Matrix Protein of Cell-associated Subacute Sclerosing Panencephalitis Viruses

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SUMMARY

The nucleotide sequence has been determined for the matrix (M) protein gene of three strains, Niigata-1, ZH and Biken, of cell-associated subacute sclerosing panencephalitis (SSPE) virus. The M proteins of the Niigata-1 and ZH strains were found to terminate prematurely as a result of nonsense mutations at nucleotide positions 68 and 96 respectively. On the other hand it was predicted that the Biken strain would express M protein with 22 amino acid differences and eight additional amino acids at its C terminus in comparison to the M protein of the Edmonston strain of measles virus. Radiolabelling of cells carrying the Biken strain showed the production of an M protein with considerably altered immunoreactivity and a marked reduction in intracellular stability. Either premature termination or rapid degradation of the M protein may underlie the defectiveness of these three strains of SSPE virus.

Subacute sclerosing panencephalitis (SSPE) is a fatal human disease caused by measles virus (MV) persisting in the central nervous system (ter Meulen et al., 1983). Most patients have been shown to have a reduced antibody response to the M protein (Hall et al., 1979; Wechsler et al., 1979). The M protein is undetectable in the brain of SSPE patients (Hall & Choppin, 1979; Lin & Thormar, 1980; Machamer et al., 1981). Because of these findings which implicate abnormality or defective synthesis of the M protein in the pathogenesis of SSPE, the expression of the M gene and the nature of its product in SSPE virus have been the subject of intensive study.

The predominant synthesis of P–M bicistronic mRNA is probably responsible for the failure of expression of the M gene in the brain of some SSPE patients (Baczko et al., 1984; Wong & Hirano, 1987). A nonsense mutation has been found in the M gene of the MV RNA from the brain of a patient (Cattaneo et al., 1986). However, the M protein was synthesized in cells carrying the genome of the IP-3CA strain of SSPE virus, but it was unstable (Sheppard et al., 1986; Cattaneo et al., 1988).

We have determined the nucleotide sequence of the M genes of three SSPE virus strains. Two of them were found to have mutations that prematurely terminated the synthesis of the M protein, whereas the mutation in the third strain was predicted to lead to the synthesis of an abnormal M protein. Analysis of the M protein synthesized in cells carrying the latter strain fulfilled the prediction.

The Niigata-1, ZH and Biken strains of SSPE virus have been established from patients’ brains as cell lines containing SSPE virus genomes but not producing infectious virus (Doi et al., 1972; Ueda et al., 1975; Mirchamsy et al., 1978). Cells carrying the Niigata-1 and ZH strains contained proteins HA, P, NP and F but M protein was not detected by immunological methods with either a polyclonal anti-MV serum or monoclonal antibodies (MAbs) against the M protein (Sato et al., 1981, 1985). In cells carrying the Biken strain, the M protein was also undetectable by immunofluorescence with anti-M MAbs (Sato et al., 1985), but has been reported to be synthesized when examined by immunoprecipitation (Breschkin et al., 1979). To study the cause
of the absence of M protein, the nucleotide sequence of the M gene was determined after gene amplification using polymerase chain reactions. Cells carrying the SSPE virus were propagated as described previously (Sato et al., 1985). RNAs were prepared from the cells using guanidinium isothiocyanate and purified by cesium chloride centrifugation (Maniatis et al., 1982). cDNAs to viral RNAs were prepared by reverse transcription from the RNA preparation using synthetic primer pairs (~74/TACAGCTCAACTTACCTGCC/~55 and

Fig. 1. Nucleotide sequences for the M protein of cell-associated SSPE virus strains Niigata-1, ZH and Biken. The sequence is shown as the plus sense DNA sequence. ED, N1 and BK represent the Edmonston (Bellini et al., 1985, 1986), Niigata-1 and Biken strains, respectively. Bars represent a match with the ED nucleotide sequence. Nucleotide number 1 is the M mRNA initiation site. Boxes and underlining indicate the translational initiation and termination sites respectively.
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Fig. 2. Comparison of the amino acid sequences of the Edmonston (ED) and Biken (BK) strains. Bars indicate a match with the ED amino acid sequence.

249/ATGTCGCCCGATTGGAGGC/230; 210/GAGGACAGCGATCCCTTAGG/229 and 436/AAGACACTCCCTGTTGTGTA/417; 397/TCACACCTTGGAGAAAGGTC/416 and 609/TCACACCTTGGAGAAAGGTC/590; 570/TCACACCTTGGAGAAAGGTC/589 and 1030/TTGAATAGTCCTTGGTCATC/1011; 991/ACGACGACGTGATCATGAAATAT/1010 and 1169/CGCTTGCCGTCGGCTGCTGG/1150. cDNAs were amplified by polymerase chain reactions (Saiki et al., 1988) with the primer pairs, purified by gel electrophoresis, inserted into the pUC118 vector and cloned into Escherichia coli DH5 cells. Two independent clones were sequenced for each region by dideoxynucleotide chain termination methods (Sanger et al., 1977). The frequency of discrepant nucleotides between any two clones was on average 0-5%. When there was a nucleotide discrepancy between two clones, a third clone was sequenced and the nucleotide common to two clones was taken to be authentic. It is not known whether this problem arises from the infidelity of reverse transcription and cDNA amplification or reflects the preexisting heterogeneity of RNA molecules contained in infected cells.

The nucleotide differences in the M gene in comparison to that of the Edmonston strain amounted to 40 (3.8%), 48 (4.5%) and 45 (4.2%) out of 1066 for the Niigata-1, ZH and Biken strains, respectively. Among them, the change from G to A at nucleotide 68 of the Niigata-1 strain and from C to U at nucleotide 96 of the ZH strain generated new termination codons (Fig. 1). Among multiple clones, there was no nucleotide discrepancy in the newly generated termination codons. In the Biken strain the change from U to C at nucleotide 1038 abolished the termination codon but the change from C to U at nucleotide 1062 created a termination codon resulting in the addition of eight amino acid residues to the C terminus (Fig. 1).

Premature termination of the M proteins of the Niigata-1 and ZH strains accounts for the lack of an immunologically identifiable product. The result is also compatible with the earlier finding that monocistronic M protein mRNA is transcribed normally in cells carrying the Niigata-1 strain (Carter et al., 1983). By contrast, the M protein was supposed to be synthesized by the Biken strain. The amino acid sequence of the M protein deduced from the nucleotide sequence is shown for the Edmonston and Biken strains in Fig. 2. Besides the additional eight amino acids in the Biken strain, there were 22 amino acid residues out of 335, scattered over the entire length, that were different between the two strains. It has been reported that a major defect of the Biken strain is in the transport of the HA protein to the cell surface, and that the M protein is synthesized (Breschkin et al., 1979). However the M protein was not detected by immunofluorescence with 14 MAbs against the M protein (Sato et al., 1985). In order to resolve the inconsistency, we reexamined whether the M protein is produced by the Biken strain. Cells were radiolabelled, the putative M protein was immunoprecipitated from the cell lysates with
Fig. 3. Fluorogram of radiolabelled M protein. Vero cells infected with the Toyoshima strain of MV and those carrying the Biken strain of SSPE virus were labelled with 120 µCi/ml of [35S]methionine (1151 µCi/mmol) for 1 h (lane P1) or 5 h (lanes P5) from 48 h after infection and seeding of cells, respectively. Cultures labelled for 1 h were chased for 1 h (lanes 1), 2.5 h (lanes 2), 5 h (lanes 3) and 7 h (lanes 4). The M protein was precipitated from cell lysates with rabbit anti-M serum and analysed by SDS–PAGE. Methods for labelling, chasing, immunoprecipitation and SDS–PAGE were described previously (Sato et al., 1988). The anti-M serum was taken from a rabbit immunized with the M protein which had been separated by SDS–PAGE from purified virions of the Toyoshima strain. Positions of M, markers are given on the right side and those of virus proteins and cellular actin (A) on the left side.

various antisera and anti-M MAbs and was analysed by SDS–PAGE. No band corresponding to the M protein was visualized from the immunoprecipitate using MAbs against the M protein, in accordance with the previous study (Sato et al., 1985) (data not shown). Monkey hyperimmune serum against MV and a convalescent serum from an atypical measles patient, both of which strongly react with all structural proteins of MV including the M protein (Sato et al., 1981, 1988), gave rise to a faint, barely recognizable M protein band (data not shown). By contrast, the M protein band was clearly resolved after immunoprecipitation with anti-M serum from a rabbit immunized with the M protein prepared after separation by SDS–PAGE (Fig. 3). These findings show that the M protein is produced but is structurally so altered that it reacts only with the antiserum prepared against its denatured form. Furthermore the M protein band disappeared during a 2.5 h chase period, indicating that it has lower intracellular stability than the M protein of the Toyoshima strain (Fig. 3). In this respect, the M protein of the Biken strain resembles that of the IP-3CA strain (Sheppard et al., 1986; Cattaneo et al., 1988). Of the 22 amino acid differences between the Biken and the Edmonston strains, the lysine to glutamic acid change at residue 89 and serine to asparagine change at residue 142 in the former strain are
unlikely to affect the integrity of the molecule, as the above two amino acid replacements are also found in the Hu2 strain of MV (Curran & Rima, 1988).

The defectiveness of all three strains of SSPE virus was thus shown to result from their failure to produce a functional M protein owing to mutations in the M gene.

Note added in proof: After the submission of this paper, the nucleotide and deduced amino acid sequences of the Biken strain of SSPE virus were reported by Ayata et al. ([1989] Journal of Virology 63, 1162–1173). Apart from minor sequence differences, the results of this study agree remarkably with those of the above report.

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REFERENCES


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