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Keeping it in check: chronic viral infection and antiviral immunity in the brain

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Abstract

It is becoming clear that the manner by which the immune response resolves or contains infection by a pathogen varies according to the tissue that is affected. Unlike many peripheral cell types, CNS neurons are generally non-renewable. Thus, the cytolytic and inflammatory strategies that are effective in controlling infections in the periphery could be damaging if deployed in the CNS. Perhaps for this reason, the immune response to some CNS viral infections favours maintenance of neuronal integrity and non-neurolytic viral control. This modified immune response — when combined with the unique anatomy and physiology of the CNS — provides an ideal environment for the maintenance of viral genomes, including those of RNA viruses. Therefore, it is possible that such viruses can reactivate long after initial viral exposure, contributing to CNS disease.

An oversimplification that is promoted in much of the scientific literature is that extracellular, receptor-binding ligands — including viruses, `cytokines` and `interferons` (IFNs) — transduce invariant signalling pathways, independent of cell type. Such generalizations limit our ability to fully appreciate the complexity and diversity of the cellular response to pathogens and potent pathogen-fighting proteins. There are also clinical ramifications of this myopic view: for example, ignoring the possibility that a particular cell population may behave uniquely upon cytokine encounter could limit drug efficacy or hinder the development of therapeutics. In this Review, we discuss some recently defined neuron-specific immune responses that broaden our view of how CNS infections, especially those caused by `RNA viruses`, are controlled.

Intuitively, the notion that neurons differ immunologically from other cell types makes sense: we cannot tolerate the loss of these generally non-renewable cells, as we can the lysis of more-easily replaceable epithelial cells. For example, herpes simplex virus (HSV)

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infection of epithelial cells results in massive immune- and virus-mediated cell death[1–3]; however, the lost cells are readily replaced, as observed in the healing that follows a cold sore. If lysis of irreplaceable neurons occurred in the same manner, neural circuits could become compromised, and, depending on the magnitude of damage, the host could be permanently impaired. Thus, the immune response to a viral challenge must be tailored to promote survival of infected neurons but to destroy infected epithelial or endothelial cells. However, such neuronal sparing might result in long-term consequences that are spatially or temporally separated from acute infection.

In this Review, we integrate insights from the fields of virology, neurobiology and immunology to provide an overview of the mechanisms by which the restricted environment of the CNS is accessed by both RNA viruses and DNA viruses, and to explain how the host response contends with such infections. We particularly focus on a developing literature that elucidates cell-specific immunity and the consequences of non-lytic viral clearance within the brain. We conclude with a forward-looking hypothesis: non-lytic clearance of neuronal infections may allow for persistence of RNA viruses that induce pathogenesis long after primary exposure.

Viral entry and spread into the CNS

Viral entry

The brain is shielded from external threats at both macro-and microscopic levels: it is encased in bone, to prevent physical injury, and separated from peripheral tissues and blood via highly specialized barriers. Although such characteristics may limit infections of CNS-resident cells, these barriers can be breached. Three major routes of viral entry into the brain have been identified: direct infection of the cells that comprise the blood–brain barrier (BBB) and blood–cerebrospinal fluid (BCSF) barrier (with consequent release of viral particles into the parenchyma), infection of cells that are able to cross these barriers, and transneuronal migration across synapses from the peripheral nervous system (PNS) into the CNS (FIG. 1).

Within the CNS, the BBB and BCSF barrier restrict the migration and diffusion of cells, pathogens, antibodies and macromolecules into the brain parenchyma. Neurotropic RNA viruses, including poliovirus (PV), measles virus (MV) and some flaviviruses, can circumvent these barriers by directly infecting the tightly associated endothelial or epithelial cells that comprise them[4]. Viral particles can then be released from the basolateral membrane into the parenchyma. For example, after MV infection of human brain microvascular endothelial cells, release of viral particles occurs from both the apical and basolateral membranes, without disrupting cell polarity or barrier integrity, allowing MV to spread into the parenchyma[5]. Alternatively, barrier integrity may be compromised when the tight junctions between cells loosen as a result of inflammation and cytokine exposure, allowing free viral particles to diffuse directly from the blood or CSF into the brain. For example, peripheral West Nile virus (WNV) infection acts through the engagement of Toll-like receptor 3 (TLR3) to induce the synthesis of cytokines — including tumour necrosis factor (TNF) — by circulating antigen-presenting cells[6]. In turn, TNF reduces BBB integrity by loosening tight junctions[7], allowing for WNV migration through the less-restrictive BBB.

In reality, however, modulating the barrier integrity is not as simple as this description implies. The balance of different cytokines can determine the extent to which the BBB is perturbed or stabilized. For example, IFNs, which are also produced in infected hosts, help to keep the barrier intact[8]; thus, the relative type and ratios of cytokines that are synthesized in response to various infections will differentially affect barrier integrity[9].

Viruses may also passively access resident CNS cells by infecting lymphocytes or monocytes that can be transported across a cellular barrier. This strategy is often referred to as the 'Trojan horse' approach, because viral particles are released once the blood cell gains access to the parenchyma. A classic example of this mode of invasion is provided by the human immunodeficiency virus type 1 (HIV-1): $CD16^+$ monocytes, permissive for HIV-1, traffic across the BBB and release virions that can then infect CNS microglia[10,11].

A third mode of CNS entry is transneuronal migration, a strategy adopted by rabies virus (RABV) and many herpesviruses, including pseudorabies virus (PRV). Intracellular trafficking in PNS neurons, which is necessary to shuttle cellular components to and from the synapse, can be commandeered to facilitate viral travel within and among synaptically connected neurons. The best-characterized examples of this type of spread are provided by herpesvirus members such as HSV type 1 (HSV-1) and the closely related PRV[4,12]. After infection of epithelial cells in the oral mucosa, HSV-1 spreads to sensory and autonomic ganglia, establishing lifelong latency. Reactivation of the virus from latency — in response to decreases in immune monitoring, other infections or stress — leads to an active infection in PNS neurons, in which viral membrane proteins (including US9, glycoprotein E and glycoprotein I) can direct movement of newly replicated viral particles in an anterograde manner[13]. During transport, viral components are shuttled along axons via microtubule tracks and in association with their dynein and kinesin motors[14,15]. Sensory neurons have a pseudo-unipolar morphology in which one axon is in contact with epithelial cells and the other synapses are in contact with CNS neurons[12]. Beyond the value of these studies to understand how neurotropic viruses are propagated, viruses that spread across synapses (including RABV and MV) have provided a valuable method to trace neural circuits *in vivo*[16,17]; that is, the use of recombinant viruses encoding fluorescent proteins. These unique virological tools may also allow the development of strategies to deliver therapeutic payloads from the periphery to the CNS.

Viral spread

Once a virus has infected a neuron, there are two primary modes of subsequent spread to other cells: the release of infectious viral particles that can infect distant permissive cells or the transfer of viral nucleic acid, subviral particles or infectious virions between infected and uninfected cells that are in direct contact. The former mechanism requires the release of viral particles through the neuronal membrane (chiefly, via budding out of the infected cell), whereas the latter mechanism is primarily dependent on viral proteins that mimic or co-opt cellular processes to direct the insertion of viral fusion proteins into a host cell membrane or to direct the spread of viral capsids, as seen with HSV[12,18]. Both modes of viral spread occur in neurons; however, in most instances, viral transfer to adjacent neurons happens in the absence of syncytia formation (BOX 1), and little or no amount of

extracellular infectious virus is detected, suggesting that neurons facilitate a distinct mode of spread for many viruses[4]. Interestingly, trans-synaptic spread of MV within primary mouse hippocampal neurons occurs independently of known MV receptors, which are crucial for syncytia formation in non-neuronal cells[19,20]. The paucity of viral particles in the extracellular space may protect the neuron from plasma membrane damage via budding and facilitate evasion of antibody detection. Although many neurotropic infections spread by direct contact at the presynaptic–postsynaptic junction, alternative modes of transport may also be used. For example, although RABV primarily spreads trans-synaptically in a retrograde manner, an electron microscopy study showed the presence of viral particles in the extracellular neuronal space, accompanied by direct neuronal budding[21].

Defining long-lasting infections

An outcome of viral neuroinvasion is that the viral genome, viral proteins and/or complete virus particles may remain in the brain long after initial exposure. To describe the myriad ways by which viruses establish enduring interactions with host neurons, numerous descriptors have been used, including ‘prolonged’, ‘persistent’, ‘latent’, ‘smouldering’, ‘quiescent’ and ‘chronic’ (REFS [4,22,23]); however, their use is not consistent. Variables — including detection threshold, target organs and cell-specific influences on the viral life cycle — collectively contribute to the challenge of establishing an agreed-upon nomenclature. Moreover, some viruses can reactivate to cause the same disease as the acute infection (such as HSV), whereas others manifest differently upon reactivation (such as varicella zoster virus (VZV), which causes chicken pox as a primary infection but typically causes shingles upon reactivation). Other viruses result in pathogenesis only after protracted infection (such as tumour-causing viruses).

For this Review, we use three classifications. Latent infections are defined as those in which the virus establishes a non-lytic state during which host-to-host transmission is not possible, unless the virus reactivates to produce infectious virions. Chronic transmissible infections are characterized by the continuous production of infectious viral progeny and their ability to be transferred to new hosts. Chronic non-transmissible infections are those in which consistent detection of viral nucleic acid over extended periods of time is observed but in which transmission to new hosts does not occur.

Latency is most frequently attributed to herpesvirus infections, such as HSV-1, HSV-2 and VZV. After initial infection of epithelial cells, these viruses become non-lytic within PNS neurons, and viral nucleic acid is maintained in a heterochromatin episomal state with negligible transcription[12]. A small number of viral transcripts are synthesized during latency and are termed latency-associated transcripts. These RNA species do not encode functional proteins but are thought to prevent neuronal apoptosis and to disrupt both innate and adaptive immune signalling through mechanisms that include inhibition of caspase activity and of granzyme B-mediated killing[24,25]. VZV also produces various proteins, including ORF63, that prevent neuronal apoptosis[24]. The term ‘latent’ accurately conveys the status of these viruses: hidden, incapable of transmission, but able to fully reactivate, spread, and be transmitted to a new host. Another type of latency, which is not typically seen in neurons, occurs after viral nucleic acid is reverse transcribed from RNA to DNA and then integrated

into the host genome. This process is unique to retroviruses, such as HIV-1 (REF.[26]). In this type of latency, integrated viral genomic DNA becomes indistinguishable from host DNA, and viral genes can be epigenetically silenced or activated throughout the cell lifetime and passed on to daughter cells.

In a chronic transmissible infection, the infectious virus can be continuously recovered from the host and can be disseminated to new hosts, as in hepatitis B and hepatitis C. Mice infected with the lymphocytic choriomeningitis virus (LCMV) offer a well-characterized model of a chronic transmissible CNS infection. LCMV infection of newborn mice leads to a non-cytopathic chronic infection in almost every tissue. Infectious LCMV particles can be recovered from multiple organs throughout life and can be shed in the faeces or transmitted vertically to offspring[23,27,28]. Although most strains of mice survive LCMV infection with no overt pathogenic consequences, some studies reported learning and memory deficits in these chronically infected animals[29], underscoring the potentially subtle effects of long-term infection on CNS function.

Chronic non-transmissible infections are also characterized by sustained viral replication or consistent detection of viral nucleic acid over extended periods of time, but further host dissemination is absent. One example may be the rare cases of MV CNS infection. Acute infection can, in some instances, lead to the development of neuropathogenic diseases, including subacute sclerosing panencephalitis (SSPE) and measles inclusion-body encephalitis. These uncommon neurological diseases often present months or years after viral exposure and are characterized either by negligible replication or by persistence of replication-competent nucleic acid in the CNS[30–32]. In both diseases, no viral dissemination to uninfected hosts has been reported. Determining whether the state of the virus that causes these sequelae is ‘latent’ or ‘chronic non-transmissible’ is difficult, owing to both the small number of clinical specimens available and the lack of small animal models that mimic SSPE disease[31,33,34]. In humans, it may be that neurological symptoms appear only once viral replication reaches a crucial threshold or when the virus infects a key site within the brain, exceeding the host’s capacity to control the infection. Alternatively, non-replicating MV genomes may be maintained for prolonged periods and reactivated later. Either way, the MV genome remains intact, in some form, long after control of the acute infection is achieved, in the absence of further viral dissemination.

A final point of clarification: not all neurotropic viruses are associated with long-lasting infections. Some viruses, such as the reovirus, can induce neuronal apoptosis through the induction of pro-apoptotic proteins such as BAX[35,36]. The reason why some infections lead to neuronal suicide, whereas others lead to a long (potentially unhappy) ‘marriage’ between the host and the virus, is a major focus in the field of neurovirology, and answering this question may lead to the discovery of virus-specific therapies to prevent or minimize infection-triggered neuropathology.

Immune clearance of neuronal infection

The various permutations of neurotropic viral infections pose unique challenges for the host, including detecting antigens within the CNS, enabling T lymphocytes to engage with

neurons that express negligible levels of proteins that are typically present on target cells, and mitigating the risks of neuroinflammation and widespread loss of generally non-renewable neurons.

Type I interferon signalling

The early response to an infection typically begins with the engagement of pathogenic motifs by pattern recognition receptors, which are expressed on (or in) virtually all cells. The binding of these receptors to conserved motifs, such as double-stranded RNA, lipopolysaccharides or glycoproteins, propagates signals that culminate in the production of type I IFNs, chiefly IFNα and IFNβ. These IFNs are secreted from the infected cell and act in both a paracrine and an autocrine manner by binding to IFN α/β receptor (IFNAR), a heterotetramer with phosphorylatable cytoplasmic domains. This engagement leads to the phosphorylation of tyrosine kinases (including Janus kinases) and the receptor itself, and is followed by tyrosine phosphorylation of cytoplasmic signal transducer and activator of transcription 1 (STAT1) and STAT2, which are usually abundant but inactive within the cytoplasm. Activated STAT1–STAT2 heterodimers couple with IFN regulatory factor 9 (IRF9) to form the complex termed ISGF3, which translocates into the nucleus and binds to IFN-stimulated response elements within the promoters of IFN-stimulated genes (ISGs). These genes encode proteins that eliminate infected cells or aid in viral clearance. Type I IFNs also bind to adjacent, uninfected cells to shield them from infection. Although this pathway is operative in many cells, alternative IFN-triggered pathways that limit viral spread but do not depend on induction of the ‘usual suspects’, the ISGs, can be induced in some cell types, including neurons[37].

Neurons also secrete type I IFNs, which can act in an autocrine or paracrine manner on neurons or other parenchymal cell types[38]. RABV, which infects muscle cells and peripheral neurons after a bite from an infected animal, induces copious IFN secretion early after infection, *in vivo* and *in vitro*[39]. By contrast, IFN-induced STAT phosphorylation in primary hippocampal neurons is delayed, with maximal activation occurring only after ~24 hours[40,41]. Delayed STAT activation coincides with delayed expression of traditional ISGs[41]. The protracted interval between receptor binding and STAT activation may be due to a greatly reduced basal expression of STATs in these hippocampal neurons, as compared with other cell types[40–42]. Interestingly, lower homeostatic STAT expression is not unique to neurons but has also been observed in another non-renewable cell type, cardiac myocytes[43]. Like neurons, cardiac myocytes have high basal IFNβ expression, which may protect them from infection[41,43]. Perhaps, the disparity between expression of IFNs and the signal transduction molecules that they induce may skew towards protection from infection rather than towards induction of a potentially cytotoxic response. Surprisingly, synthesis of ISGs can differ within a single neuron: IFNβ induces a non-canonical, local antiviral response in axons that is not observed in the neuronal soma[44,45]. The startling implication of this finding is that neurons, especially those with long processes (as in the PNS), may ‘compartmentalize’ the response to extracellular immune mediators.

Although much of this Review focuses on neuronal responses to infections and antiviral cytokines, it is important to underscore that differential responses to, and production of, type

I IFNs have been demonstrated in other parenchymal cell populations and may influence the neuronal response. For example, when comparing microglia and oligodendroglia collected from mice that were infected with a neurotropic strain of mouse hepatitis virus (MHV), it was shown that microglia are the main producers of type I IFN and downstream ISG products[46]. Overall, the fact that different cell types show distinct homeostatic expression of key signal transducers and of their downstream gene targets underscores the cellular diversity that can follow cytokine engagement.

Perhaps predictably, for many neurotropic RNA viruses, including MV, Theiler's murine encephalomyelitis virus, Murray Valley encephalitis virus, WNV and others, experimentally induced loss of type I IFN signalling results in pathogenesis, altered viral tropism (generally accompanied by increased neurovirulence) and an inability to control viral spread both *in vivo* and *in vitro*[41,47–54]. Although most of these studies were performed using IFNAR-knockout mice lacking receptor expression on all cells, selective disruption of neuronal IFN signalling (using neuron-specific knockouts of IFNAR) also results in death after vesicular stomatitis virus infection[55]. Moreover, infection of olfactory neurons and mucosa with either a neurotropic RNA virus (vesicular stomatitis virus) or a neurotropic DNA virus (cytomegalovirus) leads to a robust type I IFN response deep within the brain, preventing viral spread and attendant disease[56]. Thus, infection of cells in direct contact with the environment (including sensory olfactory neurons) can trigger a long-distance warning (production of type I IFNs) that ultimately limits or precludes viral spread to remote regions of the brain.

Antigen presentation and CNS immunity

For some time, it was known that the primary cell populations of the adaptive immune system, T cells and B cells, contributed to viral control within the brain; however, the apparent absence of a CNS lymphatic drainage system made it complicated to understand how antigens could exit the parenchyma to promote the activation and proliferation of naive antigen-specific T cells[7]. Recent findings have begun to resolve this mystery: these include the identification of lymphatic drainage portals from the CNS into deep cervical lymph nodes and the presence of a fluid gradient that flushes the brain of extracellular proteins (which are termed 'glymphatics' because of the crucial role of glia in this process)[57–59]. CSF moves towards the perivascular space, where it is transported into the dense brain parenchyma via aquaporin 4 water channels that are expressed on cortical astrocytes. The CSF movement drives the interstitial fluid towards perivenous spaces, where it then drains towards the newly identified meningeal or dura mater lymphatic vessels, and ultimately to the deep cervical lymph nodes, where T cell activation and proliferation can occur[57,59,60]. These studies indicated how antigens and professional antigen-presenting cells can exit the CNS to alert immature T cells in the lymph nodes.

T cell-mediated pathogen clearance

After T cells mature in lymphoid tissues, they enter the bloodstream, where they can interact with adhesion molecules that are expressed on the surface of blood vessel endothelia within infected tissues. Mature T cells chiefly engage with selectins (and later, integrins) on the surface of the BBB or BCSF barrier. The expression of these adhesion molecules is induced

by chemokines that are produced within the parenchyma by infected neurons and adjacent glia. This results in migration of T cells across the barrier (diapedesis). Although it was previously believed that neurons do not express major histocompatibility complex (MHC) class I molecules (and thus could not be recognized, at least in the canonical manner, by $CD8^+$ T cells), we now know that some neuronal populations constitutively synthesize these cell surface proteins and that others can induce their expression after injury or infection[61,62]. Even so, most neurons do not express typical levels of MHC class I antigens under non-inflammatory conditions[63], and thus T cell effector functions, including cytokine production, may not be triggered by the infected cell (the neuron) directly but rather by adjacent MHC class I-expressing cells (usually glia) that can display antigenic peptides via cross-presentation[64]. Although cross-presenting glia may not be directly infected, this strategy allows for elaboration of antiviral processes. Resident CNS cells may not only be invisible to immune cells as a result of reduced expression of MHC recognition molecules but may also express immunomodulatory molecules, such as programmed cell death ligand 1 (PDL1)[65], that down-modulate T effector functions. Remarkably, the MHC class I expression system that is key to T cell recognition is likely to have also other functions in neurons, including those involved in neurodevelopment and neuronal plasticity[66,67].

One of the major strategies that is used by activated T cells to combat neuronal viral infections is the production of IFNγ. Similar to type I IFNs, IFNγ transduces a signal via receptor binding, leading to STAT1 activation and homodimerization. Activated STAT1 homodimers translocate into the nucleus and bind to gamma-activated sequences (GASs) in the promoters of approximately 100 genes (which overlap with, but are generally distinct from, the ISGs that are induced by type I IFNs), promoting their transcription and translation (FIG. 2a). The products of these genes, similar to ISG proteins, combat viral infection or induce apoptosis of the infected cell[37].

STAT1 can be activated in neurons after IFNγ exposure, but the kinetics of induction are markedly slower than those observed in treated mouse embryonic fibroblasts, similar to the delayed response that is seen after type I IFN exposure[40]. In addition, IFNγ induces transcription of both traditional genes (that is, those that are typically expressed in response to IFNγ in other cellular populations) and non-traditional genes in primary hippocampal neurons after exposure[68]. This diverse profile of genes that are induced may affect the cellular outcome: although IFNγ can induce necroptosis, in neurons the virus is controlled in a non-cytolytic manner (presumably owing to the paucity of STAT1 and non-traditional GAS gene induction)[69,70] (FIG. 2b). This feature is not unique to neurons: IFNγ is also essential for controlling MHV infection of oligodendrocytes via non-cytolytic pathways[71,72]. How are genes activated when basal levels of available STAT1 are low in resting neurons? Interestingly, when challenged with a neuron-restricted MV infection, most STAT1-knockout mice survive. By contrast, all IFNγ-knockout mice show severe signs of chronic disease, with approximately 50% succumbing to infection[68,70], suggesting that the requirement for IFNγ is decoupled from the main transducer through which it signals. This observation led to the identification of an IFNγ-dependent, STAT1-independent activation of antiviral and pro-survival genes[68,73], which might be facilitated by the access of other signalling factors — including extracellular signal-regulated kinases 1 and 2 (ERK1/2) and AKT — to the activated IFNγ receptor when STAT1 is absent or not abundant (FIG. 2b,c).

IFNγ is crucial for the control of multiple neurotropic viral infections in mice and primary neuronal cultures. Recently, IFNγ was identified as a key suppressor of HSV and VZV reactivation in the trigeminal ganglion of both humans and mice[24,74–77]. What makes these studies particularly intriguing is the type of T cell that is shown to be constitutively secreting IFNγ: T resident memory cells (T_{rm})[74–79]. T_{rm} (defined by CD103 and CD69 expression) are in direct proximity to latently infected PNS neurons and do not re-enter circulation. Furthermore, these brain-resident lymphocytes have a unique molecular signature that distinguishes them from other types of `cytotoxic T cells` or from `memory T cells`[80]. T_{rm} populations expand and contract in their resident tissue, acting as a first line of defence against reinfection[81]. Moreover, as we suggest below, these cells may be crucial sentinels that keep chronic neuronal infections at bay; therefore, their loss may contribute to viral reactivation.

In addition to cytokine secretion, some T lymphocytes kill infected cells through `perforin`- and/or granzyme-mediated mechanisms. Perforins, which are found in the lytic granules of $CD8^+$ cytotoxic T cells, punch holes in the membrane of infected target cells, allowing for the delivery of granzymes that lead to lysis of the infected cell. Granzymes are serine proteases that induce caspase cleavage and activation of pro-apoptotic cellular proteins, such as BH3-interacting domain death agonist (BID). This mode of T cell-mediated killing, which efficiently eliminates ‘viral factories’, has been primarily studied in rapidly dividing cells. Interestingly, in some neuronal infections, the secretion of granzymes does not lead to lysis but rather aids in preventing viral reactivation and replication while sparing the infected neuron[82]. In addition to their ability to kill cells, granzymes can directly cleave eukaryotic translation initiation factor 4G3 (eIF4G3) (a cellular protein that is important for host and viral translation) and ICP4 (a herpesvirus-specific protein needed for the transcription of early and late viral genes)[82,83]. By cleaving eIF4G3, granzymes block viral translation but fail to induce neuronal apoptosis, further preventing viral dissemination within the host and sparing the infected neuron. Cleavage of ICP4 by granzymes directly prevents reactivation of latent HSV from infected neurons. In these instances, granzymes are acting on proteins other than their traditional protein targets to induce an alternative neuronal response.

It has also been speculated that viral RNAs and proteins can contribute to non-lytic outcomes. For instance, HSV latency-associated transcripts inhibit the action and expression of various caspase proteins, which are key mediators of the cell death process[84]. Nevertheless, in some cases, bystander immune-mediated neuronal death may occur. For example, Theiler’s murine encephalomyelitis virus infection of mice results in hippocampal neuron death through a mechanism that is dependent on inflammatory monocyte infiltration and activation[85].

Humoral responses within the CNS

The notable absence of B cells in the brain of virus-infected mice led to the misperception that B cells and the antibodies that they secrete play a minor part in viral control. In fact, numerous human CNS infections, including those caused by MV, PV, VZV, HSV and flaviviruses, are characterized by the presence of intrathecal antibodies in the CSF[86–88]. Humoral responses seem to be associated with protective rather than pathogenic functions, as

observed for Japanese Encephalitis virus and some neurotropic retroviruses[86]. Antibodies may be particularly beneficial for those infections that result in extracellular infectious virus production.

Neuronal subtypes and infection

A central theme of this Review has been the notion that infected cells, such as neurons, respond to immune effectors in cell-specific ways. However, the existence of many subpopulations of neurons, which are segregated by location and function, raises the issue of whether responses may differ within these neuronal subsets. Recent studies showed that cerebellar granule neurons and cortical neurons pretreated with type I IFNs vary in their ability to control a WNV infection[89]. Type I IFN treatment had a much greater impact on the spread of infection in cerebellar granule neurons than it did in cortical neurons (100-fold versus 15-fold reduction), and this difference correlated with discrete patterns of ISG induction[89]. Animal model studies have also shown differences in the propensity for a virus to infect individual neuronal subpopulations and regions of the brain (FIG. 3); for example, the hippocampus is heavily infected by RABV, whereas MV is more often found in the midbrain[90–95]. Whether these distinctions can be attributed to differences in viral tropism or intrinsic variations in the neuronal response to soluble immune effector proteins (or, perhaps, to the way a virus gains access to the brain) is not known. Answering this question will require further studies that must necessarily integrate virology, immunology and neurobiology.

Emerging principles in neurovirology

Preservation of virus-challenged neurons from immune-mediated lysis seems to be advantageous to the host, but this leaves open the possibility of long-term viral maintenance in surviving neurons (TABLE 1). Previously, many researchers believed that neurotropic RNA viruses were sterilely cleared from the CNS. Indeed, unlike DNA viruses or retroviruses that can establish latent infections through episome formation or integration, RNA viruses do not have known means to ‘survive’ within a host cell. This is especially relevant given the lability of RNA within the cytoplasm, which arises owing to the inherently unstable ribose subunit and the susceptibility of the 2′ hydroxyl group to deprotonation. On the other hand, RNA viral genomes are unlikely to persist in the cytoplasm as naked RNA. Ribonucleoprotein complexes would provide some protection, and viral RNAs (like other cellular RNAs) may also be sequestered in `stress granules`. Thus, mechanisms must exist to protect RNA viral genomes, allowing for their long-term stability in the cytoplasm.

Do these long-term infections have pathogenic potential? A set of studies from the late 1980s showed that MV RNA can persist in human brains for decades after resolution of the peripheral infection without causing neurological symptoms[96–100]; in these studies, organs from individuals who had died of non-viral, non-CNS-related causes were screened, and a high proportion of brain tissues were found to be MV RNA positive. In addition, some scientists have argued that MV entry into the human CNS may occur at a higher rate than previously thought[101], although only a small fraction of acutely infected people will manifest neurological consequences. Accordingly, viral RNAs were generally considered

'fossils' that were unlikely to contribute to human disease. Surprisingly, autopsy studies performed on the brain of patients that succumbed to SSPE have shown regions of the brain with no detectable MV proteins, despite the presence of MV RNA, suggesting that RNA, even with its inherent instability, can be maintained in a translationally silent state[102].

The long-term persistence of viral RNA in the CNS is not unique to MV. For example, infection of mice with the MHV strain A59, which is used to model the demyelinating disease multiple sclerosis, leads to encephalitis and hepatitis. The infectious virus is cleared from the liver and CNS in 20 days; however, the mice develop a progressive, immune-mediated demyelinating disease[103], and viral nucleic acid persists[104]. The potential importance of viral nucleic acid persistence in demyelination has been subordinated by the prevailing view that long-term disease is caused by an overactivation of the host response towards myelin proteins. Other neurotropic RNA viruses that are known to persist within the mouse brain (sometimes for periods longer than 1 year post exposure) in the absence of detectable antigen or infectious viral progeny include Sindbis virus, Sendai virus and RABV[95,105,106]. However, the lack of recoverable infectious virus does not preclude the possibility that these viruses are actively suppressed in the CNS, similar to the control of neuronal herpesvirus infections by T_{rm}. Could decreases in the magnitude or quality of the host response (for example, with ageing or after immunosuppressive therapy) lead to loss of resident memory cells and reactivation of viral replication that are temporally separated from the initial infection?

The short answer is that we do not yet know. However, it was recently shown that an endogenous retrovirus, which was integrated into the host genome millions of years ago, could contribute to human neurological disease. Amyotrophic lateral sclerosis is a progressive neurological disease of poorly understood aetiology that is characterized by consistent inflammatory response and immune-mediated pathogenesis. The expression of this human endogenous retrovirus, specifically the expression of the envelope protein, was proposed as a possible cause for the neuropathology that is seen in amyotrophic lateral sclerosis[107].

Perspectives

Limits of detection, reproducibility, consistency in the brain regions that are analysed and patient-to-patient variability all contribute to the challenges and dangers of ascribing neurotropic infections to be the aetiologic causes of poorly understood CNS diseases. Moreover, the association of 'new' viruses with CNS disease (including Zika virus, which is linked to microcephaly[108,109]) or the emergence of more neurovirulent influenza strains[110] are reminders that our understanding of the pathogenic consequences of CNS infections remain quite primitive. Translational studies have provided insights into the links between infections and disease but are not without controversy. For example, the prevalence of human cytomegalovirus in patients with glioblastoma has been hotly debated[111], although anti-cytomegalovirus treatments lead to reduction in tumour burden in some patients[111]. Furthermore, losses in host immune status due to age or chemotherapy are well known to provoke disease, as observed with JC (John Cunningham) virus infection and progressive multifocal leukoencephalopathy[112].

Whether CNS virus infections have a larger role in human diseases of unknown aetiology remains controversial. In support of this notion, CNS neurons may be an ideal harbour for long-term infections: non-lytic immune mechanisms spare neuronal loss while providing an avenue for a non-cytopathic virus to persist. Moreover, trans-synaptic spread is likely to enable viral escape from antibody recognition or phagocytosis by antigen-presenting cells. From an evolutionary perspective, neuronal survival is paramount; thus, sparing infected neurons a lytic fate may promote survival early on but could potentially open the door for viral reactivation later in life. We do not know if there are viruses that are typically found in CNS tissues of overtly healthy individuals; with the advent of the RNA sequencing technology, new RNA sequencing studies might shed light on the potential 'virome' within the brain of both asymptomatic individuals and people with neurological conditions.

One final point that is worth noting concerns the utility of mouse models (on which many of the studies cited in this Review were based) to study human CNS diseases. Scientists often make the mistake of assuming that mouse survival is equivalent to an absence of disease. This may mean that the long-term ramifications of acute virus infections, especially those of RNA viruses that are not generally considered to be lifelong, may be overlooked. However, we are increasingly becoming aware that the presence of viral fragments or latent viruses that can reactivate might evoke non-lethal pathogenic consequences resulting from either viral replication and cell damage or immune responses directed against viral antigens. Such pathogenic consequences, as seen with the learning defects in LCMV-infected mice, may be subtle. Consequently, the parallel development of more-precise approaches to assess CNS disease in mice, including tools to evaluate the impacts on learning, behaviour and memory, should refine how we describe neuropathogenesis in the many valuable mouse models that are currently in use. Finally, determining whether or not persistent viral nucleic acids detected within the brain are replication competent and how these viruses evade complete clearance could promote the development of novel antiviral therapies to treat or prevent devastating and prevalent human neurological and neurodegenerative diseases[95,113].

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Glossary

Cytokines
Small proteins released by cells that affect cell signalling and act to regulate cell growth, maturation and effector functions

Interferons
(IFNs). Signalling proteins that are released by cells in response to infection to promote an antiviral state

RNA viruses
Viruses with genomic material that is composed of RNA rather than DNA. Genomic viral RNA can be double stranded, single stranded, positive sense or negative sense

DNA viruses
Viruses with genomic material that is composed of DNA

Virions
Complete forms of an infectious viral particle

Permissive cells
Cells that actively express viral receptor proteins, thereby facilitating viral entry and infection

Budding
The final step of viral release during which a virion gains its outer membrane by bursting through the host cell membrane

Viral fusion proteins
Viral glycoproteins that are essential in mediating the virus–host interaction in which the viral membrane fuses with the host membrane releasing a virion into the host cell

Syncytia
The result of infected cells fusing with adjacent uninfected cells, producing large, multinucleated clusters

Cytotoxic T cells
A subset of T cells that are primed to kill target cells

Memory T cells
A subset of T cells that have previously interacted with their cognate antigen

Perforin
A protein that is stored by cytotoxic T cells and that creates holes in target cell membranes, allowing for the delivery of cytotoxic granzymes

Stress granules
Dense aggregates of protein and RNA that are present in the cytoplasm and are typically associated with the endoplasmic reticulum

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Box 1

Syncytia formation and trans-synaptic spread

Viruses gain entry into permissive cells through an interaction between virally encoded glycoproteins, which are expressed on the outer surface of the virus particle, and cellular receptors. Entry can be achieved through endocytosis into vesicles or via membrane fusion[114]. For fusogenic viruses, exit from the cell occurs either via budding of virus particles through the plasma membrane or via fusion of an infected cell with an adjacent, uninfected cell[114]. The latter process results in the formation of multinucleated cells, or syncytia. The formation of syncytia may support further viral production but irrevocably leads to the death of the fused cells. Similarly, release of infectious particles by budding often leads to the death of the infected cell[115].

However, viruses that are considered cytopathic in renewable cell types — including measles virus (MV), rabies virus and pseudorabies virus — can switch to a non-productive, non-syncytia-forming mode of spread when infecting neurons, promoting neuronal survival[12,19,20,116,117]. Often, this is correlated with absence of detectable extracellular viral particles. The spread of these viruses within neurons is primarily trans-synaptic, although the neuronal processes that enable a switch from viral budding and syncytia formation to non-cytolytic, trans-synaptic spread are not yet defined.

At least two possibilities might explain the viral movement across the synapse. In the first scenario, the spread of viral particles between neurons requires ligand–receptor interactions, similar to infection in non-neuronal cells. Directed transport to the synapse and focal fusion at the synaptic cleft might be required for a virus to migrate across the synapse: thus, the process that occurs in non-neuronal cells might also be operative in neurons. Trans-synaptic spread might require the same cellular and viral proteins that allow for fusion of non-neuronal cells or may be unique to the presynaptic–postsynaptic interface. For example, in MV neuronal infection, expression of the primary receptors that are used in non-neuronal cell infection is not required; however, a fusion event is still necessary for the spread to occur, perhaps, by forming a ‘pore’ through which the viral ribonucleic acid is transported[118].

In the second scenario, the close approximation between the presynaptic and postsynaptic membrane, coupled with the unique attributes of the synaptic junction, may allow for the passive transport of viruses that have trafficked or assembled there. The release of neurotransmitters and uptake of their receptors make the synaptic interface particularly fluid, which might make it uniquely able to support receptor-independent trafficking.

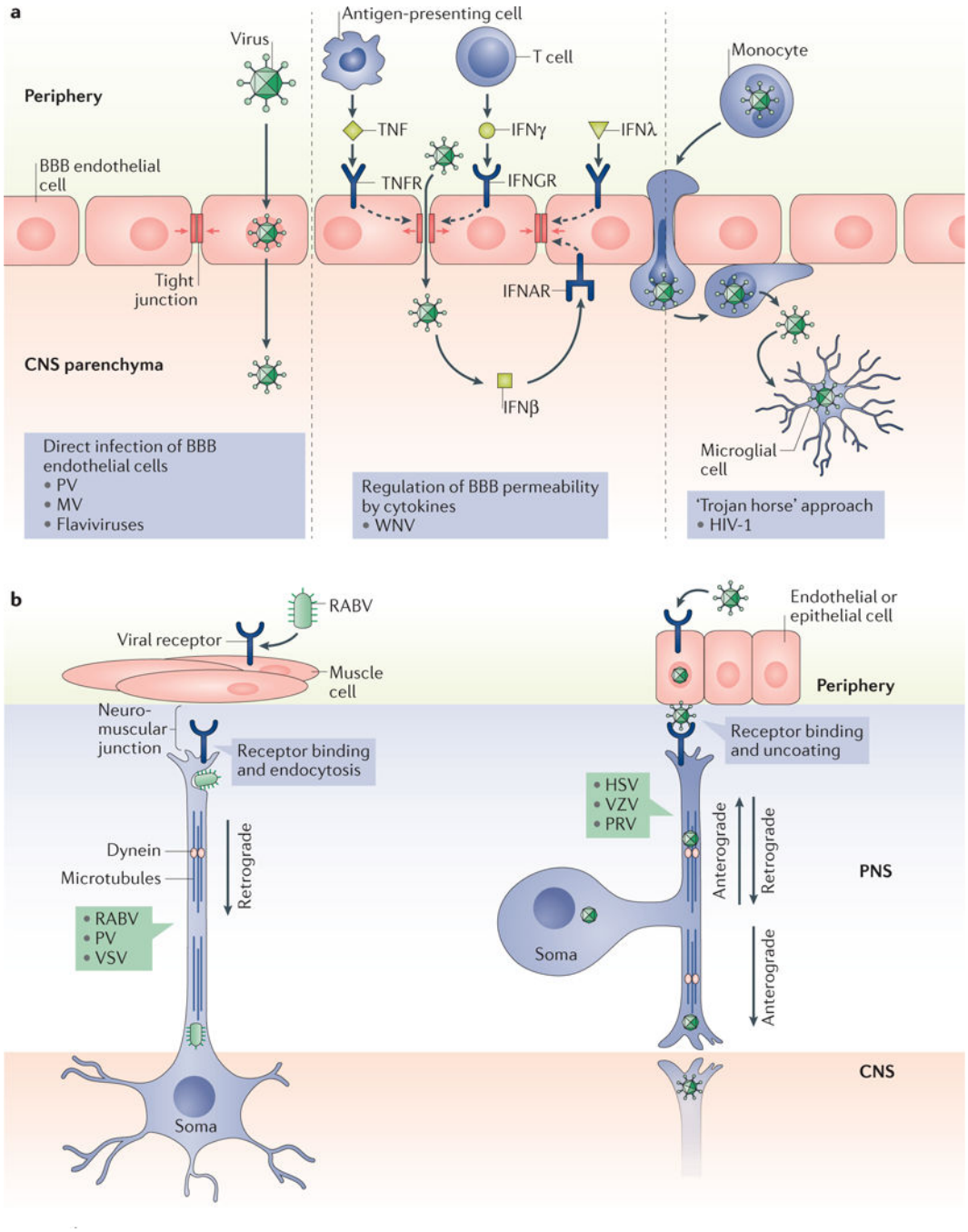

Figure 1. Viral entry into the CNS

Three modes of viral entry into the brain are shown. **a** | Viruses may directly infect the cells comprising the blood–brain barrier (BBB), followed by release into the parenchymal space (left panel). Alternatively, viruses may diffuse across permeable regions of the BBB (middle panel). Of note, BBB permeability can be influenced by cytokines, such as tumour necrosis factor (TNF) and various interferons (IFNβ, IFNγ and IFNλ), which can loosen or reinforce the barrier integrity. In the 'Trojan horse' approach (right panel), infected lymphocytes or monocytes (including macrophages) traffic across the BBB or blood–cerebrospinal fluid

barrier, releasing the virus once in the brain parenchyma. **b** | Trans-synaptic spread of viral particles involves the transport of viral genomes and associated proteins via microtubules and molecular motors. The left panel shows the movement of rabies virus (RABV) from the muscle, across the neuromuscular junction, and the dynein-mediated retrograde transport of this virus into the CNS. In the right panel, the transport of viruses (including herpes simplex virus (HSV), varicella zoster virus (VZV) and pseudorabies virus (PRV)) occurs across the epithelial or endothelial–neuron junction. In these neurons, retrograde transport brings the virus to the neuronal soma, and anterograde transport delivers the virus to the peripheral nervous system (PNS)–CNS synaptic junction. IFNAR, IFN α/β receptor; IFNGR, IFNγ receptor; HIV-1, human immunodeficiency virus type 1; MV, measles virus; PV, poliovirus; TNFR, TNF receptor; WNV, West Nile virus. Part **a** is adapted with permission from REF.[9], PLoS. Part **b** is adapted with permission from REF.[4], Elsevier.

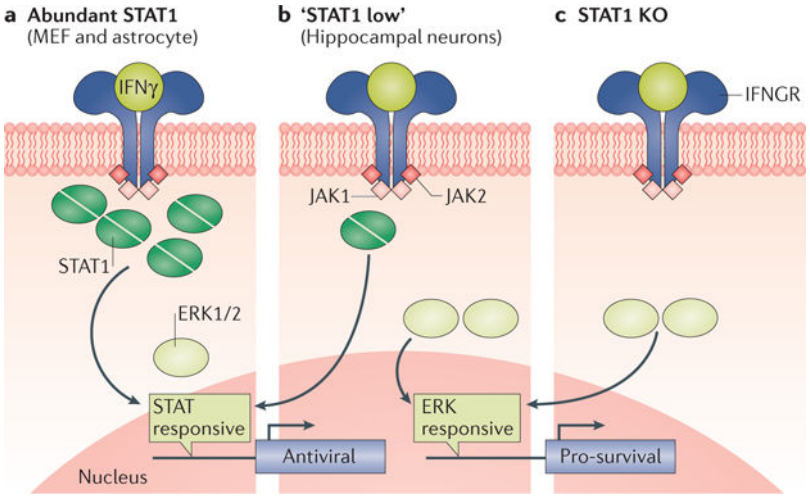

Figure 2. The receptor-occupancy hypothesis

In cells with abundant levels of signal transducer and activator of transcription 1 (STAT1) signalling proteins, engagement of the interferon-γ (IFNγ) receptor (IFNGR) by its ligand transduces a primarily STAT1-driven cellular response, leading to activation of gene products that are chiefly antiviral (part **a**). By contrast, when a particular cell population (such as hippocampal neurons) expresses reduced homeostatic levels of STAT1 (part **b**) or when STAT1 is removed by genetic deletion (part **c**), alternative signalling molecules with an affinity to the IFNGR may bind to this receptor, transducing unique cellular responses. In the case of neurons, this includes activation of extracellular signal-regulated kinases 1 and 2 (ERK1/2), which then can result in the induction of genes encoding pro-survival proteins. JAK1, Janus kinase 1; KO, knockout; MEF, mouse embryonic fibroblast.

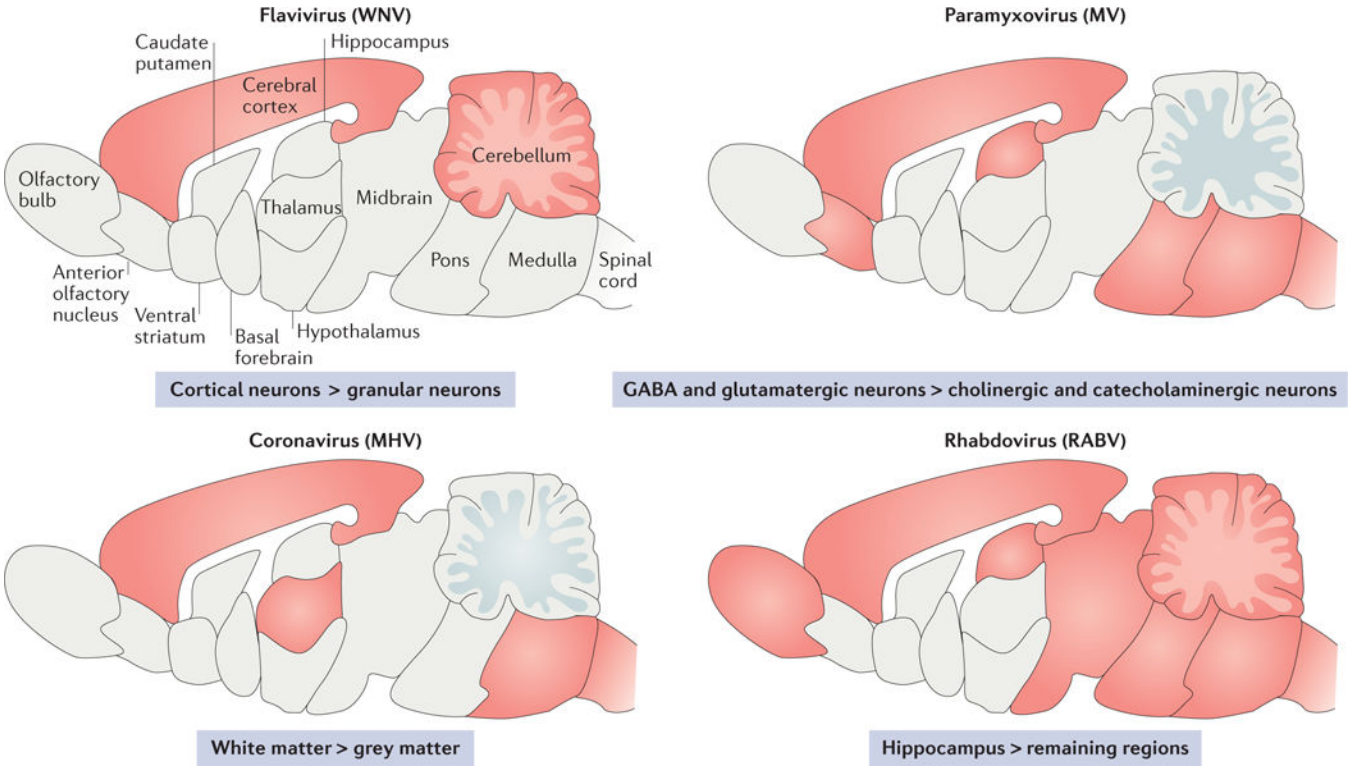

Figure 3. Tropism of neurotropic RNA viruses for distinct brain regions and neuronal subpopulations

The schematics show a simplified sagittal view of the mouse brain with the regions that are known to be infected by various viruses indicated in red. The symbol '>' indicates higher propensity for a virus to infect a certain cell type or region of the brain than another cell type or region. MHV, mouse hepatitis virus; MV, measles virus; RABV, rabies virus; WNV, West Nile virus.

Table 1

Evidence of long-term persistence of RNA viruses in CNS tissue

Virus	Model	Species detected			Infectious?	Time after infection	Tissue	Method	Refs
		RNA	mRNA	Protein					
Measles	Human	Yes	Yes	Unknown	Unknown	Decades (lifespan)	Brain	PCR	96,100
Mouse hepatitis	Mouse	Yes	Yes	NT	NT	10 months	Spinal cord and liver	*In situ* hybridization	104
Sendai	Mouse	Yes	Unknown	NT	Unknown	423 days (lifespan)	Brain	Dot blot	105
Sindbis	Mouse	Yes	Unknown	NT	Yes	17 months	Brain	PCR	106
Rabies	Mouse	Yes (genomic)	NT	Unknown	Unknown	6 months	Brain	qRT-PCR	95

NT, not tested; qRT-PCR, quantitative reverse transcription PCR.