Varicella Zoster Virus ORF9p: hijacker of the autophagy pathway?

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ORF9p (homologous to HSV-1 VP22) is a Varicella Zoster Virus (VZV) tegument protein essential for viral replication. We have previously shown that ORF9p interacts with the clathrin Adaptor Protein-1 (AP-1) complex and have identified the leucine at position 231 as important for this interaction. The mutation of this residue (VZV-ORF9-L231A-V5) strongly impairs viral replication in MeWo cells, and even more in MRC-5 cells. Further analysis suggests that the ORF9p/AP-1 interaction is important for glycoproteins trafficking. Indeed, while VZV-ORF9-V5-infected cells tend to fuse and form gigantic syncytia at 48hpi, such syncytia are rarely present in VZV-ORF9-L231A-V5-infected cells, even at 96hpi. Furthermore, flow cytometry experiments showed that at least gE is less expressed at the surface of L231A-infected cells. Additional experiments are ongoing to assess other glycoproteins such as gB, gH and gL, which are known to be implicated in cells fusion events.

In addition, transmission electron microscopy investigations revealed an accumulation of autophagic structures in L231A-infected cells, suggesting that the autophagy pathway could be differently regulated upon infection with VZV-ORF9-V5 or VZV-ORF9-L231A-V5. This idea is further supported by the fact that treatment with autophagy inhibitors has different effect on ORF9-V5- and L231A-V5-infected cells. Indeed, treatment with 3-methyladenine (3-MA), which blocks autophagosome formation, leads to 40% smaller infection foci with the ORF9-V5 at 48h post-treatment (hpt), while L231A-V5 foci are only 20% smaller compared to untreated controls. On the other hand, treatment with chloroquine, known to block autophagosome-lysosome fusion as well as lysosome acidification, has no noticeable effect on ORF9-V5 foci at 48hpt, while L231A-V5 foci are 10% smaller than untreated controls.

We thus hypothesize that the interaction between ORF9p and AP-1 is important not only for glycoproteins trafficking but also for hijacking the autophagy pathway in order to promote viral envelopment, the abrogation of ORF9p/AP-1 interaction leading to viral degradation. Further analyses are ongoing to better characterize the role of ORF9p in glycoproteins trafficking and in the autophagy pathway modulation and the link between these two processes.