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Genetic Differentiation of a Measles Virus Isolated from a Case Occurring 15 Days after Measles Vaccination

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Measles virus (MV) is an important human pathogen. Since 1954, when Enders and Peebles succeeded in isolating MV, many live measles vaccines have been developed, and are widely used today (1). Live measles vaccines are highly immunogenetic and effective in preventing a natural infection of wild-type MV. As some recipients develop vaccine-induced side-reactions such as fever and rash (2-4), it is becoming important to differentiate vaccine-derived viruses from wild-type viruses. We report here the results of genetic differentiation of an MV isolated from a case occurring 15 days after live measles vaccination.

In June 18, 2000, a 1-year 11-month-old girl with a high fever (39.0°C), cough and rash, was admitted to our hospital. A diagnosis of measles was made based on her clinical symptoms and Koplik's spots on her buccal mucosa. For virological examinations, a throat swab collected from the patient on June 22 was inoculated into B95a cells, which is a highly susceptible cell line to MV (5). Characteristic cell fusion with multinucleated giant cells appeared. The isolate was identified as an MV by an indirect immunofluorescent test and micro-neutralizing test using specific antisera. It was unknown whether the patient had been in contact with measles patients in the previous 2-3 weeks, but she had received a measles vaccine (CAM strain, Biken, Osaka) on June 7, 2000, about 10 days before the onset of the symptoms. As side-reactions of the vaccination are frequently observed around 2 weeks after vaccinations (2-4), the origin of the present isolate (F00-37041 strain) was determined by a restriction fragment length polymorphism (RFLP) assay (6). RNA was extracted from a culture fluid of F00-37041-infected B95a cells or from commercial measles CAM vaccine (Biken). The hemagglutinin (H) protein encoding region, ntd 8067-8701, of the MV genome was amplified by an RT-PCR method by using the MH2 (5′-GGGCTCCGGTGTTCCATATG-3′) and MH6 (5′-CTTGAATCTGGTATCCACTCCAAT-3′) primer pair (7). For RFLP analysis, 5 μl of the PCR product was digested by either *Dde*I, *Ava*II, or *Hae*III, and the fragments were detected by electrophoresis in 3% agarose gels with ethidium bromide staining. As shown in Figure 1, the PCR product derived from the CAM strain was cleaved into four fragments by *Dde*I, and two fragments by *Ava*II or *Hae*III. In contrast, the digestion patterns of the PCR product derived from F00-37041 strain were different from those of the CAM strain; the product was cleaved into three fragments by *Dde*I and by *Ava*II. These results suggested that the F00-37041 strain was not a vaccine-derived strain. To confirm this speculation, we determined the nucleotide sequences of the PCR product of F00-37041 strain by using a direct sequencing method, and compared the nucleotide sequence with those of the CAM strain, which was obtained from the GenBank database (accession No.U03649). We observed 24 nucleotide differences between the CAM strain and the F00-37041 strain, and 11 of them were associated with amino acid changes (Table 1). The nucleotide substitution from G to A at 8516 observed in the isolate F00-37041 was associated with an amino acid change from Asp to Asn creating a new glycosylation site (8). This change is related to the slower mobility of the H protein in gel electrophoresis and the loss of hemadsorption activity of an MV, both of which are characteristics of a recent wild-type MV (4, 7-10). Our analysis indicated that the case was a wild-type MV infection though the timing of the vaccination was compatible with the vaccine-associated case.

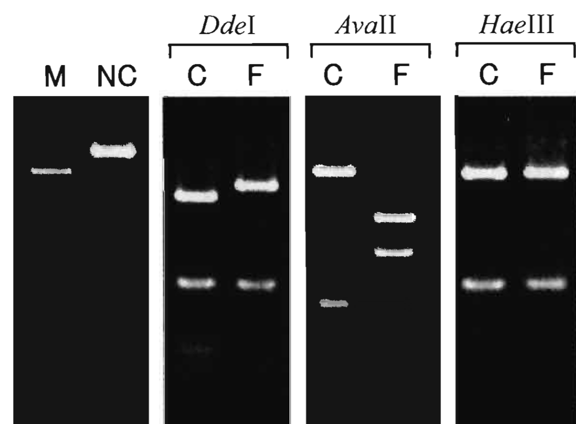

Fig. 1. RFLP analysis of the CAM strain and the F00-37041 strain. The 635-ntd long hemagglutinin (H) encoding region (ntd 8067-8701) was RT-PCR-amplified, and the products were then digested with *Dde*I, *Ava*II, or *Hae*III. M: 100 bp ladder, NC: non-cut (635 bp), C: CAM strain, F: F00-37041 strain.

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Table 1. Comparison of nucleotide and predicted amino acid substitution of HA gene (nucleotide position: 8087-8676) between the CAM strain and the F00-37041 strain

Position		Difference of nucleotide (Amino acid)	
Nucleotide	(Amino acid)	CAM	F00-37041
8096[1]	(276)	C (Leu)	T (Phe)
8105	(279)	C (Pro)	T (Ser)
8120	(284)	C (Leu)	T (Phe)
8124	(285)	G (Ser)	A (Asn)
8131	(287)	T (Cys)	C (*)[2]
8143	(291)	G (Leu)	A (*)
8156	(296)	C (Leu)	T (Phe)
8167	(299)	T (Leu)	C (*)
8174	(302)	G (Gly)	A (Arg)
8206	(312)	A (Gly)	G (*)
8282	(338)	T (Ser)	C (Pro)
8285	(339)	T (Leu)	C (*)
8311	(347)	C (Asp)	T (*)
8383	(371)	A (Arg)	G (*)
8419	(383)	A (Gln)	G (*)
8429	(387)	A (Lys)	C (Gln)
8434	(388)	T (Gly)	C (*)
8474	(402)	T (Leu)	C (*)
8482	(404)	T (Asp)	C (*)
8516	(416)	G (Asp)	A (Asn)
8539	(423)	T (Leu)	C (*)
8557	(429)	G (Ser)	A (*)
8634 8635	(455)	A T (Asn)	C C (Thr)

[1] The number of the nucleotide is based on the genomic location of the Edmonston strain.
[2] Asterisks represent an identical amino acid with the CAM strain.

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