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How Autophagy Saves Mice: A Cell-Autonomous Defense System against Sindbis Virus Infection

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Autophagy has diverse roles, including defense against infection. Levine and colleagues (Orvedahl et al., 2010) provide in vivo evidence for the antiviral function of autophagy in vertebrates: autophagy protects mice against lethal Sindbis virus CNS infection by degrading viral proteins whose accumulation would otherwise cause neuronal cell death.

In the cytoplasm of almost all eukaryotic cells, distinctive membranous structures called autophagosomes are generated de novo to engulf cellular components and deliver them into lysosomes for degradation. Such a cellular "self-eating" system, which is termed macroautophagy (herein referred to as autophagy), now draws enormous attention in diverse fields of life science, and remarkable progress in the past decade has revealed an unexpected degree of multifunctionality for autophagy. Now we know that autophagy plays roles in survival against starvation, cleaning of the cellular interior, innate and acquired immunity, lifespan extension, development, cellular differentiation, suppression of diseases (cancer, neurodegeneration, myocardial infarction, and diabetes), programmed cell death, etc. (Levine and Kroemer, 2008; Mizushima et al., 2008). Moreover, to our surprise, it has been discovered that autophagy targets intracellular invading pathogens, including bacteria, parasites, and viruses, for killing (Deretic and Levine, 2009). Thus, autophagy has been diverted from its role as a self-eating system and has taken on a role as a defense system against infection. Such defensive activity has been termed "xenophagy" (Levine, 2005).

Xenophagy has been well investigated in cultured cells. At the in vivo level, bactericidal autophagy has been studied using model organisms such as the fly and the mouse (Deretic and Levine, 2009). Regarding in vivo viral infection, whereas it has been shown that autophagy protects *Drosophila* from vesicular stomatitis virus (VSV) infection (Shelly et al., 2009), in this issue of *Cell Host & Microbe*, Orvedahl et al. (2010) report that autophagy is effective against viral infection in vertebrates. Orvedahl et al. used lethal Sindbis virus (SIN) central nervous system (CNS) infection in mice as a model system. First, they showed induction of autophagy by infection of the SIN strain SVIA in mouse embryonic fibroblasts (MEF). This induction required viral replication. The observation that autophagosomes sequestered SIN capsid or the virions together with the cytoplasm suggests that SIN is a target of autophagy rather than a hijacker of autophagy like poliovirus, which multiplies on the autophagosome membrane (Jackson et al., 2005). Indeed, the autophagic flow is enhanced in SIN-infected MEF, whereas poliovirus seems to suppress autophagosome-lysosome fusion for its benefit (Jackson et al., 2005). Autophagy induction by SIN infection and the capture of SIN proteins was also confirmed in vivo in the mouse brain.

Next, Orvedahl et al. explored the role of autophagy in the pathogenesis of SIN CNS infection in the mouse. They took three distinct strategies: (1) infection of wild-type mice with the recombinant chimeric SIN expressing a dominant-negative mutant (K130R) of Atg5, an essential protein for autophagy (Mizushima et al., 2001), (2) infection of *Atg5*$^{flox/flox}$ mice with recombinant chimeric SIN expressing Cre recombinase, and (3) infection of neuron-specific conditional *Atg5*-KO mice (*Atg5*$^{flox/flox}$;nestin-Cre mice) with SIN. In addition to the conventional method of infecting conditional *Atg5*-KO cells with SIN (strategy 3), the authors cleverly took advantage of the properties of SIN, which is a pathogen and simultaneously a vector for gene delivery in vivo, making it possible to eliminate Atg5 function only in infected neurons (strategies 1 and 2). As a result, the authors successfully demonstrated significantly increased mortality by SIN CNS infection under conditions of Atg5 inactivation in all three experiments, robustly indicating the importance of the autophagic gene in protection against virus infection in mammals in vivo.

How does Atg5 decrease mortality of SIN-infected mice? Intriguingly, there was no difference in the level of viral production irrespective of Atg5 activity both in mouse and in cultured cells (only one clone of *Atg5*$^{-/-}$ MEF showed higher titers of SVIA after infection compared to *Atg5*$^{+/+}$ MEF). Thus, Atg5 contributes to the protection against SIN CNS infection not through the control of viral multiplication, but instead, because Atg5 is essential for the clearance of SIN proteins (Figure 1). Failure to clear these proteins leads to neuronal apoptosis and then death of the mouse. Perhaps, accumulation of the surplus viral structural proteins not incorporated into virions (probably capsid protein; see below) and/or viral nonstructural proteins triggers cell death in neurons by unknown mechanisms. Autophagy may, therefore, protect neurons by sequestering and degrading these viral proteins (Figure 1). In the last part of their paper, the authors provide further insights into the mechanism for the clearance of the viral proteins. They showed that p62 is involved in SIN protein targeting to autophagosomes (Figure 1). p62 is known as an adaptor in selective

autophagy; it links the autophagosomal membrane-binding protein LC3 to targets (Pankiv et al., 2007). Coaccumulation of p62 and SIN proteins was observed in mouse neurons lacking Atg5 function, and p62 was coimmunoprecipitated with SIN capsid protein in virally infected HeLa cells. Knockdown of p62 in HeLa cells decreased the autophagic capture of capsid proteins. Finally, they demonstrated that siRNA of p62 and Atg7, another protein essential for autophagy, resulted in an increase in virus-induced cell death in vitro, supporting the idea that the antiviral function is not Atg5 specific but due to selective autophagic activity.

These seminal findings raise new questions. The observed antiviral response is not achieved by controlling the number of virions but by eliminating viral proteins that are toxic to cells. Therefore, this is not xenophagy *sensu stricto*; rather, it resembles autophagy against aggregate-prone proteins causing neurodegeneration (Mizushima et al., 2008). Is such a mechanism widely used in other viral infections, or is it unique to SIN CNS infection? Furthermore, although p62 usually binds to targets via ubiquitin, targeting of SIN capsid protein does not seem to be mediated by ubiquitination. Further investigation is required to know how p62 recognizes SIN capsid protein, whether recognition is direct or indirect, and which sequence in the capsid protein is recognized. In addition, the mechanism of cell death induced by the SIN protein remains cryptic. Can cell death be induced if only the capsid protein is expressed by gene transfection?

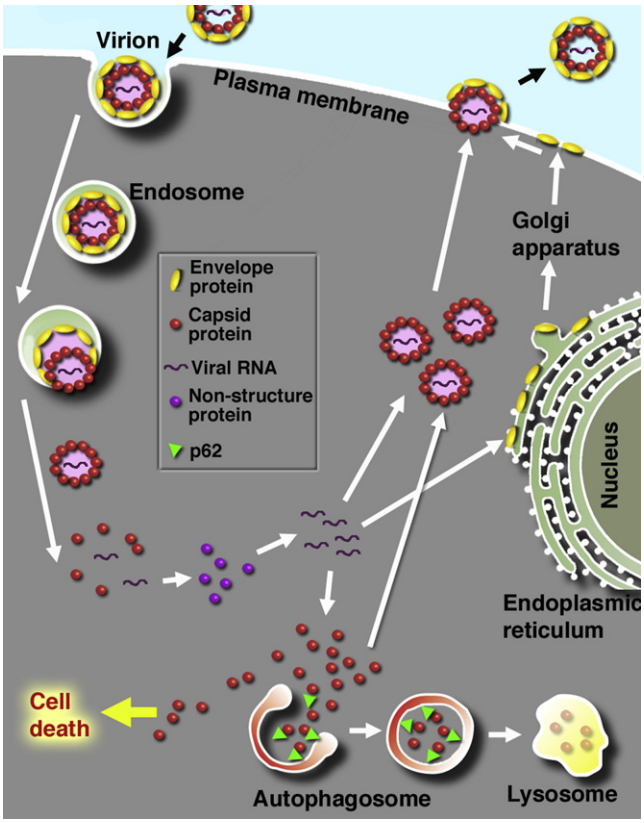

Figure 1. SIN Lifecycle and Autophagy
Endocytosis of the SIN virion is followed by fusion between viral envelope and endosome, disassembly (the core), and release of the genomic RNA. Then, nonstructural proteins (the replication proteins) are translated, which enable the replication of the genomic RNA and translation of structural proteins (capsid protein and envelope glycoproteins) from the subgenomic mRNA. Capsid proteins and the genomic RNA assemble the nucleocapsid core. Remaining capsid proteins are sequestered by p62-dependent selective autophagy and degraded upon fusion with lysosomes. If the autophagic clearance is hindered, accumulated capsid proteins induce cell death. The core associates the glycoproteins at the plasma membrane, resulting in virion budding.

It is now clear that autophagy is a host-defense response operating against infection by pathogenic microorganisms, and only in rare cases has autophagy been co-opted by the pathogens for their own benefit. The work by Orvedahl et al. opens new avenues for the study of autophagy as a cell-autonomous security system protecting against the threat of viral infection.

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