Knockout of the Sendai virus C gene eliminates the viral ability to prevent the interferon-α/β-mediated responses

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Received 31 August 1999

Abstract Sendai virus (SeV) renders cells unresponsive to interferon (IFN)-α. To identify viral factors involved in this process, we examined whether recombinant SeVs, which could not express V protein, subsets of C proteins (C, C′, Y1 and Y2) or any of four C proteins, retained the capability of impeding IFN-α-mediated responses. Among these viruses, only the 4C knockout virus completely lost the ability to suppress the induction of IFN-α-stimulated gene products and the subsequent establishment of an anti-viral state. These findings reveal crucial roles of the SeV C proteins in blocking IFN-α-mediated responses.

Key words: Sendai virus; C protein; Interferon; Interferon-stimulated gene; Stat1; p48

1. Introduction

Interferons (IFNs) play important roles in early steps of host defense mechanisms and exert their biological effects through induction of IFN-stimulated gene (ISG) products including anti-viral proteins such as double-stranded RNA (dsRNA) dependent protein kinase (PKR) and 2′,5′-oligoadenylate synthetase (2-5AS) [1]. Binding of IFN-α/β to the cell surface receptors initiates activation of the receptor-associated tyrosine kinases, Jak1 and Tyk2. These activated kinases phosphorylate specific tyrosine residues of signal transducers and activators of transcription, Stat1 and Stat2, which then combine to p48 and migrate to the nucleus to function as active ISG factor 3 (ISGF3) [2,3]. ISGF3 binds to the IFN-stimulated response element (ISRE) in target genes and activates transcription of ISGs [4].

A variety of viruses have developed sophisticated strategies to counteract IFN-mediated induction of anti-viral states [5]. For example, adenovirus [6] and Epstein-Barr virus [7,8] prevent activation of PKR by producing abundant small RNA molecules that can bind to the dsRNA-binding site of PKR, whereas vaccinia virus and reovirus express dsRNA-binding proteins to prevent dsRNA from activating PKR [9–12]. In contrast to mechanisms directed to the anti-viral proteins, several viruses developed more ingenious strategies in which the IFN signal transduction is prevented [13]. The strategies could theoretically lead to suppression of all (more than 50) ISG products. Poxviruses including vaccinia virus and myxoma virus encode soluble IFN receptor homologues [14–18]. Adenovirus inhibits formation of ISGF3 by decreasing p48 [19,20], while human cytomegalovirus decreases Jak1 as well as p48, two essential components of the IFN-α signaling pathway [21]. Little is known, however, about whether these strategies are common among viruses.

We have recently found that Sendai virus (SeV), a prototype paramyxovirus, also has the ability to suppress the anti-viral action of IFN-α [22]. SeV is likely to be one of the viruses evolving the ingenious strategies, since the activation of an IFN-α/β-responsive promoter was inhibited in the SeV-infected cells [23]. Thus, we expected that detailed analysis of the suppression mechanism of SeV would elucidate a novel viral strategy directed to the IFN signal transduction. In this study, we especially focussed on viral factors responsible for the suppression mechanism. This mechanism was very unique and required neither viral genome replication nor secondary transcription [22]. In fact, Sendai virions inactivated by short-term UV-irradiation retained the ability to interfere with the IFN-mediated induction of an anti-viral state [22]. Thus, we speculated that responsible viral factors were encoded by the 3′ upstream region of the SeV genome such as the N or P/C gene (Fig. 1A). On the other hand, recent studies using gene knockout SeVs, which were created by technology of the reverse genetics [24,25], have demonstrated that the C and V proteins play crucial roles in in vivo growth and viral pathogenicity [26–30]. These findings prompted us to examine whether the C and V knockout SeVs retained the ability to impede IFN-mediated responses.

In addition to the previously generated V(−), C/C′(−) and 4C(−) viruses, which were unable to express the V, C plus C′ and any of four C (C, C′, Y1 and Y2) proteins, respectively, we used newly created recombinant C′(−) virus, which does not express C′ protein, in this study. We show here that among these viruses, only the 4C(−) virus completely loses the ability to prevent induction of ISG products as well as the establishment of an anti-viral state.

2. Materials and methods

2.1. Generation of recombinant SeV

Generation of 4C(−), C/C′(−) and V(−) viruses was described previously [27,29]. The C′(−) virus was newly generated essentially

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PII: S0014-5793(99)01241-7

as previously described [25,29]. The initiation codon for C′ was disrupted by site-directed mutagenesis (Fig. 1B). A pair of complementary primers (CP1: ^{1830}5′-CGAAGCCGcGGCTGTCTCGAC-3′^{1809} and CP2: ^{1809}5′-GTCGAGACAGCCgCGGCTTCG-3′^{1830}), which contain a mutation (shown by lower-case letters) in the initiation codon for C′, and outer primers (OP1: ^{61}5′-CAAAGTATCCACCCT-GAGGAGCAGGTTCCAGACCCTTTGCTTTGC-3′^{105} and OP2: ^{2468}5′-TTACTCTTGCACTATGTG-3′^{2451}) were used for generation of the mutated DNA fragment by two-step PCR-based overlap primer extension [31]. Nucleotides of primers were numbered according to Shioda et al. [32]. The mutated DNA fragment was digested with *Sph*I and *Sal*I and inserted to the corresponding position in pSeV(+), which can generate a full length copy of parental SeV positive sense antigenome, to create the mutant pSeV(+)/C′(−). Using the pSeV(+)/C′(−), the recombinant C′(−) virus was recovered as previously described [25,29]. Mutagenesis in the P/C gene of 4C(−), C/C′(−), C′(−) and V(−) viruses is summarized in Fig. 1B,C.

2.2. Cell cultures and virus propagation

HeLa and Vero cells were cultured in Eagle's minimum essential medium (MEM) supplemented with 10% bovine serum, 10% tryptose phosphate broth and 60 μg/ml kanamycin. Vesicular stomatitis virus (VSV) strain New Jersey was propagated in BHK cells. Recombinant SeV stocks, cDNA-derived Z strain as a wild-type (WT), 4C(−), C/C′(−), C′(−) and V(−) viruses (Fig. 1B,C) [27,29], were prepared by harvesting culture supernatant of virus-infected Vero cells incubated for 3–7 days at 32°C in serum-free MEM in the presence of 1 μg/ml of trypsin. Hemagglutinin titers were measured as previously described [33].

2.3. IFN and antibodies

Recombinant human IFN-α-2a was purchased from Takeda Chemical Industries (Osaka, Japan). Anti-PKR rabbit polyclonal antibody (SC-707), anti-p48 rabbit polyclonal antibody (SC-496), anti-Stat1 mouse monoclonal antibody (SC-464) and anti-Stat2 rabbit polyclonal antibody (SC-476) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Mouse neutralizing monoclonal antibody against human IFN-α (MMHA-2) and rabbit polyclonal antibody against human IFN-β were purchased from Cosmo Bio (Tokyo, Japan).

2.4. Assay of virus titers

VSV was titrated by the plaque assay on BHK cells. It was critical for this study to determine conditions under which all cells were actually infected with each recombinant SeV. Since it was difficult to determine exact titers of 4C(−) virus by the plaque assay, an alternative titration method, hemadsorption test, was employed for SeV. Cells in 24 well plates were infected with sequentially diluted SeV stock. At 20 h post-infection (pi), 1% chicken erythrocytes in phosphate-buffered saline were added to the cells. After incubation at 4°C for 30 min, cells were extensively washed and then fixed with chilled methanol. The maximum dilution of virus stocks, at which 100% of cells adsorbed erythrocytes, was determined. For all the experiments in this study, cells in 24 well plates were infected with SeV at around a 3-fold higher concentration relative to the maximum dilution of virus stocks or infected with VSV at a multiplicity of infection of 10 plaque forming units/cell.

2.5. Immunofluorescent staining

Cells propagated on round cover glasses (11 mm diameter) were fixed with chilled methanol and then stained by the double immunofluorescent method with anti-SeV rabbit serum and anti-VSV mouse serum. Rhodamine-labelled anti-rabbit IgG goat serum (Tago, Burlingame) and FITC-labelled anti-mouse IgG goat serum (Tago, Burlingame) were used as the second antibodies, respectively.

2.6. Western blotting

All steps were carried out at 0–4°C. Cells in 24 well plates were lysed in 25 μl of extraction buffer (50 mM HEPES, pH 7.6, 300 mM NaCl, 1% Triton X-100, 1 mM Na_3VO_4, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin and 2 μg/ml pepstatin) according to Lee et al. [34]. Each lysate (40 or 1 μg protein) was electrophoresed in 7.5 or 10% sodium dodecyl sulfate-polyacrylamide gels [35] for analysis of ISG products (Stat1, Stat2, p48 and PKR) or virus proteins, respectively. The proteins in the gels were electrotransferred onto nitrocellulose membranes (Bio-Rad, Richmond, CA, USA) and probed with the specific antibodies. As the second antibodies, horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse antibodies (Amersham Pharmacia Biotech, UK) were used. The proteins were detected using ECL detection reagent (Amersham Pharmacia Biotech, UK). The protein concentration was determined by the BCA protein assay kit (Pierce, Rockford, USA).

3. Results

The genome construct of SeV is shown in Fig. 1A. SeV has a non-segmented negative strand RNA genome of 15 384 nucleotides, which encodes the nucleocapsid protein (N), phosphoprotein (P), matrix protein (M), fusion protein (F), hemagglutinin-neuraminidase protein (HN) and large protein (L) as structural proteins. Only the P/C gene exceptionally encodes multiple species of proteins, the V, W, X, C′, C, Y1 and Y2 proteins besides the P protein (Fig. 1A) [36,37]. The V and W mRNAs are generated by pseudotemplated addition of one and two G residue(s) at a specific P/C gene region (1051–1053) (Fig. 1A,C), termed RNA editing, while the unedited exact copy of the P/C gene encodes the P protein. Therefore, the N-terminal amino acid sequence is shared with the P, V and W proteins (Fig. 1A). On the other hand, the C proteins, a nested set of four proteins (C′, C, Y1 and Y2),

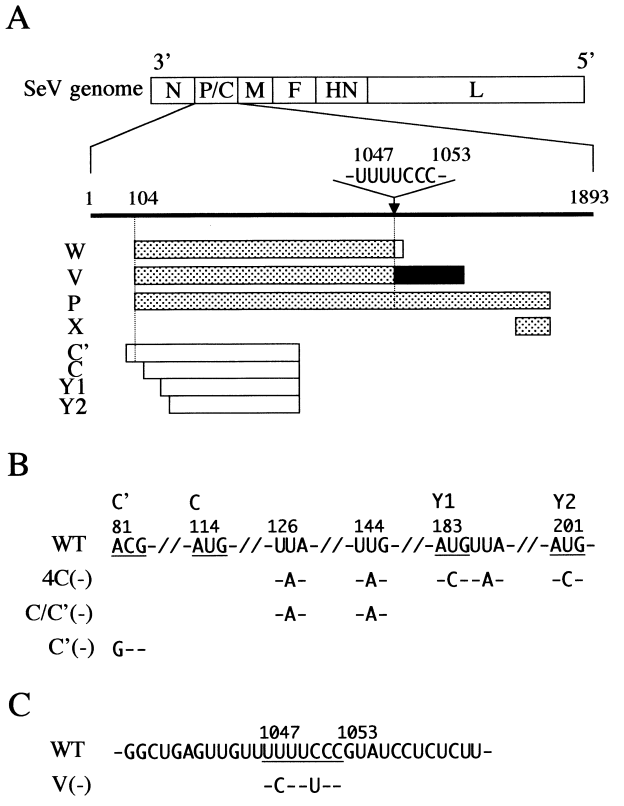

Fig. 1. Mutagenesis in the P/C gene for generation of 4C(−), C/C′(−), C′(−) and V(−) viruses. (A) The genome construct of SeV and protein species encoded by the P/C gene. The P/C gene encodes multiple proteins, V, W, X, C′, C, Y1 and Y2. The P ORF is shown as a dotted box, the V ORF in the −1 frame as a black box and the C ORF (+1) as an open box. (B) Sequence around the multiple initiation codons (underlined) in P mRNA and mutagenesis for generation of 4C(−), C/C′(−) and C′(−) viruses. Mutations were introduced to disrupt the initiation codons (114–116, 183–185 and 201–203) or to create the termination codons (126–128, 144–146 and 185–187). (C) Sequence around the RNA editing signal (underlined) in the genome and mutagenesis for generation of V(−) virus.

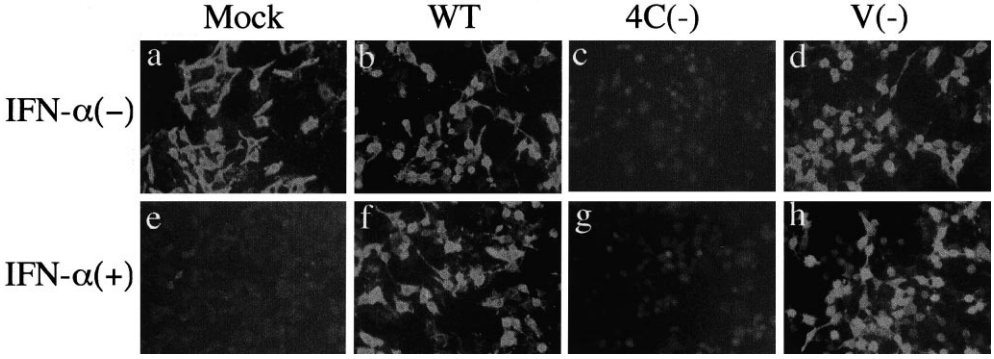

Fig. 2. Ability of WT, 4C(−) virus and V(−) viruses to rescue replication of VSV from the anti-viral action of IFN-α. HeLa cells were mock-infected (Mock) or infected with each recombinant SeV. One h after infection, the media were replaced with fresh media containing IFN-α (1000 IU/ml) or no IFN-α. Following incubation for 15 h, cells were superinfected with VSV. After further incubation for 6 h, cells were fixed with chilled methanol and stained by the double immunofluorescent method as described in Section 2. Only VSV fluorescence was shown.

initiated at different initiation codons, are encoded by an open reading frame (ORF) overlapping the P ORF in the +1 frame (Fig. 1A). The V(−), C′(−), C/C′(−) and 4C(−) viruses, which are unable to express the V, C′, C plus C′ and four C proteins, respectively, were generated as described in Section 2.

3.1. Loss of the ability of 4C(−) virus to impede the anti-viral action of IFN-α/β

VSV, one of the highly IFN-α/β-sensitive viruses, is used to assess whether an anti-viral state is established by IFN-α/β or not. First, we examined the ability of V(−) and 4C(−) viruses to rescue of VSV replication. HeLa cells were mock-infected or infected with WT, 4C(−) or V(−) virus and then treated with IFN-α (1000 IU/ml) at 1 h pi. Sixteen h after SeV infection, the cells were superinfected with VSV and further incubated for 6 h. Then, the cells were fixed and stained doubly by anti-VSV and anti-SeV antibodies. As shown in Fig. 2, VSV antigens were detected in most cells pre-infected with WT virus despite treatment with IFN-α (Fig. 2b,f) as described previously [22]. On the other hand, most of the 4C(−) virus-infected cells exhibited very little or no VSV fluorescence irrespective of treatment with exogenous IFN-α (Fig. 2c,g). In cells infected with V(−) virus (Fig. 2d,h), results were similar to those obtained in the WT virus-infected cells (Fig. 2b,f), although the ratio of cells with apparent VSV fluorescence to those with little fluorescence seemed to be lower than that in the WT virus infection on the whole. The absence of VSV antigens in the 4C(−) virus-infected cells was not due to failure of SeV infection since the double staining method confirmed that all cells were certainly infected with 4C(−) virus (data not shown). These results showed that V(−) virus retained the ability to block the IFN-mediated induction of an anti-viral state comparable to that of WT virus (Fig. 2b,d,f,h), suggesting that the V protein is not involved in this anti-IFN mechanism. On the other hand, 4C(−) virus appeared to lose the anti-IFN ability completely. It was, however, not clear why 4C(−) virus-infected cells did not allow for VSV replication even in the absence of IFN-α. We speculated that autocrine IFN-α/β induced by the SeV infection established an anti-viral state in the 4C(−) virus-infected cells because of the loss of the anti-IFN ability of 4C(−) virus. In fact, it is known that both IFN-α and IFN-β mRNAs are induced in HeLa cells in response to virus infection [38].

To check this, we examined effects of anti-IFN-α/β antibodies added to the medium on rescue of VSV replication. Fig. 3A showed Western blotting analysis of VSV proteins in infected cells in the presence or absence of anti-IFN-α/β anti-

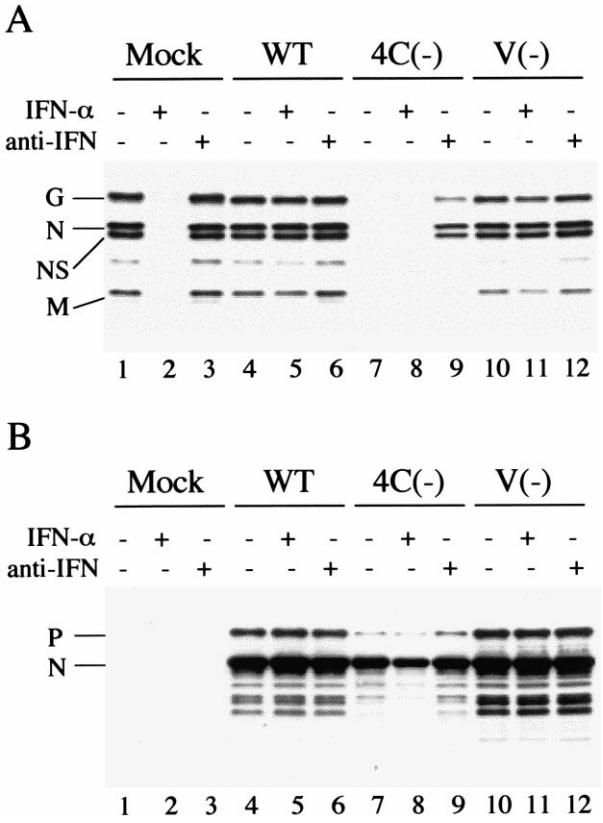

Fig. 3. Ability of WT, 4C(−) and V(−) viruses to suppress the anti-viral action of exogenous IFN-α and autocrine IFN-α/β. HeLa cells were mock-infected (Mock) or infected with each SeV. Two h after infection, the culture media were replaced with fresh media containing IFN-α (1000 IU/ml) (lanes 2, 5, 8 and 11) or no IFN-α (lanes 1, 4, 7 and 10). After incubation for 18 h, the cells were superinfected with VSV. Then, the cells were harvested at 6 h pi with VSV. The total cell extracts were subjected to Western blotting analysis with anti-VSV (A) or anti-SeV (B) serum. When cells were treated with anti-IFN-α/β antibodies (lanes 3, 6, 9 and 12), the culture media contained both neutralizing mouse monoclonal anti-IFN-α (final concentration 40 μg/ml) and rabbit polyclonal anti-IFN-β (final concentration 3000 U/ml) throughout the experiments.

bodies or exogenous IFN-α. Exogenously added IFN-α completely inhibited production of all viral components of VSV in HeLa cells (Fig. 3A, lane 2), while infection with WT or V(−) virus allowed for VSV protein synthesis (Fig. 3A, lanes 5 and 11). In the 4C(−) virus-infected cells, however, no accumulation of VSV proteins was observed irrespective of IFN-α treatment (Fig. 3A, lanes 7 and 8). These results were in good agreement with those in the immunofluorescent experiment described above.

Anti-IFN-α/β antibodies treatment did not affect notably VSV protein synthesis in WT or V(−) virus-infected cells (Fig. 3A, lanes 6 and 12). In contrast, the accumulation of VSV antigens was dramatically enhanced in the 4C(−)-infected cells (Fig. 3A, lane 9), indicating that autocrine IFN-α/β at least in part contributed to suppression of VSV protein synthesis in the 4C(−) virus-infected cells without exogenous IFN-α. We also examined SeV protein synthesis in the infected cells. Accumulation of the 4C(−) virus proteins was found to be less than that of WT or V(−) virus proteins (Fig. 3B, lanes 4, 7 and 10). Neither anti-IFN-α/β antibodies nor exogenous IFN-α affected WT and V(−) virus protein syntheses (Fig. 3B, lanes 6 and 12), while the 4C(−) virus protein synthesis was found to be suppressed or recovered to some extent by IFN-α treatment or by anti-IFN-α/β antibodies treatment, respectively (Fig. 3B, lanes 8 and 9). These results demonstrated that SeV protein synthesis was actually influenced by autocrine IFN-α/β in the absence of the SeV C proteins.

3.2. Complete loss of the ability of 4C(−) virus to suppress induction of ISG products

As described above, an anti-viral state was induced in the 4C(−) virus-infected cells, but not in the WT and V(−) virus-infected cells. To see this difference at a level of host cell gene expression, we examined the ability of each virus to impede induction of ISG products. Stat1, Stat2 and p48, components of ISGF3, were chosen for the ISG products [39] besides PKR. HeLa cells were mock-infected or infected with each SeV and treated or untreated with IFN-α at 2 h pi. Cells were harvested 18 h after IFN-α treatment. Fig. 4 showed that IFN-α treatment remarkably increased Stat1 (Stat1α and Stat1β) and p48, in the 4C(−) virus-infected cells (Fig. 4, lane 11) comparable with those in mock-infected cells (Fig. 4, lane 3), whereas in the WT or V(−) virus-infected cells, induction of Stat1 and p48 by autocrine IFN-α/β or exogenous IFN-α was strikingly suppressed (Fig. 4, lanes 6, 7, 14 and 15). As expected, even in the absence of exogenous IFN-α, Stat1 and p48 were markedly induced in the 4C(−) virus-infected cells (Fig. 4, lane 10). On the other hand, anti-IFN-α/β antibodies suppressed increase of these ISG products in the 4C(−)-infected cells (Fig. 4, lane 12), confirming that autocrine IFN-α/β actually acted to establish the anti-viral state on the 4C(−) virus-infected cells. Similar results were obtained in the induction of Stat2 and PKR (data not shown). These results, consistent with those observed in Fig. 3, suggested that the 4C(−) virus allowed for the establishment of an anti-viral state in the infected cells by the complete loss of the ability to prevent induction of ISG products.

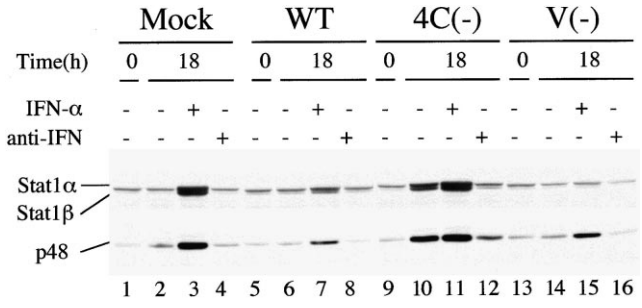

Fig. 4. Ability of WT, 4C(−) and V(−) viruses to suppress induction of Stat1α,β and p48. HeLa cells were mock-infected (Mock) or infected with each SeV. The culture media were replaced at 2 h pi with fresh media containing IFN-α (1000 IU/ml) (lanes 3, 7, 11 and 15) or no IFN-α (lanes 1, 2, 5, 6, 9, 10, 13 and 14). The cells were further incubated for 18 h until cell harvesting. When cells were treated with anti-IFN-α/β antibodies, the treatment conditions were the same as those in Fig. 3. The total cell lysates were analyzed by Western blotting with anti-Stat1 and anti-p48 antibodies.

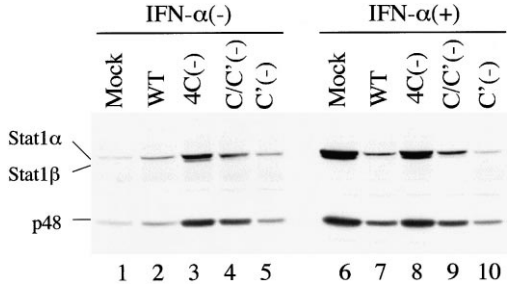

Fig. 5. Ability of C/C′(−) and C′(−) viruses to suppress induction of Stat1 and p48. HeLa cells were mock-infected (Mock) or infected with each virus. The media were replaced at 2 h pi with fresh media containing IFN-α (1000 IU/ml) (lanes 6–10) or no IFN-α (lanes 1–5). The cells were harvested after a further 18 h incubation. The total cell lysates were analyzed by Western blotting with anti-Stat1 and anti-p48 antibodies.

3.3. Ability of C/C′(−) and C′(−) viruses to suppress induction of ISG products

The ability of C/C′(−) and C′(−) viruses to suppress induction of ISG products was also examined. In the C/C′(−) virus-infected cells, Stat1 and p48 were induced to some extent probably by autocrine IFN-α/β in the absence of exogenous IFN-α (Fig. 5 lane 4), but the level of the induction was inferior to that in the 4C(−) virus-infected cells (Fig. 5 lane 3). Induction of Stat1 and p48 was strongly suppressed in the C′(−) virus-infected cells (Fig. 5, lane 5). When cells were treated with exogenous IFN-α, the difference between the 4C(−) and the other viruses became more evident. Induction of Stat1 and p48 in the C/C′(−) virus-infected cells was found to be suppressed considerably compared with that in the 4C(−) virus-infected cells. The suppression by C′(−) virus appeared to be stronger than that of WT virus. Similar results were obtained in the induction of Stat2 and PKR (data not shown). The Y1 and Y2 proteins were overexpressed in HeLa cells infected with C/C′(−) virus (data not shown) as previously described [29]. Therefore, overexpressed Y1 and Y2 proteins largely compensated for the loss of functions of the C and C′ proteins and could contribute to suppression of induction of the ISG products to some extent.

4. Discussion

The present study demonstrated that both WT and V(−)

viruses blocked the induction of ISG products (Stat1, p48, Stat2 and PKR) and consequently prevented the establishment of an anti-viral state. Didcock et al. reported that transfection of a plasmid carrying a synthetic promoter containing multimers of the well-defined ISRE linked to the luciferase reporter gene did not respond to IFN-α/β in cells infected with SeV [23]. They further showed that induction of the IFN-responsive 6-16 gene was inhibited by SeV infection. Together with their results, our findings support the idea that SeV interferes with the IFN-α/β signal transduction.

In contrast to WT and V(−) viruses, the 4C(−) virus completely lost the ability to suppress the IFN-mediated responses, demonstrating that the C proteins play crucial roles in preventing IFN-mediated induction of an anti-viral state. It is unlikely that a low accumulation of SeV proteins in infected cells (Fig. 3B, lane7) is responsible for loss of the anti-IFN ability of 4C(−) virus, because even replication-incompetent SeV can suppress the IFN-α/β-mediated anti-viral responses [22]. Rather, previous double immunostaining experiments showed that detectable levels of viral proteins were not required for the anti-IFN ability [22]. Recently, Garcin et al. have reached essentially the same conclusion using mutant viruses containing a single amino acid substitution in the C protein and similar recombinant viruses which do not express subsets of the C proteins [40]. They concluded that the AUG^{114}-initiated C protein prevents the establishment of an anti-viral state, from the results showing that not only the double mutant corresponding to the C/C′(−) virus but also the C(−) mutant allowed for IFN-α/β-mediated induction of Stat1 and lost the ability to rescue VSV. We, however, found that C/C′(−) virus retained the considerable ability to suppress the ISG products, although the suppression is slightly weak compared with WT and C′(−) viruses (Fig. 5). Therefore, our results suggest that Y1 and Y2 proteins can also play a critical role in prevention of the IFN-mediated responses. Although the reasons for the discrepancies between our and their results are unknown, they might be due to a difference in cell lines used or experimental conditions.

Growth of 4C(−) virus in ovo was very poor and was reduced by several logs, compared with that of WT virus [29]. Even CC′(−) virus was almost totally incapable of replicating in the mouse lung [29]. Therefore, 4C(−) virus is likely to be more avirulent than CC′(−) virus. This possible attenuation as well as poor growth of 4C(−) virus in ovo is explainable in part by loss of the ability of 4C(−) virus to prevent autocrine IFN-α/β from inducing an anti-viral state, as shown in our in vitro study presented here. Although SeV protein synthesis was actually suppressed by autocrine IFN-α/β in the 4C(−) virus-infected HeLa cells (Fig. 3B), its extent was not so striking when compared with the inhibition of VSV protein synthesis (Fig. 3A). This difference is probably due to the timing of the action of IFN-α/β. In the experiments shown in Fig. 3A, VSV infection followed establishment of the anti-viral state (Fig. 4), while SeV infection preceded it. Thus, the timing of virus infection to IFN treatment is an important factor for blocking IFN-mediated responses. We confirmed that SeV infection could not prevent IFN-mediated responses in cells pre-treated with IFN-α as reported by Didcock et al. [23]. On the other hand, it is well known that SeV is a good inducer of IFN-α/β. Therefore, it remains to be elucidated why WT virus can spread to neighboring cells in which an anti-viral state has already been established by the action of paracrine IFN-α/β. Why has SeV evolved the anti-IFN strategy in contrast to VSV? We previously reported that VSV protein synthesis was not inhibited at all by IFN-β treatment, if IFN-β was added to cells concomitantly with VSV infection [41]. Therefore, it is unlikely that VSV growth is affected by autocrine IFN-α/β. VSV, due to its rapid growth, may not need an anti-IFN strategy like SeV. Thus, we speculate that SeV must have evolved the anti-IFN strategy in compensation for its slower growth.

We observed acceleration of cytopathic effects in the 4C(−) virus-infected HeLa cells (IFN-α/β producing cells) compared with those in the WT virus-infected cells (unpublished results), although we did not determine whether the cytopathic effects were due to apoptosis. Therefore, the anti-IFN mechanisms may be linked to induction of apoptosis as speculated by Garcin et al. [40], since activation of PKR or 2-5AS induces apoptosis [42–45]. If the ability of SeV to inhibit IFN-α/β-mediated responses is also responsible for delay in deterioration of cell functions, it will be of advantage to the virus growth.

Acknowledgements: We thank S. Kubo for excellent technical assistance. We also thank Y. Ohnishi for technical advice and constant encouragement. This work was supported in part by a grant from the Ministry of Education, Science, Sports and Culture, Japan.

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