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Importance of the Cysteine-Rich Carboxyl-Terminal Half of V Protein for Sendai Virus Pathogenesis

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The Sendai virus V protein is a nonstructural *trans*-frame protein whose cysteine-rich C-terminal half is fused to the acidic N-terminal half of the P protein via mRNA editing. We recently created a mutant by disrupting the editing motif, which is devoid of mRNA editing and hence unable to produce the V protein, and demonstrated that this V(−) virus replicated normally or even faster with augmented gene expression and cytopathogenicity in cells in vitro, but was strongly attenuated in pathogenicity for mice (A. Kato, K. Kiyotani, Y. Sakai, T. Yoshida, and Y. Nagai, EMBO J. 16:578–587, 1997). Thus, although categorized as a nonessential protein, the V protein appeared to encode a luxury function required for the viral in vivo pathogenesis. Here, we created another version of a V-deficient mutant, $V_{\Delta C}$, encoding only the N-terminal half but not the V-specific C-terminal half, by introducing a stop codon in the *trans*-V frame, and then we compared its in vitro and in vivo phenotypes with those of the V(−) and wild-type viruses. The $V_{\Delta C}$ virus was found to be similar to the wild-type virus in vitro with no augmented gene expression and cytopathogenicity, but in vivo, it resembled the V(−) virus, displaying a similarly attenuated phenotype. Thus, the pathogenicity determinant in the V protein was mapped to the C-terminal half. The N-terminal half was likely sufficient to confer normal (wild-type) in vitro phenotypes.

Sendai virus (SeV) is a prototypic member of the family *Paramyxoviridae*, which constitutes, together with the families *Rhabdoviridae* and *Filoviridae*, the superfamily *Mononegavirales*, which is characterized by possession of a nonsegmented negative-sense RNA as the genome. In common, the genome of *Mononegavirales* is organized starting with a short 3′ leader region, followed by 5 to 10 genes, and ending with the short 5′ trailer region. They are tightly associated with nucleocapsid (N) protein subunits, forming the helical ribonucleoprotein (RNP) complex. The RNP complex but not the naked RNA genome is the template for both transcription and replication, in contrast to positive-strand RNA viruses whose naked RNA genome can serve as the functional genome and mRNA. Mononegavirus gene expression is generally monocistronic, each mRNA usually directing a single primary translation product. However, the P gene, the second proximal gene to the 3′ terminus of *Pramyxovirinae*, the major subfamily of *Paramyxoviridae*, is a notable exception, because it gives rise to multiple protein species by means of overlapping frames and by a process known as RNA editing or pseudotemplated addition of nucleotides (7, 12, 27, 30, 43 [for review, see reference 23]). In the case of SeV, the exact copy of the P gene, P mRNA, encodes the phospho- (P) protein and additionally directs the C protein in an overlapping frame by a ribosomal choice of initiation (Fig. 1). The latter frame of SeV further generates three other proteins C′, Y1, and Y2 by using initiation codons at different positions, one of which is non-AUG (8). The RNA editing involves the addition of a single pseudotemplated G residue when the RNA polymerase copies the CCC stretch in the characteristic sequence (3′-UUUUUCCC-5′) at a specific region of the genome template (Fig. 1). This pseudotemplated addition of one G residue occurs in about 25% of the P gene transcripts (17, 43) and results in a −1 frameshift. The protein encoded by this edited mRNA is named V and is characterized by a unique cysteine-rich C-terminal half (Fig. 1). The V and P proteins are N coterminal. Furthermore, the insertion of two G's at the editing site also occurs, albeit at a much reduced frequency (43, 44). The encoded protein, termed W, is thus virtually identical to the N-terminal part of the P or V protein (Fig. 1). The pseudotemplated addition of G residues has been found almost universally for all three genera, *Paramyxovirus*, *Morbillivirus*, and *Rubulavirus*, of the subfamily *Paramyxovirinae*. However, in members of the *Rubulavirus* genus, the edited version with insertion of one or two G's encodes the P protein, while the unedited version encodes the V protein (28, 42). In any case, it is interesting that the extreme C-terminal region of V protein shows a striking degree of similarity among paramyxoviruses with the highly conserved 17 residues, seven of which are cysteine residues. This conserved cysteine-rich region has been suggested to interact with either a highly conserved protein widely distributed among paramyxovirus host cells or with an invariant macromolecule such as nucleic acid (6). The V proteins of SeV and measles virus are not present in virions and are not associated with RNP (7, 16), probably because these V proteins are missing the trimerization site and L and N binding sites, all of which are localized to the C-terminal half of the P protein (16, 35, 39, 45) (Fig. 1).

While the paramyxovirus P protein is an essential component for the viral RNA synthesis and constitutes, together with the L protein, the RNA polymerase complex (15), the functions of the V and C proteins have not been established yet. However, recently developed technology to recover infectious viruses from cDNA (13, 18, 32) is shedding light on their functions. The measles virus engineered to express no C pro-

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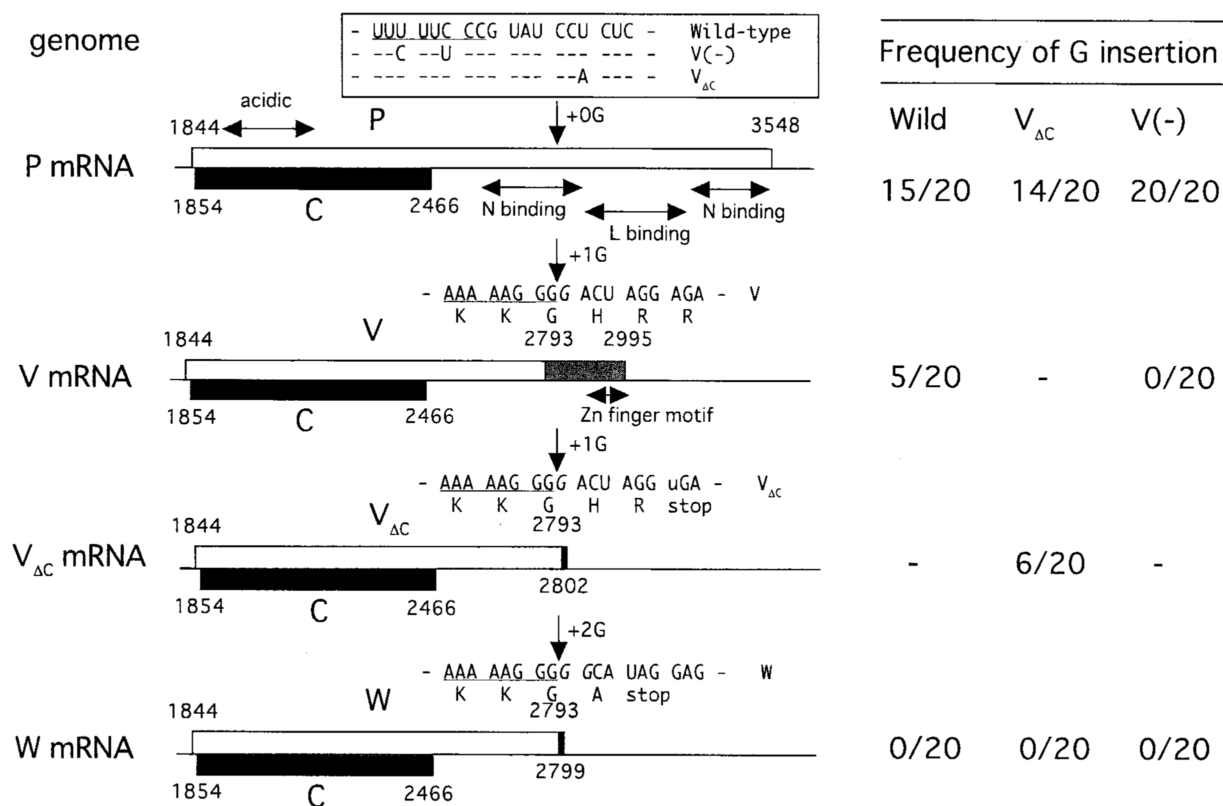

FIG. 1. SeV P gene expression and introduction of mutation. The faithful copy of the P gene encodes the P protein (open box). RNA editing with one pseudotemplated G insertion (+1G) at the editing site (arrows) produces an mRNA encoding the V protein, which is a fusion of the N-terminal portion of the P protein with the V-specific C-terminal half (shaded rectangle). Insertion of the two G's (+2G) generates the mRNA encoding the W protein. The W protein frame terminates immediately after the insertion. The extreme C-terminal portion of the V protein is cysteine rich (7), while that of P protein has the binding domains with L and N proteins as well as the trimerization site (35, 39). The P mRNA further encodes the C protein with the third reading frame (solid box) at the 5′ end independently of editing status. The characteristic sequence (UUUUUCCC) critical for the pseudotemplated G insertions and its complementary sequences in the mRNAs are underlined. Two mutations (U to C and C to U) were introduced to create V(−) virus (17). A single base substitution (U to A) to create a UGA stop codon in V frame was introduced immediately downstream of the editing locus for $V_{\Delta C}$ virus generation, which expresses a C-terminally truncated V protein. Dashes indicate residues identical to those of the wild type. The viruses recovered from the wild-type and mutated cDNAs were examined for actual frequencies of G insertions as described in Materials and Methods.

tein was found to be able to replicate normally at least in tissue culture cells, and thus the C protein was categorized as a nonessential gene product (31). The same was found to be true for SeV V protein by us (17) and others (9). However, our V-deficient SeV, V(−), was further found to be strongly attenuated in its pathogenicity for mice, indicating that SeV V protein, although nonessential for tissue culture replication, encodes a luxury function required for in vivo pathogenesis (17). In addition, the V(−) virus displayed markedly augmented gene expression, growth kinetics, and cytopathogenicity in at least some cell lines (17).

Our V(−) virus described above generates no V mRNA because of disruption of the editing signal and thus produces no V protein. In the present study, we created another version of V-deficient SeV which can normally undergo RNA editing but does not encode the cysteine-rich C-terminal half because of the stop codon introduced immediately downstream of the editing locus in the *trans* V frame. This new mutant, $V_{\Delta C}$, was compared with the previous V(−) and the wild-type viruses to learn how much the C-terminal half contributes to determining SeV's in vivo and in vitro phenotypes. The overall results have strongly suggested the importance of the V-specific C-terminal half for the viral in vivo pathogenicity. However, the acidic N-terminal half rather than the C-terminal half appeared to be more directly involved in determining in vitro replication patterns.

MATERIALS AND METHODS

Cell cultures and virus infection. Monkey kidney-derived cell lines LLCMK2 and CV1 were grown in minimal essential medium (MEM) supplemented with 10% fetal bovine serum. Monolayer cultures of these cells were infected with $V_{\Delta C}$ virus recovered from cDNA at an input multiplicity of infection (MOI) of 10 PFU/cell unless otherwise noted, and maintained in serum-free MEM. The wild-type SeV (Z strain) and the V(−) virus recovered from the respective cDNAs (17, 18) were used for comparison.

Mutagenesis of full-length SeV cDNA. The plasmid pSeV(+) contained the cDNA copy of the full-length SeV antigenome (18). A single nucleotide change was introduced immediately downstream of the editing locus (UUUUUCCC) in the P gene of pSeV(+) to create a stop codon in the *trans* V frame without affecting the P and C frames (Fig. 1). For this, a pair of primers, 5′ ^{2758}CCCGG GTCTAGAGACCGACTCAACAAAAAAGGGCATAGGtGA^{2801} 3′ ($PV_{\Delta C}$) (where the lowercase letter represents the mutation) and 5′ ^{3556}CCCGGGATC TAGTTGGTCAGT^{3536} 3′ (PR), were synthesized, and PCR was performed with the template pSeV(+) for 20 cycles of 92°C for 1 min, 55°C for 2 min, and 74°C for 3 min. The nucleotide numbering is according to that of Shioda et al. (38). The amplified fragment was digested with *Sma*I and inserted into *Sma*I-digested pSeV(+). The same mutation was introduced into the pGEM-P plasmid by the same procedure. Before use, these plasmids were purified by CsCl centrifugation.

Virus recovery from cDNAs. Viruses were recovered from cDNAs essentially according to the previously described procedures (18). Briefly, 1.2 × 10^7 LLCMK2 cells were infected with vaccinia virus (VV) vTF7-3, a gift of B. Moss

TABLE 1. Infectious virus recovery from wild-type pSeV(+) and mutant pSeV(+) after amplification[a]

Transfectant	Inoculum size (no. of cells)	Amplification virus yield	
		HAU	PFU/ml
Wild type	10^4	<2, <2	<10, <10
	10^5	256, 512 (512)[b]	3×10^8, 1×10^9 (2×10^9)[b]
	10^6	512, 512	1×10^9, 2×10^9
$V_{\Delta C}$	10^4	<2, <2	<10, <10
	10^5	64, 256 (512)[b]	9×10^7, 1×10^8 (9×10^8)[b]
	10^6	128, 256	2×10^8, 6×10^8

[a] SeV multiplication was initiated from the parental or mutated pSeV(+) that was transfected into LLCMK2 cells as described in Materials and Methods (for details, see reference 18). Transfected LLCMK2 cells were inoculated into chicken eggs after three cycles of freezing and thawing, and virus yields were determined after a 72-h incubation. Two eggs were used for each inoculum size, and titers in each egg are shown.

[b] The virus amplified in one of the eggs in each transfection recovery experiment was once again propagated in several eggs at a 10^{-7} dilution to eliminate helper VV, and the allantoic fluids were pooled and used as the stock virus; this titer is shown in parentheses.

(11), at an MOI of 2 PFU/cell. Sixty micrograms of the parental or mutated pSeV(+) and the plasmids encoding transacting proteins, pGEM-N (24 μg), wild-type pGEM-P or the mutated pGEM-P (described above) (12 μg), and pGEM-L (24 μg), were then transfected simultaneously with the aid of the lipofection reagent DOTAP (Boehringer Mannheim, Mannheim, Germany). The cells were maintained in serum-free MEM in the presence of 40 μg of araC per ml and 100 μg of rifampin per ml to minimize VV cytopathogenicity and thereby maximize the recovery rate (18). Forty hours after transfection, cells were harvested, disrupted by three cycles of freezing and thawing, and inoculated into 10-day-old embryonated chicken eggs. After 3 days of incubation, the allantoic fluid was harvested. The titers of recovered viruses were expressed in hemagglutination units (HAU) and PFU per milliliter as described previously (18). The allantoic fluid of the eggs contained 10^8 to 10^9 PFU of the recovered viruses per ml along with the helper VV in amounts of 10^3 to 10^4 PFU/ml. The latter was eliminated by the second propagation in eggs at a dilution of 10^{-7}. The second passage was used as the stock virus for all of the experiments.

Determination of the frequency of pseudotemplated G insertions. Total RNA extracted from infected CV1 cells at 26 h postinfection (hpi) was reverse transcribed with oligo(dT) primer and Superscript (Gibco-BRL, Gaithersburg, Md.) at 42°C for 90 min according to the manufacturer's recommendations. The cDNAs were amplified by PCR with the P1 (5′ ^{1843}ATGGATCAAGATGCC TTC^{1864} 3′) and PR (described above) primers. Amplified cDNA fragments were digested with *Sma*I and *Eco*RI and ligated into pGEM-4Z (Promega, Madison, Wis.) digested with the same enzymes. The cloned plasmids from 20 independent colonies were sequenced with the reverse primer by an AFLII automated DNA sequencer (Pharmacia, Uppsala, Sweden) and examined for the presence or absence of G insertions.

Radiolabelling, immunoprecipitation, and Western blotting. CV1 cells (10^6) grown in 6-cm-diameter plates were infected at an MOI of 20 with the wild-type, $V_{\Delta C}$, or V(−) virus. At 14 hpi, cells were pulse-labeled for 2 h with 0.74 MBq of Tran-^{35}S (37 TBq/mmol) (ICN, Costa Mesa, Calif.) in methionine- and cysteine-free medium. Cells were washed twice with phosphate-buffered saline, lysed in 1 ml of radioimmunoprecipitation assay buffer (10 mM HEPES [pH 7.4], 150 mM NaCl, 1 mM EDTA, 1% Nonidet P-40, 1% deoxycholate, 0.1% sodium dodecyl sulfate, 10 μg of aprotinin per ml) on ice, and centrifuged at 12,000 × *g* for 10 min. Either anti-P or anti-SeV antibody was added to the supernatants of cell lysate, which were then incubated at 4°C for several hours. Immune complexes were recovered with protein A-Sepharose (Gibco BRL), washed three times with radioimmunoprecipitation assay buffer, and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (12.5% polyacrylamide) (22). For detection of C protein, Western blotting was done with rabbit anti-C serum exactly as described previously (17, 19).

Northern hybridization. RNAs were extracted by the guanidine-isothiocyanate method from approximately 10^6 CV1 cells infected with the wild-type, $V_{\Delta C}$, or V(−) virus at various time points p.i. The RNAs were ethanol precipitated, dissolved in formamide-formaldehyde solution, and then electrophoresed in 0.9% agarose–formamide–morpholinepropanesulfonic acid gels, and capillary transferred onto Hibond-N filters (Amersham, Buckinghamshire, United Kingdom). They were probed with ^{32}P-labeled riboprobes made with T7 or SP6 RNA polymerase with pGEM-N linearized with *Nae*I or *Bam*HI, pSeV(+), and pSeV(−) (the latter two probes linearized with *Mlu*I) as the templates.

Infection of mice. Specific-pathogen-free, 3-week-old male ICR/Crj (CD-1) mice were purchased from Charles River, Japan, and used for the experimental virus infection. For the infection, five mice in each group were used and infected intranasally with 10^6, 10^7, or 10^8 PFU/mouse under mild anesthetization with ether (20, 41). Their body weights were individually measured every day up to 14 days, because disease progression well correlated with the drop in body weight (20). In a separate experiment, the virus titers in the lungs were measured at appropriate days p.i. as described previously (17). Five mice were usually used for each determination.

RESULTS

Creation of mutant SeV strain with a gene encoding the C-terminally truncated V protein and its in vitro characterization. We introduced a TGA stop codon in the V frame immediately downstream of the RNA editing site by mutating ^{2799}A to T (U to A in the genomic sense) in the plasmid pSeV(+), generating a full-length copy of SeV antigenome (Fig. 1). This mutation does not affect the P and C frames. To avoid possible reversion to the wild-type virus by recombination with the supporting plasmid, pGEM-P, during cotransfection, the same mutation was introduced into pGEM-P. Remarkably, the rate of virus recovery from the mutated pSeV(+) was comparable to that from the parental pSeV(+), yielding a full virus titer in embryonated chicken eggs following injection with approximately 10^5 freeze-thawed transfected cells (Table 1). The recovered mutant virus, $V_{\Delta C}$, and the parental virus were once again propagated in eggs to eliminate helper VV (18) and were used as stocks for all subsequent experiments (Table 1). That the introduced stop codon was retained in the $V_{\Delta C}$ virus was confirmed by nucleotide sequencing of the stock virus genome.

CV1 cells infected with $V_{\Delta C}$ virus were analyzed for P gene products by immunoprecipitation. As the controls, the wild-type SeV and V(−) virus were used. Because the P and V proteins are N coterminal, the anti-P serum precipitated not only P but also V proteins from the wild-type-infected cells and, as already shown (17), precipitated only the former but not the latter from the V(−)-infected cells (Fig. 2). The same serum also precipitated the P protein but not the authentic V protein from the $V_{\Delta C}$-infected cells. Instead, the band for $V_{\Delta C}$, well corresponding in size to the C-terminally truncated V protein, was clearly identified (Fig. 2).

The C protein was synthesized from both the $V_{\Delta C}$ and V(−)

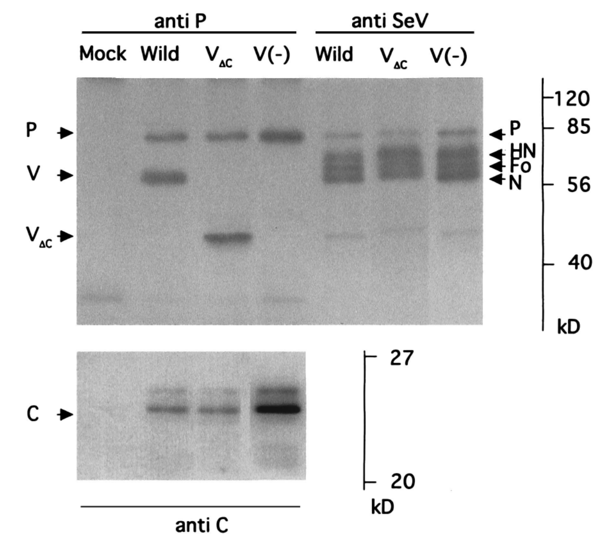

FIG. 2. Detection of P gene products in CV1 cells infected with the mutant viruses. The lysates of CV1 cells infected with the wild-type, $V_{\Delta C}$, or V(−) virus were pulse-labeled with [^{35}S]Met and [^{35}S]Cys and immunoprecipitated with anti-P or anti-SeV serum. The cell lysates were also analyzed by Western blotting with anti-C serum.

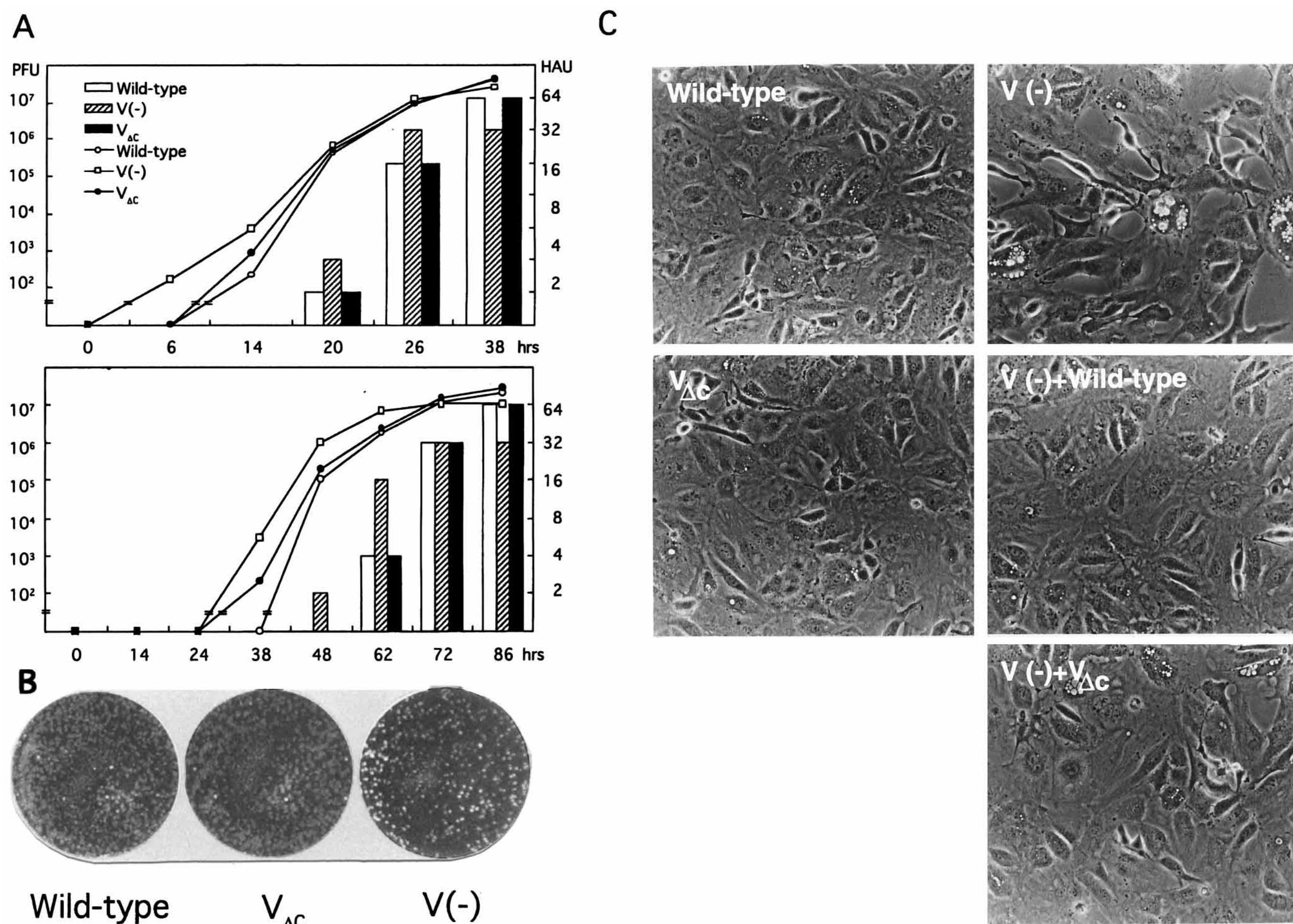

FIG. 3. Replication kinetics, plaque formation, and cytopathogenicity. (A) The wild-type and mutant viruses were inoculated into CV1 cells under single (MOI of 10 PFU/cell)- (top) or multiple (MOI of 0.01 PFU/cell)-cycle growth conditions (bottom). The virus titers are shown as PFU per milliliter (lines) and HAU (bars) of the culture supernatants. (B) Plaque formation by the wild-type, $V_{\Delta C}$, and V(−) viruses in CV1 cells at 3 days p.i. (C) Cytopathogenicity at 38 hpi following single or double infections.

viruses as well as the wild-type virus (Fig. 2). On the other hand, the frequency of insertion of G (6 of 20) into the mRNA population in $V_{\Delta C}$-infected cells was very similar to that (5 of 20) of the wild-type infection (Fig. 1). No striking differences in the immunoprecipitation patterns were found between the wild-type and $V_{\Delta C}$ viruses with the polyvalent anti-SeV serum recognizing P, HN, F, and N proteins (Fig. 2). The larger amounts of specific precipitates unique to the V(−) virus in this and the anti-C panels confirmed augmented gene expression in CV1 cells, which was characteristic of this virus (17). These results strongly suggested that the introduced stop codon indeed worked to generate the C-terminally truncated V protein without affecting the P and C frames and the frequency of cotranscriptional G insertion. The fact that there was no detectable two-G insertion in the wild-type or $V_{\Delta C}$-infected cells (Fig. 1), as well as no detectable W protein synthesis in the former (Fig. 2), confirmed that a two-G insertion leading to W protein synthesis could be quite a rare event in these cells (17).

In vitro gene expression, replication, and cytopathogenicity of $V_{\Delta C}$ virus. $V_{\Delta C}$ virus was compared with the wild-type and V(−) viruses with regard to gene expression and replication in CV1 cells as well as cytopathogenicity for the cells. In the previous work and as shown here (Fig. 3), the V(−) virus was found to be potentiated in these capacities compared with the wild-type virus. The $V_{\Delta C}$ virus was now found to display replication kinetics very similar to that of the wild-type virus and slower than that of V(−) virus (Fig. 3A). The final titers of these three viruses were, however, comparable to one another. Compared with the wild-type virus, the $V_{\Delta C}$ virus produced similar turbid plaques (Fig. 3B) and was equally cytopathic (Fig. 3C). In contrast, the V(−) virus produced clearer plaques and caused more severe cytopathogenicity than did the wild-type or $V_{\Delta C}$ virus. As already shown above, immunoprecipitation studies demonstrated comparable levels of protein synthesis between the $V_{\Delta C}$ and wild-type infections, while that of V(−) virus was augmented (Fig. 2). In accordance with this, mRNA levels in $V_{\Delta C}$-infected cells, revealed by Northern hybridization with an N-specific probe and a probe to detect all mRNA species, were comparable to those of the wild-type virus and lower than those of the V(−) virus (Fig. 4). The 50S genomic RNA was clearly detectable by Northern blotting only late in infection, and the levels were comparable among the three viruses (Fig. 4). This is consistent with the observations that their maximum virus titers were similar to one another (Fig. 3A).

When CV1 cells were doubly infected with V(−) and wild-type viruses or V(−) and $V_{\Delta C}$ viruses, the severe cytopatho-

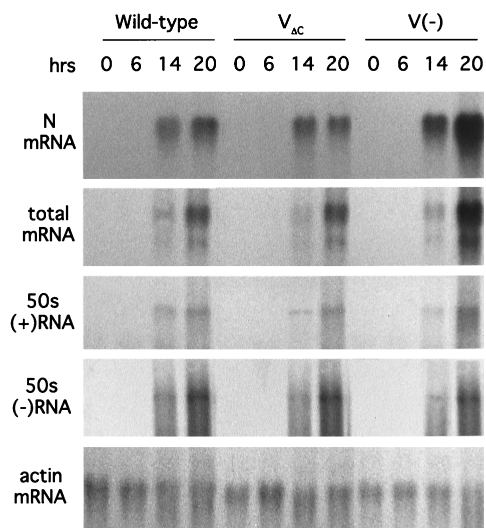

FIG. 4. Gene expression and genome replication of the wild-type and mutant viruses in CV1 cells. Infection was initiated at an MOI of 10 PFU/cell. At various hpi, as indicated on the top of each lane, the gene expression was studied by Northern blotting with negative-sense RNA probes to detect viral mRNAs. Replication was studied by Northern blotting with probes to detect either the 50S antigenome (+) RNA or the 50S genome (−) RNA.

genicity (Fig. 3C) and elevated gene expression characteristic of the V(−) virus (data not shown) were no longer seen. This dominance of the wild-type phenotype in doubly infected cells suggested the complementation (supply of V or truncated V protein) by the wild-type or $V_{\Delta C}$ virus. Thus, the N-coterminal half appeared to be sufficient to maintain the wild-type phenotypes in vitro.

In vivo replication and pathogenicity of $V_{\Delta C}$ virus. SeV causes pneumonia in the natural host, mice. We have already proved by using the V(−) virus that the V protein is indispensable for in vivo pathogenicity (17). This conclusion was drawn from the observations that the V(−) virus was cleared rapidly from the body without producing severe lesions in the target organ, the lung, and without killing the mice. In order to learn how much the lack of the cysteine-rich C-terminal half of the V protein contributes to this attenuation, we have similarly investigated the pathogenicity of $V_{\Delta C}$ virus and compared it with those of the wild-type and V(−) viruses. The viruses were intranasally inoculated into mice at three different input doses (10^8, 10^7, or 10^6 PFU/mouse). The wild-type virus significantly disturbed body weight gain and killed five (100%) and three (60%) mice by the 10th day p.i. at 10^7 and 10^6 PFU/mouse, respectively (Fig. 5). In marked contrast, the $V_{\Delta C}$ and V(−) viruses allowed all mice to survive up to 14 days following inoculation with 10^6 PFU/mouse. At 10^7 PFU/mouse, one mouse was killed at 8 days p.i. by $V_{\Delta C}$ virus, but the remaining four survived up to 14 days. None of the five mice was killed by V(−) virus under the same conditions. The profiles of body weight gain differed greatly between $V_{\Delta C}$ and the wild-type infections but were similar, although not identical, between $V_{\Delta C}$ and V(−) infections. Slight disturbance of body weight gain by the former was a constant observation. The $V_{\Delta C}$ and V(−) viruses killed five (100%) and two (40%) mice at 10^8 PFU/mouse, respectively. Fifty percent lethal doses (LD_{50}s) were calculated to be 7.9×10^5, 2.0×10^7, and 1.3×10^8 PFU for the wild-type, $V_{\Delta C}$, and V(−) viruses, respectively (Fig. 5). This indicated that $V_{\Delta C}$ virus was attenuated by as much as 25-fold, although it was still slightly (about 6.5-fold) more virulent than the V(−) virus. The main feature of V(−) virus spreading in the lungs was that it grew as efficiently as the wild-type virus in the initial 1 or 2 days of infection but was

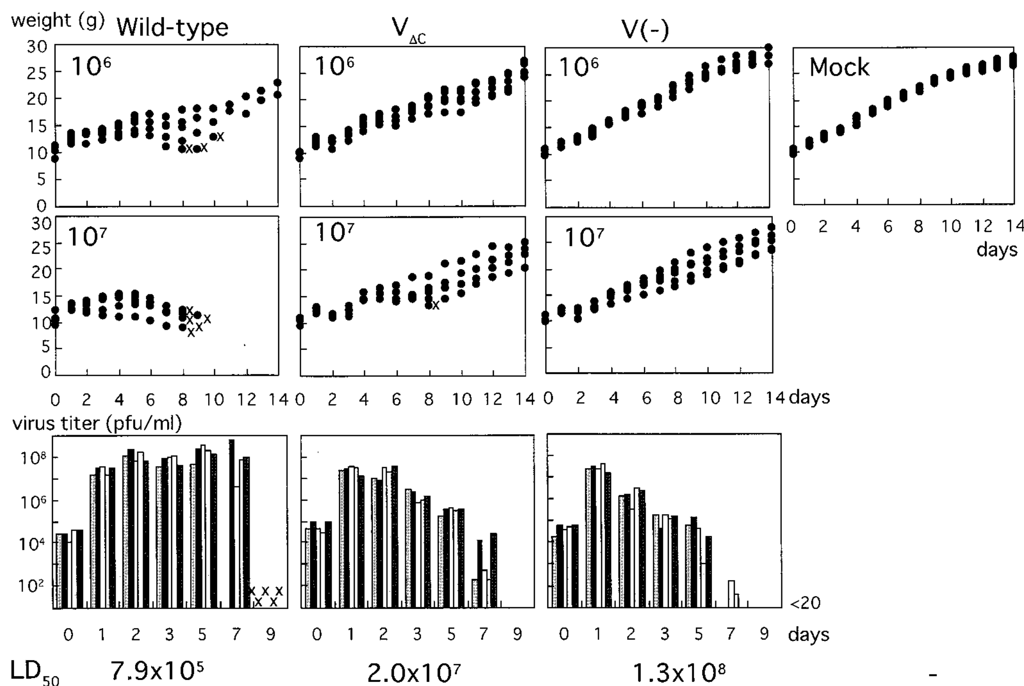

FIG. 5. Body weight gain of mice infected with the wild-type, $V_{\Delta C}$, or V(−) virus and virus multiplication in the lungs. Five mice were inoculated intranasally with 10^6 and 10^7 PFU/mouse. Every day, the weight of the mice was measured in grams. In a separate infection experiment initiated with 10^7 PFU/mouse, titers of virus in the lungs were determined. Five mice were used in each determination, and individual titers are shown. Each dead mouse is marked by an x. Some of the results for the wild-type and V(−) viruses were taken from a previous paper (17).

then rapidly cleared out (Fig. 5) as shown before (17). Interestingly, $V_{\Delta C}$ virus displayed not the same but a very similar pattern of spreading and clearance, resulting in complete clearance on day 9 (Fig. 5). A constant observation was that the clearance of the former was slightly less efficient than that of the latter, with a delay of about 1 day. This and the slightly more severe effect on body weight gain of the $V_{\Delta C}$ virus than that of V(−) virus (Fig. 5) could be associated with likewise slight differences of virulence defined by the LD_{50} between the two viruses. However, it appears to be a fair conclusion that the $V_{\Delta C}$ virus remained largely attenuated. These results clearly indicate that the cysteine-rich C-terminal region of SeV V protein is essential for maintaining a high virus burden in the lungs throughout infection and causing severe pneumonia. The introduced mutations were confirmed to be correctly retained in both of the mutant viruses recovered from the lungs.

DISCUSSION

In this and previous (17) reports, we created two different SeV V protein mutants. One is the V(−) virus, totally defective in expression of the V protein, and the other is the $V_{\Delta C}$ virus, which expresses a C-terminally truncated V protein closely resembling the W protein (Fig. 1). The mutations responsible for these changes did not affect the P and C frames and their expression (Fig. 2). The facts that both of the mutants were recovered from cDNA and replicated in eggs and tissue-culture cells as efficiently as the wild-type virus firmly established the concept that the SeV V protein falls in the category of nonessential gene product (9, 17). The same conclusion about the V protein was reached with measles virus (37). Curiously, however, the V(−) virus replicated even faster than the wild-type virus in certain but not all cell lines tested, including CV1, with augmented gene expression and cytopathogenicity (17). This was reproduced here. However, the $V_{\Delta C}$ virus showed none of such augmented in vitro phenotypes (Fig. 3 and 4). These data suggest that the expression of the N-terminal half of V protein alone is sufficient to convert the augmented phenotypes due to a total deletion of V protein to normal phenotypes characteristic of the wild-type SeV.

This conclusion was substantiated by coinfection studies showing dominance of the $V_{\Delta C}$ or wild-type phenotypes over the V(−) phenotypes (Fig. 3C). Delenda et al. (9) have also reported a SeV mutant very similar to our $V_{\Delta C}$ virus, which does not show any remarkable phenotypic changes in tissue culture cells. The mechanism responsible for maintaining the normal phenotype by the $V_{\Delta C}$ virus, probably by the N-terminal half of V protein, remains to be elucidated. This N-terminal portion is highly acidic and phosphorylated (3, 4). The C-terminally truncated W-like V protein of $V_{\Delta C}$ virus can also be phosphorylated (36). The only previously known function mapped to this portion seems to act as an acidic molecular chaperon to help N protein interact with the nascent RNA chain during the genome replication (3–5). It remains to be elucidated whether or not this function is associated with the above-described role of the N-terminal half of the V protein to maintain the normal phenotypes.

Despite the strikingly similar phenotypes displayed by the $V_{\Delta C}$ and wild-type viruses in tissue cultures in vitro, the former was found to be much less pathogenic in vivo than the latter (Fig. 5). This confirms and extends the previous notion drawn from V(−) virus that the nonessential V protein gene encodes a luxury function required for in vivo pathogenesis (17). Furthermore, the patterns of body weight gain, virus growth, and clearance were, although not identical, similar between the $V_{\Delta C}$ and V(−) viruses (Fig. 5). This suggested that the $V_{\Delta C}$ and V(−) viruses share a similar basis for attenuation. Thus, it appears to be a fair assumption that the cysteine-rich C-terminal half is a key element important for SeV pathogenesis for mice. However, the constant observation that the $V_{\Delta C}$ virus was slightly more virulent than the V(−) virus suggested a minor contribution of the acidic N-terminal half.

In conclusion, SeV V protein categorized as a nonessential protein has been shown to play an important role in in vivo pathogenesis, and the cysteine-rich C-terminal portion but not the acidic N-terminal portion could play a major role. The V protein expression could have nothing to do with the virus growth in the lung early in infection but could be essential for subsequent maintenance of a high virus load. Probably the V protein is necessary for the virus to evade host defense mechanisms which are likely recruited as early as 1 day p.i. (Fig. 5). Such an early defense could involve recruitment of NK cells and induction of interferons (1, 2, 25). In this context, SeV V protein is reminiscent of several nonessential gene products encoded by large DNA viruses such as herpes and vaccinia viruses (14, 33). Early defense may also be caused by induction of NO to kill and eliminate infected cells (26, 34). The attack by NO is associated with elevation of intracellular levels of divalent cations (10, 21), and the cations need to be chelated for the cells to survive. In this context, it is relevant to note that paramyxovirus V protein can bind zinc (24, 29, 40).

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