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# Comparative Nucleotide Sequence Analyses of the Entire Genomes of B95a Cell-Isolated and Vero Cell-Isolated Measles Viruses from the same Patient

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**Abstract.** Experimental infection of monkeys with the IC-B strain of measles virus (MV), which was isolated in marmoset B lymphoblastoid B95a cells from an acute measles patient, caused clinical signs typical for measles, while infection by the IC-V strain isolated in African green monkey kidney Vero cells from the same patient did not cause any clinical signs in infected monkeys. The IC-B strain replicated only in B95a cells, whereas the IC-V strain replicated in both B95a and Vero cells (3,6). To clarify which gene or mutation(s) was responsible for the difference in these phenotypes, the nucleotide sequences of the entire genomes of the IC-B and IC-V strains were determined. Comparative nucleotide sequence analyses revealed only two nucleotide differences, one in the P/V/C gene and the other in the M gene, predicting amino acid differences in the P, V and M proteins and a 19 amino acid deletion in the C protein of the IC-V strain. The truncation in the C protein was confirmed for the IC-V strain by immunoprecipitation using the C protein specific antiserum. No nucleotide difference was found in the envelope H gene. These results indicated that nucleotide difference(s) in the P/V/C or/and M gene, and not H gene, was responsible for the different cell tropism and pathogenicity of MV in this case.

**Key words:** measles virus, complete nucleotide sequence, B95a cell, Vero cell, C protein

## Introduction

Measles virus (MV), a member of the family *Paramyxoviridae*, genus *Morbillivirus*, causes an acute exanthematous disease that kills about one million children per year. MV virion contains the fusion (F) and hemagglutinin (H) envelope glycoproteins, the matrix (M) protein, and ribonucleocapsid complex which consists of the nucleocapsid (N) protein, the phospho (P) protein, the large polymerase (L) protein and a nonsegmented negative-stranded genome RNA of 15,894 nucleotides. In addition to the six structural proteins, MV genome codes for the nonstructural V and C proteins, which are generated from the P gene by the respective unique mechanisms. The V protein is translated from an edited mRNA which is transcribed from the P gene with a nontemplated G residue insertion at the specific editing site resulting in a frame shift downward. Thus, the amino-terminal half of the V protein is identical to that of the P protein but the amino acid sequence of the carboxy-terminal half is specific for the V protein with many cystein residues. The C protein is translated from the same mRNA for the P protein using the second AUG codon in a different coding frame. The C protein is relatively small in size and highly basic (for reviews see 1,2).

Although MV is highly contagious for humans, it was not extensively isolated from clinical specimens. Isolation of MV in primary cultures of human embryonic and monkey kidney cells or established cell lines such as African green monkey kidney Vero cells was inefficient and difficult. However, MV could be efficiently isolated from clinical samples by use of B95a cells, an adherent subline of B95-8 marmoset B-lymphoblastoid cell line (3). Thereafter, wild MV

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strains have also been isolated successfully in other human B-lymphoid cell lines such as BJAB (4,5). Moreover, wild-type MV strains isolated in B95a cells have been shown to induce clinical signs resembling to those of human measles, such as skin rashes, Koplik's spots and leukopenia, in experimentally infected cynomolgus and squirrel monkeys, whereas MV strains once adapted to Vero cells do not induce such clinical signs in monkeys (3,6).

Comparison of the nucleotide sequences of the genome of a B95a cell-isolated original virus (9301B) and its subclone (9301V) adapted to Vero cells revealed several predicted amino acid changes in P, V, C, H and L proteins and suggested the importance of amino acid changes in the polymerase and accessory proteins for the MV attenuation and different cell tropism (7).

In this paper, we have compared the nucleotide sequences of the entire genomes of another pair of MV strains isolated from the same patient in different cell lines, the IC-B strain isolated in B95a cells and the IC-V strain isolated in Vero cells, to clarify which gene or nucleotide difference(s) was responsible for the difference in the pathogenicity and cell tropism.

## Materials and Methods

### *Virus and Cells*

The IC-B and IC-V strains were isolated in B95a cells and Vero cells, respectively, from throat swab of the same patient with acute measles in 1984 (3). The IC-B and IC-V viruses were prepared by passaging in B95a cells three times and in Vero cells five times, respectively. B95a cells (3) were grown in Dulbecco's modified essential medium (DME) supplemented with 10% fetal calf serum (FCS). Vero cells were grown in Eagle's minimal essential medium (MEM) supplemented with 5% FCS and 10% tryptose phosphate broth (Difco, Detroit, MI).

### *Sequencing of the IC-B and IC-V Genomes*

For nucleotide sequence determination, MV cDNA fragments (approximately 2 Kb each) were synthesized from total RNAs from B95a and Vero cells infected with the IC-B and IC-V strains, respectively, and amplified using One step RT-PCR Kit or RNA-LAPCR Kit (Takara Biomedicals, Tokyo, Japan) and primer pairs. PCR primers were made based on the nucleotide sequence of the Edmonston strain of MV. After purification of MV gene fragments, nucleotide sequences were determined by ABI377 sequencer (PE Biosystems, Foster City, CA) using MV gene specific primers. For genome 5′ and 3′ end sequences, cDNAs of 5′ and 3′ ends were synthesized by 5′ RACE Kit (Gibco-BRL, Rockville, MD) and cloned into the plasmid pLITMUS28 (New England Biolabs, Beverly, MA) after SpeI digestion. DNA sequences were determined by sequencing several clones of the plasmids. Sequence analyses were carried out using DNASIS-Mac software (Hitachi Software Engineering, Tokyo, Japan).

### *Immunoprecipitation*

Infected B95a cells were labeled with Tran[$^{35}$S] and lysed in RIPA buffer [0.15 M NaCl, 1% Na-deoxycholete, 1% Triton X-100, 0.1% SDS, 10 mM Tris-HCl (pH 7.4)]. Radio-immunoprecipitation was done as described previously (8) using an antiserum against the $C_1$ peptide representing the amino acid 7 to 19 of the C protein, a gift from Dr. W.J. Bellini (9). SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed with a precast polyacrylamide gel SPU-15S (ATTO, Tokyo, Japan).

### *Nucleotide Sequence Accession Number*

The nucleotide sequence data reported in this paper have been deposited in the DDBJ/EMBL/GenBank nucleotide sequence databases with the accession numbers AB016162 for the IC-B strain and AB032167 for the IC-V strain.

## Results and Discussion

### *Nucleotide Sequence Analysis of the Genomes of the IC-B Strain*

First of all, nucleotide sequence of the entire genome of the IC-B strain was determined. Nucleotide sequence analysis of the IC-B strain revealed that the length of the entire genome (15,894 nucleotides) and the overall genome organizations of the IC-B strain were identical to those of the Edmonston (Ed) strain (10). The IC-B strain (referred as 84-I) has been

shown to belong to the genotype D3 (11,12) based on N gene sequences.

For the H protein, it has been reported that two amino acid residues, valine and tyrosine at positions 451 and 481, respectively, in the Ed H protein were important for hemadsorption and cell fusion of HeLa cells (5). These two amino acids also appeared to be important for the downregulation of CD46 (5,13), the complement-binding protein known as the membrane cofactor protein (MCP), which was identified to be a cellular receptor for the Ed strain (14,15). While the valine residue at 451 was conserved in the IC-B strain, the tyrosine residue at position at 481 was replaced by asparagine. Since the IC-B strains had a low hemagglutinating activity (16) and the H protein of the IC-B strain (referred as the Ichinose strain) could cause neither downregulation of CD46 nor fusion of HeLa and Cos cells when co-expressed from cDNA with the F protein (17), the tyrosine residue at 481 might be important for these activities.

*Comparison of the Nucleotide Sequences of the Genome of the IC-B and IC-V Strains*

Secondly, nucleotide sequence of the entire genome of the IC-V strain was determined. When the nucleotide sequences of the IC-B and IC-V strains were compared, only two nucleotide differences were found (Table 1), one in the P/V/C gene and the other in the M gene. The nucleotide difference at position 2,331 (G and A) in the P/V/C gene resulted in amino acid exchanges in the deduced amino acid sequence of the corresponding three proteins. An exchange occurred at position 175 (methionine and isoleucine) of both the P and V proteins and another amino acid difference was at position 168 (tryptophan and stop) resulting in a 19 amino acid deletion in the C protein of the IC-V strain. To confirm the truncation in the C protein encoded by the IC-V strain, radioimmunoprecipitation was carried out using antiserum specific for the C protein (9). As shown in Fig. 1, molecular size of the C protein of the IC-V strain was smaller than that of the IC-B strain. In addition to the C protein, prominent signals, which were considered non-specific precipitates, were observed in a relatively high-molecular weight region in our experimental condition. The C protein has been categorized as an accessory protein and in fact the recombinant MV lacking the C protein can replicate normally in tissue culture cells (18). The C protein of the IC-V strain might have lost its function involved in other than viral replication due to the 19 amino acid deletion, although the function is not clear yet.

On the other hand, the nucleotide difference at position 3,628 (C and A) in the M gene caused amino acid difference at position 64 ( proline and histidine) in the M protein. Since proline is known to be important for maintaining secondary structure of proteins, the M proteins of the IC-B and IC-V strains might have different conformation. No nucleotide difference was found in the F and H genes as well as the trailer, leader, gene start and gene end sequences.

In the previous paper, we had observed slight differences in the reactivities of the monoclonal antibodies A49 and C111 against the N and F proteins, respectively, between the IC-B and IC-V strains (3). As no nucleotide difference was detected in the N and F genes between the IC-B and IC-V strains, we reexamined the reactivities of these monoclonal antibodies by immunoprecipitation. However, we could not detect such differences observed previously (data not shown). The reactivities of these monoclonal antibodies were so weak that the amount of samples loaded or washing condition might have affected the previous results.

It was reported previously that when a B95a cell-isolated MV (9301B) was passaged in Vero cells to become a Vero cell-adapted virus (9301V), eight nonsynonymous mutations occurred in the viral genome, two in the P/V/C gene, three in the H gene

*Table 1.* Summary of nucleotide and deduced amino acid differences between the IC-B and IC-V strains

| Nucleotide | | | | Amino Acid | | | |
|---|---|---|---|---|---|---|---|
| Gene | Position | IC-B | IC-V | Protein | Position | IC-B | IC-V |
| P/V/C | 2331 | G | A | P | 175 | M | I |
| | | | | V | 175 | M | I |
| | | | | C | 168 | W | stop |
| M | 3628 | C | A | M | 64 | P | H |

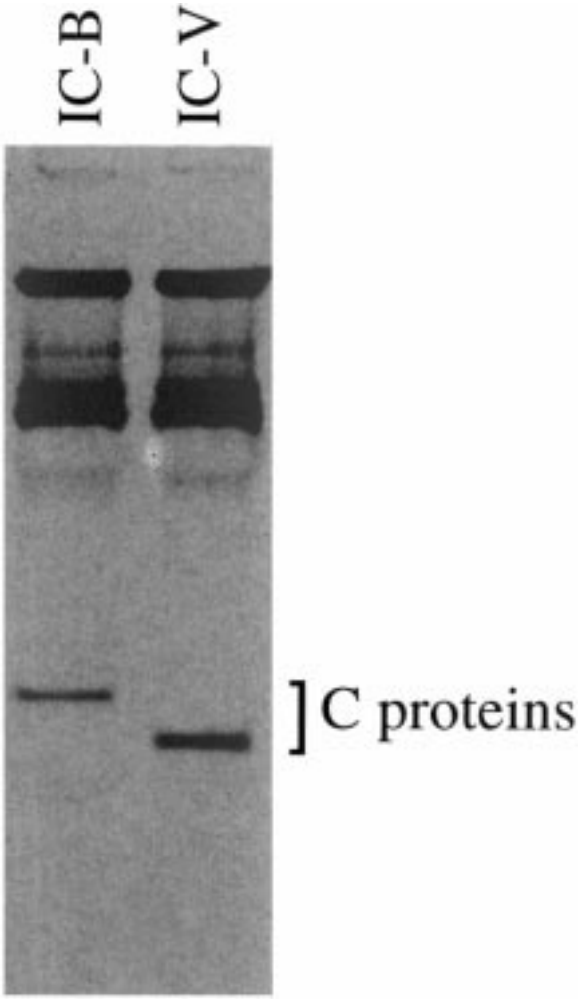


*Fig. 1.* Immunoprecipitation of the C proteins of the IC-B and IC-V strains. B95a cells infected with the IC-B or IC-V strains were labeled with Tran[$^{35}$S] and lysed. The lysates were immunoprecipitated with antiserum against the C protein and followed by SDS-PAGE.

and three in the L gene (7). Since nucleotide differences in the P/V/C gene were observed for both the IC-B/IC-V and 9301B/9301V pairs, the amino acid difference in the P, V or C proteins might be important for the cell tropism and pathogenicity of MV. Comparative analyzes of other four pairs of B95a cell-isolated and Vero cell-adapted strains indicated that every pair had at least one nonsilent nucleotide substitutions in the P/V/C gene at various positions (data not shown), supporting our hypothesis.

The involvement of the H protein in determining the cell tropism of MV is controversial. Lecouturier et al. reported that the H protein of the Hallé strain, which is fusogenic in HeLa cells, caused HeLa cell fusion when expressed with the F protein from cDNAs, whereas the H protein of the Ma93F strain, which is not fusogenic in HeLa cells, did not cause HeLa cell fusion when expressed with F protein from cDNAs (5). Tanaka et al. also reported that the H protein of the Ed strain, which is fusogenic in HeLa cells, caused HeLa cell fusion when expressed with the F protein from cDNAs, whereas the H protein of the IC-B strain (referred as Ichinose), which is not fusogenic in HeLa cells, did not cause HeLa cell fusion when expressed with the F protein from cDNAs (17). These authors proposed that the H protein plays a key role in determining MV cell tropism. An unidentified cellular receptor other than CD46 molecule for wild-type MV has been predicted on the surface of B-lymphoid cell lines such as B95a and BJAB cells (13,17,19). It might be reasonable to hypothesize that the envelope H protein determines cell tropism of MV in analogy of other viruses (20). Restricted numbers of amino acid changes in the H gene were reported between B95-8 cell-isolated and Vero cell-adapted strains (21), between a BJAB cell-grown strain and a Vero cell-grown WTF strain (22) and between a B95a cell-grown strain (9301B) and its Vero cell-adapted strain (9301V) (7). The significance of these amino acid changes in the H genes in the process of adaptation of MV to Vero cells is not yet clear, although Takeda et al. suggested from the result of cell fusion assay that the H protein is not an important factor in determining the MV cell tropism and pathogenicity (7). On the other hand, Takeda et al. also reported that no amino acid change was found in the H protein between another pair of B95a cell-isolated strain (9403B) and its Vero cell-adapted strain (9403V) (7). Recently, Johnston et al. generated recombinant Ed viruses containing the H protein of a BJAB cell-isolated strain (WTFb) using the reverse genetics system (10), and showed some growth of these recombinant viruses in Vero cells (23). These data also suggested that the H protein is not an important factor in determining cell tropism.

By comparative sequence analyses, we can clearly show that the P/V/C or/and M protein and not H protein is responsible for the different cell tropism and pathogenicity at least in the IC-B and IC-V strains. Extended comparative sequence analysis of other B95a and Vero cell-grown virus pairs should be required to elucidate this point. As the nucleotide

difference between the IC-B and IC-V strains are limited, this strain pair would be useful for studying the mechanism of the MV adaptation and pathogenicity.

At present, we do not know which nucleotide difference(s) is critical for the different cell tropism and pathogenicity between the IC-B and IC-V strains. Considering the importance of the V and C proteins for the growth and pathogenicity on Sendai virus (24–27), amino acid difference in the V and C proteins of MV might play important roles in the cell tropism and pathogenicity. In order to identify the critical nucleotide difference responsible for the different phenotypes, we have established a reverse-genetics system for the rescue of infectious MV mutants from modified cDNA of the entire genome of the IC-B strain of MV (manuscript in preparation).

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