

Interferon alpha suppresses alphaherpesvirus immediate early protein levels in sensory neurons, leading to the establishment of a latent infection

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Alphaherpesviruses are a subfamily of the herpesviruses containing closely related human and animal pathogens, including human herpes simplex virus (HSV-1) and porcine pseudorabies virus (PRV).

Cycles of latency and reactivation are a very important characteristic of these alphaherpesviruses, for example leading to recurrent episodes of cold sores and genital lesions in HSV. Neurons of the trigeminal ganglion (TG) are the predominant site of latency in both HSV-1 and PRV. Establishment of latency is generally thought to be the result of a delicate balance between virus, neuron, and unidentified immune effectors.

Using an *in vitro* 2-chamber model based on porcine TG cultures, we have identified interferon alpha (IFN) as an immune effector that is capable to drive both HSV-1 and PRV, in a latent state *in vitro*.

The IFN-induced establishment of *in vitro* latency was found to correlate with suppression of the immediate early (IE) protein ICP4 in HSV-1 and its homologue IE180 in PRV. IFN-mediated IE suppression was more efficient and rapid in HSV-1 than in PRV, correlating with a more efficient establishment of *in vitro* latency using HSV-1 versus PRV.

To further investigate the mechanism of IFN-mediated IE suppression and the differences in efficiency in IFN-mediated IE suppression between HSV-1 and PRV, we made use of rat dorsal root ganglion neuronal cells (50B11) (Chen *et al.*, 2007) because of the technical limitations associated with primary TG cultures.

At the protein level, at 4h post inoculation (hpi), for HSV-1, ICP4 protein expression was strongly reduced in IFN-treated samples (75% reduction) while for PRV, IFN treatment only slightly affected IE180 protein levels (15% reduction). At 8hpi and 12hpi the IE protein levels were significantly suppressed for both viruses. Using qRT-PCR, mRNA levels of either HSV-1 ICP4 or PRV IE180 at 4hpi were found not to be significantly different in IFN-treated samples versus control samples, whereas a strong reduction was observed at 8hpi and 12hpi (76.5 to 96%).

To investigate the lack of IE translation inhibition during PRV infection, we analyzed IFN-mediated phosphorylation and thereby inactivation of the translation initiation factor eIF2 α . As expected, treatment of cells with IFN and subsequent infection with HSV-1 resulted in a strong increase in phosphorylation of eIF2 α . However, this increase was entirely absent in PRV-infected cells, showing that PRV circumvents IFN-mediated translation inhibition interfering with phosphorylation of eIF2 α .

In summary, IFN-mediated suppression of viral IE proteins may be a key step in establishment of alphaherpesvirus latency. IFN acts at two stages to suppress IE protein levels: first at the translational level and later at the transcriptional level. However, PRV (but not HSV-1) is able to avoid IFN-mediated translational control of IE levels by phosphorylation of the translation initiation factor eIF2 α .