Virus Infections in the Nervous System

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Virus infections usually begin in peripheral tissues and can invade the mammalian nervous system (NS), spreading into the peripheral (PNS) and more rarely the central (CNS) nervous systems. The CNS is protected from most virus infections by effective immune responses and multilayer barriers. However, some viruses enter the NS with high efficiency via the bloodstream or by directly infecting nerves that innervate peripheral tissues, resulting in debilitating direct and immune-mediated pathology. Most viruses in the NS are opportunistic or accidental pathogens, but a few, most notably the alpha herpesviruses and rabies virus, have evolved to enter the NS efficiently and exploit neuronal cell biology. Remarkably, the alpha herpesviruses can establish quiescent infections in the PNS, with rare but often fatal CNS pathology. Here we review how viruses gain access to and spread in the well-protected CNS, with particular emphasis on alpha herpesviruses, which establish and maintain persistent NS infections.

Introduction

Most acute and persistent viral infections begin in the periphery, often at epithelial or endothelial cell surfaces. Infection of cells at these sites usually induces a tissue-specific antiviral response that includes both a cell-autonomous response (intrinsic immunity) and paracrine signaling from the infected cell to surrounding uninfected cells by secreted cytokines (innate immunity). This local inflammatory response usually contains the infection. After several days, the adaptive immune response may be activated and the infection cleared by the action of infection-specific antibodies and T cells (acquired immunity). Viral infections that escape local control at the site of primary infection can spread to other tissues, where they can cause more serious problems due to robust virus replication or overreacting innate immune response. This latter reaction is sometimes called a “cytokine storm” because both proinflammatory and anti-inflammatory cytokines are elevated in the serum leading to vigorous systemic immune activity (Figure 1). Such a response in the brain is usually devastating and can lead to meningitis, encephalitis, meningocerebellitis, or death.

The capacity to invade the host nervous system (NS) appears to be a rather poor evolutionary path because of the potentially damaging and lethal nature of these infections. In most cases, acute infection of the central nervous system (CNS) has no apparent selective advantage for the host or the pathogen. These infections, when they occur, often represent a zoonotic infection: an incursion of a virus from its natural host into a “dead-end host,” which has little chance to further disseminate the infection. Zoonotic virus infections are often minimally pathogenic in their natural hosts, but they can be highly virulent and neuroinvasive in nonnatural hosts. The virulence and lethality of zoonotic infections may result from the cytokine storm evoked after the primary infection. The 1918 influenza pandemic and more recent H5N1 (bird flu) outbreak in humans are good examples of this phenomenon (Teijaro et al., 2011). Patients infected with H5N1 virus die, not because of robust virus replication, but because of acute respiratory distress syndrome (ARDS) triggered by the cytokine storm. Fatal infection by rabies virus (RABV), and the less known but nonetheless devastating infections caused by West Nile virus (WNV) and Nipah virus also represent zoonotic infections. When these zoonotic RNA viruses infect fully differentiated neurons (in the brain and spinal cord), virus clearance by the immune system is a major challenge. Because CNS neurons in particular are irreplacable, canonical T-cell-mediated cytolysis of these infected cells is not a favorable strategy. Thus, as understood by animal models, recovery from viral infection and the clearance of viral RNA require a slower process involving the actions of virus-specific antibodies and interferon gamma (IFN-γ) secretion from T cells (Griffin and Metcalf, 2011).

In contrast to the dangerous zoonotic CNS infections described above, some human-adapted viruses gain access to the CNS as a result of diminished host defenses that fail to limit peripheral infections (e.g., Epstein-Barr virus, human cytomegalovirus, JC virus). Others are the result of the iatrogenic introduction of the infection directly into the CNS, bypassing peripheral barriers (e.g., use of adenovirus or lentivirus vectors, or prions). Remarkably, many alpha herpesviruses efficiently enter the peripheral nervous system (PNS) of their natural hosts and establish a quiescent infection with little or no CNS pathogenesis. Their initial peripheral infection stimulates a well-controlled intrinsic and innate immune response, as well as a long-lived adaptive immune response. The alpha herpesvirus genomes remain quiescent in PNS neurons for the life of their hosts, only occasionally reactivating to produce virions that can reinfect peripheral tissues and spread to other hosts. Perhaps the prosurvival, cytolsis-resistant nature of mature neurons facilitates establishment of such persistent and reactivatable infections.

In this review, after providing a general overview on how viruses gain access to the NS, we will focus on intra- and interneuronal virus trafficking and spread tactics and discuss how alpha herpesviruses establish persistent infections in the NS. We will finish by discussing our views on the control of virus invasion in the NS.

Entering the Nervous System

While the PNS is relatively more accessible to peripheral infections because nerves are in direct contact with tissues of all
types, the CNS proper has several layers of protection. Spread of infection from the blood to the cerebral spinal fluid and CNS cells is limited by the blood brain barrier (BBB). The BBB is mainly composed of endothelial cells, pericytes, astrocytes, and the basement membrane (Figure 2D). Pericytes project finger-like processes to ensheathe the capillary wall and coordinate the neurovascular functions of the BBB. The star shaped astrocytes (i.e., astroglia) are the major glial cell type in the CNS. The astrocyte endfeet projections ensheathe the capillary, regulating BBB homeostasis and blood flow. Brain microvascular endothelial cells (BMVECs) that line the CNS vasculature are connected by tight junctions not found in capillaries in other tissues. These junctions restrict the egress of bacteria, virus particles, and large protein molecules from the lumen of the blood vessel, while allowing the transport of metabolites, small hydrophobic proteins, and dissolved gasses in and out of the CNS. The basement membrane, a thick extracellular matrix, also surrounds these capillaries, further limiting movement of pathogens. Perivascular macrophages (i.e., microglia) residing between the endothelial and glial cells further provide immune surveillance in the CNS tissue. Virus infections that leave the periphery and find their way into the CNS do so either by direct infection of nerve endings in the tissues or by infecting cells of the circulatory system that ultimately carry the infection through the BBB into the CNS (Table 1 and Figure 2).

**Direct Infection of Nerve Endings**

**Invasion of Sensory Nerve Endings.** Some viruses can enter the PNS by binding to receptors on axon termini of sensory and autonomic neurons, which respectively convey sensory and visceral information. Most alpha herpesviruses use this route to enter the PNS and establish a life-long persistent infection (Tirabassi et al., 1998) (Figure 2A). Human alpha herpesviruses include herpes simplex type-1 (HSV-1), HSV-2, and varicella zoster virus (VZV). Well-studied animal alpha herpesviruses include pseudorabies virus (PRV) and bovine herpes virus (BHV). The cellular adhesion molecule Nectin-1 is a major neuronal receptor for these viruses (except for VZV) (Smith, 2012). Alpha herpesvirus particles enter sensory nerve endings by membrane fusion and engage dynein motors for retrograde transport to the neuronal cell body (i.e., soma) (Smith, 2012). The capsids then dock at the nuclear pore, and the viral DNA is instilled in the nucleus, where an acute or a quiescent, latent infection is established. These viruses are unique in maintaining quiescent infections in the PNS neurons of their hosts. This quiescent state can be reversed by certain stress stimuli, resulting in the production of large numbers of progeny in a relatively short period of time (1–2 days) (Camarena et al., 2010). Despite the direct synaptic connection of PNS neurons to the CNS, spread of alpha herpesvirus infection to the CNS is rare, but debilitating, in natural hosts (Tirabassi et al., 1998). Alpha herpesvirus trafficking, latency, and reactivation is further reviewed in the following sections.

**Invasion of Motor Neurons at Neuromuscular Junctions.** Neuromuscular junctions (NMJs) are specialized synapses between muscles and motor neurons that facilitate and control muscle movement. NMJs can be gateways for many viruses to spread into the CNS (Figure 2B). Most motor neurons have their cell bodies in the spinal cord, which, in turn, are in synaptic contact with motor centers in the brain. Poliovirus and RABV infections spread into the CNS through NMJs. While RABV particles enter the NMJ directly after a bite from an infected animal, poliovirus infection begins with the ingestion of virus particles followed by replication in the intestinal mucosa and secretion of new virus particles in feces, typically with no CNS infection. However, about 1%–2% of poliovirus infections...
result in the infection of motor neurons that leads to the well-known motor dysfunction poliomyelitis (Racaniello, 2006). The receptor for poliovirus is an Ig superfamily member called CD155, which is found on axonal membranes (Ren and Racaniello, 1992). Work in transgenic mice expressing human CD155 has led to a model in which the virus replicates in motor neurons within the spinal cord and subsequent retrograde spread of progeny virions to motor centers in the brain results in muscle paralysis (Kuss et al., 2008; Ohka et al., 2012).

RABV, a member of the Rhabdoviridae family, infects many animals, including bats, skunks, foxes, and dogs, and rhabdoviruses can even infect insects and plants. RABV in animal saliva spreads between hosts via bites or scratches. Animals infected with RABV can survive for months and even years secreting...
infectious particles in their saliva. In contrast, human infection results in fatal acute myeloencephalitis unless treated with rabies antiserum. RABV particles enter the axons of motor neurons at the NMJ by binding to nicotinic acetylcholine receptors (nAchRs) and neural cell adhesion molecules (NCAMs) (Ugolini, 2011). Transneuronal spread occurs exclusively between synaptically connected neurons, and the infection moves unidirectionally from postsynaptic to presynaptic neurons (retrograde spread). A unique feature of RABV infection of humans is the relatively long asymptomatic incubation period after the initial infection, which can last from weeks to 1 year. This feature provides valuable time for intervention with antiserum, which blocks CNS infection. Once rabies infection reaches the CNS, marked behavioral and neurological symptoms begin, and death almost always ensues. If the host survives long enough, virus particles can spread from the CNS to peripheral tissues, particularly to the salivary glands.

Invasion via the Olfactory Epithelium and Olfactory Neurons. The olfactory system provides a unique and directly accessible portal of entry to the CNS from the periphery. The bipolar olfactory receptor neurons have dendrites in the olfactory epithelium at the roof of the nasal-pharyngeal cavity, where odors are detected. The CNS literally is one synapse away from the environment. The olfactory nerve, which belongs to the PNS, innervates the olfactory epithelium and terminates in the olfactory bulb in the CNS proper (Figure 2C). The olfactory epithelium is well protected from most common infections by mucus and the presence of several pathogen recognition receptor systems (Kalinke et al., 2011). However, in laboratory models, the olfactory portal can be used by some viruses. There is evidence that HSV-1, vesicular stomatitis virus (VSV), Borna disease virus (BDV), RABV, influenza A virus, parainfluenza viruses, and prions can enter CNS through olfactory route (Detje et al., 2009; Mori et al., 2005).

Some emerging zoonotic infections may also spread to the CNS by the olfactory pathway. For example, the paramyxoviruses Hendra virus (HeV) and Nipah virus are natural pathogens of bats. HeV infection of humans causes lethal pneumonia but also causes encephalitis by unknown mechanisms. In mice, HeV is able to infect the CNS after intranasal infection via the olfactory system, which may also be the infection route in humans (Detje et al., 2009; Mori et al., 2005).

Table 1. Classification, Properties, and CNS Entry Routes of Viruses that Can Cause Nervous System Infections

<table>
<thead>
<tr>
<th>Genome</th>
<th>Virus Family</th>
<th>N/E Specifics</th>
<th>Viruses</th>
<th>CNS Entry</th>
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<tbody>
<tr>
<td>dsDNA</td>
<td>Adenoviridae</td>
<td>N</td>
<td>MAV-1</td>
<td>BBB and BMVECs</td>
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<td></td>
<td>Herpesviridae</td>
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<td>alpha herpesvirus HSV-1, HSV-2, VZV, PRV, and BHV sensory nerve endings and ORN</td>
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<tr>
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<td>E</td>
<td>beta herpesvirus HCMV</td>
<td>BBB and BMVECs</td>
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<tr>
<td></td>
<td>E</td>
<td>gamma herpesvirus EBV</td>
<td>BBB and BMVECs</td>
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<td></td>
<td>Polyomaviridae</td>
<td>N</td>
<td>JCV</td>
<td>BBB and BMVECs</td>
</tr>
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<td></td>
<td>Reoviridae</td>
<td>N</td>
<td>mouse model T3</td>
<td>BBB; peripheral nerve?</td>
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<tr>
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<td>E</td>
<td>mouse model</td>
<td>peripheral nerve and ORN</td>
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<td></td>
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<td>E</td>
<td>hepaviruses HCV</td>
<td>BBB and BMVECs</td>
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<td></td>
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<td>E</td>
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<td>WNV and JEV peripheral nerve, BBB, ORN, and BMVECs</td>
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<td></td>
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<td>TIMEV</td>
<td>BBB</td>
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<td>E</td>
<td>zoonotic</td>
<td>CHIKV ORN?</td>
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<td>E</td>
<td>mouse model</td>
<td>SINV ORN</td>
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<td>(+)ssRNA</td>
<td>Arenaviridae</td>
<td>E</td>
<td>zoonotic</td>
<td>LCMV BBB</td>
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<td></td>
<td>Bornaviridae</td>
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<td>BDV ORN</td>
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<td>Bunyaviridae</td>
<td>E</td>
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<td>LACV ORN and BBB?</td>
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<tr>
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<td>zoonotic</td>
<td>influenza A peripheral nerve and ORN</td>
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<td>morbillivirus MV</td>
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<td>BBB</td>
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<td>Retroviridae</td>
<td>E</td>
<td>lentivirus HIV and HTLV</td>
<td>BBB</td>
</tr>
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</table>

Table 1 continued...

*dsRNA, double-stranded RNA; ssRNA, single-stranded RNA; N, naked; E, enveloped; ORN, olfactory receptor neuron; RT, reverse transcriptase; MHV, mouse hepatitis virus.

Richardson-Burns et al. (2002).
Bergmann et al. (2006).
Emerging virus.
Tsunoda and Fujinami (2010).
Metcalf and Griffin (2011).
In a mouse model, CHIKV could enter the CNS using the olfactory path (Powers and Logue, 2007). Similarly, La Crosse virus (LACV), a mosquito-borne bunyavirus, causes rare but severe meningoencephalitis, particularly in children. How LACV spreads into the CNS is controversial, but it may occur via viremia after the initial replication in muscle cells (Johnson, 1983), or the virus may travel from the olfactory epithelium to the CNS via olfactory neurons (Bennett et al., 2008).

**Invasion by Infected Circulating Leukocytes**

Some viruses gain access to the NS without infecting neurons. Instead, these viruses infect leukocytes, which circulate in the blood, and may infiltrate in the brain parenchyma. This mechanism is known as “Trojan horse” entry since the pathogens are hidden within these immune defense cells, which are naturally able to traverse the BBB (McGavern and Kang, 2011) (Figure 2D). Lentiviruses, including simian immunodeficiency virus (SIV) and human immunodeficiency virus (HIV), and another retrovirus, human T cell leukemia virus (HTLV), may access the CNS by the Trojan horse mechanism after infection of monocytes or macrophages that infiltrate through the BBB (Kaul et al., 2001). HIV primarily targets CD4+ T lymphocytes, but it also infects macrophages/microglia, which function as specialized phagocytic and antigen-presenting cells in the CNS, and astrocytes, which express the cell-surface receptor CD4 and appropriate chemokine coreceptors (Martin et al., 1997; Schweighardt and Atwood, 2001). Viral envelope glycoproteins gp120 and gp41 mediate virion attachment to these receptors. HIV replication is extensively influenced by the immune response induced by the infection. For instance, cytokines produced during infection can either stimulate virus replication or suppress viral entry and replication, depending on the nature of the released cytokine and the state of the cell. CNS invasion occurs when infected memory T cells or infected monocyte/macrophage precursors traverse the BBB, which then differentiate into perivascular microglia. Virus replication in microglia and the subsequent induction of an inflammatory cytokine response damages the CNS, causing depression and cognitive disorders. Notably, 10%–20% of infected patients exhibit HIV-associated dementia (HAD) (McArthur et al., 1997). HIV infection also has an effect on the PNS. Between 30%–60% of AIDS patients suffer from HIV-associated sensory neuropathy characterized by the degeneration of myelinated and unmyelinated nerve fibers (Kamerman et al., 2012).

The picornavirus enterovirus 71 (EV71) (Solomon et al., 2010) and a ubiquitous human polyomavirus, JC virus (JCV) (Boothpur and Brennan, 2010), can also infiltrate into the CNS by the Trojan horse mode of entry. In the case of JCV infection, infiltration of infected B cells into the CNS in immune suppressed patients can result in the infection of oligodendrocytes (the myelin-producing cells) and astrocytes, leading to a fatal inflammatory disease in the brain called progressive multifocal leuкоencephalopathy (PML) (Boothpur and Brennan, 2010).

**Infection of Brain Microvascular Endothelium**

In some instances, virus particles in the circulatory system can reach and infect BMVECs, a major constituent of the BBB. RNA viruses such as WNV (Verma et al., 2010), Hepatitis C virus (HCV) (Fletcher et al., 2012), HTLV-1 (Afonso et al., 2008), and DNA viruses such as JCV (Chapagain et al., 2007), Epstein-Barr virus (EBV) (Casiraghi et al., 2011), human cytomegalovirus (HCMV) (Fish et al., 1998), and mouse adenovirus 1 (MAV-1) (Gralinski et al., 2009) all can infect BMVECs (Figure 2E). Infection of these cells often leads to the disruption of BBB integrity, which is accompanied by the uncontrolled migration of immune cells into the brain parenchyma. Inflammation in the brain tissue induced by the activity of these cells is usually the cause of the observed neurological disorders.

WNV, an arthropod-borne flavivirus, causes encephalitis and meningitis in a small percentage of infected humans after the initial replication in keratinocytes and Langerhans cells in the skin (Lim et al., 2011). CNS infections often are associated with inefficient immune clearance of infection in the peripheral tissues and the resulting robust virus replication and subsequent viremia (Cho and Diamond, 2012). WNV can access the CNS either by infecting sensory nerve endings or olfactory neurons or through blood circulation, but not via NMJs (Lim et al., 2011). A hallmark of WNV neuropathogenesis is the disruption of the BBB, resulting in uncontrolled entry of immune cells into the brain. The infection not only stimulates the loss of tight junction proteins in both epithelial and endothelial cells (Xu et al., 2012), but also induces production of matrix metalloproteinases that degrade the basement membrane. As a result, leukocytes traverse the capillaries into surrounding tissue and release cytokines upon recognition of WNV double-stranded RNA (dsRNA) via toll-like receptor 3 (TLR3) and tumor necrosis factor alpha (TNF-α) signaling (Verma et al., 2010; Wang et al., 2004). These events are unique to WNV and are not observed with other hemorrhagic flaviviruses such as Dengue virus (Xu et al., 2012). HIV, HTLV-1, and MAV-1 infections also may disrupt the BBB by affecting tight junction proteins (Afonso et al., 2008; Gralinski et al., 2009). MAV-1 infects both BMVECs and macrophages, leading to acute encephalomyelitis in susceptible mouse strains, which might explain how human adenovirus infections in immunodeficient patients leads to encephalitis with mortality rate approaching 60% (Ashley et al., 2009; Gralinski et al., 2009).

Another flavivirus, HCV, which primarily infects hepatocytes, has also been associated with CNS abnormalities such as cognitive dysfunction, fatigue, and depression (reviewed in Fletcher and McKeating, 2012). BMVECs are the only cell type in the brain that express all four receptors required for HCV entry (scavenger receptor BI, CD81, claudin-1, and occludin). They also support HCV infection in vitro, suggesting that they may be the CNS reservoir of HCV (Fletcher et al., 2012). CNS infection by HCV is also associated with increased expression of proinflammatory cytokines (interleukin-1 [IL-1], TNF-α, IL-12, and IL18), choline, creatine, and inositol, all of which trigger microglia and astrocyte activation, leading to pronounced neurological disorders (Fletcher and McKeating, 2012).

Prevalent human DNA viruses that establish life-long, well-controlled persistent infections in other tissues can also damage the CNS by infecting BMVECs under immune-suppressive conditions. The beta herpesvirus HCMV (Fish et al., 1998), the gamma herpesvirus EBV (Casiraghi et al., 2011), and the polyomavirus JCV (Chapagain et al., 2007) are among such pathogens. HCMV establishes life-long latency, predominantly in the cells of the myeloid lineage (Goodrum et al., 2004). If the primary infection happens during pregnancy, transmission to the not-yet-immune-competent fetus may result in a variety of fatal CNS abnormalities, such as mental retardation and hearing...
loss. Besides BMVECs, astrocytes, pericytes, neurons, microglial cells, and, importantly, neural stem cells all can be infected with HCMV (Cheenan et al., 2009; Fish et al., 1998). Astrocytes and microglial cells naturally produce copious quantities of inflammatory cytokines in response to HCMV infection, and this response can promote fetal neurodevelopmental diseases.

EBV establishes life-long latency in memory B cells (Speck and Ganem, 2010). EBV infection in young adults causes infectious mononucleosis. In rare cases, EBV infection leads to B cell lymphomas (e.g., Burkitt’s lymphoma) and epithelial cell carcinomas (e.g., nasopharyngeal carcinoma). Horwitz and colleagues showed that EBV can infect human BMVECs, where it becomes latent (Casiraghi et al., 2011). Virus reactivation in these cells increases inflammatory cytokine and chemokine expression, affecting the integrity of the local BBB, which might lead to progression of the inflammatory neurological disease multiple sclerosis (MS) (Casiraghi et al., 2011).

**Virus Induced Immune-Mediated CNS Pathogenesis**

The BBB may be disrupted by mechanisms other than endothelial cell infection. Local production of cytokines in the CNS due to viral infections affects tight junctions of the BBB as well. For example, expression of the chemokine monocyte chemoattractant protein-1 (MCP-1, CCL2), which is induced by viral infections, is sufficient to disrupt tight junction proteins in the BBB (Stamatovic et al., 2005) and predisposes the brain tissue for immune-mediated neuropathology. An interesting example of virus-induced immune-mediated CNS pathogenesis is the rare, but fatal, Borna disease virus (BDV) infection. BDV, a zoonotic RNA virus infecting warm-blooded animals, establishes persistent, noncytolytic infection in the CNS, particularly in neurons of the limbic system (cortex and hippocampus) (Planz et al., 2009). The transmission route into the CNS is probably via the olfactory system, followed by axonal transport and transsynaptic spread to other parts of the brain (Lipkin et al., 2011). Depending on the immune state of the host, BDV replication interferes with a multitude of signaling cascades, deranging the expression of cytokines, neurotrophins that control neuron survival and function, and apoptosis-related genes, leading to neuropathogenesis. Although controversial, human BDV infections might be associated with a variety of neurological disorders, including unipolar depression, bipolar disorder, and schizophrenia (Lipkin et al., 2011).

Lymphocytic choriomeningitis virus (LCMV) is a rodent-borne human pathogen that causes CNS disease. Pathogenesis occurs through the combinatorial effects of virus replication and the subsequent host immune response. Humans acquire this infection by inhalation of aerosols or dust suspensions. After the initial replication in lung cells, LCMV virions move into the circulation and can enter the CNS. In animal models, LCMV preferentially infects nonneuronal cells in the brain (i.e., glial cells), which triggers a robust inflammatory response and interferes with neuronal migration (Bonthius, 2012).

Influenza A (i.e., H5N1, H1N1), an orthomyxovirus that is both zoonotic and endemic in human population, primarily replicates in the lungs after transmission through the respiratory tract. Some of the Influenza A isolates (i.e., HK483) are neurovirulent and can spread to the CNS through olfactory, vagus, trigeminal, and sympathetic nerves in mouse models (Park et al., 2002). Interestingly, neurological and cognitive effects associated with influenza infection have also been reported after nonneurotropic H1N1 pandemics. Recent work shows that increased production of proinflammatory cytokines and activation of microglia upon peripheral, nonneurotropic influenza (A/PR/8/34) infection contributes to altered hippocampal structures and cognitive dysfunction in mice in the absence of neuroinvasion (Jurgens et al., 2012).

Paramyxoviruses, measles virus (MV), and mumps virus (MuV), which are the causative agents of highly communicable diseases, can also lead to serious CNS infections. Primary MV and MuV infections begin in the upper respiratory tract, and infection of lymphoid tissue causes viremia and spread to other tissues. MuV is highly neurotropic and can cause acute encephalopathy in children with high incidence. Elevated levels of multiple cytokines were detected in cerebrospinal fluids of children diagnosed with MuV-associated acute encephalopathy (Watanabe et al., 2013). Unlike MuV, MV infection spreads to the CNS in approximately 0.1% of the cases, causing several types of devastating neurological diseases, such as fatal subacute sclerosing panencephalitis (SSPE), which manifests weeks to years after infection (reviewed in Buchanan and Bonthius, 2012). Although the molecular mechanisms of CNS infection are not well understood, transgenic rodent models reveal that a complex interplay of innate and adaptive immune system responses are involved including disrupted Th1/Th2 balance and defective T cell proliferation (Schneider-Schaulies et al., 2003).

These selected examples of neuropathogenesis caused by diverse virus families demonstrate that, while virus replication itself may cause neuropathologies, the activated immune system also contributes to the neuronal damage in an effort to eradicate the infection. Moreover, immune-mediated changes in the CNS induced by one virus infection might stimulate the neuropathogenicity of other viruses, as is seen in coinfections with HIV and HCV, JCV, or HCMV. To better comprehend the effects of viral invasion on the NS, we must understand how viruses interact with, replicate in, and spread in between neurons.

**Cell Biology of Neuronal Infection and Spread**

Neurons are highly polarized, with distinct dendrite and axonal processes. Single axons can extend meters, many thousands of times the diameter of a typical cell. The maintenance and the communication of distal axons with their cell bodies requires highly specialized signal transduction, intracellular sorting, trafficking, and membrane systems. As obligate intracellular parasites, viruses are dependent on these cellular functions that are often cell-type specific. Accordingly, neurotropic viruses can be divided into two categories: those that accidentally or opportunistically spread to the specialized NS (e.g., WNV, HCV, and EBV) and those that have adapted to the NS (e.g., alpha herpes-viruses and RABV). From a human health perspective, these fundamental differences in virus-host interaction result in the broad range of viral pathogenesis. In this section, we will summarize how viral infections engage neuronal cell biology to enter, traffic, and spread between neurons.

**Entry**

In general, virions may enter cells by direct fusion with or penetration of the plasma membrane or by endocytosis. Alpha
Herpesviruses enter neurons by direct fusion of their virion envelopes with the plasma membrane (Figure 3A) (Lycke et al., 1988). Nectin-1, a member of the immunoglobulin (Ig) superfamily, is a receptor for HSV and PRV virions and engages the conserved viral glycoprotein gD on the virion surface to initiate the entry process. After membrane fusion, the capsid with inner tegument proteins is deposited into the axonal cytoplasm, ready for subsequent intracellular transport steps (Smith, 2012).

Most other neurotropic viruses enter neurons by endocytosis, using cellular receptors that are concentrated at nerve terminals (Figure 3A). The following neuromuscular junction proteins serve as viral receptors: NCAMs and nAChRs for RABV (Ugolini, 2011), CD155 for poliovirus (Ohka et al., 2004), and the coxsackievirus and adenovirus receptor (CAR) (Salinas et al., 2009). In addition, many viruses that do not infect neurons during natural infections can enter nerve terminals by endocytosis when artificially introduced, for example, during experimental or iatrogenic infections with adenovirus (Salinas et al., 2009), adeno-associated virus (AAV) (Cearley and Wolfe, 2007), and lentivirus vectors (Mazarakis et al., 2001).

The molecular mechanisms of endocytosis are well studied in a variety of cell types (Mercer et al., 2010). Compared to nonneuronal cells, neuronal endocytosis is highly regulated, and it is mediated by many neuron-specific isoforms of the conserved endocytosis machinery. At nerve terminals, in particular, the most prominent and best studied endocytic activity is a specialized mode of clathrin-mediated endocytosis for neurotransmitter receptor uptake and synaptic vesicle recycling (Saheki and De Camilli, 2012). These endocytosis-derived recycled synaptic vesicles typically remain near the presynaptic active zone for ongoing rounds of endo- and exocytosis, and therefore their role in mediating viral entry is unclear. Other vesicle populations, including those with canonical early and late endosomal markers like Rab5 and Rab7 GTPases, are present in axons, but their genesis, fate, and role in virus entry is also uncertain.

Upon endocytosis, the lumen of endocytic vesicles is rapidly acidified by vacuolar ATPases. In nonneuronal cell types, this pH change is often a trigger for virions to fuse with or otherwise disrupt the vesicle membrane to reach the cytoplasm (Mercer et al., 2010). Interestingly, in neurons, most virions remain inside the endocytic vesicle for subsequent long-distance retrograde trafficking to the neuronal cell body (e.g., rabies virus [Klingen et al., 2008], polio [Ohka et al., 2009], and adenoviruses [Salinas et al., 2009]). Studies of the endocytosis and trafficking of canine adenovirus 2 (CAV-2) and tetanus neurotoxin (TeNT) revealed a population of endocytic vesicles with a neutral pH, suggesting that such nonacidified vesicles may generally promote the retention and subsequent trafficking of neurotropic viruses (Salinas et al., 2010).
**Postentry Retrograde Transport**

After axonal entry, virus particles must undergo retrograde transport to the cell body using motor proteins that move along microtubules (Figure 3A). Generally, in polarized cells and particularly in axons, microtubules are oriented with the plus end at the cell periphery or axon terminal and the minus end toward the cell body. The kinesin family of motor proteins generally mediates plus-end-directed anterograde transport (from the soma to the axon terminus), and dynein motor complexes generally mediate minus-end-directed retrograde transport (from axon terminus to soma) (Kapitein and Hoogenraad, 2011).

After membrane fusion and entry into axons, alpha herpesvirus capsid-inner tegument complexes undergo very efficient retrograde transport to the cell body (Figure 3A). The viral protein VP1/2 (UL36), a major component of the inner tegument, recruits minus-end-directed dynein motors for this retrograde transport (Zaichick et al., 2013). However, retrograde axonal transport is punctuated by brief anterograde movements, purified capsid-inner tegument complexes also bind kinesin motors, and VP1/2 is a multifunctional protein involved in multiple intracellular transport steps during virus replication (Smith, 2012). Thus, motor recruitment by VP1/2, and other viral and cellular factors, is likely a complex and regulated process requiring further study.

Like alpha herpesvirus particles, RABV virions were thought to immediately undergo membrane fusion, releasing the viral nucleocapsid into the axon cytoplasm, as typically occurs in non-neuronal cell types. The nucleocapsid-associated P protein was proposed to mediate retrograde axonal transport by binding dynein light chain LC8. However, other studies demonstrated that P protein binding to LC8 is not necessary for RABV neuropathogenesis (Mebatsion, 2001), and RABV virions instead remain within endocytic vesicles during retrograde transport (Klingen et al., 2008) (Figure 3A).

The fact that most neurotropic viruses, particularly those not adapted to the nervous system, are transported within endocytic vesicles presents an inherent topological problem. Unlike alpha herpesvirus particles, which are exposed to the axon cytoplasm immediately after entry, these endocytosed virions are trapped in the lumen of an endosome and therefore can neither recruit motor complexes nor directly modulate the transport machinery, but may remain passive cargo of the pre-existing axonal transport pathways. Several viruses appear to use a common retrograde transport pathway involved in neurotrophin signal transduction. In this signaling pathway, neurotrophins (e.g., nerve growth factor [NGF] and brain-derived neurotrophic factor) and their receptors (e.g., p75NTR and TrkB) are incorporated into Rab5-positive early-endosome-like vesicles, which mature into Rab7-positive late-endosome-like vesicles. Rab7 mediates recruitment of dynactin (p50/glued), part of the dynein complex, and these vesicles undergo retrograde transport to the neuronal cell body (Deinhardt et al., 2006). This pathway is implicated in the retrograde transport of poliovirus (Ohka et al., 2009), rabies virus (Langevin et al., 2002), CAV-2, and TeNT (Salinas et al., 2009, 2010). These endosomal transport vesicles appear to remain at a neutral pH, which may stabilize pH-dependent viruses within the endosome (Salinas et al., 2009, 2010).

In some cases, the interaction between virions and their cellular receptors may direct the retrograde transport of the virion-containing endocytic vesicle. For example, the poliovirus cellular receptor CD155, which is endocytosed along with the virion, binds the dynein light chain Tctex-1 to engage the retrograde transport machinery (Ohka et al., 2004). Similarly, the envelope glycoprotein (G protein) of RABV and related rabdoviruses can confer endocytosis and efficient retrograde transport functions to heterologous lentivirus vector particles (Desmaris et al., 2001; Mazarakis et al., 2001), suggesting that interaction between the G protein and a cellular receptor (possibly p75NTR [Langevin et al., 2002]; see above) mediates retrograde transport.

While these examples provide some insight into the molecular mechanisms of postentry retrograde transport, it is not clear whether virions endocytosed at nerve terminals engage a common or default retrograde transport pathway and to what extent they alter or modulate these intracellular trafficking mechanisms.

After entry and retrograde transport to the cell body, viral nucleic acid genomes replicate and express messenger RNA (mRNA) and proteins. Remarkably, the biosynthetic machinery (i.e., ribosomes and secretory pathway organelles) of neurons is distributed in axons and dendrites, as well as in the cell bodies. It is not clear whether this distribution of cellular machinery facilitates compartment-specific viral replication and gene expression.

**Postreplication Transport, Egress, and Spread**

Many neurotropic virus infections (e.g., RABV, alpha herpesviruses, MV, and WNV) spread between chains of connected neurons. Virions do not typically spread by free diffusion, but rather between tightly connected neurons via neurochemical synapses or other close cell-cell contacts. Infected PNS neurons often are in direct synaptic contact with CNS neurons, providing a direct route of spread from the periphery. Consequently, CNS infection is determined in part by the directionality of virus spread along neuronal circuits, which is influenced by directional intracellular virus transport and egress. Motor neurons receive input from CNS neurons via synapses on the somatodendritic plasma membrane (i.e., postsynaptic contacts) and relay these signals to neuromuscular junctions at axon termini. RABV, which typically infects motor neurons, spreads exclusively in the retrograde direction along neuronal circuits, exiting from the somatodendritic plasma membrane to spread toward the CNS (Figure 2B). Most neurotropic viruses are capable of spreading retrograde in neural circuits, suggesting that there may be common or default somatodendritic transport pathways. In contrast to motor neurons, sensory neurons are typically pseudounipolar, with two axon-like projections: one innervating peripheral tissues, and the other making presynaptic contact with CNS neurons (Figure 2A). To be able to spread within this pseudounipolar architecture, the alpha herpesviruses have evolved the capacity to spread in the anterograde direction, exiting from axon termini. The alpha herpesviruses are among the very few viruses that are capable of bidirectional spread (both anterograde and retrograde direction) (Figure 3B).

Like postentry transport, postreplication transport of viral components also requires microtubule motors. The cellular mechanisms that determine whether cargo is transported into the axon or into dendrites are not entirely clear; however, several mechanisms are apparent (Kapitein and Hoogenraad, 2011). First, the axon initial segment contains a mesh of actin filaments.
proposed to function as a molecular sieve, preventing macromolecular complexes like transport vesicles or virions from entering the axon nonspecifically. Some motors, for example kinesin-1/KIF5, efficiently penetrate this barrier, providing a level of specificity so that only particular cargo/adaptor/motor combinations are transported into the axon. Second, stable microtubules acquire posttranslational modifications, most notably C-terminal deetyrosination and K40 acetylation of alpha-tubulin. Both axons and dendrites contain acetylated microtubules, and axons are enriched in deetyrosinated microtubules. Kinesin-1/KIF5 preferentially binds these modified microtubules, suggesting that posttranslational modifications may also regulate cargo transport into axons. Finally, microtubule polarity differs between axons and dendrites. While microtubules in axons have a uniform, parallel orientation, with plus ends at the axon terminus and minus ends in the soma, microtubules in dendrites have mixed, antiparallel orientations. This difference in microtubule polarity would allow only plus-end-directed kinesin motors to enter axons, whereas the minus-end-directed dynein complex could enter dendrites (Kapitein and Hoogenraad, 2011). Indeed, one study using a drug-inducible protein dimerization system showed that experimental recruitment of kinesin-1/KIF5 or kinesin-2/KIF17 induces cargo transport into axons, whereas recruitment of dynein induces transport into dendrites (Kapitein et al., 2010).

While enveloped virions may exit cells by budding through cellular membranes, which preserves membrane topology, nonenveloped viruses (e.g., poliovirus and Ad) must breach membranes to enter or exit a cell. How nonenveloped viruses cross cellular membranes, particularly neuronal membranes, without causing extensive cytopathology is not well understood (reviewed in Kirkegaard, 2009). However, a study on Théier’s murine encephalomyelitis virus (TMEV), a nonenveloped picornavirus like poliovirus, revealed that TMEV can exit from neurons into oligodendrocyte myelin sheaths without axon lysis or degeneration (Rousssarie et al., 2007). It is not clear whether other nonenveloped viruses can spread between neurons without cell lysis or axon degeneration. In the case of many enveloped viruses (e.g., RABV and MV), final assembly and egress are coordinated processes (Figure 3B). Viral components and subassemblies, such as nucleocapsids and membrane proteins, are transported separately and meet at the plasma membrane. Virions then acquire their envelope by budding through the plasma membrane, simultaneously completing assembly and egress from the cell. For decades, researchers have recognized that viral membrane proteins (i.e., glycoproteins and matrix proteins) are sorted either to the apical or basolateral side of polarized epithelial cells and that release of enveloped viruses is often polarized accordingly (see Jayakar et al., 2004, for relevant discussion). Analogously, if viral envelope proteins are preferentially sorted into the axons or dendrites, virus assembly, egress, and transsynaptic spread may also be polarized. In support of this model, a recent study showed that the directionality of viral spread along synaptic connections depends on the viral glycoprotein G (Beier et al., 2011). In this study, the authors produced recombinant VSV expressing G proteins from RABV (a related rhabdovirus), LCMV (an unrelated arenavirus), or its native VSV G protein. The RABV G protein conferred retrograde (postsynaptic to presynaptic) spread across synaptic connections, whereas the LCMV or native VSV G proteins conferred anterograde (presynaptic to postsynaptic) virus spread (Beier et al., 2011). While it is difficult to draw conclusions about the functions of viral proteins outside of their natural context, these studies contribute toward a mechanistic understanding of viral spread in the NS. But important questions remain: What are the molecular determinants of viral glycoprotein sorting in neurons? How is the transport of nucleocapsids and other viral factors coordinated to ensure efficient assembly and egress? And, does this glycoprotein-centric model hold true for other enveloped viruses?

The transport of nascent alpha herpesvirus particles is now understood in more molecular detail. These viruses replicate their genome and assemble capsids in the nucleus, traverse the nuclear envelope, acquire tegument proteins, and obtain their envelope by budding into trans-Golgi-like membranes (Smith, 2012). There has been some debate as to whether alpha herpesviruses fully assemble in the neuronal cell body prior to axonal transport or whether subassemblies are transported to distant sites of final assembly. The details of this debate are reviewed elsewhere (Diefenbach et al., 2008; Smith, 2012), but it is clear that alpha herpesviruses have evolved neuron-specific viral mechanisms to direct anterograde transport and spread along circuits of synaptically connected neurons. For both HSV and PRV, three conserved viral membrane proteins, gE, gI, and US9, are necessary for efficient anterograde transport and spread (Smith, 2012). While the role of US9 in HSV is debated, US9-null PRV mutants are completely defective in anterograde transport and spread in neurons, and these phenotypes appear to be neuron specific (Lyman et al., 2007). US9 is dispensable in nonneuronal cells and for retrograde somatodendritic egress in neurons. A recent study demonstrated that PRV US9 associates with the neuron-specific plus-end-directed microtubule motor kinesin-3/KIF1A, providing a molecular basis for this neuron-specific phenotype (Kramer et al., 2012). According to this model, PRV US9 mediates the recruitment of KIF1A, which mediates both sorting into axons and anterograde transport leading to spread of infection from presynaptic to postsynaptic cells in a neuronal circuit (Figure 3B). Thus, in bipolar neuronal circuitry, PRV serves as an illustrative example of how directional spread along neuronal circuits depends on the directionality of intracellular transport and egress. Ongoing research to identify the cellular and molecular mechanisms of viral transport and egress in neurons will be necessary to fully understand how other neurotropic viruses spread through the NS.

However, directional transport in neurons is not the sole determinant of spread in neuronal circuits. For example, PRV and other alpha herpesviruses naturally infect pseudounipolar sensory neurons that have two axon-like projections. Thus, anterograde axonal transport could direct progeny virions down either branch, into peripheral tissues or into the CNS (Figure 4). Yet, spread to the CNS is typically rare or inapparent. In this case, the directionality of spread in the nervous system may depend on factors other than intracellular transport, such as intrinsic immune responses and latency, as described in the following section.

**Alpha Herpesvirus Invasion of the Nervous System: The Potential for a Lifetime Association**

The life-long persistence of herpesvirus genomes in their natural hosts coupled with the capacity to reactivate the quiescent
infection to produce infectious particles that transmit to other hosts is remarkable in many ways (reviewed in Wagner and Bloom, 1997). Three subfamilies of the herpesviridae establish a latent persistent infection in different cell types: alphaherpesvirinae (PNS neurons), betaherpesvirinae (monocytes and macrophage precursors), and gammaherpesvirinae (B and T cells) (Speck and Ganem, 2010). In most cases, the quiescent herpesvirus genome is not integrated into the host genome and either exists as a nonreplicating, quiescent histone-covered molecule, or as a replicating extra “chromosome” (reviewed in Speck and Ganem, 2010). Recently, this paradigm was broken by human herpesvirus 6, a beta herpesvirus that can integrate into the host genome (Arbuckle et al., 2010). Alpha herpesvirus genomes can remain in a latent, but reactive, state in neurons of essentially all PNS ganglia. HSV-1 and HSV-2 are two human alpha herpesviruses causing cold sores and genital herpes. A third human alpha herpesvirus, VZV, causes chickenpox during primary infection of children and zoster (shingles) when the virus reactivates in the ganglia that transmit sensory signals toward the brain (e.g., dorsal root ganglion and trigeminal ganglion). Infection initiates in epithelial cells at a mucosal surface. The primary infection is usually well contained by host defenses, and overt pathogenic effects are rare. Some viral progeny invariably enter axon terminals that innervate the infected tissue and move on microtubules to PNS cell bodies, where a productive or latent infection may be established (Figure 4). Establishment of the quiescent infection in PNS neurons is a multistep process and depends on the efficiency of several parameters: (1) virus replication in nonneuronal cells under the pressure of local intrinsic, innate, and humoral immune responses, (2) virion entry into axon terminals and retrograde transport of nucleocapsids to the neuronal cell body, and (3) intrinsic and innate immune responses in the infected neuron and ganglia. After the latent infection is established, maintenance of this silent state requires continuous action of surrounding glial and virus-specific CD8+ T cells that suppress lytic gene expression through secretion of IFN-γ and granzyme-B (St Leger and Hendricks, 2011).

Recent studies with HSV-1 suggest that infection of neurons after axonal entry is inherently biased toward a latent infection because when the HSV-1 virions enter axons, some critical tegument proteins are transported toward the cell body separately from the capsid (Roizman and Sears, 1987). When neuronal cell bodies are exposed directly to purified virions, the infection is instead productive. This bias toward latency presumably occurs because productive infection may require that both tegument proteins and capsids containing the viral genome arrive at the nucleus at the same time (Hafezi et al., 2012; Kristie and Roizman, 1988). Besides this long-distance trafficking, other factors such as cytokine and IFN production also influence the outcome. The cytokines produced after primary infection of epithelial cells or the virion attachment to axonal receptors might induce an antiviral state in neuronal soma before the genome reaches the nucleus. Indeed, De Regge et al., using an in vitro two-chamber
neuron culture system, showed that treatment of trigeminal
ganglion neurons with IFN-α before HSV-1 or PRV infection is
sufficient to suppress lytic gene expression and induce a quiess-
cent infection (De Regge et al., 2010).

A hallmark of alpha herpesvirus latency is the expression of a
latency-associated transcript (LAT) and several microRNAs
(miRNAs) (Farrell et al., 1991; Umbach et al., 2008). Although
the exact role of the LAT transcripts is still not well understood,
there is evidence that these noncoding RNAs are involved in
blocking apoptosis of the infected neuron and in the control of
reactivation from latency (reviewed in Proença et al., 2011).
Besides LAT, HSV-1 miRNAs target the key regulators of the viral
lytic cycle (i.e., ICP0) to control and stabilize latency. Remark-
ably, for VZV, six different transcripts and proteins are produced
during latency. Interestingly, one of the most abundant proteins,
ORF63, is predominantly cytoplasmic during latency but local-
izes mostly in the nucleus during lytic infection (Cohen, 2007).
Nevertheless, almost 90% of the viral genome remains transcrip-
tionally silent during latent HSV-1, HSV-2, or PRV infection, and,
importantly, the infected neuron is not recognized or eliminated
by the immune system.

Such long-term maintenance of a silenced viral genome in a
state that can be reactivated requires precise control and a
high threshold for reactivation. Stress responses, including nutri-
tional, hormonal, and environmental, can reactivate a latent
genome. Strikingly, although reactivation can recur throughout
the life of the host, it seems to have little adverse effect on
PNS function. This is, in part, because few latently infected neu-
rons undergo reactivation: in vivo animal experiments show that
only one to three neurons, among thousands of latently infected
cells in the trigeminal ganglion, display signs of reactivation in
a single recurrence (Sawtell and Thompson, 1992).

Furthermore, neurons have evolved to inherently avoid cell
death, and viruses that persist in these have also adapted to
prevent the death of their host cells. These neuronal and viral
prosurvival strategies clearly allow neurons to survive during a
latent herpesvirus infection, but it may be that these same stra-
 tegies promote neuronal survival during reactivation as well. It
appears that a fine balance exists between the immune system
of a naturally immunized host and the virus replication machin-
ery, limiting virus replication and subsequent axon-to-cell spread
of the progeny even during reactivation (Kobiler et al., 2010; Tay-
lor et al., 2012). Indeed, it appears that neurons can tolerate not
only high viral genome inputs but also abundant viral gene
expression, which possibly is controlled by the suppressing ac-
tion of nearby CD8+ T cells (St Leger and Hendricks, 2011).
Furthermore, autophagy is a neuronal prosurvival strategy to
block several neurotropic virus infections (i.e., Sindbis virus
[SINV], HCMV, HSV-1) (Chaumorcel et al., 2008; Orvedahl
et al., 2007, 2010). The autophagy machinery normally is used
by cells to engulf cytosolic material in a double-membrane vesicle
 to be degraded by the lysosomes. HSV-1 encodes pro-
teins that modulate autophagy in a neuron-specific manner.
For example, autophagy is activated in response to HSV-1 infec-
tion through interferon-induced protein kinase R (PKR), which
induces an antiviral signaling cascade in response to viral dsRNA
(Tallóczy et al., 2002). The HSV-1 protein ICP34.5 interferes with
this pathway by binding to and inactivating Beclin-1, a host pro-
tein responsible for the recruitment of other autophagy proteins
(Orvedahl et al., 2007). This interaction has little effect or advantage in nonneuronal cells, but it promotes HSV-1 persistence in
neurons and also pathogenesis if the infection spreads to the
CNS (Yordy et al., 2012).

After reactivation, it is not clear whether progeny herpesvirus
virions are transported toward the CNS and, if so, what prevents
devastating CNS infection and pathogenesis in most immune-
competent individuals. A recent elegant study revealed the
importance of neuronal intrinsic immunity in the control of
HSV-1 spread in the NS. Children with genetic defects in the
antiviral TLR3-interferon pathway have a higher risk of herpes
simplex encephalitis (HSE) during primary infection with HSV-
1. Using pluripotent stem cells from susceptible children, the
authors showed that a TLR3-dependent intrinsic immune
response in neurons is crucial to restrict HSV-1 spread in the
NS (Lafaille et al., 2012). It is likely that the lack of this defense
system in neurons prevents the establishment of latency in the
peripheral ganglia, promoting further viral spread into the brain.
Once in the brain, where HSV-1 replication is normally tightly
restricted, TLR-3 deficient neurons may continue to support
virus replication leading to HSE.

The herpesvirus latency-reactivation cycle is understood in
principle, but important details are lacking. Small-animal models
(i.e., mouse peripheral infection and rabbit eye models to study
HSV-1 and guinea pig model for HSV-2 latency) have been quite
useful, but they do not recapitulate all the nuances of latency in
humans (Wagner and Bloom, 1997; Wilson and Mohr, 2012).
However, latency and reactivation have been addressed with
PNS neurons cultured in vitro, and these studies have provided
some mechanistic understanding of these phenomena (Camar-
ena et al., 2010; Wilcox et al., 1990). HSV-1 latency is proposed
to be maintained by the continuous binding of NGF to the TrKA
receptor tyrosine kinase (RTK) and activation of phosphatidylinos-
stol 3-kinase (PI3K)-Akt signaling pathway (Camarena et al.,
2010; Wilcox et al., 1990). Recently, Kobayashi et al. further
showed that the key target of PI3K-Akt signaling is mTORC1 in
axons (Kobayashi et al., 2012). The mTOR system regulates
the translational repressor eIF4E-binding protein (4E-BP), which
influences the state of latent viral genomes in the neuronal
nucleus. Although the cellular effectors of this pathway that
suppress the viral lytic gene expression are still unknown, any
interference with this signaling cascade (e.g., NGF depletion, inhi-
bition of mTOR activity, or hypoxia) breaks latency control and
promotes reactivation. It appears that a variety of physiological
stimuli originating in the periphery are integrated in the local
mTOR system in axons and relayed to the cell body. This local
monitoring in axons and long distance signaling to the cell
body could alarm and protect neuronal cell bodies but at the
same time might be repurposed for the replication of opportu-
nistic viruses that invade the nervous system (Figure 4).

What Controls Invasion of the Nervous System:
A Herpesvirus Perspective

Alpha herpesvirus infection of immune-competent hosts
presents an apparent two-part paradox: First, during primary
infection, the infection passes with high efficiency from epithelial
surfaces to the nerve terminals and on to the soma of PNS
ganglia neurons. Indeed, it is difficult to block primary PNS inva-
sion once epithelial cells are infected. Second, once in the PNS,
infection rarely spreads further to the brain. We learned from animal models and mutant virus infections that a complex interplay exists between the host immune system and the NS-adapted viral replication strategy. For instance, virulent PRV infection of the skin induces a violent, inflamed peripheral pruritus with almost no further spread to the brain in mice (Brittle et al., 2004). By contrast, the attenuated mutant strain PRV Bartha, which replicates as well as wild-type virus, produces almost no tissue damage and peripheral inflammation yet is massively neuroinvasive (Brittle et al., 2004). This property makes PRV Bartha an excellent neural tracer (Brittle et al., 2004).

What regulates controlled alpha herpesvirus spread in the NS, and how does the infection proceed differently in neurons, non-neuronal cells, PNS neurons, and CNS neurons? The answer may lie within the specialized signaling and gene expression patterns that maintain highly polarized morphology and function of neurons. Such a differentiated system calls for a long-distance communication between the axon terminals that are in contact with epithelial or muscle cells and the cell bodies in the ganglia. This axon-to-cell-body communication must be finely tuned, so that cell bodies are well informed about distant events near axon terminals but at the same time should not overreact. It is noteworthy that PNS neurons produce little type I IFN after HSV-1 infection (Leib et al., 1999; Szpara et al., 2010). However, when herpes viruses invade epithelial cells, prior to infecting nerve terminals, these infected cells produce a variety of inflammatory and antiviral cytokines, including type I IFN. Such potent signaling molecules are likely to have an effect on local axon function and subsequent viral invasion of PNS neurons (Cunningham and Mikloska, 2001). Indeed, previous work on human dorsal root ganglion neurons in a chambered culture system showed that IFN-α and IFN-γ, added to the epidermal cell compartment, after axonal transmission of HSV-1 infection, inhibit infection and spread (Mikloska and Cunningham, 2001).

Certainly, information about events at nerve termini (i.e., damage, inflammation, and infection) must be transmitted to the distant neuronal cell bodies to produce an appropriate response at the site of axonal insult. Because some axons can be centimeters to meters long, efficient and rapid transmission presents a potential problem for homeostasis of the neuron. How this long-distance communication is accomplished remains a fundamental problem in neuroscience and in virology. New findings suggest that new protein synthesis in axons upon environmental stimuli and subsequent transport of local signaling molecules to the nuclei might serve this function. Indeed a subset of mRNAs and the complete protein synthesis machinery are localized to axons in uninfected neurons, far away from the cell body (Vuppalanchi et al., 2009). Local translation in axons, not in the distant cell body, regulates growth cone navigation and integrity during development and regeneration, and retrograde communication with the cell body in adult neurons and plays a key role in axonal damage signaling (Jung et al., 2012).

We have found that local events involving axonal protein synthesis in or near the initially infected axon termini facilitate neural invasion and spread (Koyuncu et al., 2013). Our results also suggest that virus infection stimulates an axonal response similar to injury signaling and that these damage or danger signals induced by initial axonal infection and subsequent inflammatory events at the site of primary infection affect axonal transport of viral particles to the neuronal nuclei. Interestingly, retrograde transport of poliovirus to the CNS in mice is inefficient unless there is muscle damage at the site of infection (Lancaster and Pfeiffer, 2010). For PRV, we and others have seen almost the opposite: tissue damage blocks neural invasion (Duale et al., 2009; Koyuncu et al., 2013). Perhaps these local events in the PNS ganglia or near the infected axons contribute to determination of lytic versus latent infection states and control of further neuroinvasion.

It is still a mystery why spread of alpha herpesviruses to the CNS after the initial peripheral infection or after reactivation from latency is rare. There is no evidence that the block is at the cellular and molecular level of axonal transport to the CNS neurons. Perhaps the limitation is in the replication of virus in CNS neurons. Like PNS neurons, these neurons may be less permissive to lytic herpesvirus infection. CNS and PNS neurons may both have specialized intrinsic immune responses that promote abortive infections and long-term maintenance of latency. The suggestion that herpesvirus particles or genomes are present in the brain of latently infected individuals draws attention to possible correlation between herpesvirus infection and neuro-pathologies like Alzheimer’s disease (Hill et al., 2005). Such correlations remain difficult to study due to the lack of appropriate experimental models.

Future Perspectives

The interaction of viruses with their hosts is noteworthy in many ways. Studying the complex interplay between these tiny intracellular entities and the infected cell in a culture dish has taught us many valuable lessons in cell biology. However this interaction between virus and host in vivo is even more complicated by the hierarchical organization of cells, tissues, and systems. This complexity, while providing layers of protection against microbial invasions, also requires strictly regulated communication among cells and tissues to produce the appropriate protective response. If this combined reaction to viral attack is not sufficient, balanced, and controlled, severe pathology is likely.

It is striking that there is no prototypical virus that spreads to the CNS. Viruses with RNA or DNA genomes and enveloped or naked virions, members of diverse virus families, all can spread into the CNS under the right conditions. In general, even viruses capable of infecting a variety of cell types tend to spread from one tissue to another until they reach a barrier. If the infection is not contained locally (due to inefficient intrinsic and innate immune reaction), it can spread to vital organs, causing severe pathologies. Damage to cells can result from viral replication or by the action of the activated immune system. Virus spread to CNS can be deadly, not only because infected neurons may die, but also because of the immune-mediated inflammation in the brain. We now recognize that we are constantly infected and colonized with numerous microorganisms, some of which can affect spread and pathogenesis of invading microbes (the microbiome). This complex interaction of host and microbes was not recognized and was routinely neglected in in vitro and in vivo experimental models. However, recent pioneering work (Kuss et al., 2011; Matullo et al., 2011) suggests methods and models of how to approach these complex and multifactorial biological problems.

Historically, research on PNS-adapted alpha herpesviruses, zoonotic RABV, and enteric poliovirus taught us much of what
we know about viral spread in the NS. Developing techniques such as live-cell and intravital imaging together with neuron culturing methods (in chambers or microfluidic devices, and cocultures with other cell types) accompanied by the ability to construct recombinant viruses now allow researchers to study some of the fundamental characteristics of virus replication and spread within and between neurons. These approaches will enable new mechanistic understanding of how neurotropic viruses gain access to and spread in the NS. In vitro culture of human neuronal/pluripotent stem cells, and closer-to-natural-host models such as “humanized mice” carrying human-specific genes, cells, or tissues will reveal host-specific aspects of lytic or latent infections caused by human viruses. Novel techniques, such as deep sequencing of viral nucleic acid from clinical samples or single-molecule sequencing will facilitate identification of more neurovirulent and/or neuroinvasive virus mutants and will provide a genetic view of host barriers and viral bypass mechanisms. Screening of diseased tissues by these techniques will enable the detection of minute quantities of viral genomic material, possibly revealing more roles for viruses in a variety of neuropathologies even if we cannot propagate them in the laboratory.

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