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Parvovirus evades interferon-dependent viral control in primary mouse embryonic fibroblasts

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ABSTRACT

Engagement of innate viral sensors elicits a robust antiviral program via the induction of type I interferons (IFNs). Innate defense mechanisms against ssDNA viruses are not well defined. Here, we examine type I IFN induction and effectiveness in controlling a ssDNA virus. Using mouse embryonic fibroblasts (MEFs), we found that a murine parvovirus, minute virus of mice (MVMp), induced a delayed but significant IFN response. MEFs deficient in mitochondrial antiviral signaling protein (MAVS) mounted a wild-type IFN response to MVMp infection, indicating that RIG-I-dependent RNA intermediate recognition is not required for innate sensing of this virus. However, MVMp-induced IFNs, as well recombinant type I IFNs, were unable to inhibit viral replication. Finally, MVMp infected cells became unresponsive to Poly (I:C) stimulation. Together, these data suggest that the MVMp efficiently evades antiviral immune mechanisms imposed by type I IFNs, which may in part explain their efficient transmission between mice.

Introduction

Most cell types in mammalian hosts detect viral infection via cytosolic and nuclear pattern recognition receptors (PRRs), which sense the nucleic acid products of viral replication. The RIG-I-like receptors (RLRs), RIG-I (retinoic acid inducible gene-I) and MDA5 (melanoma differentiation-associated gene 5), are involved in sensing cytosolic RNA species, and activation via the adapter molecule MAVS leads to the production of type I IFNs, via IRF3, and of pro-inflammatory cytokines, via NF-κB (Hornung et al., 2006; Kato et al., 2005, 2006; Pichlmair et al., 2006). Viral DNA species found within the cytosol and nucleus are known to be detected by an ever-increasing group of PRRs, including RNA polymerase (Pol) III (Ablasser et al., 2009; Chiu et al., 2009), DAI (Takaoka et al., 2007), IFI16 (Unterholzner et al., 2010), and LRRFIP1 (Yang et al., 2010), which lead to downstream production of type I IFNs and pro-inflammatory cytokines in a cell- and context-specific manner.

The parvoviruses are a group of non-enveloped, single-stranded DNA viruses that infect a diverse range of species from rodents to humans. Parvoviral infections are responsible for significant clinical burden in many of the affected species. For instance, canine parvovirus and feline panleukopenia virus cause significant mortality in infected dogs (Goddard and Leisewitz, 2010) or cats (Truyen et al., 2009). There are a number of parvoviruses that infect humans, including erythrovirus B19, adeno-associated viruses, and the recently discovered human bocaviruses 1–4 and human parvovirus PARV4 (Brown, 2010). In humans, the best documented clinical manifestations occur with parvovirus B19 infection and include fifth disease, arthropathy, transient aplastic crisis, persistent anemia, and hydrops fetalis (Young and Brown, 2004). Infection with the prototype strain of MVM (MVMp) is asymptomatic in newborn mice, where it can only be detected by seroconversion, and non-pathogenic in adult mice, indicating a high degree of adaptation to its natural host (Kimsey et al., 1986).

Although, MVM has a high, RNA virus-like mutation rate, and exists as multiple in vivo and culture-adapted strains that infect a series of disparate or overlapping differentiated host cell types in vitro and in vivo (Cotmore and Tattersall, 2007), the prototype MVMp strain exhibits a pronounced tropism for fibroblasts. A recent report described activation of the innate immune response in murine embryonic fibroblasts (MEFs) infected with this virus (Grekova et al., 2010). This study found that MEFs from wild-type C57BL/6 and CD1 mice robustly produced type-I IFNs and upregulated anti-viral genes, such as protein kinase R (PKR) and 2′–5′ oligoadenylate synthetase (OAS), in response to MVMp infection (Grekova et al., 2010).

Here, we examined the role of MAVS, which is involved in the detection of RNA Pol III-synthesized RNA intermediates in

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response to dsDNA viruses (Ablasser et al., 2009; Chiu et al., 2009). We further probed the relevance of type I IFNs in the antiviral protection against MVMp infection. The results of this study suggest that parvovirus MVMp efficiently evades antiviral immune mechanisms imposed by type I IFNs in this cell type.

Results

MVMp infection in murine embryonic fibroblasts leads to delayed and limited activation of the IFN response

To examine the innate immune response to MVMp, we infected MEFs with MVMp and performed a time course experiment, which measured type I IFN mRNA induction by RT-qPCR during the first 72 h. Typical infection efficiency at this time point was around 30% (data not shown). We found a small, but statistically significant upregulation of IFN-α and IFN-β, especially at time points later than 48 h post infection (Fig. 1A). Consistent with these data, we found activation of STAT1, as measured by its phosphorylation, at later time points in MVMp-infected MEFs (Fig. 1B). The IFN response induced by MVMp infection occurred much later and was smaller in magnitude when compared to that following vesicular stomatitis virus (VSV) infection. These results demonstrated that MVMp infection in MEFs activates a delayed but measurable IFN response.

RNA pol III-dependent stimulation of the MAVS signaling pathway is not involved in the IFN response to MVMp in MEFs

We next tested whether MVMp infection results in the generation of RNA Pol III-dependent RNA recognized by RLRs. AT-rich DNA converted to 5′-triphosphate RNAs by RNA Pol III was used as a positive control. These RNAs activate RIG-I, which results in IFN production through downstream signaling via MAVS (Ablasser et al., 2009; Chiu et al., 2009). To test whether MVMp genomic DNA is able to stimulate the RNA Pol III pathway, we used HEK-293T cells, which are only able to induce IFNs in response to Poly (dA:dT) DNA through the RNA Pol III pathway (Ablasser et al., 2009; Chiu et al., 2009). These cells were transfected with a reporter plasmid that expresses luciferase under control of the IFN-β promoter. The cells were then transfected with genomic DNA purified from MVMp virions. Calf thymus DNA and Poly (dA:dT) DNA were used as negative and positive controls, respectively. When the cells were analyzed for luciferase activity, we found that transfection with MVMp genomic DNA failed to activate the IFN-β promoter in HEK-293T cells, whereas Poly (dA:dT) activated IFN-β robustly in these cells (Fig. 2A). These results indicate that the RNA Pol III-dependent IFN pathway is not activated by MVMp genomic DNA in HEK-293T cells.

Next, we examined whether MVMp-infected MEFs generate an RNA species capable of stimulating IFN through the MAVS signaling pathway. To this end, we infected wild-type MEFs with MVMp and purified total RNA from cellular extracts after 48 h. The purified RNAs were then used to transfect TLR7-deficient bone marrow cells. These cells were chosen to avoid contributions from TLR7 while probing the ability of cytosolic RNA sensors to detect purified RNA from MVMp infected cells. We found that TLR7-deficient bone marrow cells did not produce IFN-α (Fig. 2B) or IFN-β (Fig. 2C) in response to RNAs isolated by MVMp-infected cells beyond the level seen from RNAs isolated from uninfected cells. In contrast, the RNAs isolated from VSV-infected cells induced robust production of IFN-α and IFN-β in transfected TLR7-deficient bone marrow cells, as previously described (Kato et al., 2008). These results suggest that immunostimulatory RNAs are not produced in MVMp-infected MEF.

It remained possible that immunostimulatory RNAs are produced in MVMp-infected MEFs but remain undetectable in our assays. To further probe whether immunostimulatory RNAs stimulate type I IFNs in MVMp-infected MEFs, we examined MAVS-deficient MEFs. MAVS-deficient MEFs infected with MVMp generated IFN-α mRNA levels equivalent to wild-type MEFs, as measured by RT-qPCR 48 h post-infection (Fig. 2D). In addition, STAT1 phosphorylation in MAVS-deficient MEFs upon infection with MVMp was comparable to that seen upon infection of wild-type MEFs, further demonstrating that MAVS is not involved in the innate immune response to MVMp in MEFs (Fig. 2e). Taken together, these data suggest that the RNA-sensing pathways are not activated upon MVMp infection in MEFs.

MVMp infection is not blocked by type I IFNs

Next, we examined whether the low levels of secreted IFNs can actively inhibit MVMp infection of MEFs. First we examined IFN synthesis upon MVMp infection in IFN-αβR-deficient MEFs. Since IFN-α production is controlled by a positive feedback loop through

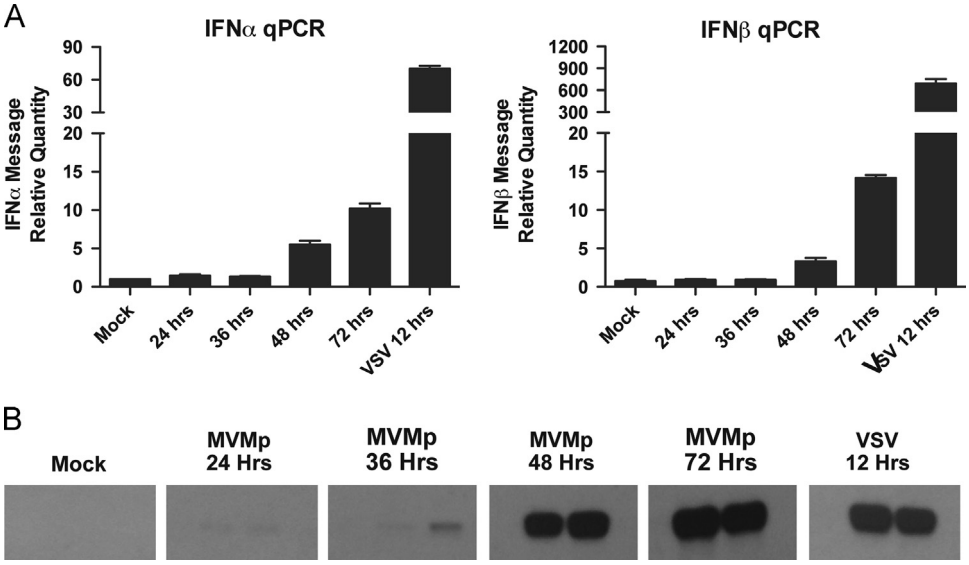

Fig. 1. MVMp activates a delayed and minor interferon response in MEFs. Wild-type MEFs were infected with MVMp (MOI 1) or VSV (MOI 1). (A) Cells were collected at the indicated time points and IFNa or IFNb mRNA levels were measured by RT-qPCR. (B) Cells were collected at the indicated time points, and lysates (in duplicate) were subjected to SDS-PAGE followed by Western blotting for phospho-STAT1.