ELSEVIER

Virus Research 86 (2002) 123–131

Virus Research

www.elsevier.com/locate/virusres

Short communication

Alterations and diversity in the cytoplasmic tail of the fusion protein of subacute sclerosing panencephalitis virus strains isolated in Osaka, Japan☆

Xiaojun Ning [a], Minoru Ayata [a], Masatsugu Kimura [a,b], Katsuhiro Komase [c], Kyoko Furukawa [a,d], Toshiyuki Seto [a,d], Nobuhisa Ito [a], Masashi Shingai [a], Isamu Matsunaga [a], Tsunekazu Yamano [d], Hisashi Ogura [a,*]

[a] *Department of Virology, Osaka City University Medical School, Asahimachi, Abeno-ku, Osaka 545-8585, Japan*
[b] *Laboratory of Biophysics, Osaka City University Medical School, Asahimachi, Abeno-ku, Osaka 545-8585, Japan*
[c] *Center for Basic Research, Kitasato Institute, Shirokane, Minato-ku, Tokyo 108-8682, Japan*
[d] *Department of Pediatrics, Osaka City University Medical School, Asahimachi, Abeno-ku, Osaka 545-8585, Japan*

Received 4 July 2001; received in revised form 16 February 2002; accepted 16 February 2002

Abstract

We determined the nucleotide sequence of the fusion (F) gene of three strains (Osaka-1, -2, and -3) of nonproductive variants of measles virus (MV). These viral strains were isolated in Osaka, Japan, from brain tissues of patients with subacute sclerosing panencephalitis (SSPE). Phylogenetic analysis revealed a close relationship among the three strains of SSPE virus. The cytoplasmic tail of the F protein, predicted from sequence analysis of the gene, is altered in all three SSPE strains when compared to the MV field strains. However, the extent and mode of alteration are different in each strain. The F protein of the Osaka-1 strain has six nonconservative amino acid substitutions and a 29-residue elongation of its cytoplasmic tail. The F protein of the Osaka-3 strain has two nonconservative substitutions and a 5-residue truncation of its C-terminus. Although the termination codon is not altered in the F protein of the Osaka-2 strain, five or six amino acids are changed in the cytoplasmic tail of the F protein of the two sibling viruses of this strain. The significance of the altered cytoplasmic domain of the SSPE viruses in the SSPE pathogenesis is discussed.

Keywords: Measles virus; Subacute sclerosing panencephalitis virus; Fusion gene; Mutation

The fusion (F) gene of measles virus (MV) (2373-nucleotides long for the Edmonston strain) encodes an inactive F precursor glycoprotein (F_0, 550-amino acids long). This spike protein is transported to the Golgi apparatus where cellular proteases cleave the F_0 into disulfide-linked subunits, F_1 and F_2. In general, the F gene has been

☆ The nucleotide sequence data reported in this paper for nucleotide sequences with accession numbers AF179430–AF179441 have been deposited in the DDBJ, EMBL, and GenBank™ databases.

* Corresponding author. Tel./fax: +81-6-6645-3910.
E-mail address: ogurah@med.osaka-cu.ac.jp (H. Ogura).

0168-1702/02/$ - see front matter
PII: S0168-1702(02)00042-4

thought to be well conserved among the paramyxoviruses (Barrett et al., 1987; Buckland et al., 1987), particularly among MVs (Rota et al., 1992). In combination with the hemagglutinin (H) protein, another glycoprotein spike of MV, the F protein is responsible for fusion of the viral envelope with the host cell membrane and for syncytium formation in the virus-infected cells. In contrast to MV isolated from acute measles, genetic information is limited on the F protein of subacute sclerosing panencephalitis (SSPE) virus. This nonproductive MV variant with defective matrix (M) protein is isolated from brains of patients with SSPE, a fatal degenerative disease of children caused by its persistent infection of the central nervous system. The molecular mechanisms of cell-to-cell spread of SSPE virus in the brain are still unclear, although a recent report (Lawrence et al., 2000) has shown that a microfusion not mediated by CD46 might be associated with in vitro transmission of MV from neuron to neuron.

The envelope genes (F, H, and M) of MV might have evolved in the transition from acute to persistent infection in infected brains. In such specific environments, some specified mutations might have selectively survived. For example, alterations of the cytoplasmic tail of the F protein have been reported in MV persistent infection (for a review, see Griffin and Bellini, 1996). However, most sequence data were obtained from directly cloned genes from SSPE brain specimens, and might not represent the replicating viral genome. Several (the MF, Biken, Niigata-1, Yamagata-1, and IP-3-Ca strains) of a few replicable isolates of SSPE virus from diseased human brains have been sequenced (Cattaneo et al., 1987, 1988a,b, 1989; Ayata et al., 1989, 1991; Komase et al., 1990; Yoshikawa et al., 1990; Wong et al., 1991). Therefore, genetic data from more isolates are needed to clarify whether the persistence and neuropathogenicity of the virus are associated with particular mutations.

We have reported three strains (Osaka-1, -2, and -3) of nonproductive SSPE virus isolated in Osaka, Japan (Ogura et al., 1997), and have characterized the defective M gene from the Osaka-1 and -2 strains (Ayata et al., 1998a; Seto et al., 1999) and compared it with those from local contemporary isolates from patients with acute measles (Ayata et al., 1998b). In this paper, we further determined the complete nucleotide sequences of the F gene of these strains of SSPE virus in order to analyze their common mutations associated with viral persistence in the brain. The several sibling viruses isolated from different portions of the same patient's brain by cocultivating with the different cell types were compared with each other in order to investigate viral spreading in the brain.

MV strains and sibling viruses of SSPE strains sequenced for this study were previously described (Ogura et al., 1997; Ayata et al., 1998b). All of the strains, including those referred to in this paper, are listed in Table 1.

Total RNA preparation from the virus-infected cells passaged in the least possible times was subjected to reverse transcription primed with a random primer (nonadeoxynucleotide mixture, Takara Shuzo, Otsu, Japan) according to the method described previously (Ayata et al., 1998b). The sequence including the open reading frame for the F protein was amplified by polymerase chain reaction (PCR) with a set of primers, MVF-m5 (5′-AATGTCCATCATGGGTCTCAAGGT-3′) and MVF-g6 (5′-CATTGTGGATGATCTTG-CACCCTA-3′) prepared on the basis of the published sequence for the Edmonston strain of MV. To amplify the F gene of the Osaka-1 strain, MVF-m6 (5′-AACGTCCATCATGGGTCTCAA-GAT-3′) was substituted for the MVF-m5 forward primer. Amplification was performed with 30 cycles of three steps (98 °C for 10 s, 55 °C for 30 s, and 72 °C for 1 min), and Pyrobest DNA polymerase was used as recommended by the manufacturer (Takara Shuzo). Amplified products were purified according to the method described previously (Ayata et al., 1998b). A portion of the F gene was sequenced directly with a Thermosequenase II dye terminator cycle sequencing kit (Amersham Phamacia Biotech, Piscataway, NJ) and a model 373S or Prism 310 sequencer (Applied Biosystems, Foster City, CA). Also, the PCR products covering the open reading frame for the F protein were cloned into a pBluescript II KS plasmid and several clones were sequenced.

The sequences for the 5′ noncoding and 3′ noncoding region of the F gene were determined by the PCR products encompassing the M–F and F–H junctions, respectively. The primer pairs used were MVM-m7 (5′-CAGCCAGCAGCCGACGGCAA-3′) and MVF-g5 (5′-GACCGGTTCAGAGTGTAGCTTCA-3′); and MVF-m4 (5′-ATCCTGATTGCAGTGTGTCT-3′) and MVH-g3 (5′-CCACTCTTCAAAATCATCGG-3′) for PCR encompassing the M–F and F–H junctions, respectively. These primers were constructed based on the published sequences for several MVs and SSPE viruses. Amplification was performed with 40 cycles of two steps (98 °C for 20 s and 68 °C for 5 min), and an LA-PCR kit was used as previously described (Ayata et al., 1998b). A portion of the PCR products was sequenced directly as described above. Additional nine primers were constructed for sequencing F genes for both orientations, on the basis of the sequences conserved among several strains of MVs and SSPE viruses. These primers were MVF-m1 (5′-GGAATCCCAKAATCAAGACTCATC-3′), MVF-m2 (5′-CTACTAATCAGGCAATTGAG-3′), MVF-m3 (5′-CAACCCAAGGGTACCTTATC-3′), MVF-g2 (5′-TTGATGACGAAGRGGAGACTTGTG-3′), MVF-g3 (5′-TGCCCGGTAGTCGAGGTGAA-3′), MVF-g4 (5′-GAGTTATCCGGGCCTTTATT-3′), and MVF-g7 (5′-TGTGGTGGATTGATCTTTCG-3′).

An unrooted phylogenetic tree drawn by a CLUSTAL W analysis, based on the sequence of the F protein coding region of the Edmonston strain (1653 nucleotides), showed all sibling viruses of the three SSPE strains, and the Nagahata and the Masusako strains of MV in the same cluster in agreement with the results shown by our previous M gene analysis (Ayata et al., 1998b) and also by the phylogenetic analysis based on the sequence of the 3′end of the N gene of selected sibling SSPE viruses (Osaka-1 Fr/V, Osaka-2 Fr/V, and Osaka-3 Bs/V) (data not shown).

Our sequence result for the F gene of the Nagahata strain was different by three nucleotides

Table 1
Measles and SSPE virus strains compared in this study

Virus	Strain	Sibling virus	Portion of brain for isolation	Cell line for isolation	Location, year of isolation[a]
Measles	Edmonston[b]			Human kidney	Boston, 1954
	Toyoshima			Vero	Osaka, 1959
	Nagahata[c]			Vero	Osaka, 1971
	Masusako			Vero	Osaka, 1983
SSPE	Osaka-1				Osaka, 1993 (1969)
		Fr/V	Frontal lobe	Vero	
		Fr/H	Frontal lobe	TIG-1[d]	
		Oc/V	Occipital lobe	Vero	
	Osaka-2				Osaka, 1994 (1984)
		Fr/V	Frontal lobe	Vero	
		Fr/B	Frontal lobe	B95a	
	Osaka-3				Osaka, 1995 (1971)
		Bs/V	Brain stem	Vero	
		Bs/B	Brain stem	B95a	
		Oc/V	Occipital lobe	Vero	

[a] Year of primary measles infection is shown in parentheses.
[b] Richardson et al. (1986).
[c] The F gene sequence of the Nagahata strain was first reported by Watanabe et al. (1995), but it differed by three nucleotides from our sequence.
[d] Diploid cells derived from human embryonic lung cells.

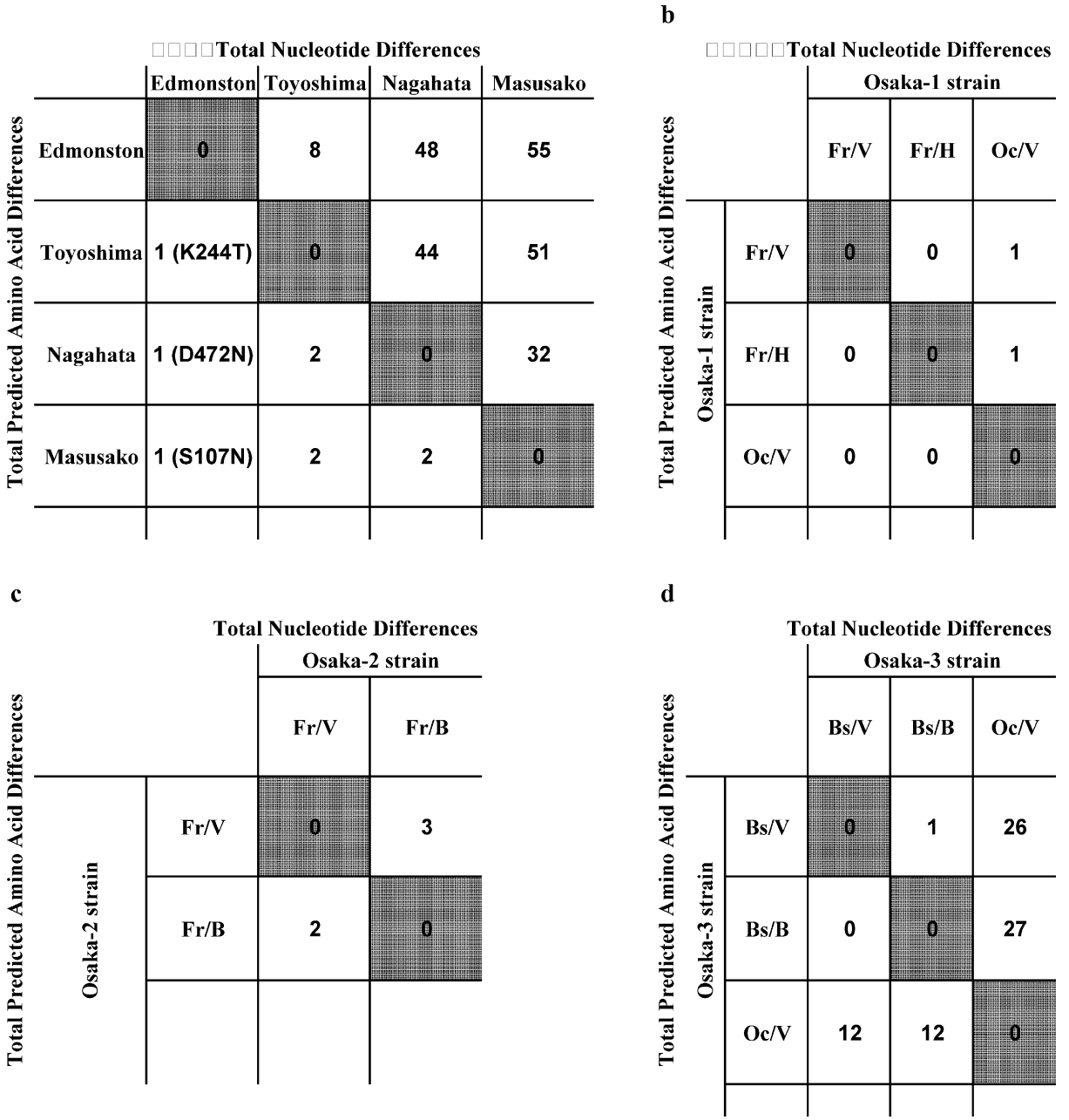

Fig. 1. Comparison of total nucleotide differences and total predicted amino acid differences among MV strains (a), among sibling viruses of SSPE virus Osaka-1 (b), -2 (c), or -3 (d) strains. Position of amino acid replacement is shown in parentheses.

from that previously reported by Watanabe et al. (1995). In their sequencing data, an A to G substitution at nucleotide number 1158 resulted in an additional amino acid substitution, a glutamine to arginine change, at residue 192 (numbered from the second AUG codon according to Cathomen et al., 1995). Total nucleotide differences and total amino acid differences among the MV strains

were shown in Fig. 1a. The Masusako strain isolated in 1983 was different from the Nagahata strain isolated in 1971 by 32 nucleotides and from the Toyoshima strain isolated in 1959 by 51 nucleotides. Despite the numerous nucleotide substitutions, only two nonconservative changes were found in the deduced amino acid sequence, showing that the F protein of the three MV strains isolated in Osaka was highly conserved. When the F protein sequences of the three MV strains were compared with that of the Edmonston strain, only a single amino acid change was noted in each strain. There were no insertions or deletions in the F gene sequence of these strains. In addition, it was confirmed that the Nagahata and the Masusako strains, which were isolated at roughly the same time and the same place as the primary measles infection in our SSPE patients, were the most adequate strains for use as a reference in comparison with the F gene of the Osaka-1, -2, and -3 strains (Table 2). This result was consistent with that from our previous M gene analysis (Ayata et al., 1998a).

When whole F gene sequences were compared, a single G insertion was observed in the 5′ noncoding region of the Osaka-1 and Osaka-3 strains (data not shown). For detailed analysis, we compared numbers of nucleotide differences of the F genes and amino acid differences of the predicted F proteins among the eight sibling viruses of the three SSPE virus strains and the two selected MV strains (the Nagahata and the Masusako) (Table 2), and among the sibling viruses of each SSPE strains (Fig. 1b, c, and d). The F gene sequence of the Osaka-1 Fr/V sibling virus of the Osaka-1 strain was different from that of the Nagahata strain by 45 nucleotides (Table 2). The sequence of the Osaka-1 Fr/H virus was identical to that of the Osaka-1 Fr/V virus, and only one nucleotide difference without amino acid replacement was found in the sequence of the Osaka-1 Oc/V virus (Fig. 1b). The F gene sequence of the Osaka-2 Fr/V sibling virus was different from that of the Nagahata strain by 51 nucleotides. The Osaka-2 strain was more closely related to the Masusako strain; the nucleotide differences of the F gene between the Osaka-2 Fr/V and the Masusako strain decreased to 27 (Table 2). There were three nucleotide differences in the sequence between the Osaka-2 Fr/V and the Osaka-2 Fr/B viruses, and two of the three differences were nonconservative changes (Fig. 1c). The F gene sequence of the Osaka-3 Bs/V sibling virus was different from that of the Nagahata strain by 46 nucleotides (Table 2). The F gene sequence of the Osaka-3 Bs/B virus was identical to that of the Osaka-3 Bs/V virus excepting a single nucleotide, which difference did not result in an amino acid change (Fig. 1d). A total of 26 nucleotide differences, 12 of which were nonconservative changes, were noted between the Osaka-3 Bs/V and the Osaka-3 Oc/V sibling viruses (Fig. 1d), whereas the F gene sequence was very similar among the sibling viruses of the Osaka-1 or -2 strains and among the Osaka-3 Bs/V and Bs/B sibling viruses (Fig. 1b, c, and d).

In all three SSPE strains no amino acid substitution was found at the glycosylation sites (amino acid residues 29, 61, and 67), at the subtilisin-related protease cleavage site (amino acid residues 108–112), at the disulfide sites (amino acid residues 68 and 195), or at the palmitoylation sites (amino acid residues 503, 515, and 521) (data not shown). In addition, the hydrophobic region located at amino acid residues 113–136 was conserved (data not shown). This conservation may be natural, given that these sites are essential for viral multiplication, especially through fusion ac-

Table 2
Comparison of nucleotide differences between SSPE strains and MV field strains

Strain	Sibling virus	Nagahata	Masusako
Osaka-1	Fr/V	45[a] (13)[b]	57[a] (13)
	Fr/H	45[a] (13)	57[a] (13)
	Oc/V	46[a] (13)	58[a] (13)
Osaka-2	Fr/V	51 (12)	27 (12)
	Fr/B	48 (10)	24 (10)
Osaka-3	Bs/V	46[a] (14)	56[a] (14)
	Bs/B	45[a] (14)	55[a] (14)
	Oc/V	48[a] (14)	59[a] (14)

[a] A single nucleotide insertion is included in the 5′-noncoding region.

[b] Amino acid differences in the region of amino acid residues 1–550 are shown in parentheses.

Virus	Strain	518	530	540	550	579
Measles	Edmonston	RGR	CNKKGEQVGM	SRPGLKPDLT	GTSKSYVRSL	
	Toyoshima	***	**********	**********	**********	
	Nagahata	***	**********	**********	**********	
	Masusako	***	**********	**********	**********	
SSPE	Osaka-1 Fr/V	***	***N******	***DPN**P*	*********R	RSSTTLETQM SHKSPLRHQA TTASSTKPT
	Osaka-1 Fr/H	***	***N******	***DPN**P*	*********R	RSSTTLETQM SHKSPLRHQA TTASSTKPT
	Osaka-1 Oc/V	***	***N******	***DPN**P*	*********R	RSSTTLETQM SHKSPLRHQA TTASSTKPT
	Osaka-2 Fr/V	***	*********I	****P***P*	**P**HA***	
	Osaka-2 Fr/B	***	**********	****P***P*	**P**HA***	
	Osaka-3 Bs/V	***	****E*****	***D******	*****	
	Osaka-3 Bs/B	***	****E*****	***D******	*****	
	Osaka-3 Oc/V	***	****E*****	***D******	*****	
	Biken	***	******			
	Yamagata-1	***	***RENKLVC	QDQA		
	IP-3-Ca	***	******			
	Patient C	***	**********	**********	**********	

Fig. 2. Deduced amino acid sequence of the carboxyl terminal region (the cytoplasmic tail) of the F protein of the measles and SSPE viruses. Asterisks indicate amino acids identical to the corresponding residues of the Edmonston strain. Positions relative to the second AUG codon of the open reading frame of the F protein are indicated at the top. The F protein sequences of the Biken, IP-3-Ca, and Yamagata-1 strains of SSPE viruses were from Watanabe et al. (1995), Cattaneo et al. (1989) and Komase et al. (1990), respectively.

tivity. There was no common amino acid substitution found only among the three SSPE strains, and the substitutions were scattered in the extracellular domain of the protein.

Concerning the cytoplasmic domain of the F protein, however, all three strains of SSPE virus were markedly affected, whereas the extent and the mode of alteration differed among the strains (Fig. 2). The predicted F protein sequences of the three sibling viruses of the Osaka-1 strain showed six nonconservative amino acid substitutions, in addition to elongation of the C-terminus by 29 residues. In contrast, C-terminal truncation with two nonconservative substitutions was found in the sequences of the three sibling viruses of the Osaka-3 strains. The Osaka-2 Fr/V and Osaka-2 Fr/B sibling viruses of the Osaka-2 strain had six and five amino acid substitutions, respectively, showing neither elongation nor truncation.

The common feature among the F genes of the SSPE viruses was the overall conservation of the F protein-coding capacity despite the predicted numerous amino acid substitutions. This contrasts with the mutation of the M gene of SSPE viruses, which severely affected the expression of the M protein (Cattaneo et al., 1986, 1987, 1988a,b; Ayata et al., 1989, 1998a,b; Seto et al., 1999). Biased hypermutation, which was commonly found in the M genes of the SSPE virus Osaka-1, -2, and -3 strains (Ayata et al., 1998a), was not obvious in the F genes of the same strains. A subtilisin-related protease cleavage site at amino acid residues 108–112 and the hydrophobic region located at amino acid residues 113–136, which has been postulated to play an important role in fusion activity (Richardson et al., 1986), was perfectly conserved. Cysteine residues used for the disulfide bond formation at amino acid residues 68 and 195 (Griffin and Bellini, 1996) were also conserved. There was no amino acid substitution at the potential sites of glycosylation (at amino acid residues 29, 61, and 67) or of palmitoylation (at amino acid residues 503, 515, and 521) (Caballero et al., 1998). An A to G transition generated an arginine to glycine substitution at residue 70, which was found in two of the three sibling viruses of the Osaka-3 strain, might be significant for viral survival in the brain based on the fact that amino acid residue 70 plays a role in the dominant antigenic site of the F protein (Fayolle et al., 1999). In addition, no common substitutions in the F protein sequences were found

among the four SSPE viruses including the Biken strain (Watanabe et al., 1995), an SSPE virus closely related to the Osaka-1 and -3 strains (Ayata et al., 1998b).

When the predicted amino acid sequences of the F proteins were compared in order to consider the evolutionary relationship between the Osaka-3 Bs/V (or the Osaka-3 Bs/B) and the Osaka-3 Oc/V sibling viruses, a newly created premature termination and eight substitutions, for 14 and 14 amino acid differences between the Nagahata strain and the Osaka-3 Bs/V, and between the Nagahata strain and the Osaka-3 Oc/V, respectively (Table 2), were common to the two sibling viruses. Six and six amino acid substitutions independently occurred in the Osaka-3 Bs/V and the Osaka-3 Oc/V sibling viruses, respectively. Therefore, it is likely that the Osaka-3 Bs/V and the Osaka-3 Oc/V viruses emerged separately, according to the progression of SSPE, from an intermediate ancestor that contained the common substitutions.

As previously indicated by Schmid et al. (1992), it is noteworthy that the cytoplasmic tail of the F protein was found to be largely affected in all three strains of SSPE viruses, whereas the extent and the mode of alteration were different among the strains. These variations might have been the result of selectivity for persistence in the brain. Thirty-three residues of the cytoplasmic tail of the F protein were identical among the known wild-type MVs, whereas the predicted F protein of three sibling viruses of the Osaka-1 strain had six nonconservative amino acid substitutions in this region. Further, a U–C transition at the authentic termination codon for the F protein of MV resulted in elongation of the C-terminus by 29 residues. Similar elongation of the cytoplasmic tail was found in the brain of SSPE patient P (Schmid et al., 1992). In contrast, in the sequence of the Osaka-3 strain, a U–G transversion generated a premature termination codon and resulted in a truncation of the C-terminus by five residues, in addition to two nonconservative substitutions. Truncations of the cytoplasmic tail were very common, and were found in the brains of SSPE patients A, B, F, G, O, R, and S (Schmid et al., 1992), as well as in the F protein of the Biken (Watanabe et al., 1995) and the IP-3-Ca strains (Cattaneo et al., 1989). The C-terminus of the Yamagata-1 strain was also truncated and had an altered reading frame (Komase et al., 1990). Although the termination codon conserved for the F protein of the Osaka-2 strain was similar to that found in the brains of SSPE patients D and K (Schmid et al., 1992), five and six substitutions were predicted in the F protein of the sibling viruses of Osaka-2 Fr/B and Osaka-2 Fr/V, respectively. These changes could greatly alter the primary and the secondary structure of the cytoplasmic tail of the F protein, and would affect possible interaction with other proteins such as M, H, and N to alter the virus assembly within membrane rafts, virus release (Naim et al., 2000; Vincent et al., 2000), and fusogenic activity. Cathomen et al. (1998b) reported that the recombinant MV with altered cytoplasmic tails of the envelope glycoproteins produced a larger syncytium than that of its parental MV. In addition, M-less MV showed enhanced syncytium forming ability and restricted virus production (Cathomen et al., 1998a), suggesting that F and H proteins need to interact M protein at the cell membrane. Since all of our SSPE strains are defective M protein expression, both acquired characters of M protein defectiveness and altered cytoplasmic tail of the F protein might be much of significance in developing of MV into SSPE virus in patient brains. The reason why the F genes with a highly mutated cytoplasmic tail are selected in the brains of SSPE patients is not fully understood. An exception was the F protein analyzed directly from a cloned gene from SSPE patient C, a patient with measles inclusion body encephalitis (MIBE), in which the cytoplasmic tail was not altered (Cattaneo et al., 1989). Although the characteristics of the viruses isolated from SSPE and MIBE patients might be similar (Roos et al., 1981; Ohuchi et al., 1987), the mechanisms of the emergence or the evolution of the virus might be different. It is of great interest that the recombinant virus with an SSPE virus-like cytoplasmic tail of the F protein penetrated more deeply into brain parenchyma but demonstrated rather reduced neurovirulence to mice when it was inoculated into the brains of CD46 transgenic mice (Cathomen et al., 1998b). Studies

of mutations commonly found in SSPE viruses using reverse genetics technology will provide further evidence to clarify our understanding of the molecular mechanisms of MV persistence in the brain.

Acknowledgements

We thank Ms M. Egami for her technical assistance and Drs M. Watanabe and S. Ueda for communicating the sequence data of the Biken strain. This work was supported by Grants-in-Aid for Scientific Research (No. 10670290 and No. 11670779) from the Ministry of Education, Science, Sports, and Culture of Japan, a Grant-in-Aid for Special Research from Osaka City University, and a grant from the Osaka Medical Research Foundation for Incurable Diseases.

References

Ayata, M., Hirano, A., Wong, T.C., 1989. Structural defect linked to nonrandom mutations in the matrix gene of Biken strain subacute sclerosing panencephalitis virus defined by cDNA cloning and expression of chimeric genes. J. Virol. 63, 1162–1173.

Ayata, M., Hirano, A., Wong, T.C., 1991. Altered translation of the matrix genes in Niigata and Yamagata neurovirulent measles virus strains. Virology 180, 166–174.

Ayata, M., Hayashi, K., Seto, T., Murata, R., Ogura, H., 1998a. The matrix gene expression of subacute sclerosing panencephalitis (SSPE) virus (Osaka-1 strain): a comparison of two sibling viruses isolated from different lobes of an SSPE brain. Microbiol. Immunol. 42, 773–780.

Ayata, M., Kimoto, T., Hayashi, K., Seto, T., Murata, R., Ogura, H., 1998b. Nucleotide sequences of the matrix protein gene of subacute sclerosing panencephalitis viruses compared with local contemporary isolates from patients with acute measles. Virus Res. 54, 107–115.

Barrett, T., Clarke, D.K., Evans, S.A., Rima, B.K., 1987. The nucleotide sequence of the gene encoding the F protein of canine distemper virus: a comparison of the deduced amino acid sequence with other paramyxoviruses. Virus Res. 8, 373–386.

Buckland, R., Gerald, C., Barker, R., Wild, F., 1987. Fusion glycoprotein of measles virus: nucleotide sequence of the gene and comparison with other paramyxoviruses. J. Gen. Virol. 68, 1695–1703.

Caballero, M., Carabana, J., Ortego, J., Fernandez-Munoz, R., Celma, M., 1998. Measles virus fusion protein is palmitoylated on transmembrane-intracytoplasmic cycteine residues which participate in cell fusion. J. Virol. 72, 8198–8204.

Cathomen, T., Buchholz, C.J., Spielhofer, P., Cattaneo, R., 1995. Preferential initiation at the second AUG of the measles virus F mRNA: a role for the long untranslated region. Virology 21, 628–632.

Cathomen, T., Mrkic, B., Spehner, D., Drillien, R., Naef, R., Pavlovic, J., Aguzzi, A., Billeter, M.A., Cattaneo, R., 1998a. A matrix-less measles virus is infectious and elicits extensive cell fusion: consequences for propagation in the brain. EMBO J. 17, 3899–3908.

Cathomen, T., Naim, H.Y., Cattaneo, R., 1998b. Measles viruses with altered envelope protein cytoplasmic tails gain cell fusion competence. J. Virol. 72, 1224–1234.

Cattaneo, R., Rebmann, G., Schmid, A., Baczko, K., ter Meulen, V., Billeter, M.A., 1987. Altered transcription of a defective measles virus genome derived from a diseased human brain. EMBO J. 6, 681–688.

Cattaneo, R., Schmid, A., Billeter, M.A., Sheppard, R.D., Udem, S.A., 1988a. Multiple viral mutations rather than host factors cause defective measles virus gene expression in a subacute sclerosing panencephalitis cell line. J. Virol. 62, 1388–1397.

Cattaneo, R., Schmid, A., Eschle, D., Baczko, K., ter Meulen, V., Billeter, M.A., 1988b. Biased hypermutation and other genetic changes in defective measles viruses in human brain infections. Cell 55, 255–265.

Cattaneo, R., Schmid, A., Rebmann, G., Baczko, K., ter Meulen, V., Bellini, W.J., Rozenblatt, S., Billeter, M.A., 1986. Accumulated measles virus mutations in a case of subacute sclerosing panencephalitis: interrupted matrix protein reading frame and transcription alteration. Virology 154, 97–107.

Cattaneo, R., Schmid, A., Spielhofer, P., Kaelin, K., Baczko, K., ter Meulen, V., Pardowits, J., Flanagan, S., Rima, B.K., Udem, S.A., Billeter, M.A., 1989. Mutated and hypermutated genes of persistent measles viruses which caused lethal human brain disease. Virology 173, 415–425.

Fayolle, J., Verrier, B., Buckland, R., Wild, T.F., 1999. Characterization of a natural mutation in an antigenic site on the fusion protein of measles virus that is involved in neutralization. J. Virol. 73, 787–790.

Griffin, D.E., Bellini, W.J., 1996. Measles virus. In: Fields, B.N., Knipe, D.M., Howley, P.M. (Eds.), Virology, third ed. Raven Press, Philadelphia, PA, pp. 1267–1312.

Komase, K., Haga, T., Yoshikawa, Y., Sato, T.A., Yamanouchi, K., 1990. Molecular analysis of structural protein genes of the Yamagata-1 strain of defective subacute sclerosing panencephalitis virus. IV. Nucleotide sequence of the fusion gene. Virus Genes 4, 173–181.

Lawrence, D.M., Patterson, C.E., Gales, T.L., D'Orazio, J.L., Vaughn, M.M., Rall, G.F., 2000. Measles virus spread between neurons requires cell contact but not CD46 expression, syncytium formation, or extracellular virus production. J. Virol. 74, 1908–1918.

Naim, H.Y., Ehler, E., Billeter, M.A., 2000. Measles virus matrix protein specifies apical virus release and glycoprotein sorting in epithelial cells. EMBO J. 19, 3576–3585.

Ogura, H., Ayata, M., Hayashi, K., Seto, T., Matsuoka, O., Hattori, H., Tanaka, K., Takano, Y., Murata, R., 1997. Efficient isolation of subacute sclerosing panencephalitis virus from patient brains by reference to magnetic resonance and computed tomographic images. J. Neurovirol. 3, 304–309.

Ohuchi, M., Ohuchi, R., Mifune, K., Ishihara, T., Ogawa, T., 1987. Characterization of the measles virus isolated from the brain of a patient with immunosuppressive measles encephalitis. J. Infect. Dis. 156, 436–441.

Richardson, C., Hull, D., Greer, P., Hasel, K., Berkovicg, A., Englund, G., Bellini, W., Rima, B., Lazzarini, R., 1986. The nucleotide sequence of the mRNA encoding the fusion protein of measles virus (Edmonston strain): a comparison of fusion proteins from several different paramyxoviruses. Virology 155, 508–523.

Rota, S.J., Hummel, B.K., Rota, A.P., Bellini, J.W., 1992. Genetic variability of the glycoprotein genes of current wild-type measles isolates. Virology 188, 135–142.

Roos, R.P., Graves, M.C., Wollmann, R.L., Chilcote, R.R., Nixon, L., 1981. Immunologic and virologic studies of measles inclusion body encephalitis in an immunosuppressed host: the relationship to subacute sclerosing panencephalitis. Neurology 31, 1263–1270.

Schmid, A., Spielhofer, P., Cattaneo, R., Baczko, K., ter Meulen, V., Billeter, M.A., 1992. Subacute sclerosing panencephalitis is typically characterized by alterations in the fusion protein cytoplasmic domain of the persisting measles virus. Virology 188, 910–915.

Seto, T., Ayata, M., Hayashi, K., Furugawa, K., Murata, R., Ogura, H., 1999. Different transcriptional expression of the matrix gene of the two sibling viruses of the subacute sclerosing panencephalitis virus (Osaka-2 strains) isolated from a biopsy specimen of patient brain. J. Neurovirol. 5, 151–160.

Vincent, S., Gerlier, D., Manie, S.N., 2000. Measles virus assembly within membrane rafts. J. Virol. 74, 9911–9915.

Watanabe, M., Wang, A., Sheng, J., Gombart, A.F., Ayata, M., Ueda, S., Hirano, A., Wong, T.C., 1995. Delayed activation of altered fusion glycoprotein in a chronic measles virus variant that causes subacute sclerosing panencephalitis. J. Neurovirol. 1, 412–413.

Wong, T.C., Ayata, M., Ueda, S., Hirano, A., 1991. Role of biased hypermutation in evolution of subacute sclerosing panencephalitis virus from progenitor acute measles virus. J. Virol. 65, 2191–2199.

Yoshikawa, Y., Tsuruoka, H., Matsumoto, M., Haga, T., Shioda, T., Shibuta, H., Sato, T.A., Yamanouchi, K., 1990. Molecular analysis of structural protein genes of the Yamagata-1 strain of defective subacute sclerosing panencephalitis virus. II. Nucleotide sequence of a cDNA corresponding to the P plus M dicistronic mRNA. Virus Genes 4, 151–161.