Detection of measles virus nucleoprotein mRNA in autopsied brain tissues

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By means of RT-PCR, a portion of measles virus (MV) mRNA encoding nucleoprotein (NP) could be detected in 11 (18%) of 61 brain tissue samples obtained from administrative autopsy cases, who apparently had not suffered from subacute sclerosing panencephalitis (SSPE)-like central nervous system disorders. Most of the brain-derived NP sequences showed significant synonymous nucleotide substitutions when compared with wild-type MV isolates and SSPE virus. Our present results suggest that MV commonly persists in the human brain without causing apparent clinical symptoms, probably due to decreased virus replication.

Subacute sclerosing panencephalitis (SSPE) is a rare and incurable neural disease that occurs several years after recovery of a patient from measles virus (MV) (ter Meulen et al., 1983). Mutated MV (SSPE virus) has been isolated from brain tissues of SSPE patients by cocultivation of infected brain cells with tissue culture cell lines (Homma et al., 1982; Kristensson & Norrby, 1986). SSPE virus is different from ordinary MV in several ways. SSPE virus barely produces free virus and is highly neurovirulent in mice (Ohuchi et al., 1981; Billeter et al., 1989). Some of the molecular biological aspects of the differences between SSPE virus and MV have been recently reported (Hirano et al., 1993; Schneider-Schaulies et al., 1995). Little is known, however, about the progression from acute measles to SSPE. In experimental infection in monkeys, MV was frequently detected in the brain during the acute phase, after subcutaneous inoculation (F. Kobune, personal communication). This result led us to hypothesize that MV could easily reach the brain in humans as well. However, whether MV could persist in the brain for a long period after recovery from acute measles remained to be clarified. We therefore obtained 61 brain tissue samples from administrative autopsies and tried to detect MV mRNA using RT-PCR. We report here that MV mRNA was detected in 11 (18%) of the 61 samples.

Brain parenchymal tissues of the right frontal lobe were collected from 61 administrative autopsy cases within 24 h post mortem during the period 1992 to 1994. These autopsy cases consisted of 40 males and 21 females between 5 months and 83 years old (mean 54 years). After rinsing in PBS to remove blood cells, the brain parenchymal tissues were stored at −130 °C until use. Total cellular RNA was extracted from the brain tissues using the acid guanidinium–phenol–chloroform method (Chomczynski & Sacchi, 1987). Ten μg of total RNA was reverse-transcribed into cDNA as described previously (Shibahara et al., 1994) with some modifications, using primer NP4 specific for MV nucleoprotein (NP) mRNA (Fig. 1). NP mRNA has been shown to be the most abundantly expressed mRNA of MV (Cattaneo et al., 1987; Schneider-Schaulies et al., 1990). The cDNA was then amplified by nested PCR. The primers used in this study were based on the reported MV sequences (Moil et al., 1993; Rozenblatt et al., 1985) and are depicted in Fig. 1. First-round PCR was performed over 35 cycles, each consisting of 1 min at 94 °C, 1 min at 55 °C or 45 °C and 2 min at 72 °C using Tth DNA polymerase (Toyobo) with outer primer set NP1 and NP4. Using a 2 % volume of the first-round PCR products, second-round PCR was carried out with the same programme, using inner primer set NP2 and NP3. As an internal control, RT–PCR for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was carried out using 10 ng of total RNA as described previously (Gao et al., 1993). When MV genomic RNA was analysed, the primer NP1 was used in the RT reaction and the cDNA thus obtained was amplified as described above. The PCR products were electrophoresed in a 3 % agarose gel containing ethidium bromide and visualized by UV illumination.

One of the 61 brain tissue samples (number 7) showed positive amplification of NP mRNA when the annealing
Fig. 1. Positions and sequences of the primers used in RT-PCR to detect NP mRNA or the NP gene of MV. The nucleotide numbering is as described by Mori et al. (1993).

**First PCR**

NP1

5' -CCTCAATTACCACTCGATCC-3' (235–254)

NP4

5' -TTGATGCGAAGGTAAGGCCA-3' (443–462)

**Second PCR**

NP2

5' -TAATTGGAAACCCGGATGTG-3' (280–299)

NP3

5' -ACTCTGGACAACCTCTAACA-3' (409–428)

Fig. 2. RT-PCR for (a) MV and (b) GAPDH. Lane 1, DNA marker (φX174 DNA digested with HaeIII), lanes 2 to 17; brain sample numbers 31 to 46, respectively.

Temperature was set at 55 °C in the first- and second-round PCR. When the temperature was lowered to 45 °C, some of the other samples became positive for amplification. Fig. 2(a) illustrates representative results obtained with samples 31–46. RT-PCR for GAPDH mRNA served as an internal control (Fig. 2b). In total, 11 of the 61 samples showed positive amplification for MV NP mRNA. The positive samples were from seven males and four females. All but one of the positive cases were aged 37 or older (mean 60 years) and like most Japanese people of this age group were thought to have had MV infection in their childhood. No information was available as to whether they had suffered from complications during and/or after the episode of measles. The cause of death of these 11 cases was acute cardiac failure (five cases), gastric cancer or serious gastric ulcer, subarachnoid haemorrhage, acute alcohol intoxication, carbon monoxide poisoning and traffic accident (one case each). None of them had suffered from SSPE-like central nervous system disorders. The brain tissues were macroscopically normal, although the possibility of minor microscopic changes was not excluded.

Nucleotide and deduced amino acid sequences of the brain-derived MV clones were determined by direct sequencing with a Taq DyeDeoxy Terminator Cycle Sequencing kit and an ABI 373A autosequencer (Applied Biosystems). The sequences were compared with those of the Edmonston strain, a standard strain of MV (Fig. 3). As a control, the NP genes from wild-type MV isolates were amplified under the same conditions (annealing temperature 45 °C) and sequenced. These wild-type isolates were obtained from typical measles patients, either directly from throat swabs (RI12 to RI20) or after cultivation of throat swab samples in B95-8 cells (T4 and N13). NP gene sequences of the wild-type MV isolates (T4, N13 and RI12 to RI20) were identical and had a single substitution at the 404th residue compared with the Edmonston strain. Recently sequenced MV isolates in the United States and Canada have the same nucleotide substitution at this position compared with the Edmonston strain (Rota et al., 1994). Two of the brain-derived MV clones (numbers 7 and 36) had the same nucleotide sequence as that of the wild-type MV isolates. The remaining nine clones obtained from the brain tissues had unique sequences, differing by 1–4 nucleotides and 1–3 amino acids from those of the wild-type MV isolates.
Our present results demonstrate the presence of a portion of MV mRNA in the brains of autopsied individuals with no apparent history of SSPE. Nine of the 11 MV sequences amplified from brain tissues had significant nucleotide substitutions compared with the wild-type MV (Fig. 3). As another control, we amplified the corresponding portions of NP mRNA synthesized in B95-8 cells that had been infected with the wild-type MV isolates (T4, N13, RI12, RI13 and RI16). The same conditions were used for PCR (annealing temperature 45°C). Nucleotide sequences of the NP mRNA completely matched with each other and were identical to that of the wild-type virus (data not shown). Taken together, these results exclude the possibility that the mutations observed with the brain-derived sequences were introduced during RT–PCR procedures, thereby strongly suggesting that the mutated NP sequences were present in the brain tissues. The remaining two sequences derived from the brain samples were identical to that of the wild-type virus. It is possible, however, that nucleotide alterations are present in other portions of the genome of the brain-derived MV clones.

The region analysed in the present study (residues 310 to 408) has been reported to be highly conserved among MV and SSPE viruses, showing few, if any, synonymous nucleotide substitutions and no asynonymous ones (Cattaneo et al., 1989; Rota et al., 1994; Radecke & Billeter, 1995). In view of this sequence conservation, asynonymous substitutions in the MV sequences obtained from the brain tissues are significant. Amino acid alterations in NP as well as other viral proteins would possibly affect replication and expression of the viral genome so that production of progeny virus and induction of CPE are minimized. This idea may explain why the presence of MV in the brain tissues of the individuals analysed in this study did not cause apparent signs of infection even after long-term persistence. In fact, our attempts to isolate infectious MV from brain tissues have failed (data not shown). Also, decreased gene expression would mean that the virus-infected cells might escape detection and destruction by cytotoxic T lymphocytes, especially in the brain where the immunological surveillance is rather ineffective. It is generally accepted that MV infection occurs mostly in childhood.

![Fig. 3. Alignment of (a) nucleotide and (b) deduced amino acid sequences of MV NP mRNA obtained from the brain tissue samples (brain 7–61) and the NP gene of fresh wild-type MV isolates (T4–RI20) and the Edmonston strain. All the nucleotide sequences are shown in the orientation of mRNA sequences. The nucleotide numbering is as described by Mori et al. (1993).](image-url)
and that the virus is eliminated from an individual by potent anti-MV immunity after recovery from acute measles. However, the present results suggest the possibility that MV can persist in the brain for a long period of time with reduced levels of replication and expression. In this regard, it was recently reported that mesenteric microvascular endothelium and macrophage-like cells, and that it could be an aetiologic agent of Crohn’s disease (Wakefield et al., 1993; Ekbom et al., 1994). In view of these findings, the concept of viral clearance after recovery from acute MV infection needs to be modified. The process from MV persistence to the clearance after recovery from acute MV infection needs investigation.

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References


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