CellPress

A RIPtide Protects Neurons from Infection

Ryan P. Gilley[1] and William J. Kaiser[1,*]
[1]Department of Microbiology, Immunology, and Molecular Genetics, University of Texas Health San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78229, USA
*Correspondence: kaiserw@uthscsa.edu
http://dx.doi.org/10.1016/j.chom.2017.03.014

RIPK3 and RIPK1 limit virus spread by executing either apoptotic or necroptotic cell death in response to infection. In a recent issue of *Cell*, Daniels et al. (2017) unveil an unexpected cell death-independent requirement of RIP kinase activity in coordinating neuroinflammation, restricting West Nile virus pathogenesis in neurons.

Necroptosis is an autonomous form of cell death that eliminates virally infected cells to prevent replication and dissemination. Diverse pathogen-sensing and inflammatory pathways induce necroptosis by engaging receptor-interacting protein kinase (RIPK)3. Activation of RIPK3 requires oligomerization facilitated by RIPK1, TIR-domain-containing adaptor-inducing IFN (TRIF), or the Z-nucleic acid binding protein DAI/ZBP1/DLM-1. Activated RIPK3 subsequently phosphorylates the pseudokinase mixed-lineage kinase domain-like protein (MLKL), leading to a collapse in cell membrane integrity and subsequent necroptotic cell death (Weinlich et al., 2017). The central host-defense role of RIPK3-driven necroptosis is highlighted by the number of herpesviruses that have evolved countermeasures to directly thwart RIPK3 activation. For example, murine cytomegalovirus (MCMV) sustains cell viability by encoding a viral inhibitor of RIP activation (vIRA) that binds RIPK3 and disrupts viral sensing by DAI. A recombinant MCMV encoding a mutant vIRA is severely attenuated both in vitro and in vivo, and infection is normalized in mice lacking RIPK3 (Upton et al., 2010, 2012). RIPK3 mice are also more susceptible to lethal vaccinia virus (VV) and influenza infection (Cho et al., 2009; Thapa et al., 2016). In the context of infection with MCMV, VV, or influenza, RIPK3-driven cell death appears to be the primary mechanism that restricts viral pathogenesis (Figure 1).

In a recent study, Oberst and colleagues unveil an unanticipated antiviral neuroinflammatory role of RIPK3 that functions independently of cell death (Daniels et al., 2017). Using a murine model of West Nile virus (WNV) infection, they demonstrate that RIPK3 limits WNV pathogenesis. In contrast, WNV infection of *Casp8*$^{-/-}$*Mlkl*$^{-/-}$ double knockout mice was remarkably similar to infection of wild-type mice, indicating that neither extrinsic apoptosis nor necroptosis contributed to the RIPK3 antiviral phenotype. In contrast, infection of RIPK3 null mice with WNV led to increased mortality that correlated with a defect in WNV clearance from the central nervous system (CNS). Despite a transient increase in WNV burden in the spleen and serum of *Ripk3*$^{-/-}$ mice, viral loads in the periphery ultimately returned to levels similar to control mice, aligning with a normal antiviral T cell response as well as generation of neutralizing antibodies. In contrast, multiple CNS tissues sustained high viral loads, indicating a specific RIPK3 defect in viral clearance from this compartment. The role of RIPK3 was not intrinsic to the infected cell, as WNV replicated to similar levels in cultured neurons, and direct intracranial inoculation of WNV revealed no difference in viral titers at early times. Rather than eliminate infected cells via necroptosis, RIPK3 was crucial for neurons to produce key inflammatory mediators including the chemokines CCL2 and CXCL10. Ultimately, deficient chemokine levels in the *Ripk3*$^{-/-}$ mice compromised recruitment of anti-WNV CD8^{+} cells and myeloid cells into the CNS. RIPK1 kinase-dead knock-in mice phenocopied RIPK3 null mice with diminished levels of protective chemokines and an enhanced mortality following infection with WNV. Thus, both RIPK3 and RIPK1 collaborate for efficient chemokine induction within the CNS to control viral infection.

The majority of studies in recent years have focused on the role of RIPK3 in orchestrating necroptosis; however, RIPK3 also fulfills additional signaling functions, including the production of inflammatory cytokines and control of the inflammasome (Najjar et al., 2016; Vince et al., 2012). To evaluate RIPK3 in neurons, Daniels et al. (2017) generated mice that express a modified form of RIPK3 that can be activated synthetically in the absence of any upstream stimulus. Enforced RIPK3 oligomerization rapidly induced necroptosis when expressed in mouse embryonic fibroblasts. In contrast, RIPK3 oligomerization in neurons produced a profile of chemokines similar to that observed during WNV infection and did not lead to necroptosis. This strikingly different response to RIPK3 activation in neurons likely stems from limiting levels of MLKL; thus, other roles of RIPK3 manifest when necroptosis is unable to dominate as a signaling outcome. RIPK3-deficient neurons also showed defective chemokine and cytokine expression following stimulation of toll-like receptors (TLRs) 3, 4, and 7. Thus, this study reveals a surprising neuron-specific function for RIPK3 in regulating gene expression downstream of diverse innate immune signals as well as viral infection.

The study by Daniels et al. (2017) highlights that death-independent functions for RIPK3 may differ significantly by inflammatory stimulus and cell type. An unresolved line of study includes the precise signaling events upstream of the RIPKs during WNV infection in neurons. As noted, RIPK3 activation typically requires oligomerization with other cellular RIP homotypic interaction motif (RHIM)-containing proteins including RIPK1, TRIF, and DAI. Death receptors such as TNFR1 utilize RIPK1 to activate RIPK3, TLR3/TLR4 employ TRIF, and DAI bridges sensing of influenza and herpesvirus to

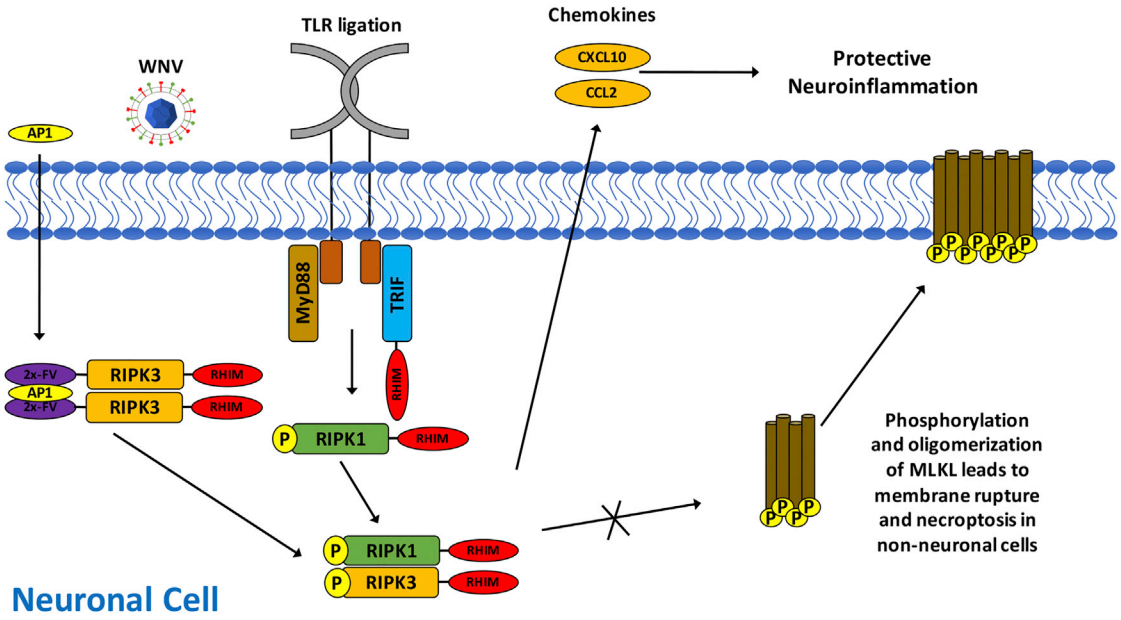

Figure 1. RIPK3 Coordinates a Cell Death-Independent Immune Response to Viral Infection in the CNS
Infection of neuronal cells with West Nile Virus (WNV) or TLR activation drives RIPK1/RIPK3 kinase-dependent generation of proinflammatory chemokines to promote an antiviral leukocyte response. Mice lacking *Ripk3* were deficient in controlling viral replication and spread within the CNS.

RIPK3. It will be important to determine the potential contribution of these various drivers of RIPK signaling and whether RIPK3 restricts other neuroinvasive viruses including Zika and HSV1. Additionally, it is remarkable that RIPK1 and RIPK3 kinase activity is required for expression of a limited subset of genes, while genes including TNFα and IL-1β remain unaffected. Thus, RIPK deficiency does not result in a global defect in, for example, NF-κB signaling. In the setting of TLR4 activation, RIPK3 and RIPK1 promote proinflammatory gene expression by sustaining the activation of Erk1/2, cFos, and NF-κB (Najjar et al., 2016). It is tempting to speculate that a similar pathway may operate not only in neurons to sculpt antiviral gene expression, but also more broadly during necroptosis to enhance the necroinflammatory response of virally infected cells. Though the antiviral role of RIPK3 in MCMV, HSV1, and influenza infection has focused to date on cell death, it is likely that enhanced chemokine expression may also occur, as revealed by Daniels et al. (2017) for WNV. Altogether, identifying the vital role of RIPK3 in coordinating antiviral neuroinflammation confers a purpose for this enzyme beyond death, underscores the myriad roles these kinases play in host defense, and highlights considerations for RIPK-targeted therapy to treat inflammatory disease.

ACKNOWLEDGMENTS

Work in the authors' laboratory is supported by funding from the NIH (DP5 OD012198) and the William and Ella Owens Medical Research Foundation.

REFERENCES

Cho, Y.S., Challa, S., Moquin, D., Genga, R., Ray, T.D., Guildford, M., and Chan, F.K. (2009). Cell *137*, 1112–1123.

Daniels, B.P., Snyder, A.G., Olsen, T.M., Orozco, S., Oguin, T.H., III, Tait, S.W.G., Martinez, J., Gale, M., Jr., Loo, Y.-M., and Oberst, A. (2017). Cell. Published online March 30, 2017. http://dx.doi.org/10.1016/j.cell.2017.03.011.

Najjar, M., Saleh, D., Zelic, M., Nogusa, S., Shah, S., Tai, A., Finger, J.N., Polykratis, A., Gough, P.J., Bertin, J., et al. (2016). Immunity *45*, 46–59.

Thapa, R.J., Ingram, J.P., Ragan, K.B., Nogusa, S., Boyd, D.F., Benitez, A.A., Sridharan, H., Kosoff, R., Shubina, M., Landsteiner, V.J., et al. (2016). Cell Host Microbe *20*, 674–681.

Upton, J.W., Kaiser, W.J., and Mocarski, E.S. (2010). Cell Host Microbe *7*, 302–313.

Upton, J.W., Kaiser, W.J., and Mocarski, E.S. (2012). Cell Host Microbe *11*, 290–297.

Vince, J.E., Wong, W.W., Gentle, I., Lawlor, K.E., Allam, R., O'Reilly, L., Mason, K., Gross, O., Ma, S., Guarda, G., et al. (2012). Immunity *36*, 215–227.

Weinlich, R., Oberst, A., Beere, H.M., and Green, D.R. (2017). Nat. Rev. Mol. Cell Biol. *18*, 127–136.