Outcomes of RIP Kinase Signaling During Neuroinvasive Viral Infection

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Abstract Neuroinvasive viral diseases are a considerable and growing burden on global public health. Despite this, these infections remain poorly understood, and the molecular mechanisms that govern protective versus pathological neuroinflammatory responses to infection are a matter of intense investigation. Recent evidence suggests that necroptosis, an immunogenic form of programmed cell death, may contribute to the pathogenesis of viral encephalitis. However, the receptor-interacting protein (RIP) kinases that coordinate necroptosis, RIPK1 and

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RIPK3, also appear to have unexpected, cell death-independent functions in the central nervous system (CNS) that promote beneficial neuroinflammation during neuroinvasive infection. Here, we review the emerging evidence in this field, with additional discussion of recent work examining roles for RIPK signaling and necroptosis during noninfectious pathologies of the CNS, as these studies provide important additional insight into the potential for specialized neuroimmune functions for the RIP kinases.

1 Introduction

1.1 Viral Infections of the CNS

Diseases associated with neuroinvasive viral infections are a significant and growing burden on global health. The central nervous system (CNS) is normally protected from infection by physical and biological barriers that limit viral neuroinvasion (Daniels and Klein 2015). Severe neuroinvasive viral infections occur with higher frequency in very young and elderly patients, as well as those who are immunocompromised (Murray et al. 2006; Singh et al. 2015). Genetic factors can also predispose otherwise healthy adult patients to neuroinvasive infection, though the mechanisms that drive susceptibility in these cases are poorly understood (Kallianpur and Levine 2014; Long et al. 2016). Viral infection of the CNS often results in life-threatening pathological outcomes, including encephalitis, meningitis, and neuronal death (Salimi et al. 2016). As the CNS is highly susceptible to injury and limited in its capacity for repair, even those patients who survive viral encephalitis frequently experience long-term sequelae, including emotional dysregulation, altered personality, memory loss, and dementia (Ronca et al. 2016; Patel et al. 2015; Klein et al. 2017).

Beyond infection in the adult CNS, neuroinvasive viral infection in utero is also associated with severe pathological outcomes, including spontaneous abortion, developmental abnormalities, and lifelong intellectual disability (Coyne and Lazear 2016; Neu et al. 2015). Indeed, alarmingly high rates of neonatal microcephaly have driven much of the recent public and scientific interest in Zika virus (ZIKV), an emerging neuroinvasive flavivirus (Lazear and Diamond 2016). As few effective treatments exist for either adult or congenital neuroinvasive infections, identifying new therapeutic targets for both prevention and intervention is a matter of great interest to investigators (Lazear et al. 2016; Kok 2016).

1.2 Programmed Cell Death During Infection

Among the potential therapeutic targets for neuroinvasive viral infection are the signaling molecules that orchestrate the various forms of programmed cell death. Programmed cell death is a common host response to infection, as it cuts short virus production by infected cells and facilitates key immunological processes such as phagocytosis and antigen presentation (Jorgensen et al. 2017; Brault and Oberst 2017). However, the activation of cell death programs in the CNS involves a unique set of outcomes compared to other tissues. Adult neurons are considered to be predominantly postmitotic and are therefore not easily replaced (Anda et al. 2016). Moreover, individual neurons typically possess highly elaborate and overlapping dendritic arbors whose formation and maintenance rely on both intracellular and intercellular regulation. These synaptic networks are likely permanently compromised following cell death, and, while some redundancy and compensatory mechanisms exist, this loss comes with a significant cost to the organism (Koleske 2013; Klein et al. 2017). Death of myelinating oligodendrocytes is similarly problematic, as structural and biochemical features of the adult CNS prevent the full regeneration of functional myelin sheaths (Franklin and Gallo 2014). Thus, the potential immunological benefits of programmed cell death in the CNS must be weighed against the cost of losing irreplaceable cells and critical cytoarchitecture.

In all tissues, the specific consequences of cell death are influenced by which of the various forms of programmed cell death are triggered (Blander 2014). Apoptosis is the canonical form of programmed cell death, characterized by activation of the caspase proteases and the nonlytic fragmentation and shrinkage of dying cells that are recognized for removal by phagocytes (Jorgensen et al. 2017). While apoptosis can occur in response to infection or stress, it is also the predominant form of developmentally programmed cell death such that the death of cells by apoptosis sculpts developing embryos and contributes to tissue homeostasis throughout life. This elimination of cells that pose no risk to the host proceeds without activating inflammatory effector functions, leading to the impression that apoptosis is immunologically silent (Yamaguchi et al. 2014).

In contrast to apoptosis, programmed cell death pathways leading to cell lysis, such as necroptosis and pyroptosis, occur in response to pathogen infection or cellular stress and do not contribute directly to either development or tissue homeostasis. Both pyroptosis and necroptosis result in the release of intracellular contents as well as the production and release of inflammatory cytokines and other mediators. For these reasons, pyroptosis and necroptosis are considered to be pro-inflammatory. The death of infected cells by these programs likely contributes to initiation of host immune responses (Orozco and Oberst 2017; Bergsbaken et al. 2009). Here, we focus on necroptotic signaling within the CNS.

Necroptosis is defined by activation of receptor-interacting protein kinase (RIPK)3 and RIPK3-dependent phosphorylation of the pseudokinase MLKL (Linkermann and Green 2014). Phosphorylated MLKL can translocate to and apparently disrupt the plasma membrane resulting in cellular swelling and lysis.

A common form of necroptosis occurs downstream of TNF family death receptors, via RIPK1 recruitment of RIPK3. Two RIPK3-independent pathways may also trigger necroptosis. Toll-like receptor (TLR3) and TLR4 adaptor TRIF and Z-nucleic acid-binding protein (ZBP)1 (also known as DAI and DLM1) may also recruit RIPK3. Unlike either apoptosis or pyroptosis, necroptosis is caspase-independent and is inhibited by the apoptotic protease caspase-8. This feature of necroptotic signaling, combined with the observation that numerous pathogens encode inhibitors of the caspases, has led to the idea that necroptosis represents an alternate form of cell death that is engaged by the inhibition of apoptosis (Mocarski et al. 2014). While this is likely true in some contexts, emerging evidence indicates more complex roles for the necroptotic pathway in immune responses to infection as well as sterile injury (Oberst 2015).

The heterogeneous roles of necroptotic signaling result from differential functions of the RIP kinases, which drive both cell death-dependent and cell-independent signaling outcomes. Notably, the RIP kinases contribute to the initiation of inflammatory transcription pathways, among other functions, in addition to their role in the initiation of necroptosis (Orozco and Oberst 2017). RIPK1 has a well-characterized role in the activation of NF-κB and MAPK in response to TNF, although this signaling proceeds independently of RIPK1 kinase activity (Silke et al. 2015). Emerging evidence also indicates that RIPK3 contributes to inflammatory responses, independent of the initiation of necroptosis. Supporting this idea is the finding that mice lacking the terminal necroptotic effector MLKL, in which the execution of necroptosis is impossible, differ in phenotype from mice lacking its upstream activator RIPK3, or in which the kinase activity of RIPK1 is ablated (termed $Ripk1^{KD}$) (Newton et al. 2016). Although $Ripk3^{-/-}$ and $Ripk1^{KD}$ mice are susceptible to infection by WNV (Daniels et al. 2017b) and influenza (Nogusa et al. 2016), they resist TNF-induced shock and ischemia-reperfusion injury (Newton et al. 2016), phenotypes that are distinct from MLKL-deficient mice. These observations suggest that RIPK3 has additional biological roles beyond activation of MLKL and necroptosis. Thus, the distinction between a role for necroptosis and cell death-independent functions of the RIP kinases within the CNS requires differentiation of outcomes.

Initial work suggests that RIP kinase signaling is a key component of the host response to viruses in the CNS, but that the outcome of this signaling is not necroptosis. In this chapter, we will focus on recent work identifying diverse roles for the necroptotic signaling axis during neuroinvasive viral infection. Beyond the setting of infectious disease, RIPK-driven necroptosis and inflammation have also been implicated in disease associated with various non-infectious neurological pathologies and neurodegenerative disorders. Indeed, a majority of studies of necroptosis within the CNS have been performed using non-infectious disease models (Zhang et al. 2017), with most of these studies identifying necroptosis as highly detrimental to the CNS. Many reports demonstrate remarkable amelioration of CNS inflammation and injury using pharmacological inhibition and/or genetic ablation of RIPK signaling. Thus, we will also discuss recent studies of the impact of necroptosis during non-infectious CNS pathologies, as these studies provide

important additional insights concerning the immunological outcomes of RIPK signaling and necroptosis within the CNS and potential clues as to how they may function during CNS infection.

2 Neuroinvasive Viral Infections

2.1 Coronaviruses

Coronaviruses are positive-stranded RNA viruses that are common respiratory pathogens in mammals and birds. However, some coronaviruses, such as human coronavirus OC43 (OC43) and mouse hepatitis virus (MHV), are capable of spreading to the CNS, where they replicate in neurons, resulting in neuronal death and degeneration (Desforges et al. 2014). Coronavirus neuropathology may arise via different mechanisms, including neuroinflammation (Li et al. 2004), unfolded protein responses (Favreau et al. 2009), and glutamate excitotoxicity (Brison et al. 2011). MHV can also chronically persist in the murine CNS, resulting in viral and immune-mediated demyelination (Weiss and Leibowitz 2011). Indeed, coronaviruses are among the infectious agents that have been implicated in idiopathic demyelinating disorders of humans, such as multiple sclerosis (Venkatesan and Johnson 2014; Mentis et al. 2017).

Programmed cell death and RIPK signaling may also drive coronavirus-induced neuropathology. Recent studies suggest that coronaviruses are capable of inducing necroptotic cell death in infected neurons in vitro, and that this neuronal death may contribute to the disease burden observed in mouse models of neuroinvasive coronavirus infection. OC43 infection in primary mouse neurons has been shown to induce RIPK1 and RIPK3 expression, and a mutant strain of OC43 exhibiting increased neurovirulence induced significantly higher expression of both RIPK1 and RIPK3 in neurons compared to a non-mutant reference virus (Meessen-Pinard et al. 2017). Similar results were observed in a differentiated human neuroblastoma cell line (LA-N-5), where OC43 was also shown to induce cell death that was RIPK1- and MLKL-dependent, but Bax- and caspase-independent (Meessen-Pinard et al. 2017; Favreau et al. 2012). While coronavirus-induced neuronal necroptosis in vivo has yet to be reported, the increased propensity for the neurovirulent strain of OC43 to induce neuronal necroptosis in vitro may underlie the greater mortality and neurologic disease burden observed in mouse models using this virus.

One key finding from these studies is that the antiviral outcomes of RIPK signaling in neurons may be separable from the activation of programmed cell death. Knockdown of RIPK1 expression resulted in decreased cell death, along with increased viral replication in OC43-infected LA-N-5 cells (Meessen-Pinard et al. 2017). Remarkably, however, chemical inhibition of MLKL ameliorated cell death but had no significant impact on viral replication, suggesting that cell death-independent outcomes of RIPK1 signaling, such as cytokine production, were

necessary to restrict viral replication. While the protective versus pathologic outcomes of necroptosis during neuroinvasive coronavirus infection require more extensive study, these initial findings suggest that programmed cell death may not be absolutely necessary for neuronal control of viral replication, and that therapies that target the proximal effectors of cell death while leaving upstream signaling intact may preserve essential immune functions while limiting neuronal injury and degeneration.

2.2 *Japanese Encephalitis Virus*

Flaviviruses are another family of positive-stranded RNA viruses that exhibit neuroinvasive potential. As major encephalitic arboviruses disseminated via insect vectors, most notably mosquitoes, flaviviruses are endemic in most temperate and tropical regions of the world, where seasonal outbreaks and epidemics of viral encephalitis are increasingly common (Salimi et al. 2016). Indeed, flaviviruses are the most common cause of viral encephalitis globally, with Japanese encephalitis virus (JEV) being the most prevalent. JEV infection causes a mild febrile illness in most patients but can result in fatal meningoencephalitis in vulnerable populations. Neuroinvasive JEV infection results in massive neuronal death that is driven by viral replication as well as immunopathology caused by activated glia and infiltrating peripheral leukocytes (Myint et al. 2014; German et al. 2006). Because of this extensive neuronal death, survivors of JEV encephalitis very commonly experience profound and persistent neurologic and psychiatric sequelae (Wang and Liang 2015).

Hallmarks of JEV encephalitis in both rodents and humans are perivascular and parenchymal necrosis within the CNS (German et al. 2006), as well as TNFα-dependent neuroinflammation (Chen et al. 2012). Because TNFα is a canonical inducer of necroptosis, these observations suggest that necroptosis may be an important component of the neuroimmune response to JEV. Using a mouse model of JEV encephalitis, Bian and colleagues recently demonstrated considerable upregulation of MLKL expression and phosphorylation within the CNS following JEV infection (Bian et al. 2017). Enhanced MLKL expression was also noted in JEV-infected, $NeuN^+$ neurons in vivo, as well as Neuro2a cells in vitro, though direct evidence of MLKL-dependent neuronal cell death was not reported. However, despite robust engagement of MLKL following infection, loss of MLKL did not impact mortality following JEV infection in mice. CNS viral burden was also unchanged in $Mlkl^{-/-}$ mice compared to WT controls in both intraperitoneal and intracranial infection models. Remarkably, however, although endpoint mortality was unchanged, $Mlkl^{-/-}$ mice exhibited delayed weight loss and progression of clinical signs of disease compared to controls. $Mlkl^{-/-}$ mice also exhibited significantly lower CNS expression of inflammatory cytokines, including TNF, IL-1β, IFNγ. These findings suggest that engagement of MLKL within the JEV-infected CNS resulted in neuroinflammation that did not contribute to control of JEV

replication, but instead worsened disease pathogenesis. Neuroinflammation in response to viral infection commonly results in bystander injury to uninfected neural tissue and, on balance, may be harmful rather than protective (Klein et al. 2017; Swanson and McGavern 2015). Similarly, this initial study suggests that the outcomes of MLKL activation/necroptosis in the JEV-infected CNS also primarily result in immunopathology rather than viral control, and may, in fact, be a promising target for the alleviation of neuroinflammation and neurodegeneration during viral encephalitis. This idea is supported by many recent studies demonstrating the pathological outcomes of necroptosis in the CNS during noninfectious disease states, to be discussed later.

Importantly, although this study examined the impact of the effector protein MLKL during JEV encephalitis, there are other, possibly MLKL-independent, functions of upstream signaling by RIPK1 and RIPK3 in this setting that have not yet been investigated. As mentioned above, RIPK signaling regulates multiple, distinct signaling outcomes, above and beyond the initiation of necroptosis (Meessen-Pinard et al. 2017; Daniels et al. 2017b; Nogusa et al. 2016). RIPKs may, therefore, have additional functions that serve to restrict JEV infection and pathogenesis; these may include induction of apoptosis (Nogusa et al. 2016) as well as cell death-independent contribution to transcription of inflammatory cytokines.

2.3 West Nile Virus

Recent work studying RIP kinases in the context of infection with a related flavivirus, West Nile virus (WNV), suggests that inflammatory transcription, rather than necroptotic cell death, may be the prevalent outcome of RIPK activation in the CNS. WNV is included in the JEV serogroup of mosquito-borne flaviviruses of significant global health concern. WNV began to receive significant attention upon its introduction into the USA in 1999 (Lanciotti et al. 1999) and has since become the leading cause of epidemic viral encephalitis, accounting for >90% of neuroinvasive arboviral infections each year (Lindsey et al. 2014). Neuroinvasive WNV infection results in considerable viral and immune-mediated pathology, including neuronal cell death (Daniels et al. 2017a; Samuel et al. 2007; Salimi et al. 2016). A high proportion of patients that survive neuroinvasive WNV infection suffer long-lasting cognitive sequelae, with an estimated 10,000 survivors currently living with chronic neurocognitive impairments in the US (Salimi et al. 2016). Chronic impairment appears to result from neuroinflammatory insults that persist beyond the resolution of infection (Vasek et al. 2016; Klein et al. 2017). Despite this high propensity for immunopathology, robust innate and adaptive immune responses are required for effective viral clearance and host survival (Suthar et al. 2013; Cho and Diamond 2012). Thus, as with other neuroinvasive flavivirus infections, distinguishing the mechanisms that promote protective versus pathologic neuroinflammation is a central goal of current research (Klein and Hunter 2017).

Neuronal apoptosis is a pathologic rather than protective feature of West Nile virus encephalitis, as mice lacking key apoptotic signaling components exhibit decreased tissue injury with no apparent impact on viral dissemination or burden within the CNS (Samuel et al. 2007; Clarke et al. 2014). Recent work in our laboratory sought to investigate necroptotic and non-necroptotic RIPK signaling during WNV infection. In contrast to neuroinvasive coronavirus and JEV, we found no evidence for the initiation of necroptosis following WNV infection (Daniels et al. 2017b). Viral pathogenesis in WNV-infected *Mlkl*$^{-/-}$ mice did not significantly differ from WT controls across a broad array of virologic and immunologic parameters. However, both *Ripk3*$^{-/-}$ and *Ripk1*^{KD} mice exhibited markedly greater susceptibility to WNV, with severe clinical signs of disease and 100% mortality following subcutaneous infection. This increased susceptibility was ascribed to the absence of RIPK-dependent neuronal chemokine expression and a consequently diminished recruitment of antiviral immune cells to the CNS.

Our study supports the importance of diverse signaling outcomes via RIPK1 and RIPK3, particularly as they affect the immune response in the CNS. Moreover, our study establishes a key protective role for the RIP kinases independent of their canonical role as mediators of programmed cell death. Protection was associated with coordinated chemokine expression but appeared to proceed independently of key inflammatory cytokines, such as TNF and IL1β. Chemokines are extraordinarily important neurobiological mediators during both homeostasis and disease, regulating diverse processes that include development, neurotransmission, synaptic maintenance, and myelination, in addition to immunological responses to infection and injury (Williams et al. 2014; Reaux-Le Goazigo et al. 2013; Durrant et al.2014). Our findings suggest that RIPK may serve additional roles in the CNS associated with the coordination of chemokine expression.

2.4 Zika Virus

In a subsequent study, we examined roles for the RIP kinases in the control of Zika virus (ZIKV), another mosquito-borne flavivirus that has received significant recent attention due to its global spread, potential for human-to-human transmission, and the severe congenital neurologic pathology associated with vertical transmission from mother to fetus (Miner and Diamond 2017; Pardy and Richer 2019). Unexpectedly, we found that primary cortical neurons derived from mice lacking ZBP1, RIPK3, or the kinase activity of RIPK1 displayed a cell-intrinsic defect in the control of viral replication, while no such defect was observed upon infection of neurons with WNV (Daniels et al. 2019). We traced this cell-intrinsic defect to the metabolic enzyme IRG1, which is upregulated in neurons upon ZIKV infection in a ZBP1- and RIPK-dependent manner. IRG1 catalyzes the production of the metabolite itaconate, which alters cellular metabolism by inhibiting succinate dehydrogenase (SDH) activity (Lampropoulou et al. 2016; Murphy and O'Neill 2018). Suppression of SDH activity by IRG1-dependent itaconate was sufficient to

reduce ZIKV replication in neurons. Interestingly, we found that the IRG1/itaconate pathway was also able to suppress WNV replication, but that upon WNV infection, IRG1 could be redundantly upregulated by either the ZBP1/RIPK pathway or by the TLR7/Myd88 pathway. These findings implicated alterations in neuronal metabolism as an unexpected adaptation to limit viral replication in these cells, suggesting that these alterations can occur downstream of RIPK activation, among other innate immune signaling pathways.

Though only a handful of studies have examined roles for RIPK signaling and necroptosis in the CNS during viral infection, two key themes have emerged (Fig. 1). First, MLKL-dependent necroptosis is only one possible outcome of RIPK signaling, particularly in neural tissue. On balance, necroptosis appears to be pathologic rather than protective. Second, RIPK signaling in the CNS appears to serve important, protective antiviral and immunologic functions by enhancing inflammatory chemokine expression and altering neuronal metabolism to suppress viral replication and dissemination. An attractive hypothesis to explain these findings is that alternative mechanisms of RIPK-mediated pathogen control have evolved in highly specialized tissues such as the CNS, in which there are significant constraints on repair and in which individual cells serve unique, networked, and irreplaceable functions. Consistent with this idea, in our study of RIPK signaling during WNV encephalitis, we did not observe evidence for death-independent and RIPK-mediated chemokine expression in peripheral myeloid cells. RIPK signaling

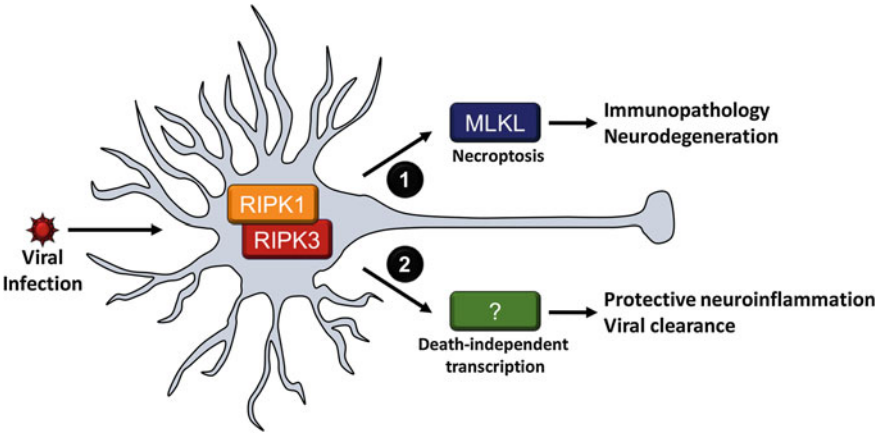

Fig. 1 Diverging outcomes of RIPK signaling during neuroinvasive viral infection. Emerging evidence suggests two possible outcomes of RIPK activation in neurons during viral infection. 1 Some viruses may engage canonical necroptotic cell death via the activation of the executioner protein MLKL. However, MLKL-dependent cell death has so far been shown to be an ineffective antiviral strategy in the CNS, resulting instead in immunopathology and neurodegeneration. 2 RIPK signaling in neurons also appears to serve inflammatory roles that are independent of programmed cell death, though the molecular mechanisms that orchestrate this role remain unknown. Death-independent RIPK signaling has so far been shown to coordinate the transcription of inflammatory chemokines, resulting in protective neuroinflammation and viral clearance

was dispensable for virologic control and adaptive immunity in peripheral compartments (Daniels et al. 2017b). Consistent with these observations peripheral cells lacking IRG1 did not display the defects in control of ZIKV replication observed in neurons lacking this enzyme (Daniels et al. 2019). Studies in non-CNS model systems have also identified necroptosis-independent outcomes of RIPK signaling, including RIPK-dependent apoptosis and noncanonical inflammasome activation (Nogusa et al. 2016; Najjar et al. 2016; Newton et al. 2016; Lawlor et al. 2015), though neither of these outcomes were observed in the CNS in our studies. Defining general versus CNS-specific outcomes of RIPK signaling during viral infection will be an intriguing area for future study.

3 Lessons from Noninfectious CNS Pathologies

Comparatively, more has been done to investigate the roles of RIPK signaling and necroptosis during noninfectious pathologies of the CNS. While there are obvious differences between infection and sterile disease states, CNS injury and neurodegeneration are marked by considerable inflammation and immunopathology, suggesting parallels to infection (Faden et al. 2016; Sankowski et al. 2015). As during CNS infection, necroptosis during noninfectious CNS pathologies also appears to contribute significantly to neurodegeneration and chronic neuroinflammation (Zhang et al. 2017). Recent studies of necroptosis and RIPK signaling in the context of these pathologies provide important additional insight into how these processes function in the CNS more generally and suggest several promising avenues of inquiry for future studies of viral infection.

3.1 Spinal Cord Injury

Mechanical trauma to the spinal cord results first in a primary lesion composed of dying cells at the site of trauma, followed by progressive secondary injury to surrounding tissues resulting from ongoing inflammation (Schwab et al. 2014). Cell death within primary lesions has been primarily attributed to non-programmed, mechanically induced necrosis as well as apoptosis (Byrnes et al. 2007). Apoptosis has been observed in both neurons and oligodendrocytes following spinal cord injury (SCI) and can be observed within hours following injury in rodent models (Zhang et al. 2012). The contribution of neuronal and oligodendroglial apoptosis to events that follow SCI is well established, as numerous studies have demonstrated through the use of inhibitors that apoptosis contributes to secondary injury, increased lesion size, and reduced motor function (Han et al. 2012; Yin et al. 2012; Tang et al. 2014; Zhao et al. 2017; Chen et al. 2017a).

Recent evidence, however, suggests that necroptosis also contributes to the pathogenesis of SCI. RIPK3 expression is highly upregulated in both primary and

secondary SCI lesion tissue and can be observed in neurons, oligodendrocytes, and astrocytes (Kanno et al. 2015; Wu et al. 2016). Moreover, propidium iodide^{+} necrotic cells can be observed within SCI lesions long after the initial mechanical insult, suggesting that ongoing programmed necrosis contributes to secondary injury (Kanno et al. 2015; Fan et al. 2016). Several recent studies have demonstrated that pharmacological blockade of the necroptotic signaling axis significantly improves outcomes following SCI. For example, administration of the canonical RIPK1 inhibitor necrostatin-1 (Nec-1) has been shown to effectively increase neuronal survival, reduce tissue damage, and improve recovery of motor function in rodent SCI models (Liu et al. 2015; Wang et al. 2014). Pharmacological inhibition of necroptosis may also prevent injury to the structural tissues of the spine, such as the nucleus pulposus of the intravertebral disk (Chen et al. 2017b).

In addition to serving as a proximal mechanism of neuronal pathogenesis following primary injury, necroptosis also appears to contribute to secondary injury via multicellular processes. In particular, necroptosis within inflammatory M1 microglia and reactive astrocytes has been associated with ongoing immunopathology and poor outcomes following SCI (Fan et al. 2016). A hallmark of secondary injury in the CNS is the initiation of reactive gliosis, characterized in part by the proliferation of microglia, astrocytes, and oligodendrocyte precursor cells (OPCs) within and around lesion tissue. Gliosis is a complex process that has both beneficial and detrimental effects at the site of injury. Proliferating glia promote a return to homeostasis within injured tissue by inhibiting excitotoxicity, restoring blood–brain barrier function, and physically sequestering injured tissue from adjacent healthy tissue via formation of a "glial scar." However, gliosis and glial scar formation also actively inhibit axon regeneration and contribute to the formation of "cavities" around injured tissue that preclude functional recovery (Burda and Sofroniew 2014).

Recent studies demonstrate that reactive glia undergo multiple forms of programmed cell death, including necroptosis, during gliosis and that different cell death programs contribute to the diverse regulatory outcomes of gliosis following SCI. For example, inflammatory M1 macrophages and microglia have been shown to induce necroptosis in reactive astrocytes, and astrocytic necroptosis, in turn, contributes to immunopathology and spinal cord cavitation (Fan et al. 2016). Inhibition of necroptosis in a rodent model of SCI has been shown to not only significantly ameliorate injury and cavitation, but also to skew the gene expression of reactive astrocytes toward a beneficial, neurotrophic phenotype (Fan et al. 2016). Necroptotic M1 microglia have also been observed in spinal cord lesions in both rodents and humans, where they likely also contribute to ongoing neuroinflammation and injury (Fan et al. 2015, 2016; Dhib-Jalbut and Kalvakolanu 2015). These studies and others establish that necroptotic glia contribute significantly to ongoing inflammation and neuropathology during CNS injury and may serve as important additional targets for clinical intervention during neuroinfectious diseases in which reactive gliosis contributes to neuropathogenesis (Burda and Sofroniew 2014).

3.2 Traumatic Brain Injury

Similarly to SCI, trauma to the higher CNS also results in a biphasic response of primary cell death followed by neuroinflammation and secondary injury. In contrast, to SCI, however, traumatic brain injury (TBI) has fewer options for surgical intervention, and, in addition to the sensorimotor impairments observed following SCI, TBI can result in long-term cognitive, emotional, and behavioral dysfunction (Walker and Tesco 2013). Moreover, TBI is a purported risk factor for the later development of neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and Amyotrophic Lateral Sclerosis (ALS) (Gardner and Yaffe 2015; Faden and Loane 2015). Emerging evidence demonstrates that several classes of neural cells are susceptible to necroptosis following TBI (Walker and Tesco 2013). RIPK1, RIPK3, and MLKL expression have been shown to increase in forebrain regions including cerebral cortex and hippocampus in rodent controlled cortical impact (CCI) models (Zhou et al. 2012; Liu et al. 2016), and Nec-1 administration has been shown to significantly reduce neuroinflammation and ameliorate functional impairment following CCI (You et al. 2008; Wang et al. 2012).

Notably, studies of RIPK signaling during TBI suggest that necroptosis can occur in adult neural progenitor cells. Using a CCI model, Zhou and colleagues observed that the majority of degenerating cells in the hippocampal dentate gyrus, a major site of adult neurogenesis, were immature granular neurons (Zhou et al. 2012). Most of these dying immature neurons did not exhibit classical morphological and biochemical characteristics of apoptosis, but did exhibit high levels of RIPK1 expression. While more explicit confirmation of RIPK-mediated necroptosis in neuronal progenitors is needed, this study, combined with others demonstrating improved functional recovery from TBI following pharmacological blockade of RIPK1 (You et al. 2008; Wang et al. 2012), suggests that RIPK signaling may further exacerbate neurologic disease by inhibiting CNS repair. Perturbations of neurogenesis during neuroinvasive infections of both fetuses and adults have been described in recent reports, including several notable studies of ZIKV infection, and likely contribute to long-term cognitive and functional impairments observed in survivors of these infections (Sun et al. 2014; Liang et al. 2016; Bayless et al. 2016; Brnic et al. 2012; Li et al. 2016; Tang et al. 2016). Thus, studies examining the susceptibility of neural progenitors to necroptosis during viral infection are warranted and may yield important new insight into the poorly understood etiology of microcephaly and other neurologic impairments resulting from ZIKV infection.

3.3 Neurodegeneration

The CNS is also susceptible to chronic neurodegenerative diseases such as Alzheimer's Disease and Parkinson's Disease, which remain poorly understood despite widespread public awareness. Neurodegenerative disease is most often

observed in aging populations and is characterized by the progressive atrophy and death of neurons, ultimately manifesting in motor dysfunction, dementia, or other significant neurologic impairments (Hindle 2010; Reitz and Mayeux 2014). Several distinct stimuli have been found to trigger neurodegeneration, including dysregulated protein folding, axonal transport, and lysosomal storage, among others (Bourdenx et al. 2017; Onyenwoke and Brenman 2015). The pathophysiological mechanisms of neurodegeneration are also complex and include excitotoxicity (Mehta et al. 2013), inflammation (Sankowski et al. 2015), and programmed cell death (Fan et al. 2017). Remarkably, despite the extraordinary heterogeneity of these diseases, a growing body of recent research has established that pharmacological inhibition of RIPK1 signaling ameliorates pathology in a diverse set of neurodegenerative disease models, including models of Alzheimer's Disease (Yang et al. 2017; Qinli et al. 2013), Parkinson's Disease (Wu et al. 2015; Dionisio et al. 2019), ALS (Ito et al. 2016; Re et al. 2014), Huntington's Disease (Zhu et al. 2011), and others (Cougnoux et al. 2016; Zhang et al. 2017), though some of these findings remain controversial (Wang et al. 2019). While not all of these studies directly implicate necroptosis as a pathological driver of neurodegeneration, it is now clear that RIPK signaling is capable of orchestrating several distinct degenerative processes in the CNS, through programmed cell death, neuroinflammation, and other processes (Zhang et al. 2017).

One particularly intriguing finding of recent studies of necroptosis during neurodegeneration is the apparent central involvement of RIPK signaling across a number of neuronal subtypes and brain regions. The brain exhibits remarkable immunologic heterogeneity, as cell type and region-specific immune responses have been observed during both neuroinvasive infection and CNS autoimmunity (Daniels et al. 2017a; Cho et al. 2013; Stromnes et al. 2008; Lees et al. 2008; Durrant et al. 2016; Williams et al. 2020). However, inhibition or genetic ablation of RIPK signaling has been shown to limit degeneration and/or cell death in diverse neuronal populations, including cultures of spinal cord motor neurons (Re et al. 2014; Ito et al. 2016), cerebral cortical neurons (Qinli et al. 2013), striatal neurons (Zhu et al. 2011), and dopaminergic PC12 cells (Wu et al. 2015). Similar findings have been observed in vivo across several functionally and anatomically distinct CNS regions, including the cerebral cortex (Yang et al. 2017; Vitner et al. 2014), hippocampus (Yang et al. 2017), cerebellum (Cougnoux et al. 2016), and spinal cord (Ito et al. 2016). These findings implicate RIPK signaling as a central driver of pathological processes across a broad set of CNS tissues with diverse functional profiles and developmental ontogenies.

The implication of this signaling axis in the pathogenesis of neurodegenerative disease across the CNS suggests that RIPK signaling may also be a highly relevant mediator of host responses to a broad array of neuroinvasive infections targeting many different CNS regions and cell types. However, the central involvement of this pathway across CNS pathologies may complicate efforts to target it for therapeutic intervention. On one hand, the necroptotic signaling axis may provide broad-spectrum targets that improve the feasibility of therapeutic development for neuroinvasive viral infections. Neuroinvasive viruses collectively represent a major

source of human disease burden; however, individual viral species often cause only a small number of clinical cases, limiting the cost-effectiveness of developing virus-specific therapies (Kok 2016). Therapeutic intervention with RIPK inhibitors to treat neurodegeneration may also increase susceptibility to neuroinvasive viral infection. Further work uncovering how these processes operate across different CNS cell types, regions, and pathogens will be necessary to guide the development and delivery of effective therapies.

4 Conclusions

Necroptosis has been traditionally understood as an alternative, immunogenic form of programmed cell death and an important component of the host response to pathogens. Emerging evidence suggests, however, that necroptosis during acute viral infection of the CNS is, at best, an ineffective antiviral response and, at worst, a significant driver of neuropathology. This idea is supported by a larger body of evidence demonstrating the diverse contributions of RIPK signaling and necroptosis to the neuropathogenesis of CNS injury and neurodegeneration. Despite this detrimental role, the RIP kinases also appear to coordinate unexpected, necroptosis-independent antiviral immune responses in the CNS, promoting protective rather than pathological neuroinflammation.

Future research should work to more fully uncover the extent to which RIPK signaling serves specialized functions in the CNS during viral infection, as well as the physiologic and evolutionary drivers of these functions. The continuing development of improved genetic and other tools, such as *Ripk3*$^{flox/flox}$ mice (Strilic et al. 2016), will allow more careful examination of cell- and tissue-specific roles for the necroptotic signaling axis across experimental systems. Studies using a broader range of neurotropic viruses are also needed, as this pathway may function differently in response to variations in pathogen detection, route of neuroinvasion, or differential cellular tropism of individual viruses. Understanding the specific mechanisms that govern RIPK-driven neuroimmune responses may be of great benefit in the study of neuroinvasive viral infection, for which new molecular targets for prevention and therapy are urgently needed.

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