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Myxoma virus infection of primary human fibroblasts varies with cellular age and is regulated by host interferon responses

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Abstract

Recent studies have indicated a critical role for interferon (IFN)-mediated antiviral responses in the host range of myxoma virus (MV), a pathogenic poxvirus of rabbits. To investigate the contribution of IFN to MV tropism in nonleporine cells, primary human dermal fibroblasts (HDFs) were tested for permissiveness to MV infection. Low-passage HDFs that underwent fewer than 25 population doublings (PD) were fully permissive for MV infection, supporting productive virus replication and cell-to-cell spread. In contrast, early and late viral gene expression was detectable in high-passage HDF (>75 PD), but MV failed to generate infectious progeny and could not form foci in these cells. Vesicular stomatitis virus (VSV) plaque reduction assays confirmed that constitutive IFN production progressively increased as HDFs were passaged, concurrent with an increase in the expression of transcripts for type I IFN and IFN-responsive genes involved in antiviral responses. These findings correlated with the enhanced sensitivity of higher-passage HDF to inducers of type I IFN responses, such as dsRNA. Furthermore, pretreatment of low-passage HDF with type I IFN abrogated MV spread and replication while treatment of mature HDF with neutralizing antibodies to IFN-β, but not IFN-α, restored the capacity to form foci. These findings emphasize the importance of post-entry events in determining the permissiveness of human cells to MV infection and support a critical role for innate type I IFN responses as key determinants of poxvirus host range and species restriction.

Keywords: Poxvirus; Myxoma virus; Tropism; Host range; Interferon; Antiviral responses

Introduction

Myxoma virus (MV) is a leporipoxvirus that is the causative agent of myxomatosis, a lethal disease of European rabbits that presents with extensive fulminating lesions, immune dysfunction, and secondary bacterial infections of the respiratory tract (Fenner and Ratcliffe, 1965). Although MV exhibits strict species specificity for the rabbit and is nonpathogenic in other vertebrates, growing evidence indicates that MV can productively infect cells from diverse species in vitro. For example, MV has been recently shown to replicate in select murine cells (Johnston et al., 2003), as well as a broad spectrum of human tumor cell lines (Sypula et al., 2004). For most pathogens, the requirement for species-specific cell surface receptors that mediate virus entry is a critical determinant of host range (Mims, 1989; Schneider-Schaulies, 2000). However, no specific host cell receptor has ever been identified as being obligatory for infection by any poxvirus (Moss, 2001), suggesting that virus–host interactions that follow adsorption and entry of target cells determine permissiveness to poxvirus infection.

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MV has adapted to replicate successfully in the presence of vigorous host immunity by employing numerous strategies to evade, obstruct, or subvert elements that mediate antiviral responses to infection (Kerr and McFadden, 2002; Seet et al., 2003). In addition to sharing the ultimate goal of creating an intracellular environment that promotes productive virus infection, a central component of many of these strategies is the ability to manipulate cell signaling pathways critical to host antiviral responses (Greber, 2002). For example, MV infection rapidly activates kinase-mediated signal transduction that facilitates downstream events leading to productive infection (Johnston et al., 2003; Masters et al., 2001), inhibition of which renders cells nonpermissive (Johnston et al., 2003; Lalani et al., 1999). More recently, MV has also been shown to target signaling pathways involving nuclear factor (NF)-κB (Camus-Bouclainville et al., 2004). Although the actions of many of the immunomodulatory proteins encoded by MV are dispensable for replication in culture, they are often essential for replication within the host (Kerr and McFadden, 2002). Thus, the pathways targeted by MV to manipulate innate and adaptive host defense mechanisms have important consequences for viral tropism and the pathogenic effects of viral infections and may provide insight into the molecular mechanisms underlying poxvirus host range.

Recently, host antiviral responses mediated by type I interferon (IFN), which includes IFNα and IFNβ (Lau and Horvath, 2002), have been implicated as key determinants of the restrictive host range exhibited by MV in murine cells (Wang et al., 2004). Infected cells are likely capable of multiple response pathways to induce type I interferon-regulated genes, but it is believed that the virus–host tug-of-war is exerted in particular at the level of interferon regulatory factor (IRF)-3 activation, which is one of the key regulators of IFN-β gene expression (reviewed in Barnes et al., 2002; Levy et al., 2003; Malmgaard, 2004; Servant et al., 2002; Williams and Sen, 2003). If type I IFN is produced as a consequence of virus infection, the ligand is released into the extracellular milieu where it acts in both an autocrine and a paracrine fashion, binding to specific receptors on the surface of both infected and uninfected cells. This interaction activates signaling cascades, such as the signal transducer and activator of transcription (STAT)-janus kinase (JAK) pathway, leading to the expression of interferon-responsive genes. The activity of the resulting gene products promotes an antiviral state that decreases the susceptibility of uninfected cells to subsequent infection and impedes virus spread.

In fact, the actions of IFN are so effective at controlling the spread of viruses that all poxviruses employ at least one mechanism to disrupt their activity (Seet et al., 2003). These mechanisms primarily target host responses activated by dsRNA produced within infected cells during virus transcription, such as the IFN-dependent enzymatic cascades mediated by double-stranded RNA (dsRNA)-dependent protein kinase R (PKR) and the 2′,5′-oligoadenylate synthetase (OAS) (reviewed in Katze et al., 2002; Malmgaard, 2004; Taniguchi and Takaoka, 2002; Williams and Sen, 2003). PKR and OAS regulate response pathways that impede viral replication at multiple levels, promoting cell cycle arrest, a shutdown of host and virus protein synthesis and apoptosis. In an effort to inhibit these pathways, MV is predicted to encode a competitively inhibiting structural mimic of eukaryotic initiation factor (eIF)-2α, a substrate of PKR that induces growth arrest (Ramelot et al., 2002), as well as a dsRNA binding protein (Barrett et al., 2001). However, the exact mechanism of action for these proteins remains unclear.

In the current study, primary human dermal fibroblasts (HDFs) derived from neonatal foreskin explants were used to further investigate the contribution of type I IFN to MV host range and cell tropism. Neonatal HDFs initially exhibit decreased basal level IFN activity following explantation that increases with progressive population doublings (PD) as cells are passaged in vitro, a process commonly used as a model for cellular aging and the progression toward senescence (Komatsu et al., 1981; St. Geme and Horrigan, 1969). This progression manifests with increased type I IFN production, elevated levels of transcripts for IFN-responsive genes, and increased sensitivity to inducers of type I IFN responses. Of note, these properties are not unique to human fibroblasts, and similar responses have been reported for fibroblasts from other species, such as chick embryo fibroblasts (Lockart, 1968; Marcus and Carver, 1967) and murine L-cells (Jordan and Merigan, 1974).

Consistent with a critical role for IFN responses as determinants of MV host tropism, we report that primary HDFs are initially permissive to MV replication, but become refractory to MV infection with repeated passaging in culture. This loss of permissiveness correlates with increased basal levels of IFN activity in high-passage HDF compared to low-passage cells and can be completely reversed by inhibiting host IFN responses. These findings emphasize the importance of early events following recognition and entry of target cells in determining permissiveness to poxvirus infection and suggest a major role for IFN responses in the maintenance of the species barrier exhibited by many poxviruses.

Results

Human dermal explant cultures are permissive to MV infection

Primary HDFs were selected as a model system for these studies for several reasons. First, virus-enriched discharge from skin lesions that characterize MV infection of rabbits supports transmission of the virus by direct contact with an infected host, or more typically, via arthropod vectors such as the mosquito (Fenner and