HCMV, human cytomegalovirus; HSV, herpes simplex virus; LCMV, lymphocytic choriomeningitis virus; JEV, Japanese encephalitis virus; MCMV, mouse cytomegalovirus; PVR, poliovirus receptor; RCMV, rat cytomegalovirus; RT, reverse transcribed; SHIV, simian–human immunodeficiency virus; SIV, simian immunodeficiency virus; ssRNA, single-stranded RNA; WNV, West Nile virus.