Neuronal Survival Strategies in the Face of RNA Viral Infection

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Neurons of the mammalian central nervous system (CNS) are an essential and largely nonrenewable cell population. Thus, viral infections that result in neuronal depletion, either by viral lysis or by induction of the cytolytic immune response, would likely lead to profound neurologic impairment. However, many viral infections that result in tissue destruction elsewhere in the host produce few overt symptoms in the CNS, despite readily detectable virus expression. This observation has lead to the speculation that neurons possess strategies to limit the replication and spread of otherwise cytopathic viruses. These strategies either favor the clearance of virus in the absence of appreciable neuronal loss or promote the establishment of noncytolytic persistent infections. This review discusses some of these strategies, with an emphasis on how such survival techniques lessen the potential for CNS neuropathology.

Background: Virus Particle versus Infectious Particle

Over the past decade, a major focus in virology has been to identify the roles that host cell proteins play in the execution of the virus life cycle. As obligate intracellular parasites, viruses depend on cellular cofactors, and in that sense, a definition of the "infectious particle" would include not just the virally encoded proteins but also the cellular proteins that enable replication to occur. Moreover, the cellular tools that are available to the virus will depend on cell type, cell cycle status, differentiation state, and extracellular mediators that may influence metabolism (e.g., cytokines and hormones). Such differences may affect how the virus replicates and spreads. For example, certain cell types may be able to alter viral replication in an effort to limit the cytopathicity that might otherwise result from an unrestricted infection. Recent evidence indicates that central nervous system (CNS) neurons possess survival skills that influence the replication of neurotropic RNA viruses and moderate the activity of the resulting antiviral response.

The Immune-Privileged CNS and RNA Viral Infection

CD8 cytotoxic T lymphocytes (CTL) mediate their antiviral effects by recognizing viral peptides presented by class I major histocompatibility complex (MHC) molecules on infected target cells. Engagement of the T cell receptor with the peptide-loaded MHC leads to perforation of the target cell membrane, delivery of cytolytic granules, and apoptosis of the infected cell [1]. In most tissues, the loss of infected cells is accompanied by enhanced uninfected cell division to replenish the cell population. However, this may not be an optimal strategy for clearance of all viral infections, especially those in tissues with little capacity for renewal, such as the CNS [2, 3].

To limit immune cell entry into the CNS and restrict T cell–neuron interactions, multiple anatomic and biochemical barriers exist within the brain, including the presence of the blood-brain barrier, limited lymphatic drainage, and the paucity of class I MHC on resident brain cells [2, 4]. Collectively, these properties of the CNS contribute to an "immune privileged" environment under normal conditions. Nevertheless, activated T lymphocytes patrol the CNS parenchyma [5, 6] and are recruited into the brain after infection by many neurotropic viruses [7–9]. In some circumstances, including herpes simplex virus (HSV) encephalitis, such inflammatory responses are associated with marked CNS damage [8]. In others the immune response is able to resolve an infection without apparent damage to the host (e.g., the immune-mediated clearance of lymphocytic choriomeningitis virus [LCMV]) [10]. These data suggest that the CNS has unique strategies to recruit the immune response following a pathogenic challenge and to promote clearance mechanisms that prevent or limit immunopathology. Understanding how these defenses work and how chronic CNS disease occurs when they fail is of paramount importance in the development of appropriate therapies to prevent or reverse virus-induced neuropathology.

Role of neuron-derived chemokines in the recruitment of the immune response to the CNS. Chemokines are a superfamily of small secreted proteins that promote recruitment of leukocytes to the site of a pathogenic challenge by signaling through G protein–coupled receptors [11, 12]. Such engagement results in leukocyte activation and increased adhesion to capillary endothelia. In the brain, infection by a number of viruses has been correlated with a rapid and robust induction of chemokines from multiple resident cells, including astrocytes, microglia, and endothelial cells [13–17]. Moreover, specific chemokine neutralizing antibodies and knockout mice have been

valuable tools in demonstrating the crucial role chemokines play in leukocyte recruitment to the CNS. For example, Lane et al. [15] showed that administration of RANTES neutralizing antisera to C57BL/6 mice infected with neurovirulent mouse hepatitis virus (MHV) reduced macrophage accumulation and resultant demyelination as compared with control mice, implicating a protective role for RANTES in this model system.

Whether neurons contribute to the recruitment of leukocytes to the CNS after viral infection has not been established. To address this issue, we used a transgenic mouse model in which viral infection was restricted to CNS neurons. In this system, a human measles virus (MV) receptor, CD46, was expressed specifically in CNS neurons under the transcriptional control of the rat neuron-specific enolase (NSE) promoter [18]. NSE-CD46 transgenic mice supported infection by MV-Edmonston and adult mice mounted aggressive CD4 and CD8 T cell responses that protected them from neurologic impairment and death [19]. We recently showed that MV challenge of NSE-CD46$^+$ mice results in a potent induction of T cell–recruiting chemokines in infected neurons. We used RNAse protection assays to show induced expression of the chemokines IP-10 and RANTES in infected adult brains and in infected primary hippocampal neuron cultures. Confocal microscopy studies further demonstrated that viral antigens and chemokines colocalize in infected neurons (unpublished data). In vivo depletion of IP-10, RANTES, and monokine-induced interferon (IFN)-γ from immunocompetent adult mice reduced the infiltration of T cells in infected mouse brains by up to 60% and was correlated with increased neuronal MV infection. Thus, these studies suggest that neurons may play a crucial and early role in the host response to infection by triggering the rapid production of chemokines that help to alert and focus the antiviral response to the infected CNS.

Noncytolytic role for T cell–derived cytokines in viral clearance from neurons. It is increasingly apparent that the term "cytotoxic" T lymphocyte is somewhat of a misnomer, since in addition to their ability to lyse target cells, CTL also secrete cytokines that may clear an infection noncytolytically. Walker et al. [20] first speculated that CD8 T cells produce a factor that slows human immunodeficiency virus (HIV) replication in the absence of cell death. Subsequently, Guidotti and colleagues [21, 22], by using transgenic mice that expressed the hepatitis B virus (HBV) genome in hepatocytes, showed that CTL-derived tumor necrosis factor (TNF)-α and IFN-γ noncytolytically inhibited HBV replication [21, 22].

The utilization of a noncytolytic arm of the cellular immune response would be especially advantageous in neuronal infections, since neurons are generally considered nonrenewable. Thus, their loss by a lytic immune response could be as deleterious to the host as the viral infection itself. Indeed, data from multiple laboratories suggest that the host response can mediate viral clearance from the CNS via noncytolytic strategies. For example, immune-mediated clearance of a persistent LCMV

infection within the CNS is noncytopathic [3, 10] and requires the presence of T cell–elaborated cytokines such as IFN-γ [23]. Other viruses with similar dependence on noncytolytic clearance are Sindbis [7], MHV [24, 25], vesicular stomatitis [26–28], and MV [29, 30].

While it is not yet fully clear how cytokines cause nonlytic viral clearance, recent studies provide some insight into this process and show a critical role for Th1 cytokines (e.g., IFN-γ and TNF-α). IFN-γ is crucial for the noncytopathic suppression of HSV-1 infections in trigeminal ganglia [31] and photoreceptor cells in the eye [32] and in neuron infections caused by Borna virus [33], MHV [34], and Theiler's virus [35]. Neurons express IFN-γ receptors [36], providing support for the idea that neurons respond to this cytokine. For MV, the importance of IFN-γ has been noted in both humans [37] and mice [29, 30].

Recent work from our laboratory showed that addition of recombinant murine IFN-γ to MV-infected primary neurons reduced viral RNA load by as much as 50% [30]. Whether the same pathways that are activated by type I and II IFN to induce an antiviral state are also involved in the clearance of an established viral infection is an area of intense investigation. Monocyte/macrophage-elaborated cytokines such as interleukin (IL)-12 are also involved in the resolution of viral infections. Not only does IL-12 skew the T helper response toward a Th1 profile, it can directly promote noncytopathic recovery from neurotropic infection by regulating nitric oxide synthesis [26, 28, 38]. Thus, cytokines contribute to viral clearance directly (by inhibiting virus replication) and indirectly (e.g., by inducing other inflammatory cytokines). Defining the cellular pathways that result in viral loss without concomitant neuronal death and establishing which factors govern how the host response "chooses" between cytopathic and noncytopathic effector mechanisms are major research areas in neurovirology and neuroimmunology.

Intraneuronal Restrictions on Viral Replication and Spread

Many of the viruses that infect the CNS establish latent or persistent infections in neurons despite being highly cytopathic or inducing vigorous antiviral immune responses in peripheral tissues [9]. These viruses include members of the paramyxovirus, enterovirus, retrovirus, arenavirus, coronavirus, and alphavirus families [8]. Emerging observations with these viral systems support the interpretation that neurons have developed strategies to prevent their destruction by otherwise cytopathic viral infections.

Restricted viral budding. One block to viral replication in neurons is at the level of viral egress. Studies of viruses from several families show that the normal mode of viral release is blocked or altered in neurons. For example, in the persistent infection caused by the murine pathogen LCMV, viral RNA is

readily detected in neurons by in situ hybridization [39], but electron microscopic (EM) examination does not reveal budding viral particles [40], which are readily visible in susceptible fibroblasts [41].

In studies with MV, a similar cell type-specific difference was observed. In susceptible fibroblasts, MV infection is highly productive and cytolytic; viral spread occurs by the production of extracellular progeny and by the fusion of infected cells with adjacent uninfected cells. In neurons, MV spread occurs in the absence of extracellular virus, syncytia formation, or neuronal lysis. Decreased virus production correlates with an inability of MV to form buds at the neuronal plasma membrane [42]. MV transmission throughout these neuron cultures is not inhibited by neutralizing antibodies, paralleling rabies studies in which neutralizing antibodies are less effective in preventing spread in neurons than in nonneuronal cells [43]. Together, these data indicate a novel mechanism of MV transmission in neurons [42, 44]. EM studies of neuronal infection with wild type MV strains revealed the presence of viral ribonucleoproteins (RNPs) at the neuronal synapse [42, 45], supporting earlier observations by researchers who used mouse-adapted strains [46, 47].

Pasick et al. [48] also observed viral RNPs at the synaptic membranes in the neuronal infection caused by the MHV and showed compelling evidence for homology between the MHV nucleoprotein and the microtubule protein tau and suggested that this homology allows the RNP to be actively translocated from the neuronal cell body to the axon terminus [48]. Thus, while neurons may place restrictions on viral replication to limit cytopathicity, viruses may in turn encode proteins that facilitate neuronal transport. Of interest, while the CD46 receptor is required for initial MV entry in the NSE-CD46 transgenic system, it is not needed for subsequent neuron-neuron spread [42]. The lack of requirement for CD46 may mean that the hemagglutinin protein that interacts with the cellular receptor may also be dispensable for neuronal infection, although for other RNA viruses that spread via the synapse, such as rabies, a requirement for the viral surface proteins was noted [49].

Whether MV utilizes an alternative receptor at the synapse or sequesters the transsynaptic machinery to be transported across the synapse to infect the postsynaptic neuron is not yet resolved. While promotion of transsynaptic viral spread may prevent direct lysis of neurons, such an altered mode of transmission may benefit the virus as well. For example, viruses that spread transsynaptically and do not release viral particles may be invisible to the humoral immune response [50].

Age dependence and neuronal maturity. Often the outcome of infection with neurotropic RNA viruses differs depending on the age of the infected host (e.g., intracerebral infection of neonatal mice with LCMV results in a lifelong infection, whereas a similar infection of adults is lethal [41]). The opposite occurs in NSE-CD46 transgenic mice after MV infection: Infection of neonates is lethal but infection of adults results in viral clearance [18, 19]. In both cases, the differential outcome

of infection is due to immunologic differences: In the LCMV-infected adult mice, for example, death is due to a vigorous CTL response that results in choriomeningitis, edema, and mortality [41]. However, age-related differences in response to viral infection may also be due to the differentiated status of the infected neuron [51]. For example, infection of mouse neurons with the A7(74) strain of Semliki Forest virus causes mortality in neonatal animals but not in adults due to the inability of virus to bud from adult neurons [52, 53]. While the exact nature of this abortive infection remains under study, the metabolism of rapidly dividing neurons differs substantially from that of fully differentiated nondividing neurons. Differences such as axonogenesis and myelination likely have a significant impact on the mechanics of viral replication.

CNS Diseases Associated with Chronic RNA Viral Infections

Although we propose that neurons may be more able to deploy antiviral tactics than generally believed, we do not suggest that viral infections of neurons are not a significant public health problem. Even though infections may be held off from inducing immediate damage, many chronic CNS infections eventually result in some form of CNS disease, months or even years after exposure. Such CNS damage can occur as a consequence of neuronal dysfunction, blood-brain barrier damage, glial activation, immune cell infiltration, or some combination [8]. In most cases, it is not known whether these lesions develop slowly after CNS infection or are rapidly manifested after a prolonged period of quiescent infection. Human diseases associated with chronic infection of the brain include subacute sclerosing panencephalitis (SSPE) following acute MV infection, the spongiform encephalopathies caused by some lentiviruses, chronic neurodegeneration after Borna disease virus (BDV) infection, postinfluenza encephalitis, mumps meningoencephalitis, and CNS neoplasms possibly resulting from the human polyomaviruses JC and BK [8].

In addition to these serious but relatively rare disorders, there is considerable debate in the literature concerning the possible association of viruses with CNS disorders of unknown etiology. These include highly controversial reports linking BDV with schizophrenia [54], echoviruses with amyotrophic lateral sclerosis [55], a number of viruses with demyelinating diseases [8], and inoculation of the measles-mumps-rubella vaccine with autism [56]. While a formal association between these diseases and viral infection awaits further study, it is certain that we do not yet know the degree to which the CNS can be influenced by persistent infections.

Even when a viral etiology is suspected, the basis for neurologic impairment may not be apparent. For example, viral infection may impair differentiated or "luxury" functions in the absence of neuronal death [57]: Mice persistently infected with LCMV show marked neurobehavioral deficits [58] despite the

absence of inflammation or necrosis in the brain [10]. These deficits correlate with neurochemical alterations, including transcriptional suppression of the neurotransmitter somatostatin [59] and the synaptic structural protein GAP-43 [60]. Thus, while these mice survive a persisting LCMV infection with little overt neuropathology, the influence of viral infection on cellular gene expression may compromise the functional capacity of infected neurons.

Chronic infections may also cause neurologic impairment indirectly. For example, the major targets of HIV-1 infection in the human CNS are microglia, but the dominant lesion that leads to AIDS dementia is neuronal loss [8]. While the precise basis of this indirect neuropathology is unresolved, it seems likely that virus-mediated impairment of microglia (which play an important supportive role for neurons) could lead to a depletion of neurotropic factors or an accumulation of neurotoxic factors [61].

While evidence of the AIDS dementia complex does not usually appear until the terminal stages of the disease, CNS infection often occurs soon after seroconversion [8]. Thus, either infection and the onset of pathology occur simultaneously and the apparent delay between infection and clinical symptoms reflects the requirement for a threshold level of CNS damage or some change occurs months to years after quiescent infection and results in rapid onset of neuronal destruction. This "sudden change" in the dynamics of CNS infection is a third possible way in which persisting neurotropic infections may cause disease. In this scenario, restrictions imposed by the host cell on virus production or cytopathicity may be bypassed by the spontaneous generation of neurovirulent variants that could initiate a rapid disease course.

A controversial study showed a possible example of a shifting virus-host cell relationship: About 20% of adults who died of non–CNS-related disease had evidence of persistent MV infection within the CNS [62], suggesting that the presence of viral RNA in the CNS may not be sufficient to result in CNS disease. Viral RNA isolated from patients who died of MV-induced SSPE consistently contained biased hypermutations in the envelope-associated genes, matrix, fusion and hemagglutinin [63, 64], which may be associated with induction of CNS disease. These data support the hypothesis that cytopathic variants may arise in patients who develop SSPE, although it is not known whether these defective virions are involved in MV-associated neuropathology.

Outlook

Even though our appreciation of the interplay between viruses and neurons is quite recent, a substantial body of evidence suggests that viral infections of neurons differ substantially from infection of nonneuronal cells. Neurons possess strategies to curtail viral replication, rapidly recruit the antiviral immune response to the brain parenchyma, and exploit noncytolytic effector mechanisms of viral clearance. Further efforts to understand how the neuronal microenvironment alters the virus life cycle will affect our understanding of both viruses and neurons. Moreover, the presence of many subpopulations of CNS neurons implies that neurons in different regions of the brain may respond to viral infections in unique ways. A better understanding of the complex interaction between pathogens and the immune response in the brain will contribute to the development of therapies to prevent or reverse viral infections of neurons and the diseases they cause.

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