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Case report

Acute ascending necrotizing myelitis in Okinawa caused by herpes simplex virus type 2

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Summary. A case of rapidly progressing ascending myelitis was necropsied. Necrosis was present throughout the whole length of the spinal cord and involved both the grey and white matter randomly. The perivascular lymphocytic infiltration in the spinal cord in the present case was more pronounced than that in the previously reported two cases of necrotizing myelopathy associated with malignancy. Using immunoperoxidase staining the presence of herpes simplex virus type 2 (HSV 2) antigen was demonstrated. Electron microscopic examinations revealed large numbers of HSV particles in the spinal cord. HSV 2 may be a common aetiological agent of necrotizing myelopathy and myelitis in Okinawa, an HSV 2 endemic area. In the present case, the necrosis was mainly found in the spinal cord but was also observed, to a very limited extent, in the brain.

Key words: Necrotizing myelitis – Herpes simplex virus type 2

Introduction

Mancall and Rosales (1964) made a detailed report of necrotizing myelopathy associated with visceral carcinoma, and proposed that the disorder might be due to the disturbed metabolic processes related to the neoplasm. Since 1903, about 29 cases of necrotizing myelopathy associated with malignancy (Mancall and Rosales 1964; Ojeda 1984; Iwamasa et al. 1989) have been reported in English language journals. In 1984, Ojeda reported two similar cases and pointed out that this rare disorder should be diagnosed only when all known possible causes of spinal cord necrosis, including aortic disease producing ischaemia, radiation therapy to the spinal cord and metastatic disease have been excluded.

We recently reported two cases of necrotizing myelopathy associated with malignancy which were caused by herpes simplex virus type 2 (HSV 2; Iwamasa et al. 1989). Independently of these reports, Wiley et al. (1987) reported a case of acute ascending necrotizing myelopathy associated with diabetes mellitus caused by HSV 2 infection. They suggested that HSV 2 was a common aetiological agent in necrotizing myelopathy. It was considered that latent infection of HSV 2 in the dorsal root ganglia was followed by spread to the spinal cord when the immunodefence system was disturbed by diabetes mellitus or other conditions. One additional case of progressive myelitis associated with AIDS caused by HSV 2 was reported by Britton et al. (1985). However, it is not clear whether there are any differences between the necrotizing myelopathy associated with malignancy and that associated with other severe conditions such as diabetes mellitus. HSV 2 latently infecting the dorsal root ganglia is generally considered to be an aetiological agent of recurrent inguinal skin eruptions, whereas in the present case and in other reported cases of necrotizing myelopathy (Klastersky et al. 1972; Britton et al. 1985; Wiley et al. 1987; Ahmed 1988; Iwamasa et al. 1989), HSV 2 produced the necrosis mainly in the spinal cord. Why HSV 2 mainly produces spinal cord necrosis in such cases is also obscure.

Taha et al. (1989) recently reported neurovirulence of HSV 2 using HSV 2 strain HG 52 and its variant virus JH 2604. They made the JH 2604 virus from HG 52 strain by deletion of the genome between 0 to 0.02 and 0.82 to 0.83 map units which contained *p* and *v* and *p*, *v*, *u* and *g* regions (*Bam*H1 cleavage) respectively. The JH 2604 was not neurovirulent for mice inoculated intracranially, while HG 52 showed strong neurovirulence. We have also investigated the restriction endonuclease cleavage analysis of HSV 2 DNA which was isolated in Okinawa. The HSV 2 DNA in Okinawa showed a difference at *p* and *v* regions when compared with the standard HSV 2 strains SAV and UW 333, and HSV 2 from the mainland of Japan, which were not strongly neurovirulent. Epidemiologically, the HSV 2 infection rate in Okinawa gradually increases with age, and the general incidence in Okinawa is 38%, which is higher

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than that in other prefectures (e.g. 25% in Kagoshima). Moreover, the general incidence of HSV 1 infection in Okinawa reaches 100% at age 40 (Yoshitake et al., in preparation 1990). Okinawa is an island in the southernmost part of Japan.

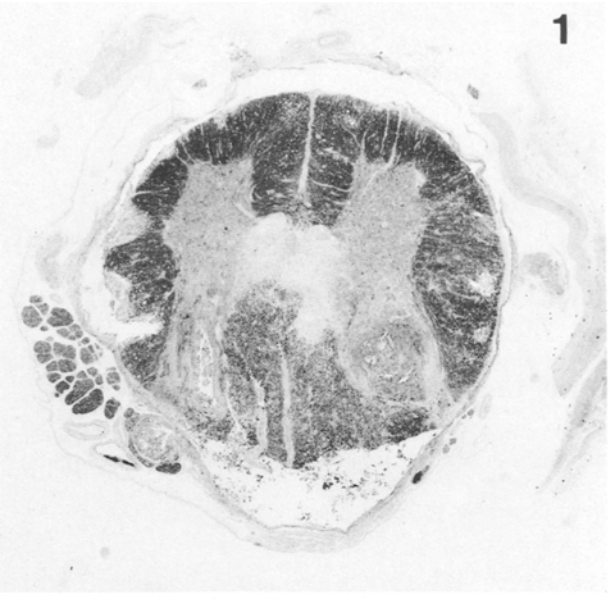
Case report

On 28 June 1989, a 64-year-old diabetic man was admitted to the National Okinawa Hospital with a 10-day history of numbness and weakness of the lower extremities, and urinary bladder dysfunction. He suffered from significant weight loss due to loss of appetite from grief for a spouse who had died the previous month. He had been suffering from diabetes mellitus for 12 years. Laboratory investigations, a chest X-ray, CT examinations and a ^{67}Ga scintigram revealed no significant findings except for the blood sugar and serum protein values (160 mg/dl and 6.3 g/dl, respectively). The protein content and cell count of the cerebrospinal fluid were 75 mg/dl and 6 white blood cells/mm^3 respectively with no abnormal cells. The peripheral white blood cell count was 11700/mm^3, 78% of which were polymorphonuclear cells. Guillain-Bárre syndrome or other unknown myelopathy was suspected. Treatment with steroids (prednisolone, 60 mg/day) and vitamins was initiated. On 20 July, the steroid treatment was terminated, because the peripheral white blood cell count had risen to 24600/mm^3. The serum hepatic enzyme levels were increased (SGOT, 179 IU; SGPT, 223 IU). Serum HTLV-1 antibody was negative. Rapidly ascending flaccid paraplegia was observed. However, myelography revealed no abnormal findings. On 30 July, the patient became delirious and small eruptions appeared on his back and abdomen. On 1 August, he died of respiratory insufficiency associated with intercostal paresis.

Materials and methods

A complete necropsy, including the brain and spinal cord, was performed 4 h after the patient's death. The brain and spinal cord were examined after 10 days when completely fixed with formalin. Samples from all organs were routinely processed in paraffin, sectioned at 4 μm and stained by conventional methods. The brain was sectioned in the coronal plane. The spinal cord was cut in the horizontal plane. The samples from the brain and spinal cord were processed in paraffin. Sections of 4 μm and 10 μm were stained with haematoxylin and eosin (H&E), phosphotungstic acid haematoxylin (PTAH), Klüver-Barrera, Bodian and Gomori's silver impregnation staining. Also, 6-μm frozen sections were stained with Sudan III. For immunohistochemical examinations, 4-μm deparaffinized sections were stained by the routine immunoperoxidase method. Polyclonal antisera for HSV 2, HSV 1 and cytomegalovirus (CMV) were obtained from Dako (Kyoto). According to the technique of Martin et al. (1988), the specificity of the antisera was tested at various dilutions by using Vero cell and HSV 2 (SAV strain) and HSV 1 (HS strain). Monoclonal antibodies for HSV 2 (Chemicon International Los Angeles, California, USA) and HSV 1 (Cooper Biomedical, West Chester, PA, USA) were also employed. Furthermore, sections were examined after staining for C3, IgG, IgM and fibrin immunohistochemically using polyclonal antibodies (all obtained from Dako Kyoto). For electron microscopic examination, formalin-fixed samples of the spinal cord (L3 level) were refixed in 2% osmium tetroxide and embedded in Epon 812. Ultrathin sections were examined under a Hitachi 12 A electron microscope.

Frequency of perivascular lymphocytic infiltration (cuffing) and necrosis were analysed by computer image analysis (Nikon Cosmozone 98 System). Five of the serial horizontal plane sections (H&E staining) of each spinal cord level were investigated after photographic magnification (100×).



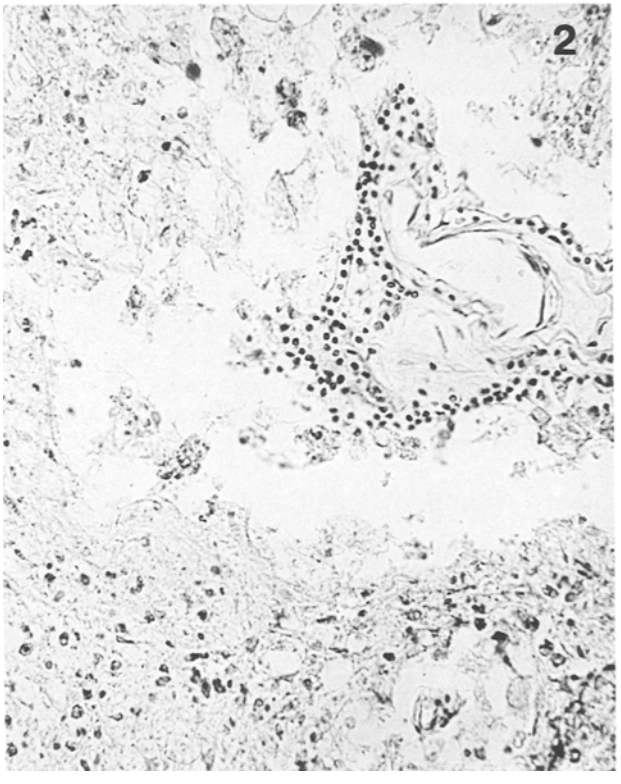


Fig. 1. Necrosis of the spinal cord (L2). Klüver-Barrera staining

Fig. 2. Perivascular lymphocytic infiltration (L3). Klüver-Barrera staining, ×260

Necropsy findings

Both lungs were congested and atelectatic with small foci of bronchopneumonia. A small haemorrhagic ulcer was observed in the duodenum. A small erosin was found at the middle portion of the oesophagus. In the liver, mild central fatty degeneration was observed, but there was no evidence of hepatitis. The adrenal glands showed no remarkable finding and there were no tumours.

Table 1. Distribution of perivascular lymphocytic infiltration, necrosis and HSV 2 antigen demonstrated using anti-HSV 2 monoclonal antibody in the cerebrum, cerebellum and spinal cord

			PLI	Necrosis	HSV 2 antigen
Cerebrum, lobus frontalis			−	−	−
lobus parietalis			+	<1%	+
lobus temporalis (r)			+	<1%	+
lobus temporalis (l)			±	−~<1%	±
lobus occipitalis			−	−	−
Cerebellum			+	<1%	+
Medulla oblongata			+	<1%	+
Spinal cord	C	1–2	+	<5%	+
		3–4	+	<1%	+
		5–6	+	<1%	+
		7–8	+	<1%	+
	T	1–2	+	<1%	+
		3–4	+	<5%	+
		5–6	+	<1%	+
		7–8	++	<5%	++
		9–10	+	<5%	+
		11–12	++	<5%	++
	L	1	++	5%~30%	++
		2	+++	5%~30%	+++
		3	+++	5%~30%	+++
		4	+++	5%~30%	++
		5	+++	5%~30%	++

±, Very weak; +, weak; ++~+++, moderate; ++++, strong

PLI, Perivascular lymphocytic infiltration. Frequency of perivascular lymphocytic infiltration and necrotic area of sections were measured by computer image analysis (Nikon Cosmozone 98 System)

The spinal cord was soft without macroscopic haemorrhage. The leptomeninges and dura mater were normal. The nerve roots were equal in size. Sagittal sections of the cerebellum demonstrated slight softening in the deep white matter on both sides. Light microscopy of the brain sections revealed occasional acute petechial haemorrhages in the cortex, with perivascular lymphocyte infiltration. A few small necrotic foci were scattered in the temporal area of the cerebral cortex and cerebellum. However, there was less microglial hyperplasia, and very few lipid-laden macrophages were present in the brain. Histological sections of the spinal cord demonstrated numerous, and randomly scattered areas of necrosis (Fig. 1) with small occasional haemorrhages in the grey and white matter. Within the necrotic areas, scattered lipid-laden macrophages were observed by Sudan III staining. The distributions of necrosis and degeneration in each segment of the spinal cord and the brain are listed in Table 1, after measurement by computer image analysis (Nikon Cosmozone 98 System). There was also slight perivascular lymphocytic infiltration (Fig. 2), of which evidence was found in the leptomeninges. Perivascular lymphocytic infiltration (cuffing) in the present case was more frequent than in our two previous cases of necrotizing myelopathy associated with malignancy (Iwamasa et al. 1989). However, in the present case, the necrosis was more closely confined than in the previous two cases. The posterior root fibres (L1-3) were also affected (Fig. 3), whereas the dorsal ganglia showed very weak degeneration. Fibrin thrombi and vascular abnormalities were not detected in the spinal cord when investigated using PTAH and Gomori's silver impregnation staining. Detailed examinations of all organs revealed virus inclusion bodies (Cowdry type A) on the skin of the lower back, the squamous epithelium of the oesophagus at the erosive middle portion and the spinal cord (L2) (Fig. 4). Immunoperoxidase staining of HSV 2,

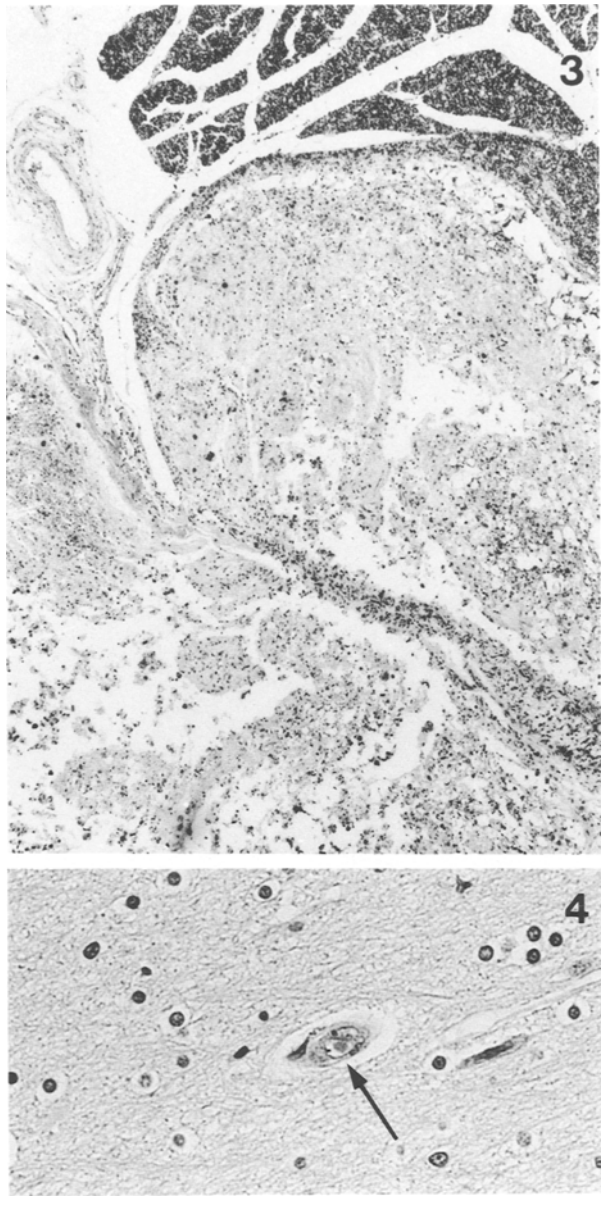


Fig. 3. Necrosis of the posterior root fiber (L3). Klüver-Barrera staining, ×50

Fig. 4. Cowdry type A inclusion body (*arrow*) in the spinal cord (L2). H&E staining, ×260

HSV 1 and CMV antigens was performed in the spinal cord, cerebral cortex and cerebellum. HSV 2 antigen was strongly demonstrated in the grey and white matter of the spinal cord (Fig. 5a), although these areas stained negative for CMV. HSV 2 antigen was occasionally found in the temporal area of the cerebral cortex, cerebellum and posterior root fibres (L1-3). However, the antigens in the dorsal ganglia were quite sparse. Polyclonal antibody for HSV 1 showed positive reactions when examined at a 300-fold dilution. However, at match dilution (i.e. 1000-fold), it was negative. Polyclonal antibody for HSV 2 revealed strong positive reactions even when diluted to over a 1000-fold dilution, similarly to the report of Martin et al. (1988). Monoclonal antibody for HSV 2 also showed a strong positive reaction (Fig. 5b), whereas that for HSV 1 did not. The positive reaction was observed in the nuclei and the cytoplasm of nerve cells and also in the central canal

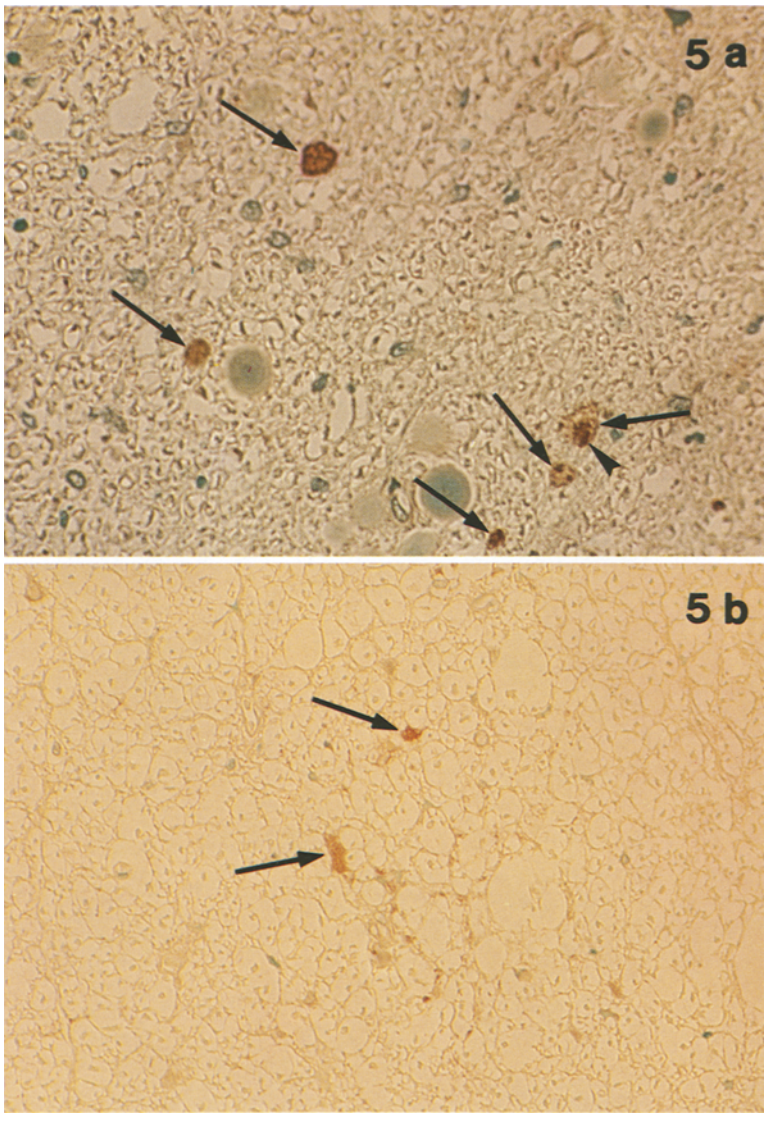


Fig. 5. a Immunohistochemical demonstration of HSV 2 antigen using polyclonal antibody in the spinal cord (L3). *Arrows* indicate positive reaction of necrotic cells. *Arrowhead* indicates the positive reaction in the nucleus. ×260.
b Immunohistochemical demonstration of HSV 2 antigen using monoclonal antibody in the spinal cord (L3). Positive reactions were found in necrotic cells (*arrows*). ×270

epithelium. Immunohistochemical examinations for C3, IgG, IgM and fibrin did not reveal any remarkable findings.

Electron microscopic investigation of the spinal cord demonstrated numerous HSV particles (Fig. 6). Encapsulated virions surrounded by single membranous structures were observed.

Discussion

Clinically, the present case was one of acute ascending myelopathy followed by slight brain involvement. The patient died of respiratory insufficiency caused by intercostal paresis. Pathologically, the spinal cord revealed numerous areas of necrosis in the grey and white matter. Perivascular lymphocytic infiltration (perivascular cuffing) was found in the spinal cord and occasionally in the cerebral cortex and cerebellum. Such infiltration was more frequent in the present case than in our two previously reported cases (necrotizing myelopathy associated with malignancy), which were also caused by HSV 2 (Iwamasa et al. 1989). The present case may thus be regarded as one of necrotizing myelitis. Detailed examinations of all the organs revealed Cowdry type A inclusion bodies in the skin, oesophagus and spinal cord (L2). Hitherto, due to the difficulty of isolating HSV in autopsy cases, and also the absence of viral inclusions on light microscopy, acute necrotizing myelopathy and myelitis caused by HSV 2 infection has been estimated to occur at a low rate (Wiley et al. 1987). In fact, to date there have been few reported cases which were not associated with malignancy (Britton et al. 1985; Wiley et al. 1987; Ahmed 1988). However, Wiley et al. (1987) described one case in which HSV 2 was an important aetiolgoical agent. The present case presented clinically acute ascending myelopathy of unknown aetiology and at the terminal stage the patient became delirious and displayed skin eruptions. Treatment with steroids was given. It might be considered that the steroid accelerated the dissemination of HSV 2 at the terminal stage. Virological examinations were not requested by the neurologist. However, immunoperoxidase staining using anti-HSV 2 polyclonal and also monoclonal antibodies re-

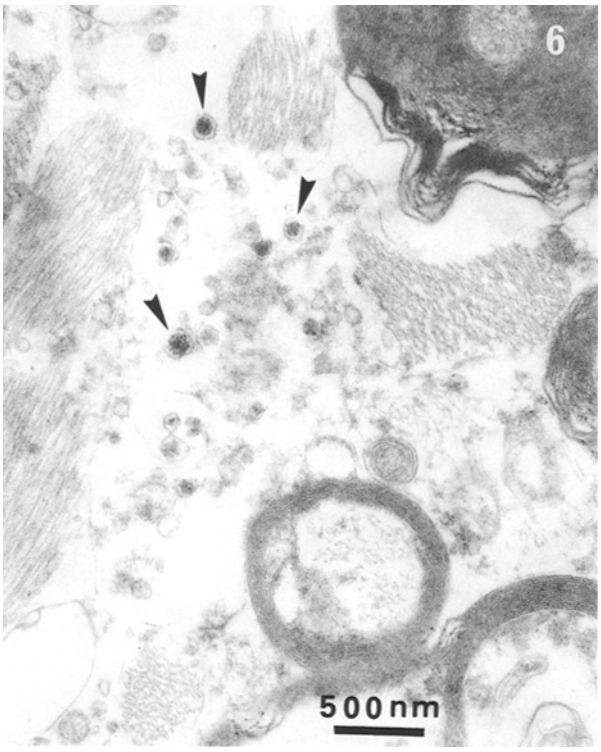


Fig. 6. Electron micrograph of the spinal cord (L3). Specimens which were fixed with 10% formalin at necropsy were refixed in 2% osmium tetroxide. Routinely processed Epon sections were then observed under a Hitachi 12 A electron microscope. Numerous HSV particles (*arrowheads*) were observed. However, the particles were degenerated and broken by formalin fixation

vealed HSV 2 antigen in the spinal cord, and large numbers of HSV particles were demonstrated electron microscopically. Okinawa is an HSV 2 endemic area (Yoshitake et al., in preparation) and HSV 2 is considered to be a common aetiological agent of necrotizing myelopathy and myelitis in Okinawa. We isolated HSV 2 (Yoshitake et al., in preparation) in seven cases of inguinal herpes in Okinawa. HSV 2 DNA from these seven cases was analysed electrophoretically after treatment with restriction enzymes. The size of *p* and *v* *Bam*H1 cleavage fragments of all isolates of HSV 2 showed larger molecular size than those of SAV, UW 333, and HSV 2 from the mainland of Japan, which were not strongly neurovirulent. However, DNA of HSV 1 in Okinawa did not show any remarkable difference. Recently, Taha et al. (1989) reported that the HSV 2 DNA region composed of *p, v, u* and *g* fragments (*Bam*H1 cleavage) closely related to the neurovirulence. They made the variant HSV 2 strain JH 2604 from HG 52 by deletion of the genome between 0 to 0.02 and 0.81 to 0.83 map units in which *p, v, u* and *g* regions were included. The HG 52 was strongly neurovirulent, but JH 2604 showed no neurovirulence. Therefore, it may be considered that the change of the molecular size of *p* and *v* fragments relates to the necrotizing myelopathy and myelitis. Furthermore, perivascular lymphocytic cuffing was frequently observed in this case, differing from our two previous malignancy-associated cases (Iwamasa et al. 1989), where very little was observed. The presence of perivascular lymphocytic cuffing may therefore be considered to depend upon the condition of the patient, and in particular on his/her immunological condition. The mechanism of viral spread in the spinal cord, however, remains obscure. Wiley et al. (1987) described reactivation of latently infected HSV 2 in the dorsal ganglia. In the present case, the patient had suffered from diabetes mellitus for 12 years and loss of appetite for about 1 month. Myelopathy was observed at an early stage, and then skin eruptions at the terminal stage. Reactivation of latently infected HSV 2 in the dorsal ganglia may have occurred.

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