Paramyxovirus Sendai virus V protein counteracts innate virus clearance through IRF-3 activation, but not via interferon, in mice

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Abstract

The present study was undertaken to clarify the role of Sendai virus (SeV) V protein, which has been shown to downregulate IFN-β induction through inhibition of IRF-3 activation, in viral pathogenesis. Mice infected with rSeV mutants, deficient in V expression or expressing V lacking the C-terminus, had several-fold higher IFN activity levels in the lungs than those in wild-type virus-infected mice, and the mutant viruses were rapidly excluded from the lung from the early phase of infection before induction of acquired immunity. In addition, the unique early clearance of the mutants did not occur in IRF-3 knockout (KO) mice. However, high titers of IFN were detected even in the infected KO mice. Furthermore, early clearance of the mutant viruses was also observed in IFN signaling-deficient mice, IFN-α/β receptor KO mice and STAT1 KO mice. These results indicate that SeV V protein counteracts IRF-3-mediated innate antiviral immunity for efficient virus replication and pathogenesis in mice, but it is not IFN.
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Introduction

The subfamily *Paramyxovirinae* of the family *Paramyxoviridae* includes many human and animal pathogens such as measles virus as well as the newly emergent Hendra and Nipah viruses, which can cause fatal zoonotic infections (Lamb and Kolakofsky, 2001). Viruses are subjected to various antiviral host responses upon infection, and interferon (IFN) responses play important roles in early innate immunity and in modulation of subsequent acquired immunity (Goodbourn et al., 2000; Sen, 2001; Taniguchi and Takaoka, 2002). Recent extensive studies have revealed that most paramyxoviruses encode specific viral proteins, accessory V and/or C proteins encoded by the *P* gene to circumvent the IFN system by blocking IFN signaling and limiting the production of IFN in infected cells (Garcia-Sastre, 2001, 2004; Goodbourn et al., 2000; Gotoh et al., 2001, 2002;

Horvath, 2004; Nagai and Kato, 2004). For instance, in simian virus 5 (SV5), which belongs to the genus *Rubulavirus* of *Paramyxovirinae,* the V protein targets STAT1 for proteasome-mediated degradation, thereby blocking both IFN-α/β and IFN-γ signaling within infected cells (Didcock et al., 1999a, 1999b), while the C protein, instead of the V protein, counteracts IFN signaling in the case of Sendai virus (SeV) of the genus *Respirovirus* of *Paramyxovirinae* (Didcock et al., 1999a, 1999b; Garcin et al., 1999; Gotoh et al., 1999). SeV C protein blocks IFN signaling by inducing abnormal phosphorylation and dephosphorylation of STAT1 and STAT2 and its binding to STAT1 (Gotoh et al., 2003a, 2003b; Kato et al., 2004; Komatsu et al., 2000, 2002; Takeuchi et al., 2001). In addition, it has become evident that V and C proteins downregulate the production of IFN in infected cells as well. The V proteins of SV5 and SeV have been shown to inhibit induction of IFN-β through blocking interferon regulatory factor (IRF)-3 and NF-κB activation (He et al., 2002; Komatsu et al., 2004; Poole et al., 2002). SeV C protein also blocks the signaling pathway leading

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to IRF-3 activation (Komatsu et al., 2004). Furthermore, RNA helicases, retinoic acid inducible gene I (*RIG-I*) and melanoma differentiation-associated gene 5 (*mda-5*) products, have recently been identified as the host factors for detecting intracellular dsRNA produced by virus replication and initiating antiviral responses (Andrejeva et al., 2004; Yoneyama et al., 2004). In addition, the cysteine-rich C-terminal domain of V protein, highly conserved among paramyxoviruses, has been demonstrated to bind mda-5, resulting in inhibition of activation of the IFN-β promoter through IRF-3 (Andrejeva et al., 2004; Yoneyama et al., 2005). Thus, the V and C proteins of paramyxoviruses are thought to play essential roles for evading host innate immunity, especially the IFN system, upon virus infection. However, it is still unclear how the inhibitory capacity of V protein against IRF-3 activation revealed *in vitro* is actually involved in *in vivo* viral replication and pathogenesis.

SeV infects exclusively respiratory epithelial cells of rodents and causes fatal bronchopneumonia (Ishida and Homma, 1978), and experimental infection of mice with SeV has provided a useful model for investigation of viral pathogenesis (Fujii et al., 2002; Kato et al., 1997a, 1997b; Kiyotani et al., 1990; Kurotani et al., 1998; Sakaguchi et al., 2003; Tashiro and Homma, 1983). SeV is an enveloped virus possessing a non-segmented single-stranded negative-sense RNA genome containing six genes in the order 3′-(leader)-*N-P-M-F-HN-L*-(trailer)-5′. The accessory proteins, V and C, are also expressed from the genome in infected cells. The V protein is generated from a *P* gene transcript subpopulation possessing an insertion of a nontemplated G residue at a specific editing site, and hence the V protein consists of a P/V common amino-terminal half and a V unique carboxyl-terminal half. The V-unique C-terminal region contains 15 amino acid residues highly conserved among almost all paramyxoviruses, including 7 cysteine residues that form a zinc finger-like motif and, indeed, bind Zn^{2+} (Fukuhara et al., 2002; Huang et al., 2000; Liston and Briedis, 1994; Paterson et al., 1995; Steward et al., 1995). On the other hand, C′, C, Y1 and Y2 proteins, collectively called C proteins, are encoded by an overlapping, shifted open reading frame of the upstream regions of the P and V mRNAs with multiple translational start codons and a common termination codon. The C protein is expressed by viruses belonging to three of the five genera of *Paramyxovirinae*: *Respirovirus*, *Morbillivirus* and *Henipahvirus* (Lamb and Kolakofsky, 2001; Nagai, 1999; Nagai and Kato, 2004).

We demonstrated previously by using a reverse genetics system that SeV V and C proteins are categorically nonessential gene products for viral replication but that silencing their expression severely impairs viral replication and pathogenesis, and we have suggested that the V and C proteins function for evading host innate immunity (Kato et al., 1997a, 1997b; Kurotani et al., 1998). However, C-knockout (KO) SeV [rSeV C(−)] and V-KO SeV [rSeV V(−)] were different with respect to their replication in cultured cells and in the mouse lung: replication of rSeV C(−) was severely impaired both in cultured cells and in mice, whereas rSeV V(−) propagated in cultured cells as efficiently as or more efficiently than the parental wild-type (WT) virus but showed a unique attenuated replication phenotype in mice. V(−) virus replicated in the mouse lung as efficiently as WT virus until

1 day after infection but was cleared from the lung thereafter far more rapidly than WT virus, indicating that SeV V protein encodes a *luxury function* required for *in vivo* viral replication and pathogenesis (Kato et al., 1997a). The pathogenicity determinant in the V protein was mapped to the highly conserved cysteine-rich C-terminal half (Fukuhara et al., 2002; Huang et al., 2000; Kato et al., 1997b). The unique characteristics of rSeV V(−) replication have been confirmed with a highly virulent SeV field isolate (Sakaguchi et al., 2003). The poor replication capacity of rSeV C(−) both in cultured cells and in mice could be explained by the multifunctional capacity of the C protein, with probable roles in virus assembly (Hasan et al., 2000; Sugahara et al., 2004) and control of viral transcription (Curran et al., 1992), in addition to inhibition of IFN signaling. It is unclear, however, whether the *luxury function* of SeV V protein required for *in vivo* viral replication and pathogenesis can be explained by its inhibitory effect on IRF-3 activation leading to negative regulation of IFN-β production because rSeV V(−) can replicate well even in cultured cells producing IFN, and IRF-3 also functions as a key activator of multiple cellular genes other than the immediate early IFN-α/β genes (Grandvaux et al., 2002).

In the present study, we investigated replication and pathogenesis of rSeV mutants of the V protein in mice possessing various deficiencies in innate immunity to clarify roles of the inhibitory capacity of V protein against IRF-3 activation in virus replication and pathogenesis. The results obtained indicate that propagation of the rSeV mutants in the mouse lung is suppressed by a cellular factor(s) induced by IRF-3, but it is not IFN, and that SeV V protein is required for counteracting IRF-3-induced innate immunity other than IFN to replicate efficiently in mice.

Results

Inhibition of rSeV V(−) replication at the early phase of mouse infection occurs independently of T cell functions

To investigate the involvement of T cell functions in rSeV V(−) clearance from infected mice, replication of V(−) virus was examined in T cell-deficient BALB/c[nu/nu] mice. rSeV WT propagated efficiently in BALB/c mice and was cleared from the lung by day 9 post infection (p.i.) (Fig. 1A). In BALB/c[nu/nu] mice, WT virus replicated more efficiently than it did in BALB/c mice, and a high virus load was maintained throughout the observed infection period (Fig. 1B). In contrast, in BALB/c mice, V(−) virus began to be cleared from day 2 p.i. in a stepwise manner and became undetectable on day 7 p.i., though the infectivity of V(−) virus on the first day p.i. was almost the same as that of WT virus (Fig. 1A), confirming previous results obtained in ICR mice (Kato et al., 1997a). In BALB/c[nu/nu] mice, rSeV V(−) grew as well as WT virus until the first day p.i. and then decreased rapidly to approximately 1/1000 of WT virus in infectivity by day 3 p.i. (Fig. 1B), as was observed in BALB/c mice. However, infectivity of V(−) virus in the lung began to increase again from day 5 p.i., and the titers remained thereafter in the order of 10^6 cell-infecting units (CIU), less than 1/10 of that of WT virus. These results indicate that clearance of rSeV