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Dissection of measles virus V protein in relation to its ability to block alpha/beta interferon signal transduction

Shinji Ohno,[1] Nobuyuki Ono,[1]† Makoto Takeda,[1] Kaoru Takeuchi[2] and Yusuke Yanagi[1]

[1]Department of Virology, Faculty of Medicine, Kyushu University, Fukuoka 812-8582, Japan

[2]Department of Infection Biology, Institute of Basic Medical Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8575, Japan

Correspondence
Yusuke Yanagi
yyanagi@virology.med.kyushu-u.ac.jp

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Interferon (IFN)-α and -β are the main cytokines for innate immune responses against viral infections. To replicate efficiently in the hosts, viruses have evolved various countermeasures to the IFN response. The V protein of measles virus (MV) has been shown to block IFN-α/β signalling. Here, the wild-type IC-B strain of MV was shown to grow comparably in the presence and absence of IFN-α, whereas replication of the Edmonston tag strain recovered from cloned DNA was strongly suppressed in its presence. The V protein of the IC-B strain, but not the Edmonston tag strain, blocked IFN-α signalling. The V protein of the Edmonston strain from the ATCC also inhibited IFN-α signalling. There were three amino acid differences between the V proteins of the Edmonston ATCC and tag strains, and substitutions of both residues at positions 110 and 272 were required for the Edmonston ATCC V protein to lose IFN-antagonist activity. The P protein of the IC-B strain, which shares the N-terminal 231 aa residues with the V protein, also inhibited IFN-α signalling. Indeed, fragments comprising only those 231 residues of the IC-B and Edmonston ATCC V proteins, but not the Edmonston tag V protein, were able to block IFN-α signalling. However, the N-terminal region of the Edmonston tag V protein, when attached to the C-terminal region of the Edmonston ATCC V protein, inhibited IFN-α signalling. Taken together, our results indicate that both the N- and C-terminal regions contribute to the IFN-antagonist activity of the MV V protein.

INTRODUCTION

Interferons (IFN)-α and -β act as essential mediators of antiviral innate immunity in the host. Upon infection, viral double-stranded RNA (dsRNA) induces the activation of dsRNA-dependent protein kinase R (PKR) and phosphorylation of IFN-regulatory factors, leading to the production of IFN-α/β in infected cells. Secreted IFN-α/β binds to the common IFN-α/β receptor on the surface of adjacent cells, activating the Jak/Stat signalling pathway. The outcome of this signalling is the transcriptional activation of target genes that contain the IFN-stimulated response element (ISRE) in their upstream regulatory sequences. Products of IFN-α/β-inducible genes make the cells resistant to viral infection by various mechanisms including cleavage of viral mRNA, inhibition of virus translation and inhibition of cell growth (Goodbourn *et al.*, 2000).

To replicate efficiently in their hosts, many viruses have evolved strategies to overcome the host's antiviral defence mechanisms (Goodbourn *et al.*, 2000; Gotoh *et al.*, 2002; Katze *et al.*, 2002). Virus countermeasures to the IFN response have been classified into three types. Some viruses have the ability to block the induction of IFN-α/β, while other viruses inhibit signal transduction in response to IFN-α/β by affecting the molecules involved in the Jak/Stat pathway. Yet other viruses can inhibit IFN-induced antiviral enzymes such as PKR and 2′,5′-oligoadenylate synthetase. Some viruses have multiple countermeasures. The influenza virus NS1 protein is able to inhibit the induction of IFN as well as the activities of antiviral enzymes by binding and sequestering dsRNA (Garcia-Sastre, 2001).

Measles virus (MV) is a member of the genus *Morbillivirus* in the family *Paramyxoviridae*. Measles remains an important cause of childhood mortality mainly due to secondary infections caused by MV-induced immunosuppression (Griffin, 2001). The Edmonston strain of MV isolated in 1954 (Enders & Peebles, 1954) is the progenitor of currently used live vaccines and has been extensively studied in laboratories. The Edmonston B strain is one of the vaccine

†Present address: Departments of Immunology and Medical Biophysics, University Health Network, Ontario Cancer Institute, Toronto, Ontario, Canada M5G 2M9.

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strains, for which a rescue system to generate infectious virus from cloned DNA is available (the rescued virus is called the Edmonston tag strain) (Radecke *et al.*, 1995). The Epstein–Barr virus-transformed marmoset B-cell line B95-8 and its subline B95a are highly sensitive to MV in clinical specimens, and virus strains isolated in B95a cells retain pathogenicity to monkeys (Kobune *et al.*, 1990). Thus, B95a is now commonly used to isolate wild-type MV strains. There is, however, a distinct difference in cell tropism between MV vaccine strains and B95a-isolated wild-type strains. We and others have successfully explained this by demonstrating that wild-type strains isolated in B95a cells usually use signalling lymphocyte activation molecule (SLAM) as a cellular receptor, whereas the Edmonston and some wild-type strains use both CD46 and SLAM as receptors (Manchester *et al.*, 2000; Tatsuo *et al.*, 2000b; Erlenhoefer *et al.*, 2001; Hsu *et al.*, 2001; Schneider *et al.*, 2002; Yanagi *et al.*, 2002).

Like other paramyxoviruses, MV produces two non-structural accessory molecules, the C and V proteins, encoded within the phosphoprotein (P) gene (Griffin, 2001). The P and C proteins are translated from overlapping reading frames on a functionally bicistronic mRNA and the V protein is translated from V mRNA, which is formed by insertion of a single nucleotide as a result of RNA editing. Thus, the amino acid sequence of the C protein is entirely different from that of the P protein, whereas the V protein shares the N-terminal 231 aa residues with the P protein but has a unique C-terminal region. The functions of these accessory molecules are not completely understood, but the V and C proteins of some paramyxoviruses have been shown to have IFN-antagonist activity. The V proteins of simian virus 5 (SV5) (Didcock *et al.*, 1999; Andrejeva *et al.*, 2002b; Chatziandreou *et al.*, 2002), mumps virus (Kubota *et al.*, 2001; Nishio *et al.*, 2002), human parainfluenza virus 2 (hPIV2) (Nishio *et al.*, 2001; Parisien *et al.*, 2001), Nipah virus (Rodriguez *et al.*, 2002) and Hendra virus (Rodriguez *et al.*, 2003) and the C protein of Sendai virus (Kato *et al.*, 2001; Garcin *et al.*, 2002; Saito *et al.*, 2002) inhibit IFN-α/β signalling by affecting the Jak/Stat pathway. Recently, the V protein of MV was reported to block signal transduction in response to IFN-α/β (Palosaari *et al.*, 2003; Takeuchi *et al.*, 2003). Another study showed that the C protein of MV also inhibits the IFN response (Shaffer *et al.*, 2003). Recombinant MV deficient in either the C or V protein propagates efficiently in cultured cells (Schneider *et al.*, 1997; Escoffier *et al.*, 1999), but not in animal models *in vivo* such as CD46-transgenic mice, cotton rats or SCID mice engrafted with human thymus/liver implants (Tober *et al.*, 1998; Valsamakis *et al.*, 1998; Patterson *et al.*, 2000). These results have suggested that the MV C and V proteins play important roles in the replication and pathogenicity of MV *in vivo*.

In this study, we have demonstrated that the V protein of the Edmonston tag strain is incapable of inhibiting IFN-α signalling, unlike the V proteins of other MV strains, presumably accounting for the strain's high sensitivity to IFN action. We identified two amino acid residues in the Edmonston tag V protein responsible for its loss of IFN-antagonist activity. We also found that both the V and P proteins of the MV wild-type IC-B strain possessed the ability to block IFN-α signalling. In fact, a molecule comprising the N-terminal 231 residues common to the V and P proteins was able to block signal transduction in response to IFN-α. Our results indicate that both the N- and C-terminal regions play a role in the IFN-antagonist activity of the MV V protein.

METHODS

Cells and viruses. Derivations and culture conditions of B95a, 293T, Vero and Vero/hSLAM cells have been previously described (Tatsuo *et al.*, 2000a; Ono *et al.*, 2001). The IC-B strain of MV was originally isolated in B95a cells from a patient with measles (Kobune *et al.*, 1990, 1996). The Edmonston tag (Radecke *et al.*, 1995) and IC-B (Takeda *et al.*, 2000) strains were recovered from cDNA clones and grown on Vero and B95a cells, respectively. The Edmonston strain was also obtained from the ATCC (designated the Edmonston ATCC strain in this report) and grown on Vero cells. The infectivity of all MV strains was determined in Vero/hSLAM cells by plaque titration.

Construction of expression plasmids. cDNAs encoding the V and P proteins of the various MV strains were obtained by reverse transcription of total RNA from virus-infected cells, followed by PCR using specific primers. V cDNAs were identified by the presence of a non-templated nucleotide insertion at the RNA editing site. Expression of the C protein from these cDNAs was abolished by introducing two stop codons immediately downstream of the C protein initiation codon such that they did not affect amino acid sequences of the P and V proteins. The absence of the C protein was confirmed by Western blot analysis. Site-directed mutagenesis was performed using gene splicing by overlap extension (Horton *et al.*, 1990) with primers in which mutations were introduced. cDNA clones encoding truncated V proteins containing only the N-terminal 231 residues were obtained using PCR by introducing a stop codon following aa 231. These cDNAs were all cloned into the expression vector pCAGGS (Niwa *et al.*, 1991) and their sequences were verified by DNA sequencing. cDNA encoding the C protein of Sendai virus was kindly provided by A. Kato (Kato *et al.*, 2001). The IFN-α/β-inducible plasmid pISRE-Luc has three tandem repeat sequences of the ISRE followed by the firefly luciferase gene and was kindly provided by K. Ozato (Wang *et al.*, 1996). The plasmid pRL-TK has the herpes simplex virus (HSV) thymidine kinase promoter followed by the *Renilla* luciferase gene (Promega).

IFN-α sensitivity of MV strains. Vero/hSLAM cells were infected with the IC-B, Edmonston ATCC or Edmonston tag strain of MV at an m.o.i. of 0·001. One hour after infection, IFN-αA/D (Sigma), the recombinant human IFN-α, was added to the cells at a concentration of 1000 IU ml^{-1}. Some infected cells were not treated with IFN-αA/D. At 48 h after infection, cells were harvested together with culture media and sonicated for 20 s. Virus titres in the cells and media were determined on Vero/hSLAM cells.

Western blot analysis. 293T cells were transfected with empty pCAGGS vector or with pCAGGS encoding the appropriate protein using Lipofectamine 2000 (Invitrogen). At 24 h after transfection, proteins from whole-cell extracts were separated by SDS-PAGE and transferred to a PVDF membrane (Amersham). The membrane was blocked in 5 % skimmed milk in Tris-buffered saline containing 0·05 % Tween 20 (TBST) for 1 h at room temperature and incubated with rabbit anti-V polyclonal antibody (Takeuchi *et al.*, 2003)

or serum from a patient with subacute sclerosing panencephalitis (SSPE) (Yanagi *et al.*, 1992) overnight at 4 °C. The membrane was washed six times in TBST for total of 30 min and treated with peroxidase-conjugated donkey anti-rabbit immunoglobulin (Ig) antibody (Amersham) or peroxidase-conjugated goat anti-human Ig antibody (EY laboratories) for 1 h. After washing six more times, the membranes were treated with the ECL Plus reagent (Amersham) and luminescence was detected using VersaDoc 3000 (Bio-Rad).

Reporter assay for the inhibition of IFN-α signalling. 293T cells were transfected with empty vector or with pCAGGS encoding the appropriate protein, together with pISRE-Luc and pRL-TK, using Lipofectamine 2000. At 36 h after transfection, the medium was changed to one supplemented, or not, with 1000 IU IFN-αA/D ml^{-1}. After a further 8 h of incubation, the luciferase activity of the cells was measured using the Dual-Luciferase Reporter assay system (Promega). Transfection efficiencies of different samples were normalized against the *Renilla* luciferase activity, which is regulated by the HSV thymidine kinase promoter and therefore unaffected by IFN treatment. Relative luciferase activity was calculated by dividing the firefly luciferase activity of IFN-α-treated cells by that of untreated cells. Data represent the mean values $\pm$ SD for triplicate samples.

RESULTS

Differences in the sensitivity to IFN-α action among MV strains

It has been reported that vaccine and Vero cell-adapted strains of MV induce much higher levels of IFN-α/β compared with wild-type MV strains isolated in peripheral blood mononuclear cells (PBMC) or B95-8 cells (Naniche *et al.*, 2000). Although the strong induction of IFN-α/β can account for the attenuated phenotype of vaccine strains, we wondered whether there might also be differences in the sensitivity to the effect of IFN among MV strains. To test this possibility, we studied the replication of different MV strains in Vero/hSLAM cells (a clone of Vero cells stably transfected with the human SLAM cDNA; Ono *et al.*, 2001) treated with IFN-α. Vero/hSLAM cells express both SLAM and CD46, and the parental Vero cells have a defect in IFN production (Emeny & Morgan, 1979), thereby enabling us to examine the IFN sensitivity of MV strains without complications arising from differences in the induction of IFN production. We examined the Edmonston ATCC, Edmonston tag and wild-type IC-B strains of MV.

Vero/hSLAM cells were infected with one of these MV strains at an m.o.i. of 0·001. Recombinant IFN-α (1000 IU ml^{-1}) was added to the infected cells 1 h after infection and viral titres were determined at 48 h post-infection (Fig. 1). The IC-B strain gave almost identical titres in the presence and absence of IFN-α. The titre of the Edmonston ATCC strain was approximately 1 log lower in the presence of IFN-α than in its absence. By contrast, the titre of the Edmonston tag strain was almost 5 logs lower in the presence of IFN-α than in its absence. Thus, the Edmonston tag strain was much more sensitive to the effects of IFN-α than the IC-B and Edmonston ATCC strains. Although the Edmonston tag and ATCC strains are presumably closely related, they appeared to have distinct abilities to resist IFN action.

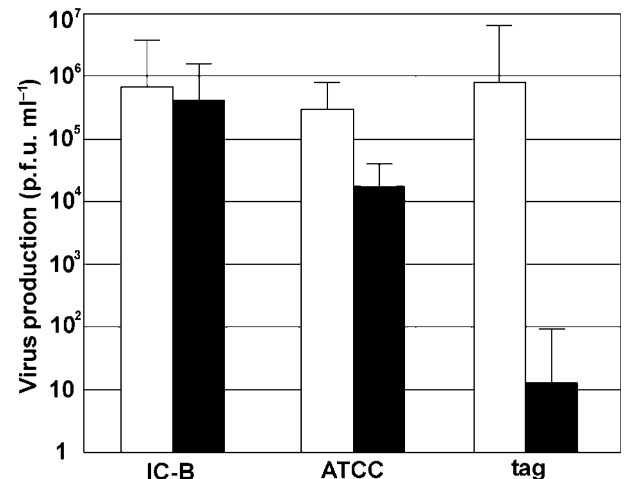

Fig. 1. Differences in sensitivity to IFN-α among MV strains. Vero/hSLAM cells were infected with the IC-B, Edmonston ATCC or Edmonston tag strain of MV at an m.o.i. of 0·001 and cultured in the presence (filled bars) or absence (open bars) of 1000 IU recombinant IFN-α ml^{-1}. Virus titres at 48 h post-infection were determined on Vero/hSLAM cells and expressed as p.f.u. ml^{-1}. Data represent the mean values $\pm$ SD for triplicate samples.

Ability of MV V proteins to inhibit IFN signal transduction

The V and C proteins of MV have been reported to inhibit IFN signalling (Palosaari *et al.*, 2003; Shaffer *et al.*, 2003; Takeuchi *et al.*, 2003). The difference in the abilities of these accessory proteins may account for the observed differences in sensitivity to IFN among MV strains. Since there was no sequence difference in the C protein between the Edmonston tag and ATCC strains, we focused on the V protein in this study.

We examined whether there was any difference in the IFN-antagonist activity among V proteins of different MV strains. To evaluate the activity of the V protein, we performed a reporter assay in which signal transduction in response to IFN-α/β could be assessed. We transfected 293T cells with an expression plasmid encoding the MV V protein, or with the empty vector, together with pISRE-Luc (encoding firefly luciferase under the control of the ISRE) and pRL-TK (encoding the *Renilla* luciferase under the control of the HSV thymidine kinase promoter). At 36 h after transfection, IFN-α was added to the cells and firefly luciferase activity was measured 8 h later and normalized using the activity of the *Renilla* luciferase. To eliminate a possible contribution of the C protein to IFN-antagonist activity, we introduced stop codons into the reading frame encoding the C protein in all plasmids used in this study.

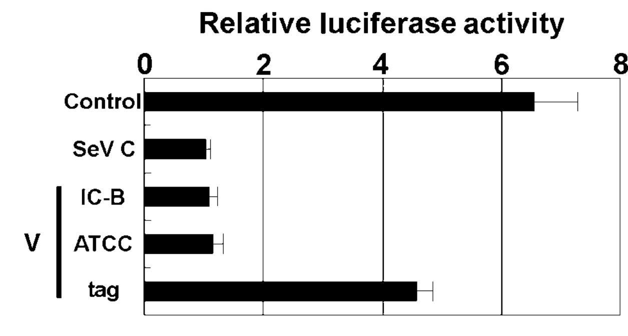

Fig. 2. Ability of MV V proteins to block IFN-α signalling. 293T cells were transfected with pISRE-Luc, pRL-TK and the empty vector (Control) or the expression plasmid encoding the indicated protein. At 36 h after transfection, the medium was changed to one with or without 1000 IU IFN-α ml^{-1}. After a further 8 h of incubation, the relative luciferase activities of IFN-treated cells were determined as described in Methods. Data represent means ± SD for triplicate samples. A representative of several experiments is shown. SeV C, Sendai virus C protein.

As expected, IFN-α treatment induced activation of the ISRE promoter in cells transfected with the empty vector, while expression of the Sendai virus C protein, an accessory molecule known to inhibit IFN-α/β signalling, abolished IFN-α-induced activation of the ISRE (Fig. 2). The V proteins of the IC-B and Edmonston ATCC strains also inhibited activation of the ISRE in response to IFN-α, confirming previous studies performed under somewhat different experimental conditions (Palosaari *et al.*, 2003; Takeuchi *et al.*, 2003). By contrast, the V protein of the Edmonston tag strain did not effectively block IFN signal transduction. Thus, there were differences in the ability of the V protein to inhibit IFN signalling among the MV strains.

Identification of amino acid residues responsible for loss of the IFN-antagonist activity

Nucleotide sequencing indicated that the predicted amino acid sequences of the IC-B and Edmonston tag V proteins (299 aa) used in this study were identical to those previously reported (Radecke *et al.*, 1995; Takeuchi *et al.*, 2000), with 16 aa differences between them. We also determined the sequence of the Edmonston ATCC V protein. There were three amino acid differences between the Edmonston ATCC and Edmonston tag V proteins: tyrosine at position 110, cysteine at position 272 and tyrosine at position 291 in the Edmonston ATCC V protein were replaced by histidine, arginine and histidine, respectively, in the Edmonston tag V protein (Table 1). At these three positions, the Edmonston ATCC strain had the same residues as the IC-B strain.

In order to identify amino acid residue(s) responsible for the difference in the IFN-antagonist activity between the Edmonston ATCC and tag V proteins, we expressed V protein mutants with substitutions at the three positions in all possible combinations and examined their ability to block IFN-α signalling (Fig. 3a). The mutants were designated according to the location(s) where substitutions were introduced. Expression of these mutant proteins was confirmed by Western blot analysis using anti-V polyclonal

Table 1. Differences in the predicted amino acid sequence of the V protein between the MV Edmonston ATCC and tag strains

Amino acid residues are indicated by single letter codes. Only those residues that are different between the two strains are shown.

Position	Edmonston ATCC	Edmonston tag
110	Y	H
272	C	R
291	Y	H

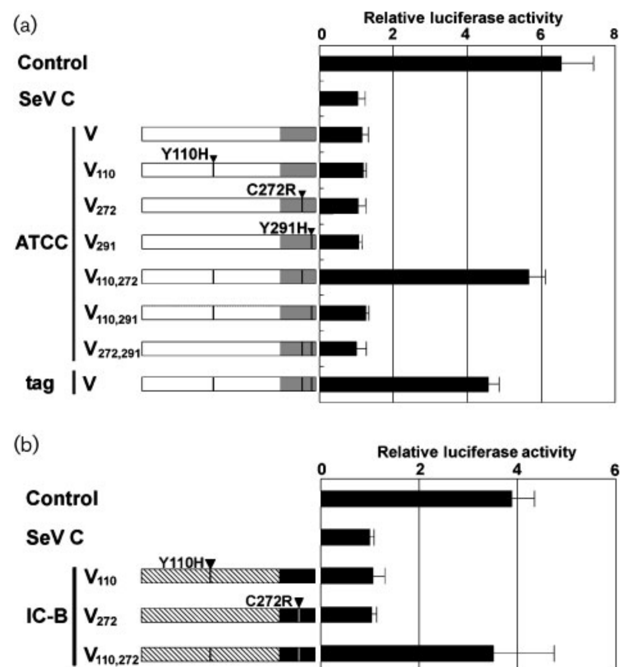

Fig. 3. Amino acid residues critical for the ability of the MV V protein to block IFN-α signalling. (a) Structures of the Edmonston ATCC and tag V proteins and the V protein mutants. Open and shaded bars indicate the N- and C-terminal regions of the Edmonston ATCC V protein, respectively, and vertical lines indicate the location of the substitutions Y110H, C272R and Y291H in the Edmonston tag V protein or mutant V proteins. Substitutions were defined by comparison with the sequence of the Edmonston ATCC V protein. We transfected 293T cells with empty vector (Control) or the expression plasmid encoding the indicated protein and determined their relative luciferase activity, as indicated in Fig. 2. (b) Hatched and filled bars indicate the N- and C-terminal regions of the IC-B V protein, respectively, and vertical lines indicate the location of the substitutions Y110H and C272R in the mutant V proteins.

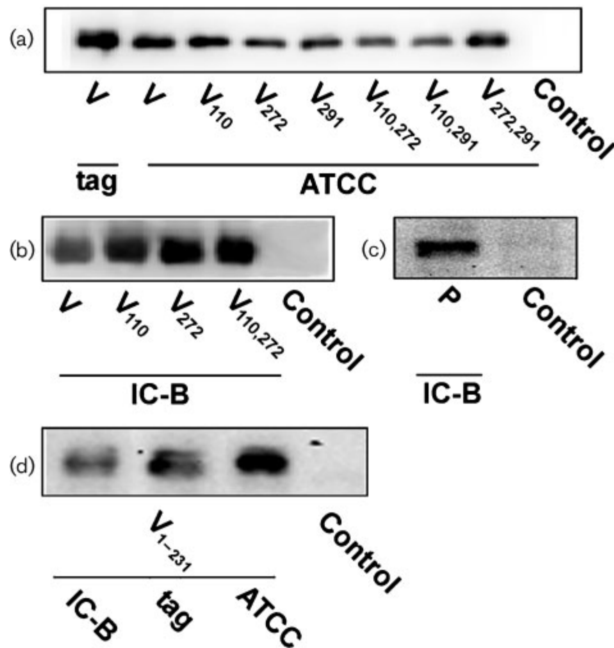

Fig. 4. Western blot analysis of expressed MV proteins. 293T cells were transfected with empty vector (Control) or the expression plasmid encoding the indicated protein. Proteins from whole-cell extracts were separated by 12 % SDS-PAGE and transferred to PVDF membrane. (a) The Edmonston ATCC V protein and its mutants with one or two amino acid substitutions at position 110, 272 or 291, as well as the Edmonston tag V protein, were detected using an anti-V polyclonal antibody. (b) The IC-B V protein and its mutants with substitutions at positions 110 and/or 272 were detected using an anti-V polyclonal antibody. (c) The IC-B P protein was detected using serum from a patient with SSPE. (d) Truncated N-terminal regions (V_{1-231}) of IC-B, Edmonston tag and Edmonston ATCC V proteins were detected using serum from a patient with SSPE.

antibody (Fig. 4a). Notably, the Edmonston tag V protein, which did not possess IFN-antagonist activity, was expressed at a higher level than the Edmonston ATCC V protein and its mutants. When the Edmonston ATCC V protein was endowed with a single amino acid substitution at position 110, 272 or 291 (Y110H, C272R or Y291H), it retained its IFN-antagonist activity (Fig. 3a). Only when both Y110H and C272R substitutions were present (ATCC $V_{110,272}$) did the V protein lose its ability to block IFN signalling (Fig. 3a). Although the expression level of ATCC $V_{110,272}$ was rather low, ATCC $V_{110,291}$, which was expressed at an even lower level, clearly exhibited IFN-antagonist activity. Thus, we concluded that the inability of ATCC $V_{110,272}$ to inhibit IFN signalling was not due to its low level of expression.

We also produced IC-B V protein mutants with substitutions at positions 110 and/or 272 and detected similar levels of expression (Fig. 4b). Again, a single amino acid substitution, Y110H or C272R, did not affect the ability of the IC-B V protein to block IFN-α signalling (Fig. 3b). However, when both Y110H and C272R substitutions were introduced, the protein failed to exhibit IFN-antagonist activity.

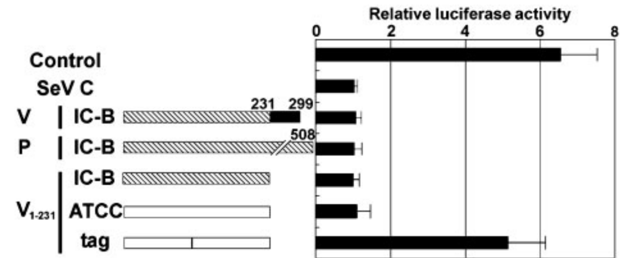

Fig. 5. IFN-antagonist activity of the N-terminal region of the MV V protein. N-terminal regions (V_{1-231}) of IC-B, Edmonston ATCC and Edmonston tag V proteins as well as intact IC-B V and P proteins were examined for their ability to block IFN-α signalling. Within the mutant constructs, open bars indicate the N-terminal region of the Edmonston ATCC V protein, hatched bars indicate the P protein and N-terminal region of the V protein, and filled bars indicate the C-terminal region of the V protein of the IC-B strain. The vertical line indicates the location of the substitution Y110H. 293T cells were transfected with the empty vector (Control) or the expression plasmid encoding the indicated protein and their relative luciferase activities were determined, as indicated in Fig. 2.

IFN-antagonist activity of the N-terminal region of the V protein

In the luciferase reporter assay, we found that the P protein of the IC-B strain blocked IFN-induced activation of the ISRE (Fig. 4c, Fig. 5). As both the V and P proteins of the IC-B strain exhibited IFN-antagonist activity, the N-terminal region common to these two proteins was expected to contain sequences capable of blocking the signalling. Therefore, we tested the IFN-antagonist activity of truncated V proteins containing only the N-terminal 231 residues. We confirmed similar levels of expression of the truncated V proteins by Western blot analysis (Fig. 4d). The truncated V proteins of the IC-B and Edmonston ATCC strains (designated IC-B V_{1-231} and ATCC V_{1-231}), but not the Edmonston tag strain (designated tag V_{1-231}), were able to block IFN signalling as efficiently as the V protein of the IC-B strain (Fig. 5), indicating that the N-terminal region common to the P and V proteins is sufficient to inhibit IFN-α signalling. Within these 231 residues, there was only a single amino acid difference between the Edmonston tag and ATCC strains. Thus, tyrosine at position 110 appears to be critical for the IFN-antagonist activity of the N-terminal region of the V protein.

DISCUSSION

The V protein of the MV Edmonston strain (Palosaari *et al.*, 2003) and that of the wild-type IC-V strain (Takeuchi *et al.*,

2003) have been shown to block signal transduction in response to IFN-α/β. Six wild-type MV strains (AK-1 to AK-6) isolated in B95a cells were also found to suppress the IFN-α signalling pathway although their V proteins were not examined for their ability to inhibit IFN signalling (Yokota *et al.*, 2003). Our results with the wild-type IC-B and Edmonston ATCC strains were consistent with these recent studies (the IC-B and IC-V strains were isolated in B95a and Vero cells, respectively, from the same patient and the IC-B V protein has a single amino acid difference compared with the IC-V V protein; Takeuchi *et al.*, 2000). By contrast, we found that the V protein of the Edmonston tag strain rescued from cloned DNA did not exhibit IFN-antagonist activity.

There were three amino acid differences (positions 110, 272 and 291) in the V protein between the Edmonston tag and ATCC strains. Notably, the predicted amino acid sequence of the Edmonston ATCC V protein was identical to that of other vaccine strains derived from the Edmonston strain: Moraten, Schwarz, Zagreb, Rubeovax (Edmonston B) and AIK-C strains (Parks *et al.*, 2001). Although the Edmonston tag strain is based on the Edmonston B strain, it was recently reported to diverge from the Edmonston B strain by 10 aa substitutions, including those in the V protein (Combredet *et al.*, 2003). These changes presumably occurred because of adaptation of the Edmonston B-derivative virus to growth on Vero cells. Thus, the inability of the V protein to block IFN signalling is restricted to the Edmonston tag strain. These inadvertent mutations, however, allowed us to locate amino acid residues in the V protein critical for its IFN-antagonist activity. Interestingly, the Y110H or C272R substitution did not affect the ability of the Edmonston ATCC V protein to block IFN signalling if introduced individually. Only when these substitutions coexisted did the V protein lose its IFN-antagonist activity. The substitution at position 291 did not appear to affect the ability of the V protein to block IFN signalling.

The C-terminal region of the V protein, which is relatively conserved among paramyxoviruses, is cysteine-rich and has zinc-binding properties (Griffin, 2001). Previous reports have implicated the C-terminal region in the IFN-antagonist activity exhibited by the V proteins of SV5, mumps virus, hPIV2 and Newcastle disease virus (Didcock *et al.*, 1999; Kubota *et al.*, 2001; Nishio *et al.*, 2001, 2002; Park *et al.*, 2003). Accordingly, the P proteins of these viruses do not possess the ability to block IFN action. On the other hand, it was recently reported that the N-terminal region of the Nipah virus V protein, which is shared with the P and W proteins, had IFN-antagonist activity (Park *et al.*, 2003).

Our structural studies revealed that substitutions of tyrosine at position 110 in the N-terminal region together with cysteine at position 272 in the C-terminal region were required for the Edmonston ATCC V protein to lose its IFN-antagonist activity. Furthermore, the truncated N-terminal regions (231 residues) of the Edmonston ATCC and IC-B strains were able to exhibit IFN-antagonist activity. As expected, the N-terminal region of the Edmonston tag strain with histidine at position 110 failed to block IFN signalling. However, the chimeric protein comprising the N-terminal region of the Edmonston tag V protein and the C-terminal region of the Edmonston ATCC V protein (ATCC V_{110} in Fig. 3a) blocked IFN signalling as efficiently as the V proteins of the IC-B and Edmonston ATCC strains, indicating that the inability of the N-terminal region to block IFN signalling can be compensated by the presence of the C-terminal region of the Edmonston ATCC V protein. Similarly, another chimeric molecule comprising the N-terminal region of the Edmonston ATCC strain and the C-terminal region of the Edmonston tag strain (ATCC $V_{272,291}$ in Fig. 3a) was able to block IFN signalling. Thus, while either the N- or C-terminal region usually plays a role in the IFN-antagonist activity of V proteins of other paramyxoviruses, both the N- and C-terminal regions appear to do so in the MV V protein. In the case of SV5, amino acid substitutions in the N-terminal region (Chatziandreou *et al.*, 2002; Young *et al.*, 2001) or mutations of any of the conserved cysteine residues in the C-terminal region (Andrejeva *et al.*, 2002a) can prevent the V protein from blocking IFN signalling.

While the wild-type IC-B strain grew comparably in the presence and absence of IFN-α, replication of the Edmonston ATCC strain was suppressed by approximately 1 log in the presence of IFN-α (Fig. 1). This occurred despite the fact that the V proteins of the IC-B and Edmonston ATCC strains similarly blocked IFN signalling. This finding suggests the presence of additional mechanisms suppressing IFN action in wild-type MV strains. Naniche *et al.* (2000) reported that wild-type MV strains are more sensitive to IFN action than the Edmonston strain. The reason for the discrepancy between their observation and ours is unknown, but it may be due to different conditions used in their experiments, including the use of PBMC, the measurement of virus replication at a later time point (4 days post-infection) and methods used for assessing virus replication. Furthermore, CD46, a cellular receptor for the Edmonston strain, is constitutively present in all PBMCs, whereas SLAM, a receptor for both Edmonston and wild-type strains, is expressed on only a proportion of PBMCs (Aversa *et al.*, 1997). Thus, their conditions may have favoured replication of the Edmonston strain over that of the wild-type strains.

Previous studies have shown that a recombinant MV defective in the V protein replicated normally in cultured cells and PBMCs (Schneider *et al.*, 1997; Escoffier *et al.*, 1999), but replicated less efficiently and did not exhibit strong pathogenicity in CD46-transgenic mice, SCID mice engrafted with human thymus/liver implants or cotton rats (Tober *et al.*, 1998; Valsamakis *et al.*, 1998; Patterson *et al.*, 2000). It should be noted that all these studies were performed using the recombinant virus based on the Edmonston tag strain. Since our results showed that the V protein of the Edmonston tag strain does not possess

IFN-antagonist activity, it is not surprising that the V-deficient Edmonston tag strain grew as efficiently *in vitro* as the parental virus. In fact, Patterson *et al.* (2000) reported that, after treatment of HeLa cells with 1000 IU IFN, there was no difference in the IFN sensitivity between the Edmonston tag strain and its V-deficient mutant. However, the observation that the V-deficient Edmonston tag strain does not replicate well *in vivo* implies a function(s) of the V protein besides IFN antagonism.

Recently, the V protein of SV5 was shown to have the ability to inhibit induction of IFN-α/β, in addition to its IFN-antagonist activity (He *et al.*, 2002; Poole *et al.*, 2002; Wansley & Parks, 2002). The V protein of MV may also control the induction of IFN. Furthermore, it was reported that the V protein of MV forms complexes with the N protein and acts to control accumulation of viral RNA and proteins (Tober *et al.*, 1998). These and other functions of the MV V protein await further studies. The C protein of Sendai virus has the capacity to block IFN-α/β signalling (Kato *et al.*, 2001; Garcin *et al.*, 2002; Saito *et al.*, 2002). A recent study showed that the C protein of MV also inhibits the IFN response (Shaffer *et al.*, 2003). In fact, it has been reported that replication of the C-deficient Edmonston tag strain was compromised in PBMC and animal models (Valsamakis *et al.*, 1998; Escoffier *et al.*, 1999; Patterson *et al.*, 2000). We also detected weak IFN-antagonist activity in the C proteins of MV strains examined in this study, although there was no significant difference among the strains (data not shown).

How did the Edmonston B-derivative virus, used to produce the Edmonston tag strain, lose the IFN-antagonist activity of its V protein? A plausible explanation is that during *in vitro* passaging in Vero cells, mutations accumulate in the P gene of the virus such that the resulting P protein allows better virus replication in the cells by sacrificing the IFN-antagonist activity of the V protein encoded in the same gene. Wansley and Parks (2002) reported that a recombinant SV5 whose P/V gene was replaced with that of an attenuated strain lost the ability to block IFN signalling and induction, but gained the ability to produce viral proteins and mRNA more efficiently than the wild-type virus. Takeda *et al.* (1998) used a wild-type MV isolate in B95a cells and its Vero cell-adapted attenuated strain to show that a few amino acid changes in the polymerase (L and P proteins) and/or accessory V and C proteins were responsible for the attenuation through adaptation to efficient growth in Vero cells. Our view appears consistent with these observations.

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