Nuclear IFI16 induction of IRF-3 signaling during herpesviral infection and degradation of IFI16 by the viral ICP0 protein

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AUTHOR SUMMARY

Sensing of microbial infection by the innate immune system is well documented at many cellular sites, including at the cell surface, in the cytosol, and in intracellular vesicles, but there is limited evidence of nuclear innate signaling. Innate sensing is mediated by binding of microbial macromolecules to host cell receptors, which trigger signaling pathways that lead to the expression of proinflammatory cytokines and interferons. Because of the similarities between viral and cellular DNA, innate sensing of DNA viruses is thought to be excluded from the nuclear compartment. However, herpesviruses replicate mainly in the nucleus of infected cells, and their DNA is a potent activator of the IFN regulatory factor-3 (IRF-3) signaling pathway, suggesting that these viruses may be sensed in the nucleus during infection. Using HSV-1 infection of normal human fibroblasts as a model system for the activation of IRF-3 signaling, we found that HSV DNA is sensed by the nuclear IFN inducible protein 16 (IFI16) DNA sensor, which is necessary to induce an innate immune response to HSV-1. Moreover, viral-encoded infected cell protein 0 (ICP0) counteracts this response by inhibiting IFI16 in the nucleus, preventing its activity as a transcription factor, and targets IFI16 for proteasomal degradation.

HSV-1 induces an innate immune response in human fibroblasts in the absence of viral gene expression (1). To investigate the initial activation of the IRF-3 signaling pathway in response to HSV-1, we infected primary human foreskin fibroblasts (HFF) with a replication-defective HSV-1 virus that does not express viral gene products and induces an innate immune response (2). We observed that inhibition of viral DNA release into the nucleus from incoming capsids by a serine protease inhibitor blocks virus-induced IRF-3-responsive gene expression, suggesting that viral DNA is sensed upon accumulation in the nucleus.

The IFI16 DNA sensor is important for sensing HSV-1 infection in human cells (3). Although initially described as a cytosolic sensor (3), IFI16 was found to be localized to the nucleus of HFF cells, suggesting that it might be involved in sensing HSV-1 viral DNA. Knockdown of IFI16 or STING by siRNA decreased IFN-β gene expression in response to HSV-1 infection but had no effect on the response to an RNA virus. CRM1-mediated nuclear export by leptomycin B treatment inhibited phosphorylation of the IRF-3 signaling component TBK-1; however, we did not observe export of IFI16 from the nucleus to the cytoplasm during infection, as has been described in Kaposi sarcoma-associated herpesvirus activation of inflammasome and NF-κB signaling (4).

The importance of the IRF-3 signaling pathway in restricting virus replication is strongly suggested by observations that HSV-1 gene expression inhibits IRF-3 signaling through multiple mechanisms (5). Expression of the viral immediate-early ICP0 E3 ubiquitin ligase is sufficient to inhibit this pathway (2), as indicated above; however, the mechanism of its inhibition is, to our knowledge, unknown. We therefore examined the localization of IRF-3 during infection with a virus that expresses ICP0. We observed that ICP0 inhibits signaling at two distinct steps during infection: (i) following IRF-3 activation and (ii) by inhibiting the pathway upstream of IRF-3 activation at later stages of infection. This later inhibition was observed concomitantly with the relocation of IFI16 to the cytoplasm and subsequent degradation of IFI16 in an ICP0-dependent manner. Either inhibition of the proteasome or infection with an ICP0 E3 ubiquitin ligase-defective mutant prevented the loss of IFI16.

Based on our results, we propose the following model for the activation and ICP0-mediated inhibition of the IRF-3 signaling pathway during HSV-1 infection (Fig. P1). In HFFs, HSV-1 infection is sensed initially by IFI16 upon the release of viral DNA into the nucleus. A nuclear-to-cytoplasmic signaling cascade initiates that activates IRF-3 and induces its accumulation in the nucleus. ICP0 expressed at early times during infection targets IFI16 for degradation, inhibiting additional signaling and activation of IRF-3. Our results define a pathway for nuclear innate sensing of HSV DNA by IFI16 in infected HFFs and reveals how a virus can block this nuclear innate response.