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Diane E. Alexander
Washington University School of Medicine in St. Louis

David A. Leib
Washington University School of Medicine in St. Louis

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Addendum

Xenophagy in herpes simplex virus replication and pathogenesis

Diane E. Alexander[1] and David A. Leib[1,2,*]

[1]Departments of Ophthalmology and Visual Sciences and [2]Molecular Microbiology; Washington University School of Medicine; St. Louis, Missouri USA

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Autophagy functions in part as an important host defense mechanism to engulf and degrade intracellular pathogens, a process that has been termed xenophagy. Xenophagy is detrimental to the invading microbe in terms of replication and pathogenesis and many pathogens either dampen the autophagic response, or utilize the pathway to enhance their life cycle. Herpes simplex virus type 1 (HSV-1) counteracts the induction of xenophagy through its neurovirulence protein, ICP34.5. ICP34.5 binds protein phosphatase 1α to counter PKR-mediated phosphorylation of eIF2α, and also binds the autophagy-promoting protein Beclin 1. Through these interactions, ICP34.5 prevents translational arrest and downregulates the formation of autophagosomes. Whereas autophagy antagonism promotes neurovirulence, it has no impact on the replication of HSV-1 in permissive cultured cells. As discussed in this article, this work raises a number of questions as to the mechanism of ICP34.5-mediated inhibition of autophagy, as well as to the role of autophagy antagonism in the lifecycle of HSV-1.

Autophagy, or perhaps more correctly a specific type of autophagy termed xenophagy,[1] is an important host defense mechanism against a number of chronic intracellular pathogens.[2-15] Typical of many microbial countermeasures against innate immunity, recent work has shown that autophagy is either inhibited by the invading pathogen, or exploited to actually enhance its replication cycle. The processes of autophagy and xenophagy and their alteration by microbes is, therefore, a newly-discovered pivotal aspect of the fascinating cat-and-mouse game of microbial pathogenesis and host-pathogen interactions.

One well-studied example of a xenophagy-altering factor is the herpes simplex virus type-1 (HSV-1) neurovirulence protein, ICP34.5 (Fig. 1). For some years ICP34.5 was known to counteract the host innate immune response mediated by PKR by directing the dephosphorylation of the translation initiation factor eIF2α through its ability to bind protein phosphatase 1α (PP1α).[16,17] This was shown to counter the translational arrest concomitant with PKR activation and eIF2α phosphorylation, thereby promoting viral replication and virulence. Recent data, however, consistent with autophagy being an eIF2α- and PKR-dependent pathway, shows that ICP34.5 antagonizes induction of xenophagy following HSV-1 infection.[13-15] It was originally thought that ICP34.5 controlled xenophagy solely through its ability to bind PP1α, but it was further discovered that a 20 amino acid stretch of ICP34.5 is responsible for its binding to Beclin 1, a protein required for autophagosome formation.[13] An HSV-1 recombinant lacking these 20 amino acids of ICP34.5 is severely neuroattenuated and fails to inhibit xenophagy, demonstrating that the interaction between ICP34.5 and Beclin 1 is required for full HSV-1 neurovirulence.

These findings raised a number of questions. First, are the severe replication defects in vitro and in vivo of ICP34.5-null viruses due to their inability to counteract autophagy, or their inability to dephosphorylate eIF2α and prevent the subsequent shutoff of protein synthesis? Second, what are the relative roles of the ICP34.5 Beclin 1- or PP1α-binding domains in the control of xenophagy? Finally, are other HSV-1 proteins involved in the regulation of xenophagy?

To answer the first question, we utilized primary *atg5*$^{-/-}$ murine embryonic fibroblasts (MEFs) which are unable to undergo autophagy[18,19] in conjunction with an ICP34.5-deficient virus.[20] This virus has a severe replication defect in primary wild-type MEFs that is completely restored in *pkr*$^{-/-}$ MEFs.[15] We hypothesized that the inability to control autophagy may contribute to the severe replication defect of an ICP34.5-null virus in wild-type MEFs and expected to observe partial restoration of replication in autophagy defective MEFs. Surprisingly, the replication of this ICP34.5-null virus was unchanged in *atg5*$^{-/-}$ MEFs suggesting that an inability to control xenophagy does not affect virus replication in cultured cells. In further support of these data, the Beclin 1 binding mutant and control wild-type viruses grew comparably in wild-type MEFs. These data are consistent with the idea that counteracting translational shutoff via regulation of the PKR pathway is the primary role for ICP34.5 in mediating efficient viral replication in vitro. This raises yet another question. Why is the inability to control xenophagy so important for replication in vivo, especially in the brain? The answer may lie in inherent differences between primary cultured cells and cells in vivo. Alternatively, xenophagy may be a more potent and critical anti-viral pathway in certain cell types, providing the host with a distinct advantage of having a relatively selective and non-destructive way to clear intracellular pathogens. This may be

*Correspondence to: David A. Leib; Department of Ophthalmology; Box 8096; Washington University School of Medicine; 660 South Euclid Avenue; St. Louis, Missouri 63110 USA; Tel.: 314.362.2689; Fax: 314.362.3638; Email: leib@wustl.edu

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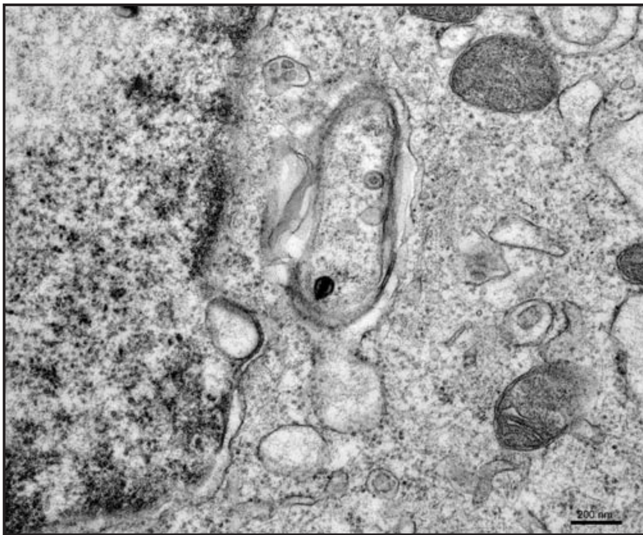

Figure 1. Representative electron micrograph of *atg5*$^{+/+}$ MEFs infected with an HSV-1 recombinant lacking ICP34.5. Higher numbers of autophagosomes and numbers of virions within autophagosomes were observed for ICP34.5-deleted viruses relative to wild-type. Scale bar = 200 nm.

especially important in organs such as the brain where cells are largely post-mitotic, and cytokine- and inflammatory cell-mediated damage would have an irreversible, devastating outcome. In support of this hypothesis, the Beclin 1 binding domain mutant was neuroattenuated and unable to efficiently replicate in the brains of mice. From the teleological perspective of the pathogen, therefore, control of autophagy may be more critical for growth in tissues with high constitutive autophagy levels.[21]

To answer the second question regarding the relative roles of Beclin 1- and PP1α-binding in ICP34.5-mediated control of xenophagy, we generated an ICP34.5 mutant that lacks the PP1α binding domain, but its Beclin 1-binding domain remains intact. By comparing the phenotypes of the ICP34.5 Beclin 1 binding mutant, the PP1α binding mutant, and that of a null mutant we are determining the roles of these domains in viral replication, pathogenesis, and control of xenophagy. Finally, we think it is likely that another HSV-1 protein, US11, contributes to the control of xenophagy. US11 blocks the activity and/or activation of PKR mediated by dsRNA or PACT.[22,23] Since autophagy is PKR-dependent, US11 likely acts in concert with ICP34.5 to control autophagy through counteracting the PKR pathway. As opposed to HSV-1 strains which lack ICP34.5, US11-null viruses grow normally in vitro and are slightly attenuated in vivo.[24-26] These small phenotypes may be due to compensation by the dominant effect of extant ICP34.5. We have therefore generated an HSV-1 double mutant that is unable to bind Beclin 1 and lacks US11and are determining whether US11 contributes to control of xenophagy.

It is clear that our understanding of the interrelationship between viruses and the autophagy pathway is in its infancy, but given the broad impact of xenophagy on many viruses, and their intricate subversive countermeasures, it seems likely that a wealth of information is likely to emerge in the coming years.[27] It is also possible that a better understanding of these pathways could lead to a whole new class of antiviral therapies, designed to augment the xenophagic degradation of otherwise hard-to-treat intracellular pathogens, or to deny certain pathogens access to the autophagic machinery which serves to promote their replication and disease.

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