Type I IFN response to *Papiine herpesvirus* 2 (*Herpesvirus papio* 2; HVP2) determines neuropathogenicity in mice

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A B S T R A C T

Isolates of baboon α-herpesvirus *Papiine herpesvirus* 2 (HVP2) exhibit one of two distinct phenotypes in mice: extremely neurovirulent or apathogenic. Previous studies implicated the type I interferon (IFN) response as being a major factor in controlling infection by apathogenic isolates. To further investigate the possibility that the host IFN-β response underlies the pathogenicity of the two HVP2 subtypes, the susceptibility of mice lacking the IFN-β receptor (IFNAR$^{-/-}$) to infection was examined. Apathogenic isolates of HVP2 (HVP2ap) replicated in IFNAR$^{-/-}$ primary mouse dermal fibroblast (PMDF) cultures as well as neurovirulent (HVP2nv) isolates. IFNAR$^{-/-}$ mice were also susceptible to lethal infection by HVP2ap isolates. Unlike Balb/c or parental 129 mice, LD$_{50}$ and ID$_{50}$ values for HVP2ap were the same in IFNAR$^{-/-}$ mice indicating that in these mice infection always progressed to death. HVP2ap replicated in the skin at the site of inoculation and invaded dorsal root ganglia as efficiently as HVP2nv in IFNAR$^{-/-}$ mice. Since the virion host shutoff (vhs) protein encoded by the *UL4*1 gene of herpes simplex virus has been implicated in circumventing the host IFN-β response and the phenotype of *UL4*1 deletion mutants of HSV is very similar to that of HVP2ap isolates, the *UL4*1 gene was deleted from HVP2nv (Δ41) and replaced with the *UL4*1 ORF from HVP2ap (Δ41C). Like the parental HVP2nv virus, the Δ41C recombinant replicated efficiently in Balb/c PMDFs and did not induce a strong IFN-β response. The neuropathogenicity of the Δ41C recombinant was also the same as the parental HVP2nv virus in Balb/c mice, indicating that the vhs protein does not underlie the different neuropathogenic phenotype of HVP2ap and HVP2nv. In contrast, the Δ41 deletion virus induced a strong IFN-β response but was still able to undergo multiple rounds of replication in PMDF cultures, albeit at a slower pace than the parental HVP2nv. This was reflected in vivo as the *Δ41* mutant had an LD$_{50}$ equivalent to that of the parental HVP2nv virus although the time to death was longer. These results indicate that while the vhs protein is involved in preventing and/or suppressing an IFN-β response, it is not responsible for the ability of HVP2nv to overcome IFN-β induced resistance of uninfected cells and does not underlie the divergent pathogenicity of the two HVP2 subtypes in mice.

Introduction

The baboon herpesvirus *Papiine herpesvirus* 2 (*Herpesvirus papio* 2; HVP2) is very closely related to both herpes simplex virus (HSV) of humans and *Macacine herpesvirus* 1 (monkey B virus; BV) of macaques (Eberle and Hilliard, 1995; Huff and Barry, 2003; Keeble, 1960; Palmer, 1987; Weigler, 1992; Whitely and Hilliard, 2001). While BV causes a severe and usually fatal encephalomyelitis when transmitted to humans or other non-macaque primates (Huff and Barry, 2003; Loomis et al., 1981; Sabin and Wright, 1934; Thompson et al., 2000), there are no reported incidents of HVP2 infection or death in humans. However, in Balb/c mice one subtype of HVP2 (HVP2ap) does not produce clinical signs of disease and infection results in only minimal tissue destruction at both the site of inoculation and within both the peripheral and central nervous system (PNS and CNS). In contrast, the second subtype (HVP2nv) produces a fulminant, rapidly fatal CNS infection (Ritchey et al., 2002; Rogers et al., 2003, 2006). HVP2 has however caused fatal infections in young baboons (Wolf et al., 2006) and an HVP2nv isolate was also recently reported to be the cause of a fatal neurological infection in a Colobus monkey (Troan et al., 2007). The clinicopathogenesis of HVP2nv in mice closely parallels what has been observed in human BV infections and provides an excellent model system for examining host-virus interactions within the context of cross-species infections (Rogers et al., 2006).

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Within a particular species, it is the sum of multiple complex interactions between virus and host that determines the pathogenesis and clinical outcome of infection (Brandt, 2005; Enquist et al., 1998; Mossman and Ashkar, 2005; Rouse and Lopez, 1984). Thus, virtually any viral factor which affects the ability of a virus to replicate and/or spread within a host can be considered a virulence factor. In addition, both viral dose and route of inoculation can influence the ability of a virus to establish a productive infection within a given host (Breshears et al., 2005; Weeks et al., 2000). In HSV infection of mice, the efficiency of viral replication at the peripheral site of infection is directly related to the ability of the virus to enter sensory neurons and invade the CNS (Yamada et al., 1986). However, once a virus has gained entry to a host organism, the most important host determinant of pathogenicity is likely the host innate immune response (Mossman and Ashkar, 2005; Simons and Nash, 1984).

A critical component of the innate anti-viral response is production of the type I interferons α and β (IFN-α, IFN-β). IFN-α is rapidly produced by infected plasmacytoid dendritic cells (pDCs) or when they are stimulated by recognition of viral components via toll-like receptors (TLRs) either through endocytosis of viral particles or autophagy of infected cells (Lee et al., 2007). The ability of pDCs to mount an early IFN-α response is due to constitutive expression of the interferon regulatory factor (IRF)-7 while in other cell types, baseline IRF7 expression is weak and only moderately induced by viral infection (Dai et al., 2004; Lee et al., 2007).

IFN-β is rapidly induced in many cell types (including those infected by herpesviruses such as fibroblasts and epithelial cells) due to their constitutive expression of IRF-3. IFN-α and -β produced by infected cells binds to the IFN-α/β receptor (IFNAR) initiating a signal transduction cascade via the Janus associated kinase — signal transducer and activator of transcription (Jak-Stat) pathway (Darnell et al., 1994; Goodbourn et al., 2000). The end result of this pathway is that infected cells are recognized and destroyed while uninfected cells are protected from viral infection, thereby limiting the ability of the viral infection to spread. Although infected IFNAR$^{-/-}$ cells are capable of expressing IFN, the lack of the receptor for IFN-α/β prevents the signal transduction cascade via the Jak/Stat pathway, resulting in no amplification of the IFN-β response, and so neighboring uninfected cells remain sensitive to viral infection.

The pathogenic phenotype of HVP2ap in mice is similar to that described for HSV: normal replication in vitro but reduced neuro-pathogenicity in vivo (Rogers et al., 2003). HVP2ap-infected primary mouse dermal fibroblast (PMDF) cultures have also been shown to produce more IFN-β than HVP2nv-infected PMDF cultures (Rogers et al., 2007). Further, pretreatment of Balb/c PMDF cell cultures with exogenous murine IFN-β significantly decreased titers of HVP2ap but not HVP2nv, suggesting that HVP2nv is not effectively controlled by the mouse IFN-β response. Similar phenotypes have been observed with other alpha-herpesviruses and experimental animal models have proven invaluable in dissecting the mechanisms of numerous viral anti-IFN genes.

The virion host shutoff (vhs) protein of HSV1 and HSV2 encoded by the UL41 gene is probably the best known example of a herpesvirus protein responsible for abrogating the type I IFN response in infected cells (Duerst and Morrison, 2004; Korom et al., 2008; Suzutani et al., 2000). The vhs protein is a component of the virion tegument, and so is released into the cytoplasm of cells immediately on infection. The HSV vhs has RNase activity and functions to degrade certain mRNAs in infected cells (Esclatine et al., 2004; Everly et al., 2002; Karr and Read, 1999). Late in infection the vhs protein interacts with the viral α-transducing factor VP16. This interaction reduces the RNase activity of vhs, allowing accumulation of viral transcripts (Lam et al., 1996; Schmelter et al., 1996; Strand and Leib, 2004). Deletion of the UL41 gene results in higher levels of IFN-β production in infected cell
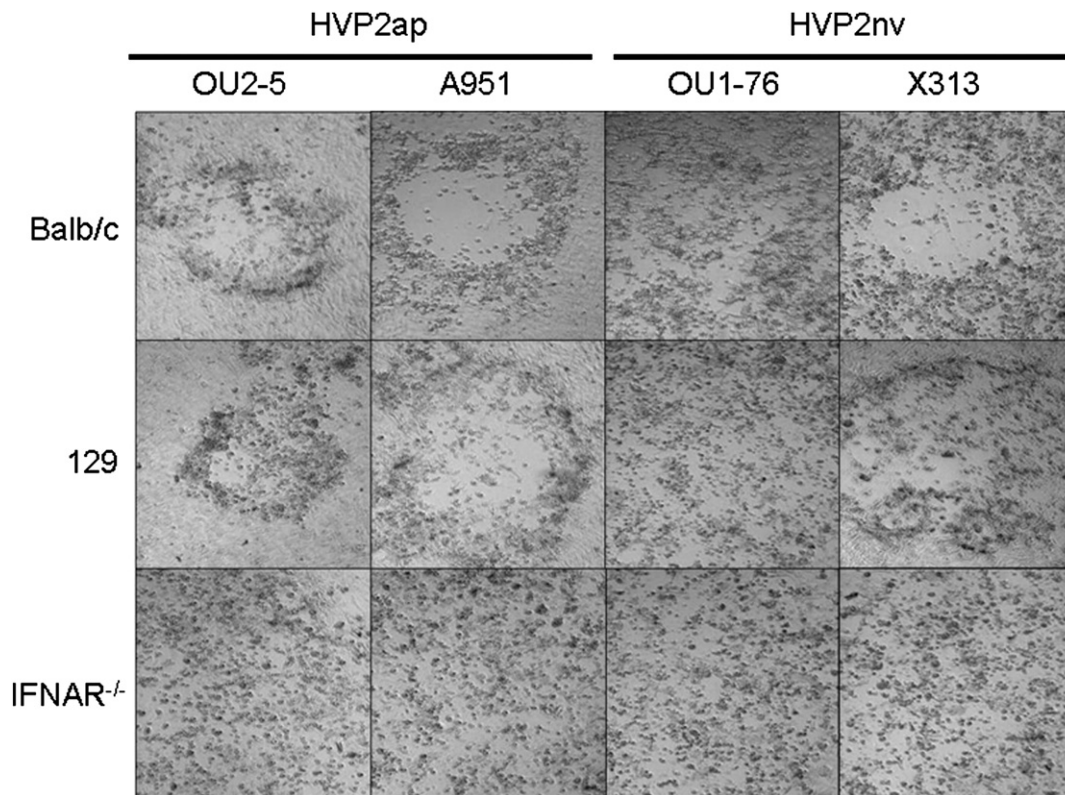
Fig. 1. Plaque formation by HVP2 subtypes in BALB/c, 129 and IFNAR$^{-/-}$ PMDF cultures. Confluent PMDF monolayers were inoculated at an MOI of 0.25 PFU/cell and photographed at 48 h PI. HVP2nv isolates produced complete CPE within 48 h in all three cell types. HVP2ap isolates produced discrete plaques by 24 h in Balb/c and 129 cell cultures, but these failed to enlarge with time. In contrast, HVP2ap isolates completely destroyed IFNAR$^{-/-}$ cultures by 48 h PI.