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COMMENTARY

Zika virus infection activates sting-dependent antiviral autophagy in the *Drosophila* brain

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ABSTRACT

Viral infection of neurons is pathogenic, yet the factors that govern innate control of neurons remain incompletely understood. Using a *Drosophila* model we have defined the essential role that inflammatory-dependent Sting activation plays in inducing antiviral macroautophagy/autophagy to restrict Zika infection in the fly brain. Our discovery that Sting is an essential mediator of innate defenses in *Drosophila* provides further support for an ancient and conserved pathway that likely evolved to target intracellular pathogens for autophagic destruction.

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Canonical autophagy (or 'macroautophagy') is an ancient and conserved intracellular pathway that recycles nutrients, and degrades protein aggregates, and damaged organelles to maintain cellular homeostasis. Autophagy is activated by a variety of stressors, and leads to the targeted sequestration of cytoplasmic contents within double-membrane vesicles, termed autophagosomes, which fuse with a lysosome to degrade the engulfed cargo. Autophagy also plays a critical role in the innate immune defense against diverse intracellular pathogens from flies to mammals. Autophagy can be activated by pathogen infection and innate immune signaling leading to the degradation of intracellular bacteria, viruses and eukaryotic parasites.

In particular, autophagy can be induced by a variety of viral infections, and can restrict the replication or pathogenesis of viruses including the arthropod-borne viruses Rift Valley fever virus, vesicular stomatitis virus, sindbis virus, and chikungunya virus. In addition, studies have shown that herpes simplex virus type 1 evolved mechanisms to evade antiviral autophagy. Many of these viruses infect neurons, and autophagy has emerged as an essential component of antiviral defenses against encephalitic viruses, as nonlytic clearance of pathogens is particularly important in mature neurons which cannot self-renew.

Zika virus (ZIKV) is another arthropod-borne virus, which has recently emerged. Fetal infections lead to severe complications as neuroprogenitors are lytically infected. In contrast, mature neurons are refractory to infection due to unknown mechanisms. We set out to develop a *Drosophila* model for ZIKV infection, and found that ZIKV specifically infects and replicates in adult fly brains, albeit to low levels [1]. Perhaps surprisingly, many arboviruses, including the related West Nile virus, infect the mosquito central nervous system, and are controlled by incompletely understood innate immune mechanisms. We explored the innate mechanisms that control ZIKV infection of the brain and found that despite the fact that the antiviral RNA interference (RNAi) pathway is active in the fly brain, ZIKV is not controlled by this pathway. This suggests that ZIKV encodes a suppressor of RNAi silencing. Because inflammatory Rel/NFKB pathways TOLL and IMD participate in diverse immune responses, we explored whether these pathways are engaged during ZIKV infection. We found that the Rel/NFKB IMD pathway is induced upon ZIKV infection in the brain. Moreover, Rel/NFKB signaling controls infection, as Rel/NFKB transcription factor mutants display increased viral infection and now succumb to the otherwise non-lethal infection. Furthermore, specific depletion of Rel/NFKB in neurons leads to increased ZIKV infection, suggesting that the inflammatory response in neurons is critical to limit infection and pathogenesis. This Rel/NFKB pathway is canonically induced by the pattern recognition receptors peptidoglycan recognition proteins LC (PGRP-LC) or PGRP-LE, which bind to bacterial peptidoglycans either extracellularly or intracellularly, respectively. Surprisingly, we found that flies mutant for both receptors (PGRP-LC and -LE) are more susceptible to ZIKV infection. Further studies will determine if PGRP-LC or PGRP-LE interact with ZIKV directly or indirectly to induce this protective inflammatory cascade.

To identify the antiviral effectors transcriptionally induced by Rel/NFKB, we compared the transcriptome of uninfected and virally infected flies. In addition to many well-known Rel/NFKB-dependent genes (e.g. anti-microbial peptides) we identified the fly ortholog of TMEM173/STING, as being induced by viral infection. We confirmed that Sting is induced in the fly brain upon ZIKV challenge, and that this is dependent on Rel/NFKB. Using a transposon insertion mutant we also demonstrated that Sting is antiviral against ZIKV infection in the brain, and that neuronal-specific depletion of Sting leads to increased replication of ZIKV, suggesting Rel/NFKB-induced Sting expression is specifically required in neurons to control ZIKV infection (Figure 1).

While this was the first demonstration of an innate role for Sting in flies, mammalian TMEM173/STING is well-known to control viral infections. TMEM173/STING activates multiple downstream antiviral pathways including IRF3-dependent

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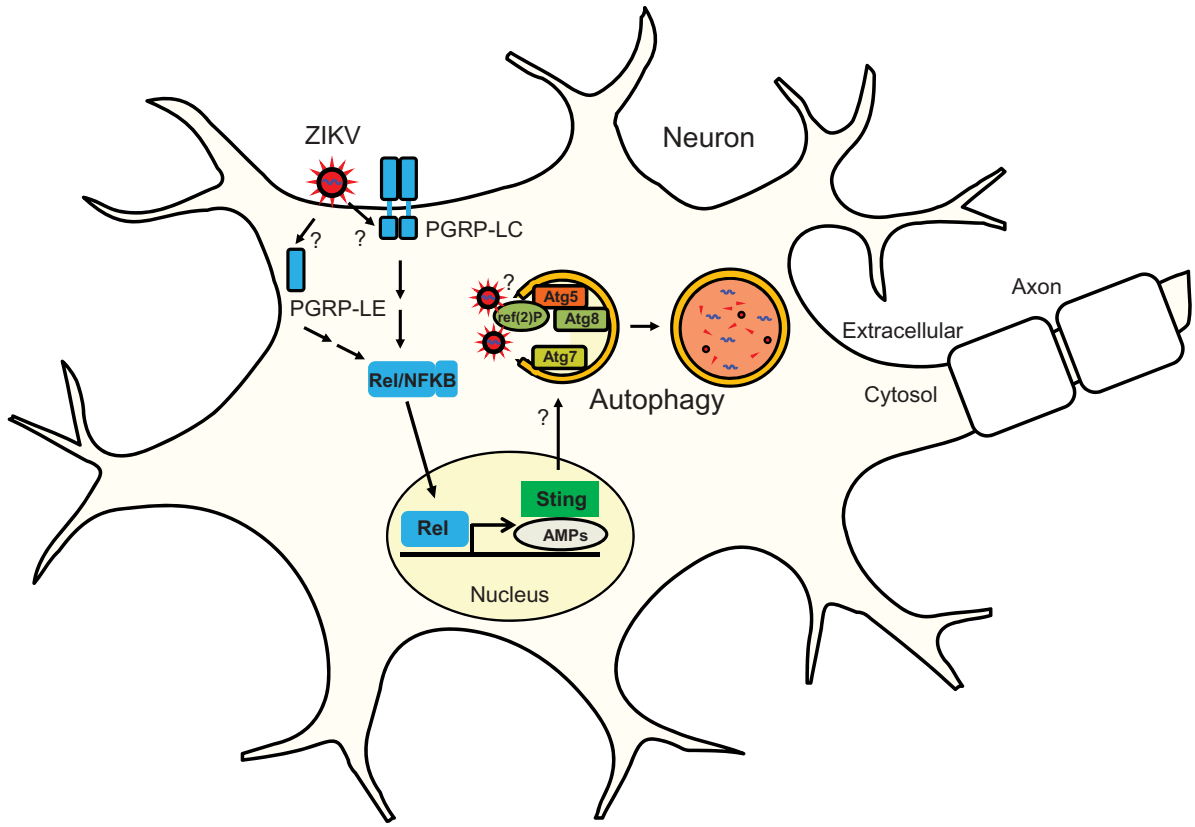

Figure 1. Schematic representation of the mechanism by which Rel/NFKB-dependent STING expression activates antiviral autophagy to restrict ZIKV infection in fly brain. ZIKV infects mature neurons, and is controlled by Rel/NFKB inflammatory signaling that activates the expression of STING. This pathway is required for antiviral autophagy activation in neurons, which is necessary and sufficient to block infection. AMPs, anti-microbial peptides.

type I interferons, NFKB signaling and autophagy. Because we found that Sting is downstream of Rel/NFKkB, and because flies do not encode IRFs, we hypothesized that Sting may control antiviral autophagy. Indeed, we found that ZIKV infection induces autophagy in the fly brain as measured by increases in Atg8-II by immunoblot, as well as increased mCHERRY-Atg8 puncta by confocal microscopy. Using RNAi we found that loss of autophagy genes (*Atg5, Atg7*) leads to increased viral infection of the brain. In addition, we found that the well-known autophagy cargo receptor ref(2)P/SQSTM1/p62 is also required for antiviral control. Importantly, we found that flies null for *Atg5* support increased infection and rapidly succumb to ZIKV infection. Moreover, we demonstrated that neuronal autophagy controls ZIKV replication because neuronal depletion of Atg5 leads to increased viral infection of the brain. Lastly, we found that both Rel/NFKB and Sting are required for the induction of antiviral autophagy, as the ZIKV-dependent Atg8-II accumulation is lost in the Rel/NFKB and Sting mutants. Altogether, these results suggest that Rel/NFKB-dependent Sting -dependent autophagy is essential for control of ZIKV infection in neurons, and limits pathogenesis in the fly brain. The mechanism by which Sting induces autophagy, and the viral cargo targeted for degradation are unknown.

While autophagy limits infection, wild-type animals support a low level of infection in the brain. Therefore, we hypothesized that ectopic activation of autophagy would be protective. Indeed, pharmacological activation of autophagy by feeding flies rapamycin protects the brain from ZIKV infection. This demonstrates that boosting autophagy is sufficient to protect the central nervous system from ZIKV infections. Whether autophagy plays a role in protecting mature mammalian neurons from ZIKV infection is unknown. Nevertheless, this work highlights a novel role for the Rel/NFKB-Sting-autophagy pathway in the control of neuronal infections and suggests new strategies for interventions.

Disclosure statement

No potential conflict of interest was reported by the authors.

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