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Equine herpesvirus-1 infection disrupts interferon regulatory factor-3 (IRF-3) signaling pathways in equine endothelial cells

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ABSTRACT

Equine herpesvirus-1 (EHV-1) is a major respiratory viral pathogen of horses, causing upper respiratory tract disease, abortion, neonatal death, and neurological disease that may lead to paralysis and death. EHV-1 replicates initially in the respiratory epithelium and then spreads systemically to endothelial cells lining the small blood vessels in the uterus and spinal cord leading to abortion and EHM in horses. Like other herpesviruses, EHV-1 employs a variety of mechanisms for immune evasion including suppression of type-I interferon (IFN) production in equine endothelial cells (EECs). Previously we have shown that the neuropathogenic T953 strain of EHV-1 inhibits type-I IFN production in EECs and this is mediated by a viral late gene product. But the mechanism of inhibition was not known. Here we show that T953 strain infection of EECs induced degradation of endogenous IRF-3 protein. This in turn interfered with the activation of IRF-3 signaling pathways. EHV-1 infection caused the activation of the NF-κB signaling pathways, suggesting that inhibition of type-I IFN production is probably due to interference in IRF-3 and not NF-κB signal transduction.

1. Introduction

Equine herpesvirus-1 (EHV-1) is a member of the family *Herpesviridae* and subfamily *Alphaherpesvirinae*, which also include herpes simplex virus type-1 (HSV-1), bovine herpesvirus type-1 (BHV-1), pseudorabies virus and varicella zoster virus (VZV) (Pellett et al., 2012). EHV-1 infection causes mild to severe respiratory distress, early neonatal death in foals, sporadic abortions or abortion storms in pregnant mares, or the neurologic disease known as equine herpesvirus myeloencephalopathy (EHM) in adults (Allen et al., 2008; Kydd et al., 2006; Lunn et al., 2009; Nugent and Paillot, 2009; Pusterla et al., 2009). After initial replication in the respiratory epithelium, the virus is carried into regional lymph nodes and infects lymphocytes which enter the blood stream causing viremia (Kydd et al., 1994a,b). Through the circulation the virus reaches and infects the endothelial cells of the blood vessels of the placenta or spinal cord leading to vasculitis and thrombosis (Allen et al., 2004; Allen and Bryans, 1986) which causes abortion or EHM (USDA-APHIS, 2007). Although EHV-1 induced abortions are seen primarily in the late gestation, cases of abortions during mid-gestation have also been reported where mares aborted as early as 5 months of gestation (Doll and Bryans, 1963). Possible reasons for the reduced susceptibility to endometrial infection and abortion during early gestation relate to the developmental status of the placenta, as discussed by Allen et al. (1998). Once the horses are recovered from an EHV-1 infection, the virus may establish latency in these infected animals (Allen and Bryans, 1986). The latent virus can recrudesce at any time, especially during stress, and can initiate a new outbreak of EHV-1 infection.

In response to many viral infections the host cells mount an antiviral innate immune response by secreting type-I interferons (IFNs) such as IFN-α and IFN-β (Isaacs and Lindenmann, 1957). During viral infections, the viral motifs or pathogen associated molecular patterns are recognized by cellular pattern recognition receptors (PRRs) including toll-like receptors, retinoic acid inducible gene-I (RIG-I) like receptors, or melanoma differentiation associated gene-5 (MDA-5). These PRRs recruit several kinases which phosphorylate and activate transcription factors including interferon regulatory factor-3 (IRF-3), activating transcription factor-2/c-JUN (ATF-2/c-JUN) and nuclear factor κB (NF-κB). These activated transcription factors associate with CREB binding protein/p300 (CBP/p300) acting as co-activators in the nucleus to form the IFN-β enhanceosome, which induces transcription of IFN-β genes (Mossman and Ashkar, 2005; Pichlmair and Reis e Sousa, 2007). Secreted IFN-β signals through the JAK-STAT signaling path-

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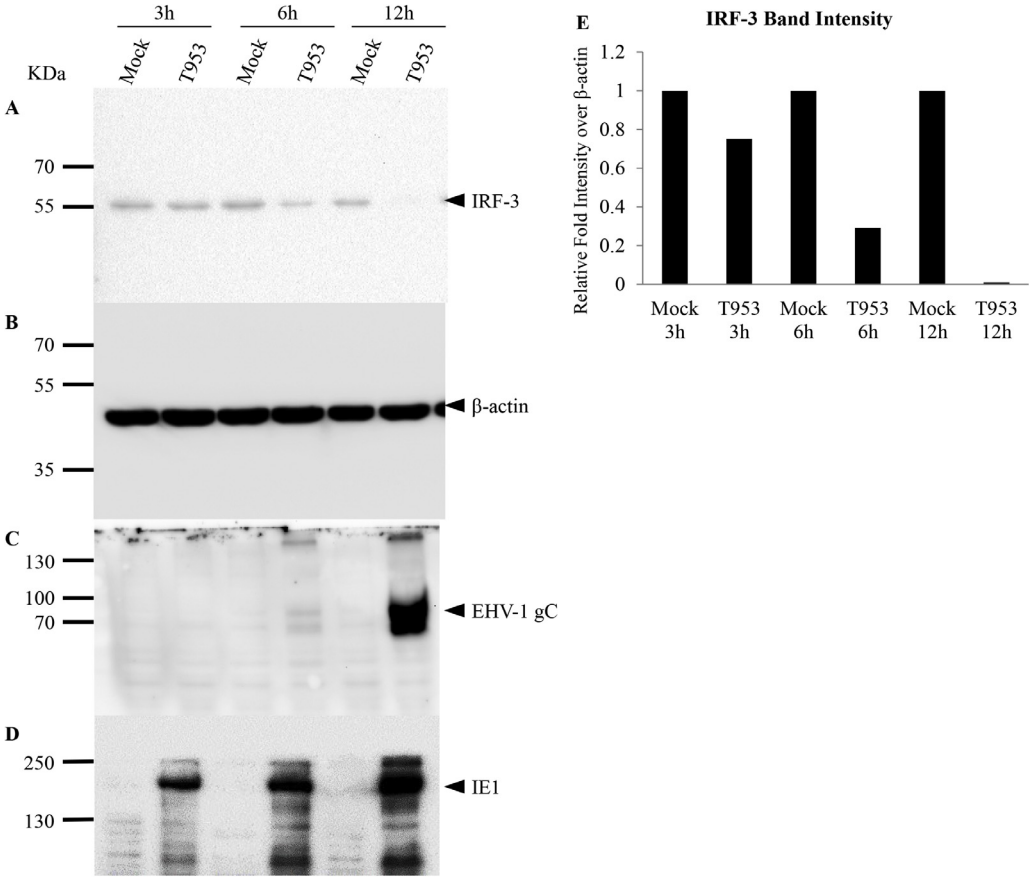

Fig. 1. Effect of EHV-1 T953 strain infection on endogenous level of IRF-3 protein. EECs were either mock infected or infected with T953. At indicated time points whole-cell lysates were prepared and equal amounts (40 μg) of lysates were separated by 10% SDS-PAGE and blotted with (A) anti-IRF-3, (B) anti-β- actin, (C) EHV-1 gC and (D) EHV-1 IE1 antibodies. β-actin was used as endogenous loading control. (E) Densitometric analyses were done by NIH ImageJ software and the IRF-3 band intensity was normalized to β-actin band intensity. The Western blot images were representative of 3 independent experiments.

ways to induce transcription of a number of interferon stimulated genes (ISGs) providing global antiviral resistance that limits virus replication and virus spread to surrounding cells (Mossman et al., 2001).

While some viruses like Sendai virus (SeV), vesicular stomatitis virus, transmissible gastroenteritis virus (Akerlund et al., 1996; Beura et al., 2010; Iannacone et al., 2010) induce host cells to produce IFNα/β, others like dengue virus, porcine reproductive and respiratory syndrome virus (PRRSV), and Rift Valley fever virus suppress IFNα/β induction (Aguilar et al., 2007; Beura et al., 2010; Billecocq et al., 2004; Jordan, 1973; Rodriguez-Madoz et al., 2010a,b). Some viruses like HSV-1, BHV-1, VZV, mouse cytomegalovirus (MCMV), lymphocytic choriomeningitis virus and influenza viruses first induce and then inhibit IFN-α/β production (Ambagala and Cohen, 2007; Le et al., 2008; Melroe et al., 2007; Mossman et al., 2001; Saira et al., 2007; Yu et al., 2011; Zhou et al., 2010). Because IFN-α/β production is a very important host defense mechanism, several viruses have evolved single or multiple strategies to evade this response. Thus, some viruses inhibit IRF-3 activation pathways by inhibiting IRF-3 phosphorylation (Basler et al., 2003), dimerization (Jennings et al., 2005), nuclear translocation (Wilson et al., 2008), or formation of IFN-β enhanceosome (Jennings et al., 2005). Viruses like PRRSV can also interfere with NF-κB signaling pathways in addition to the interference in IRF-3 signaling pathways (Song et al., 2010). Furthermore, herpesviruses can inhibit IFN-α/β induction by preventing IRF-3 phosphorylation, dimerization, nuclear translocation, and association with CBP/p300, or can enhance IRF-3 degradation (da Silva et al., 2011; Melroe et al., 2007; Paladino et al., 2010; Paladino and Mossman, 2009; Saira et al., 2007), or prevent NF-κB activation (Jones and Arvin, 2006).

EHV-1 also employs several mechanisms to evade host immune responses including inhibition of complement-mediated or T-cell mediated clearance, and suppression of MHC-I expression (Huemer et al., 1995; Rappocciolo et al., 2003; Sarkar et al., 2015; van der Meulen et al., 2006). We have previously shown that EHV-1 suppresses type-I IFN induction in infected equine endothelial cells (EECs) and this is possibly mediated by a viral late gene product (Sarkar et al., 2015). However, the mechanism of this suppression is not known. We hypothesized that EHV-1 infection could block the IRF-3 signaling pathways by either direct degradation of IRF-3 protein, or by blocking its activation or nuclear translocation. To address this, here we investigated the effect of EHV-1 infection on the IRF-3 and NF-κB signaling pathways in EECs.

2. Materials and methods

2.1. Cells and viruses

EECs derived from equine pulmonary artery endothelial cells (Hedges et al., 2001), were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Fisher Scientific, Rockford, IL) with 10% fetal bovine serum (FBS, Hyclone Laboratories, Inc., Logan, UT), 100 U/ml penicillin-streptomycin, 1 mM sodium pyruvate, 0.1 mM non-essential amino acids and 200 mM L-glutamine (Life Technologies, Carlsbad, CA) in a humidified incubator at 37° C with 5% CO_2. Rabbit kidney cells (RK-13, American Type Culture Collection (ATCC) CCL-