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Measles virus C protein suppresses gamma-activated factor formation and virus-induced cell growth arrest

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ABSTRACT

Measles virus (MeV) produces two accessory proteins, V and C, from the P gene. These accessory proteins have been reported to contribute to efficient virus proliferation through the modulation of host cell events. Our previous paper described that Vero cell-adapted strains of MeV led host cells to growth arrest through the upregulation of interferon regulatory factor 1 (IRF-1), and wild strains did not. In the present study, we found that C protein expression levels varied among MeV strains in infected SiHa cells. C protein levels were inversely correlated with IRF-1 expression levels and with cell growth arrest. Forced expression of C protein released cells from growth arrest. C-deficient recombinant virus efficiently upregulated IRF-1 and caused growth arrest more efficiently than the wild-type virus. C protein preferentially bound to phosphorylated STAT1 and suppressed STAT1 dimer formation. We conclude that MeV C protein suppresses IFN-γ signaling pathway via inhibition of phosphorylated STAT1 dimerization.

Introduction

Measles is a highly contagious acute febrile disease characterized by Koplik's spots and generalized maculopapular rash. Despite the availability of live attenuated vaccines, measles is still a severe problem and the cause of childhood mortality in developing countries. Alveobronchiolitis and encephalitides are the major causes of death from measles infection. Measles virus (MeV) is a member of the genus Morbillivirus of the family Paramyxoviridae. The MeV genome consists of six genes encoding the proteins N (nucleoprotein), P (phosphoprotein), M (matrix protein), F (fusion protein), H (hemagglutinin), and L (large protein) (Griffin, 2007). In addition, the P gene also encodes two accessory proteins, V and C. V is generated by RNA editing through the insertion of guanine at position 751 of the P gene nucleotide sequence (Cattaneo et al., 1989). As a result, the N-terminus of V protein has the same amino acid sequence as that of P protein. C protein is translated from another initiation codon (Bellini et al., 1985). A flame shift occurs so that the amino acid sequence of C protein is distinct from those of P and V proteins. V and C proteins have been reported to contribute to virulence and efficient viral replication. V protein has been shown to suppress interferon (IFN) signaling via interaction with Jak1 and STAT1 (Caignard et al., 2009; Caignard et al., 2007; Takeuchi et al., 2003) and to suppress IFN production via interaction with MDA-5, a cytosolic RNA sensor (Nakatsu et al., 2008; Ramachandran and Horvath, 2010). C protein supports virus replication, host cell growth, and apoptosis. It contributes to genome replication (Bankamp et al., 2005) and virus assembly (Devaux and Cattaneo, 2004). It releases the translational inhibition of viral RNA and suppresses IFN induction (Nakatsu et al., 2006; Nakatsu et al., 2008). C protein was also reported to inhibit IFN signaling (Shaffer et al., 2003); however, V protein was shown to be a more potent inhibitor of IFN-inducible gene expression than C protein (Fontana et al., 2008). On the other hand, some reports indicate that C protein has no effect on IFN-α/β or IFN-γ signaling (Nakatsu et al., 2008; Takeuchi et al., 2003).

Two types of MeV strains exist, namely the wild strains and Vero cell-adapted (Vero-adapted) strains (Yanagi et al., 2009). Wild strains infect host cells via the SLAM (CD150) as a receptor. On the other hand, MeV can adapt to SLAM-negative cells, such as Vero cells. The resulting Vero-adapted strains infect cells via CD46, which is expressed in various types of primate cells, as a receptor. Attenuated strains are Vero-adapted type. Strains isolated from clinical specimens using B95a cells or Vero cells with the human SLAM gene (Vero-hSLAM) are wild strains. In cynomolgus and squirrel monkey experimental infection models, the wild strains induce clinical symptoms that resemble human measles (Kobune et al., 1996). An alternative and still unidentified receptor for MeV is suggested to exist on epithelial cells (Takeda, 2008). Cell tropism and pathogenicity are considered to be different between wild strains and Vero-adapted strains (Takeda, 2008).

Our previous study indicated that epithelial cell lines infected with Vero-adapted strains showed growth arrest caused by the induction of interferon regulatory factor-1 (IRF-1) in an IFN-independent manner (Yokota et al., 2004). However, a wild strain showed less

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IRF-1 induction and less growth arrest than Vero-adapted strains. IRF-1 is an IFN-γ-inducible protein (Imanishi et al., 2000; Kroger et al., 2002). IFN-γ induces the activation of the transcription factor, gamma-activated factor (GAF), which is a homodimer of Tyr-phosphorylated STAT1, and the STAT1 is phosphorylated by Jak1 and Jak2 protein kinases which associated with IFN-γ receptor (reviewed in Goodbourn et al., 2000, and Stark et al., 1998). GAF translocates into the nucleus and binds to the gamma activation sequence (GAS) motif of the IFN-γ inducible gene promoters. Our previous data described that the induction of IRF-1 in Vero-adapted MeV-infected epithelial cells occurred in an IFN-γ-independent manner and constitutive phosphorylation of STAT1 and Jak1 is observed (Yokota et al., 2004). In this paper, we show that C protein suppresses the induction of IRF-1 via the inhibition of GAF formation.

Results

Viral protein expression in SiHa cells infected with various strains of MeV

All SiHa cells infected with MeV except for CAM70 showed similar levels of H, P, and V proteins determined by Western blotting (Fig. 1A). SiHa cells infected with CAM70 (SiHa-CAM70) expressed significantly lower levels of these proteins, and H and V proteins of CAM70 had a higher molecular weight than those of other MeV strains. The low levels in SiHa-CAM70 cells were consistent with the lower production of infectious particles reported previously (Yokota et al., 2004). Similar observation of higher molecular weight CAM70 V protein was reported (Fontana et al., 2008). The authors speculated that V protein of CAM70 had received post-transcriptional modifications, such as phosphorylation.

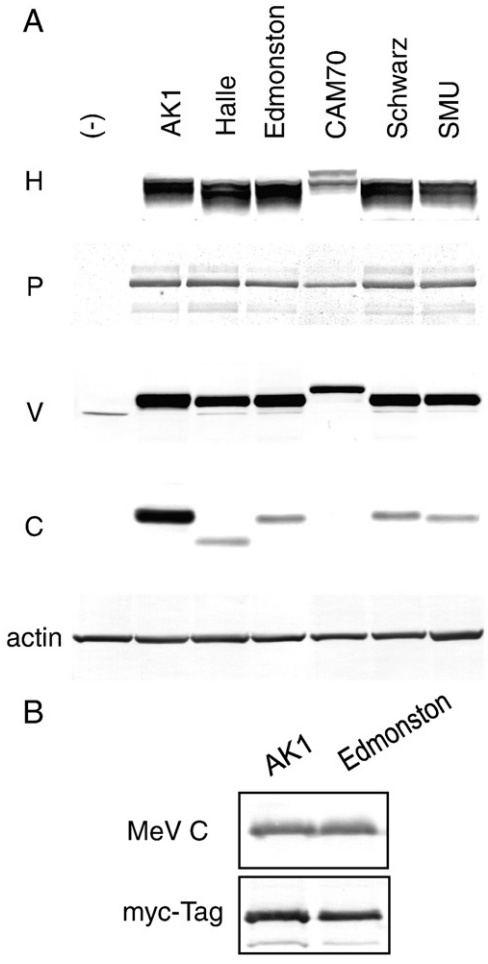

Fig. 1. Viral protein expression in SiHa cells infected with various MeV strains. A) Expression of viral proteins (H, P, V, and C) by Western blotting. Actin was used as a control for protein loading. B) Comparison of anti-C protein antibody reactivity toward C proteins derived from wild strain (AK1) and Vero-adapted strain (Edmonston). Recombinant myc-tagged C proteins were prepared using the rabbit reticulocyte in vitro translation/transcription system. C protein was detected by Western blotting using anti-C antibody and anti-myc-TAG antibody.

C protein expression varied among strains as shown by Western blotting (Fig. 1A). The anti-C antibody used in this study was prepared by immunization with a peptide consisting of amino acid residues no. 20 to 40 of a wild strain, IC-B (Takeuchi et al., 2003). Two amino acid residues in this region are different between the wild and Vero-adapted strains (Table 1). First, we examined the reactivity of anti-C antibody in C proteins derived from the wild strain AK1 and Vero-adapted strain Edmonston. Myc-tagged C protein derived from AK1 and Edmonston were prepared by in vitro translation. Western blotting with anti-C antibody and anti-myc antibody showed similar band intensity (Fig. 1B). This suggested that the anti-C antibody had a similar reactivity to wild strain C protein and Vero-adapted strain C protein.

C protein was not detected in SiHa-CAM70 by Western blotting. A smaller molecular weight C protein was detected in SiHa-Halle cells. Analysis of the nucleotide sequence of MeV P cDNA revealed a point mutation that introduced a stop codon at position of no. 168 of the amino acid sequence of C protein. Therefore, C protein was truncated in SiHa-Halle cells. Other cell lines infected with Halle showed ordinary molecular weight C protein or both the ordinary and truncated form (data not shown). The mutant of Halle strain with the truncation seemed to be selectively proliferated in SiHa cells. SiHa-AK1 cells showed a remarkably high level of C protein compared with cells infected with other MeV strains.

SiHa cells infected with MeV showed growth suppression. The degree of suppression varied among MeV strains (Fig. 2A) as we reported previously (Yokota et al., 2004). Growth suppression and the expression levels of C protein seemed to be inversely correlated (Fig. 2B). Basal expression levels of IFN-γ inducible genes, IRF-1 and CIITA, were determined by real time reverse transcription–PCR (RT–PCR) and Western blotting in MeV-infected SiHa cells (Figs. 2C and 3A). Levels varied among MeV strains and were inversely correlated with C protein levels and cell growth rate. These IRF-1 expressions were independent to IFN produced by the infected SiHa cells as previously reported (Yokota et al., 2004). In addition, IFN-γ-induced IRF-1 expression in SiHa-AK1 cells was significantly suppressed compared with uninfected SiHa cells and SiHa-CAM70 cells (Fig. 3B). These results suggested that SiHa-AK1 cells, which expressed higher levels of C protein, showed suppressed IFN-γ inducible gene expression both at basal and at IFN-γ-induced levels.

C protein suppresses MeV-induced IRF-1 transcription and growth arrest

We examined the effect of C protein expression on cell growth. Upon infection with a recombinant C-deficient MeV mutant derived from wild strain IC-B (ΔC), SiHa cells showed significant growth arrest (Fig. 4A) and IRF-1 upregulation (Fig. 4B). On the other hand, wild-type recombinant virus (wt) did not significantly affect cell growth or the expression of IRF-1. Infection and replication efficacy of the recombinant

Table 1
Differences in C protein amino acid sequences.

Strain	Virus type	Amino acids different from the Edmonston strain
Edmonston	Vero-adapted	–
SMU	Vero-adapted	–
Schwarz	Vero-adapted	M104T
Halle	Vero-adapted	W168Stop
CAM70	Vero-adapted	R13K
AK1	Wild	L25P, S39T, G44R, R78K
IC-B	Wild	L25P, S39T, G44R, R78K