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Case report

Efficient isolation of subacute sclerosing panencephalitis virus from patient brains by reference to magnetic resonance and computed tomographic images

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Subacute sclerosing panencephalitis virus has been isolated with difficulty from brains of infected patients. More strains are needed for the study of the pathogenesis of this virus. To make the isolation more efficient, we selected portions to be examined from the brains of three patients with reference to findings of repeated magnetic resonance and computed tomographic imaging. Three cell lines susceptible to measles virus field strains were used. In all three cases viruses were isolated most effectively from recent lesions and with Vero cells. Our results suggested that these imaging methods and Vero cells could be used for improvement in the efficiency of isolation of this virus from patient brains.

Keywords: SSPE virus; Virus isolation; MR image; CT image

Subacute sclerosing panencephalitis (SSPE) is a fatal degenerative disease caused by persistent infection of the central nervous system (CNS) with measles virus (MV). The disease usually affects children and young adults. The SSPE virus was first isolated by cocultivation by Horta-Barbosa *et al* (1969) almost 30 years ago. With even this simple method, recovery of the virus was difficult (Fraser and Martin, 1978). Wechsler and Meissner (1982) reported failure to recover the virus in some 80% of attempts. Few reports of SSPE virus isolation have appeared (Doi *et al*, 1972; Burnstein *et al*, 1974; Ueda *et al*, 1975; Makino *et al*, 1977; Kratzsch *et al*, 1977; Mirchamsy *et al*, 1978; Homma *et al*, 1982), although numerous attempts have been made. The low efficiency of isolation suggested that the virus strains that could be isolated had some unusual properties. Only a few such isolates (Niigata-1, Biken, Yamagata-1, MF, and IP-3-Ca strains) have been cloned, sequenced and expressed. Information obtained from these isolates is slight, so it is necessary to improve the efficiency of isolation of the SSPE virus from patient brains: not for diagnosis, but because gathering of biological, biochemical, and genetic data from more isolates is needed for understanding of the persistence and pathogenicity of the virus and for the development of new therapies and methods of prevention. Here, we selected brain portions likely to contain infectious virus by reference to findings of abnormalities by imaging methods. We used three cell lines susceptible to measles virus field strains.

Vero cells, TIG-1 cells (diploid cells derived from human embryonic lung), and B95a cells (a monolayer culture derived from B95-8 cells, marmoset B lymphocytes transformed by Epstein-Barr virus; Kobune *et al*, 1990) were used to make isolation more likely than if a single cell line was used. The cells were cultured in Dulbecco's modified Eagle medium supplemented with 1% fetal bovine serum (FBS) and 4% newborn calf serum (NCS) for Vero cells, 5% FBS and 5% NCS for TIG-1 cells, and 5% FBS for B95a cells. Samples were taken from two patients during autopsy. Within 30 min after brain dissection, three samples from the right hemisphere, each a cube measuring about 1 cm on all

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sides, were taken, washed with phosphate-buffered saline, cut into small pieces, and treated with trypsin to give single cells. The cells obtained were cocultivated with each of the three cell lines with incubation at 35°C under an atmosphere of 5% CO_2 in air. From the third patient, a small biopsy specimen from the right frontal lobe was obtained (see below), cut into fine pieces, and cocultivated with Vero cells and B95a cells without trypsin treatment because of its size. The cultures were observed every day under a microscope for virus-induced syncytial foci. Subculturing was done once a week for Vero cells and B95a cells and once every 10–14 days for TIG-1 cells. Identification of the isolates as SSPE virus was done by indirect fluorescent antibody (IFA) staining, and also by radioimmunoprecipitation (RIP) and by sodium dodecyl sulfate-polyacrylamide gel electrophoresis with MV monoclonal antibodies and MV hyperimmune monkey serum.

Case 1 was of a 25-year-old woman who died 8 years after the onset of SSPE. Immune responses to measles virus antigens were found in the cerebrospinal fluid (CSF) and serum by hemagglutination inhibition (HI) and complement fixation (CF) tests. Macroscopic examination at autopsy showed atrophy of the brain (830 g) and enlargement of the cerebral ventricles. Neuropathological findings showed perivascular infiltration and diffuse gliosis with Cowdry type A inclusion bodies in the cerebrum and brain stem. Case 2 was of a 12-year-old boy found to have SSPE when 10 years old. Immune responses to MV antigens were found in the CSF and serum by HI and CF tests at admission to hospital. When an Ommaya reservoir was put in the right lateral ventricle for administration of interferon-β 8 months after onset, the small piece of right frontal brain tissue within the needle was obtained and used for virus isolation. Neuropathological examination showed Cowdry type A inclusion bodies in some oligodendrocytes together with proliferation of astrocytes and microglia. Case 3 was of a 25-year-old man who died 14 years after the onset of SSPE. Immune responses to MV antigens were found in the CSF and serum by HI tests. Macroscopic examination at autopsy showed atrophy of the brain (805 g) and enlargement of the cerebral ventricles. Neuropathological findings were of diffuse perivascular lymphoid cuffing and gliosis with Cowdry type A inclusion bodies in some neurons throughout the brain and spinal cord.

The clinical course in each case by Jabbour's classification is summarized in Figure 1. In magnetic resonance (MR) images of case 1, soon after onset in 1985, lesions were observed as high-intensity areas in T_2-weighted images bilaterally in the brain stem (22 Oct 1985 in Figure 2a). After temporary improvement in the findings, new lesions appeared in 1988 and progressed rapidly in the frontal lobes and slowly in the occipital lobes (11 Oct 1988). Later, MR images showed severe atrophic changes (18 Sep 1990). In case 2, no lesions were detected in MR imaging (22 Mar 1994 in Figure 2b) before the Ommaya reservoir was installed and a biopsy specimen was taken (on 1 Jun 1994). One month after the operation, high-intensity areas in T_2-weighted images were found in the frontal lobe, including the surroundings of the reservoir tube, and in both occipital lobes (27 Jun 1994). However, it was possible that the findings in the frontal lobe were a result of the operation. Later MR images showed all of these lesions to be progressing (14 Jul 1994), and still later, atrophic changes occupied the whole brain. In case 3, computed tomographic (CT) imaging was done more often than MR imaging. CT images showed lesions first in the right parieto-occipital region (21 Aug 1981) and later in the same year in the temporal lobes as well. The lesion became smaller before long, and brain atrophy developed. Two years after onset, new lesions appeared in the brain stem bilaterally (7 Feb 1984) but disappeared by late in that year. Lesions appeared again about 2 years later together with bilateral lesions in the parieto-occipital regions in CT images (9 Jun 1987), and brain atrophy progressed rapidly overall. No significant findings were obtained from the frontal lobes in the serial CT images before atrophy became severe.

The findings of MR and CT images were used in selection of three brain portions for virus isolation before autopsy. Lesions that had appeared early, in the mdidle of the course, and late were selected so that we could find if infective SSPE viruses could be recovered from lesions of different ages. If so, we wanted to find if the virus isolates had different properties. The results are summarized in Table 1. In case 1, two sibling viruses were isolated with Vero cells from the right frontal and occipital lobes, and one sibling virus was isolated with TIG-1 cells, from the right frontal lobe only. They were designated the Osaka-1 strain. No virus was recovered from any brain region with B95a cells. In case 2 only, Vero cells and B95a cells were used, as mentioned above. Two sibling viruses were isolated, and they were designated the Osaka-2 strain. In case 3, four sibling viruses, designated the Osaka-3 strain, were recovered: with Vero cells, viruses were isolated from the right occipital lobe and the right brain stem, and with TIG-1 cells and B95a cells, viruses were isolated from the right side of the brain stem. All three strains were identified by their immunoreactivities to antibodies specific to MV with IFA staining or RIP (data not shown), and were found to be nonproductive, defective viruses.

In our study, replicable SSPE viruses were recovered in all three cases as nonproductive cell-associated viruses. In the two autopsy cases, isolation was successful especially from lesions recent in onset as identified by MR or CT imaging. Perhaps some SSPE viruses in such lesions are still

infective even when the brain at autopsy is completely atrophic (when it weighs about 800 g). In the patient who underwent biopsy, viruses were recovered even from the extremely small biopsy specimen from a region that looked intact on MR images. Later, MR images showed the appearance of a lesion in the biopsy region; perhaps the specimen was from a fresh lesion just before it became detectable.

Of the cell lines used for virus isolation, Vero cells seemed to be best. Viruses were isolated with Vero cells from all cases and from a number of portions of the brain. With B95a cells, we isolated SSPE virus from two of the three cases, so B95a cells are useful for such isolation. However, with these cells, isolation was not always successful. This result is different from that when MV is isolated from an acute case of measles. Kobune *et al* (1990) found that B95a cells are 10 000-fold more susceptible to field MV than Vero cells. Kobune *et al* (1990) first isolated MV with B95a cells in 1984, and recent MV field isolates have biochemical and genetic characteristics different from those of the still earlier Edmonston strain (Rota *et al*, 1992; Sakata *et al*, 1993). Therefore the susceptibility to B95a cells to field MV before 1984 is not known. Our

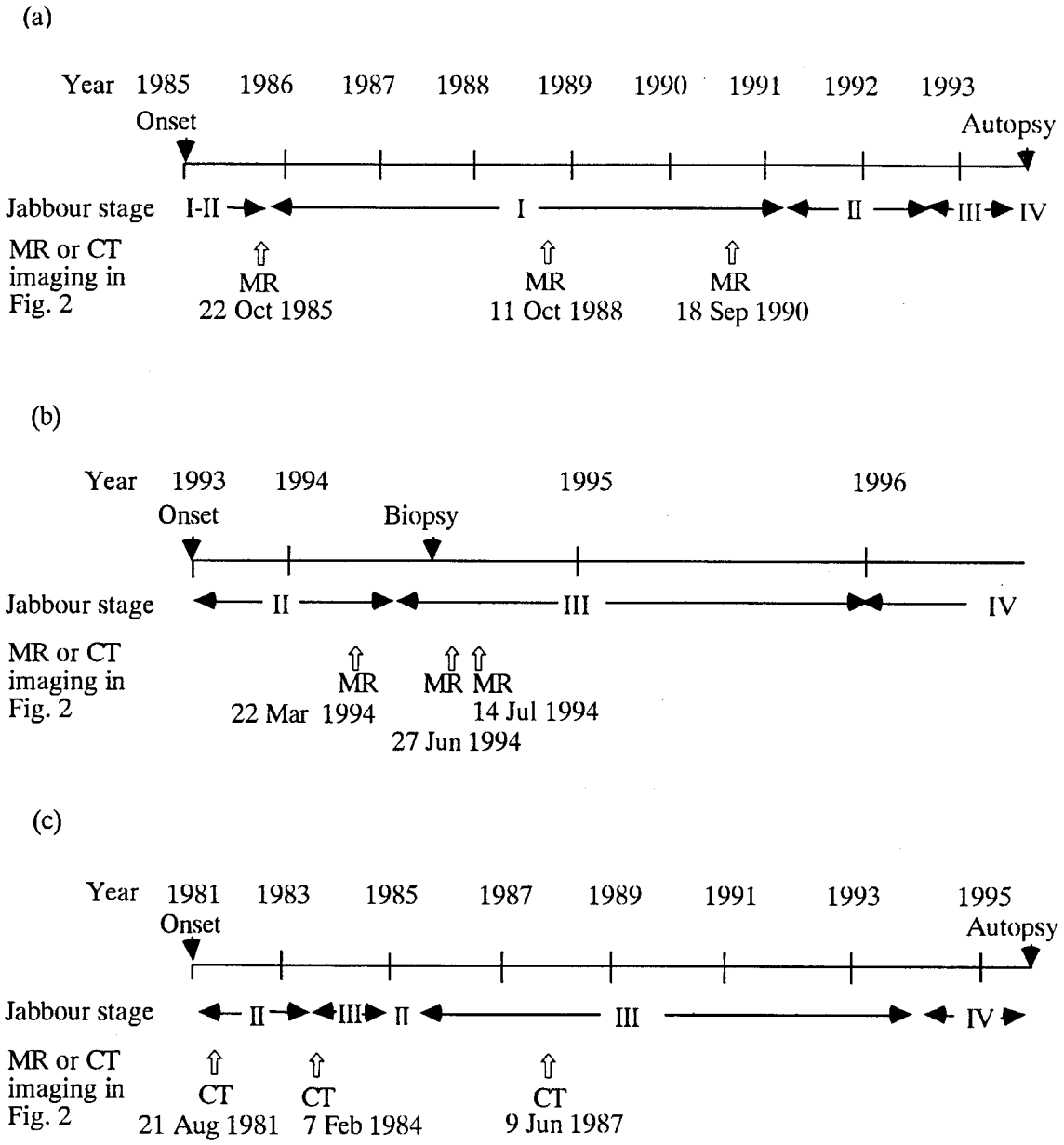

Figure 1 Clinical courses of the three SSPE patients. (a) Case 1. (b) Case 2. (c) Case 3.

patients with SSPE had acute measles in 1969, 1984, and 1971 (cases 1, 2 and 3, respectively). B95a cells might not be susceptible to progenitor MV in case 1. Alternatively, MV may change in its cell tropism; it may become incapable of infecting B95a cells and instead come to infect cells of epidermal origin (like

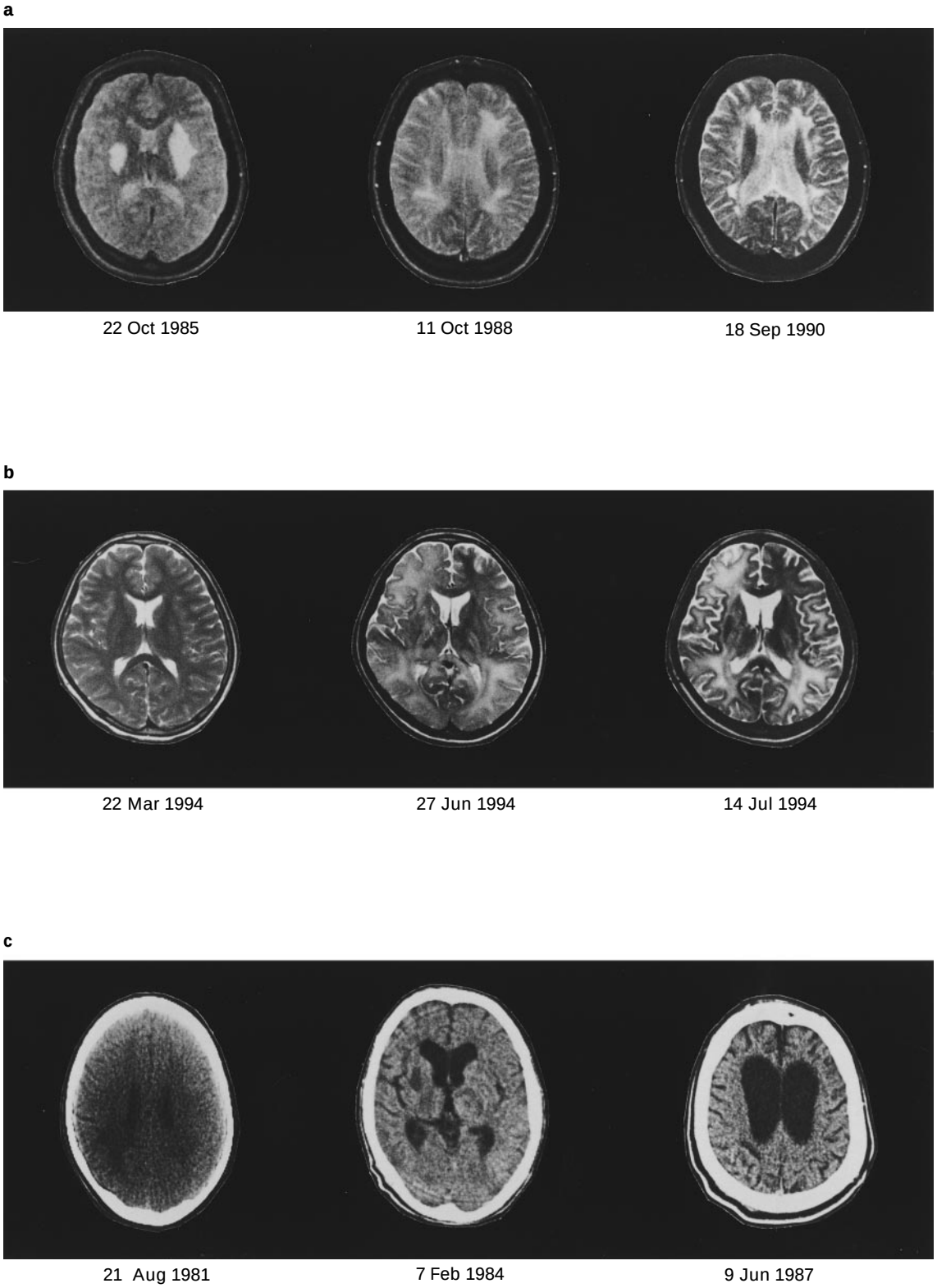

Figure 2 Serial magnetic resonance (T_2-weighted) or computed tomographic images of the three SSPE patients. (a) Case 1. (b) Case 2. (c) Case 3.

Table 1. Results of SSPE virus isolation from three patients

Case	Strain	Brain region sampled[a]	Tme SSPE lesions first appeared in MR or CT imaging	Days until the first detection of virus-induced syncytium formation: Cells used for isolation: Vero	TIG-1	B95a
1	Osaka-1	Frontal lobe	Oct 1988	9	13	–
		Occipital lobe	Oct 1988	14	–	–
		Brain stem	Oct 1985	–	–	–
2	Osaka-2	Frontal lobe	Jun 1994	9	NT	5
3	Osaka-3	Frontal lobe	ND	–	–	–
		Occipital lobe	Aug 1981	13	–	–
		Brain stem	Oct 1983	12	19	10

[a] The right hemisphere of the brain was sampled in all cases.
ND, Not detected.
NT, Not tested.
–, Not isolated. Cocultivated cells were observed for at least 4 weeks through blind passages.

Vero cells) after prolonged presence in the CNS. TIG-1 cells seemed to give less satisfactory results than Vero cells, including the time taken before the first virus-induced syncytium was detected.

Only a few nonproductive SSPE virus isolates from infected brains have been partially sequenced and expressed: strains Niigata-1 (Ayata *et al*, 1991; Wong *et al*, 1991; Hirano *et al*, 1993), Biken (Ayata *et al*, 1989; Wong *et al*, 1991; Hirano *et al*, 1993), Yamagata-1 (Wong *et al*, 1989, 1991; Yoshikawa *et al*, 1990; Ayata *et al*, 1991; Komase *et al*, 1992; Hirano *et al*, 1993), MF (Cattaneo *et al*, 1987), and IP-3-Ca (Cattaneo *et al*, 1988, 1989). The various studies examined replicable and pathogenic viruses within brains affected by SSPE, and valuable information was obtained. Still, SSPE virus is isolated with a low rate of success and with much labor. Some kinds of strains may be easier to isolate than others, and mutation may occur during *in vitro* passage. Therefore, in another procedure, full-length cDNAs of SSPE virus genes have been cloned directly from specimens from an infected brain for use in identification of the structures and functions of variant mRNAs arising from mutation (Schmid *et al*, 1987). This approach involves some problems. Cloned genes may or may not be from the most numerous variant infecting the brain. They may be derived from the pathogenic virus that caused the disease to start and progress with some intervals of no change, or may be derived from another pathogenic or nonpathogenic virus. Cloned genes cannot be assigned to a specific genome. The two currently used methods therefore have a number of disadvantages. The procedure suggested in this report makes it easier to isolate SSPE virus from infected brains and should be useful in investigation of the persistence and pathogenesis of the SSPE virus in the CNS.

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