ANTIVIRAL IMMUNITY

Neuronal itaconate restricts viral infection

> RIPK signalling restricts ZIKV pathogenesis independently of MLKL-dependent or caspase 8-dependent cell death pathways

Cell death in the form of necroptosis can be triggered by various viral infections as an innate host defence pathway. However, it is intuitive to suppose that cell death as a mechanism for pathogen control should be restricted in highly sensitive organs such as the central nervous system (CNS). Indeed, a previous study by Oberst and colleagues described a cell death-independent function of the necroptotic kinases RIPK1 and RIPK3 in protection against neuroinvasive West Nile virus (WNV) infection. The same group now extend this finding to show that neuronal Zika virus (ZIKV) infection triggers a cell-intrinsic pathway through these kinases that alters neuronal metabolism to create a cellular environment suppressive for viral replication.

Mice lacking RIPK3 or mice expressing a kinase-dead version of RIPK1 (*Ripk1*$^{KD/KD}$) developed clinical signs of paresis after subcutaneous infection with ZIKV, whereas wild-type mice or mice lacking the necroptotic effector protein MLKL did not. Furthermore, the phenotype of *Ripk3*$^{-/-}$ mice was not affected by caspase 8 deficiency. The data therefore indicate that RIPK signalling restricts ZIKV pathogenesis independently of MLKL-dependent or caspase 8-dependent cell death pathways.

Whereas there was no difference in viral burden between the peripheral tissues of *Ripk3*$^{-/-}$ mice and wild-type mice after subcutaneous ZIKV infection, copy numbers of ZIKV RNA in CNS tissues were higher in *Ripk3*$^{-/-}$ mice (but not *Mlkl*$^{-/-}$ mice). Thus, the cell death-independent, RIPK3-mediated pathway for viral restriction seems to be CNS intrinsic. This supposition was supported by intracranial ZIKV infection of *Ripk3*$^{-/-}$ mice and *Ripk1*$^{KD/KD}$ mice, which had increased CNS viral titres and accelerated and increased mortality. Constitutive overexpression of RIPK3 decreased the brain viral burden independently of MLKL expression. Further experiments in vitro showed that *Ripk3*$^{-/-}$ and *Ripk1*$^{KD/KD}$ primary cortical neurons, but not *Mlkl*$^{-/-}$ neuronal cultures, supported enhanced ZIKV replication compared with wild-type neurons. However, primary bone marrow-derived myeloid cells or microglial cultures did not show increased ZIKV replication in the absence of RIPK3, which indicates that this viral restriction pathway in the CNS is neuron specific. In support of this, mice with forebrain neuron-specific overexpression or deletion of RIPK3 had increased or decreased survival, respectively, after intracranial infection.

To investigate the upstream pathways that activate RIPK3 in response to ZIKV infection, the authors used mice lacking key components of pattern recognition receptor signalling. They showed that mice and neuronal cultures lacking the nucleic acid sensor ZBP1 (also known as DAI) were more susceptible to ZIKV infection. *Zbp1* mRNA was upregulated in brain homogenates after ZIKV infection and the phosphorylation (activation) of RIPK3 was decreased in infected *Zbp1*$^{-/-}$ neurons in vitro.

Downstream of RIPK3, Oberst and colleagues showed that the antimicrobial gene *Irg1* is induced by ZIKV infection in wild-type but not *Ripk3*$^{-/-}$, *Ripk1*$^{KD/KD}$ or *Zbp1*$^{-/-}$ neurons. Knockdown of *Irg1* in RIPK3-overexpressing primary neurons abrogated the anti-ZIKV activity of RIPK3. The metabolic product of IRG1, itaconate, has been shown to have various immunoregulatory effects in myeloid cells, including metabolic reprogramming through inhibition of succinate dehydrogenase (SDH) activity. Increased concentrations of itaconate and succinate were observed in infected wild-type neurons but not *Ripk3*$^{-/-}$ or *Irg1*$^{-/-}$ neurons. Treatment with a competitive inhibitor of SDH activity inhibited ZIKV replication in wild-type, *Ripk3*$^{-/-}$ and *Irg1*$^{-/-}$ neuronal cultures. SDH inhibition or administration of itaconate also rescued viral burden in vivo after intracranial ZIKV infection.

In summary, this study describes an immunometabolic mechanism for the restriction of ZIKV infection that is preferentially engaged by neurons and not myeloid cells, perhaps reflecting the need to avoid cell death in crucial tissues such as the CNS. Further data, not discussed here, also show that IRG1 has antiviral activity against neuronal WNV infection and in human neural lineage cells.

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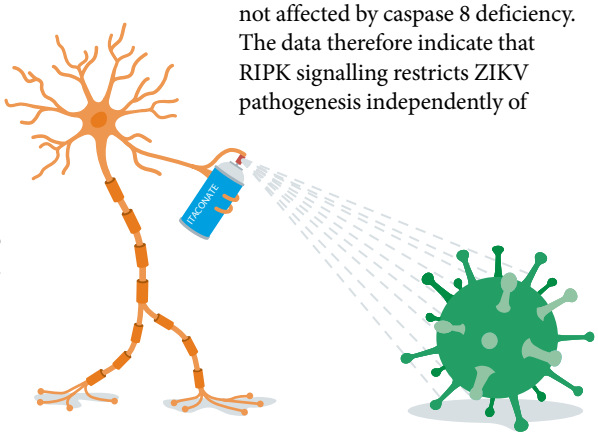

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ORIGINAL ARTICLE Daniels, B. P. et al. The nucleotide sensor ZBP1 and kinase RIPK3 induce the enzyme IRG1 to promote an antiviral metabolic state in neurons. *Immunity* https://doi.org/10.1016/j.immuni.2018.11.017 (2019)