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Herpes Simplex Myelitis: Differences in Clinical Manifestations Between Herpes Simplex Virus Type 1 and Type 2

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1. Introduction

Various clinical types of central nerve infection caused by herpes simplex virus (HSV) have been identified, and the clinical features of not only encephalitis and meningitis, but also Mollaret meningitis and Elsberg syndrome, have been clarified (Tyler, 2004; Eberhardt et al., 2004). Myelitis is rare, and it has been mostly reported as ascending myelitis, necrotizing myelitis or myeloradiculitis (Klastersky et al, 1972; Koskiniemi et al., 1982; Britton et al., 1985; Tucker et al., 1985; Wiley et al., 1987; Ahmed,1988; Nakagawa et al., 1991; Iwamasa et al., 1991). The initial symptoms of herpes simplex myelitis (HSM) are sensory-motor disturbance of lower limbs and urinary disturbance. Then, transverse myelopathy ascends from level of the lumbosacral spinal cord to level of the cervicothoracic spinal cord. As ascending necrotizing form of HSM often accompanied encephalitis, quadriplegia, or respiratory muscle paralysis, the prognosis of this condition is poor (Table 1). In terms of pathological features, necrotic changes are common, and hemorrhagic lesions are occasionally seen. As a result of technical advances in the 1990s, including diagnostic imaging using MRI and virological diagnosis using PCR, HSM is now more frequently identified. Consequently, in addition to acute ascending myelitis, patients with transverse

Clinical symptoms:	
(initially)	Sensorimotor disturbances of lower limbs, Urinary disturbance
	Ascending progression of myelopathy (1-2 weeks)
Complication:	Genital herpes
Associated condition:	HIV, Malignancy, Diabetes mellitus (immunocompromised)
HSV typing:	HSV-2
Diagnosis:	Autopsy
Prognosis:	Fatal

Table 1. Characteristic of acute ascending necrotizing myelitis

myelitis that arises in the cervicothoracic spinal cord and does not ascend, mild myelitis, and recurrent myelitis, have been reported. The present article summarizes the clinical features of HSM and discusses diagnosis and therapy.

2. Case presentation

2.1 Case 1: Acute ascending myelitis by HSV-2

A 44-year-old man with diabetes mellitus presented with urinary retention and weakness in both legs following scrotal numbness. He had genital herpes, and sensory-motor disturbance of his lower limbs ascended to the level of thoracic spinal cord in 5 days. Neurological examination revealed paraparesis and sensory disturbances below the T6 level. The urodynamic study showed atonic contraction. Analysis of his CSF showed 105 WBC (all mononuclear cells), protein of 68 mg/dl, and the test for serum antibody to human T lymphotropic virus type I (HTLV-I) antibody and results of collagen vascular screening test were both negative. He was treated with acyclovir (1,500 mg/day) and betamethasone (8mg/day) for 4 weeks. He showed some improvement in urinary retention, but the paraparesis persisted as sequelae. The anti-HSV antibody (neutralizing test) in his serum antibody was 1:4, and did not change in paired samples. On admission, the test for anti-HSV antibody (ELISA) in the CSF showed positive IgM-HSV 2.000 (4+) and negative IgG-HSV IgG 0.076 (-). Following CSF tests showed the decrease of the IgM-HSV level 0.234(+) and the increase of the IgG-HSV 0.366(+). HSV-2 DNA was detected in his CSF sample by PCR.

2.2 Case 2: Recurrent HSM by HSV-2

A 49-year-old female presented with urinary retention, back pain, numbness and a sudden onset of weakness in both legs. Neurological examination showed paraparesis, positive Babinski sign, and sensory disturbances below the T5 level on the right and below the T7 level on the left. The urodynamic study showed atonic contraction. Analysis of her CSF showed 30 WBC (all mononuclear cells), protein of 79 mg/dl, IgG index of 0.32, myelin basic protein of 3.1 µg/ml, and an absence of oligoclonal IgG bands. A myelogram revealed cord enlargement at the levels of T3 to T7 segments. Anti-HSV antibody by EIA was positive in her CSF. After treatment with intravenous acyclovir (1,500 mg/day) and betamethasone (8 mg/day) for 4 weeks, she recovered significantly. One year and 10 months later, she again developed myelopathy at the T6 level, and she showed Romberg's sign. CSF analysis showed 196 WBC (100% mononuclear cells), protein of 90 mg/dl, IgG index of 0.51, and myelin basic protein of 6.7 µg/ml. MRI of her thoracic cord indicated an increased signal intensity in the posterior portion of spinal cord at the 2nd thoracic vertebra Tl-weighted image with Gd-DTPA enhancement. Brain MRI was normal. In her CSF, anti-HSV antibody using EIA remained positive. No herpetic vesicles were observed in either episode. The test for serum antibody to HTLV-I antibody and results of collagen vascular screening test were both negative. Flow cytometry showed no abnormality in lymphocyte subset. She was treated with acyclovir (1,500 mg/day) and betamethasone (8mg/day) for 3 weeks and again showed complete recovery. HSV-2 DNA was detected at both first and second episode.

2.3 Case 3: Chronic myelitis by HSV-2

A 38-year-old male presented with progressive weakness in both legs, urinary retention, and numbness, for one month. Neurological examination revealed paraparesis and sensory disturbances below the T6 level. Babinski's sign was negative. His bladder was distended.

Analysis of his CSF showed 11 WBC (all mononuclear cells), protein of 75 mg/dl, IgG index of 0.44, and a positive oligoclonal IgG band. The myelogram revealed the cord enlargement at the T2 to T6 segments. The anti-HSV-1 antibody in his CSF determined by immunofluorescence (IF) was 1:4, and in serum it was 1:640. After administration of intravenous acyclovir (750 mg/day for 4 weeks) and prednisolone (60 mg/day for 2 weeks), his paraparesis improved. The anti-HSV-1 antibody in his CSF decreased 1:2, and serum antibody was 1:320. Five months later, however, myelopathy developed again at the same level. Analysis of his CSF showed 2 WBC (all mononuclear cells), protein of 42 mg/dl, IgG index of 0.25, and negative oligoclonal IgG bands. Brain and thoracic MRI showed no abnormalities. The anti-HSV-1 antibody (IF) in his CSF was 1:8, and serum antibody was 1: 1,280. Serum antibody against HTLV-I was negative. Intravenous methylprednisolone (1 g/day for 3 days) exerted beneficial effects. During the second episode genital herpetic vesicles were observed, and antibody to HSV remained positive in the CSF. He was then administered intravenous acyclovir (750 mg/day for 4 weeks), with improvement in numbness and urinary retention, but the paraparesis persisted. HSV-2 DNA was detected by PCR.

3. Clinical features

Table 2 lists the clinical features of patients with HSM, including our own patients. Klastersky and colleagues (Klastersky et al, 1972) reported the first patient with HSM in 1972, and HSM was only reported as ascending myelitis until the 1980s. The initial symptoms of HSM included lumbar pain, leg pain, and urinary disturbance. Sensory and motor disturbance has been observed to begin in the leg and ascend to the cervicothoracic spinal cord in 1-2 weeks. Like encephalitis or meningitis caused by HSV, disease onset or progression was sometimes accompanied by fever, and in about half of the patients, eczema herpeticum was seen on the lips or genitals. In most patients, the cause of ascending myelitis was HSV-2. During the 1970s and 1980s when these patients were reported, it was difficult to diagnose HSM based on clinical findings. Therefore, pathological diagnosis based on autopsy findings was required for the diagnosis of myelitis and the identification of the causative virus. Many patients with ascending myelitis had such underlying disease as AIDS or immunosuppression due to cancer or diabetes (Koskiniemi et al., 1982; Britton et al., 1985; Tucker et al., 1985; Wiley et al., 1987; Nakagawa et al., 1991; Iwamasa et al., 1991), and HSV-2 myelitis had poor prognosis. Hence, lethal ascending necrotizing myelitis due to HSV-2 in an immunocompromised host was considered the typical clinical feature of HSM, and also HSM was considered an opportunistic infection. In the 1990s, MRI began to be used for diagnostic imaging of the spine, and PCR began to be used for virological diagnosis (Aurelius et al., 1991; Nakajima et al., 2005), which greatly contributed to the diagnosis of HSM. Consequently, clinical features associated with HSM, other than ascending myelitis, were identified. Therefore, patients with transverse myelopathy in the cervicothoracic spinal cord, myelitis without ascending inflammation (non-ascending myelitis), polio-like atrophy that developed as unilateral arm sensory and motor disturbance (Kyllerman, 1993), or recurrence (Shyu, et al., 1993), have been reported. In addition, the frequency of fever and eczema herpeticum has decreased, and the frequency of HSV-1 has increased as a causative virus. Today, it is generally accepted that the frequency of previously reported immuno-

suppressive underlying diseases in HSM is somewhat low. Moreover, cases of HSM with good prognosis have been reported because of the early diagnosis and the early induction of antiviral agent, acyclovir.

Age/ sex	Neurological symptom Clinical course	Fever	Rash	HSV subtype	Associated condition	Prognosis Sequelae	Author
45/F	Ascending myelitis	+	–	HSV-1	–	Death*	Klastersky 1972
21/F	Ascending myelitis	+	–	HSV**	Pregnancy	Severe	Koskiniemi 1982
M	Subacute ascending myelitis	–	+	HSV-2	AIDS	Death *	Britton 1985
36/M	Ascending myelitis	+	+	HSV-2	AIDS	Death *	Tucker 1985
57/M	Ascending myelitis	+	+	HSV-2	DM	Death *	Wiley 1987
29/F	Ascending myelitis	+	–	HSV-2	–	Severe	Ahmed 1988
64/M	Ascending myelitis	–	–	HSV-2	LK	Death *	Nakagawa 1991
74/F	Ascending myelitis	–	+	HSV-2	ATL	Death *	
64/M	Ascending myelitis	–	–	HSV-2	DM	Death *	Iwamasa 1991
7/M	Non-ascending myelitis	+	–	HSV-1	–	Severe	Kyllerman 1993
72/M	Recurrent ascending myelitis	–	+	HSV-1	–	Severe	Shyu 1993
59/M	Ascending myelitis	+	+	HSV-2	–	Death *	Folpe 1994
76/F	Subacute ascending myelitis	+	–	HSV-2	–	Death	Ellie 1994
51/M	Ascending myelitis	–	–	HSV**	–	Death	Radhakrishnan 1994
42/M	Non-ascending myelitis	–	–	HSV**	–	Recovery	Petereit 1996
44/M	Ascending myelitis	–	+	HSV-2	DM	Severe	Nakajima 1998
39/M	Ascending myelitis	+	–	HSV-2	–	Severe	
69/M	Ascending myelitis	–	–	HSV-2	–	Severe	
50/F	Ascending myelitis	+	–	HSV-2	–	Severe	
68/M	Ascending myelitis	–	–	HSV-2	–	Recovery	
38/M	Subacute ascending myelitis	–	+	HSV-2	–	Severe	
49/F	Recurrent non-ascending myelitis	–	–	HSV-2	–	Recovery	
69/M	Non-ascending myelitis	+	–	HSV-1	–	Severe	
58/M	Subacute non-ascending myelitis	–	–	HSV-1	–	Recovery	
48/M	Ascending myelitis	–	–	HSV-1	–	Recovery	Kuker 1999
6/M	Ascending myelitis	–	–	HSV**	–	Recovery	Galanakis 2001
70/F	Recurrent ascending myelitis	–	+	HSV-2	–	Recovery	Gobbi 2001
60/M	Non-ascending myelitis	–	–	HSV-1	–	Recovery	Azuma 2001
59/M	Encephalo-myelo-radiculitis	+	–	HSV-2	–	Severe	Kusuhara 2002

* Autopsy case, **Unknown HSV subtype, AIDS; Acquired immunodeficiency syndrome, DM; Diabetes mellitus, LK; Lung cancer, ATL; Adult T cell leukemia

Table 2. Clinical features of HSM

4. MRI findings

As MRI findings, spinal lesions of HSM are generally either hypointense or isointense on T1-weighted images and hyperintense on T2-weighted images similar to images obteined in HSV encephalitis (Ellie et al., 1994; Petereit et al, 1996; Nakajima et al., 1998; Kuker, et al., 1999; Galanakis et al., 2001; Gobbi et al., 2001; Azuma et al., 2001) (Fig. 1). Furthermore, spinal lesions occasionally accompany gadolinium enhancement (Fig. 2), and gadolinium enhancement of spinal nerve root and dura mater has been reported (Kuker, et al., 1999). In some cases, spinal lesions were exhibited as hyperintense on both T1 and T2-weighted images, which show hemorrhagic lesions (Nakajima et al., 1998) (Fig. 2). The size of spinal lesions varies, however, lesion in a patient with HSM is single and do not occur in multiple as seen in acute disseminated encephalomyelitis (ADEM) or multiple sclerosis.

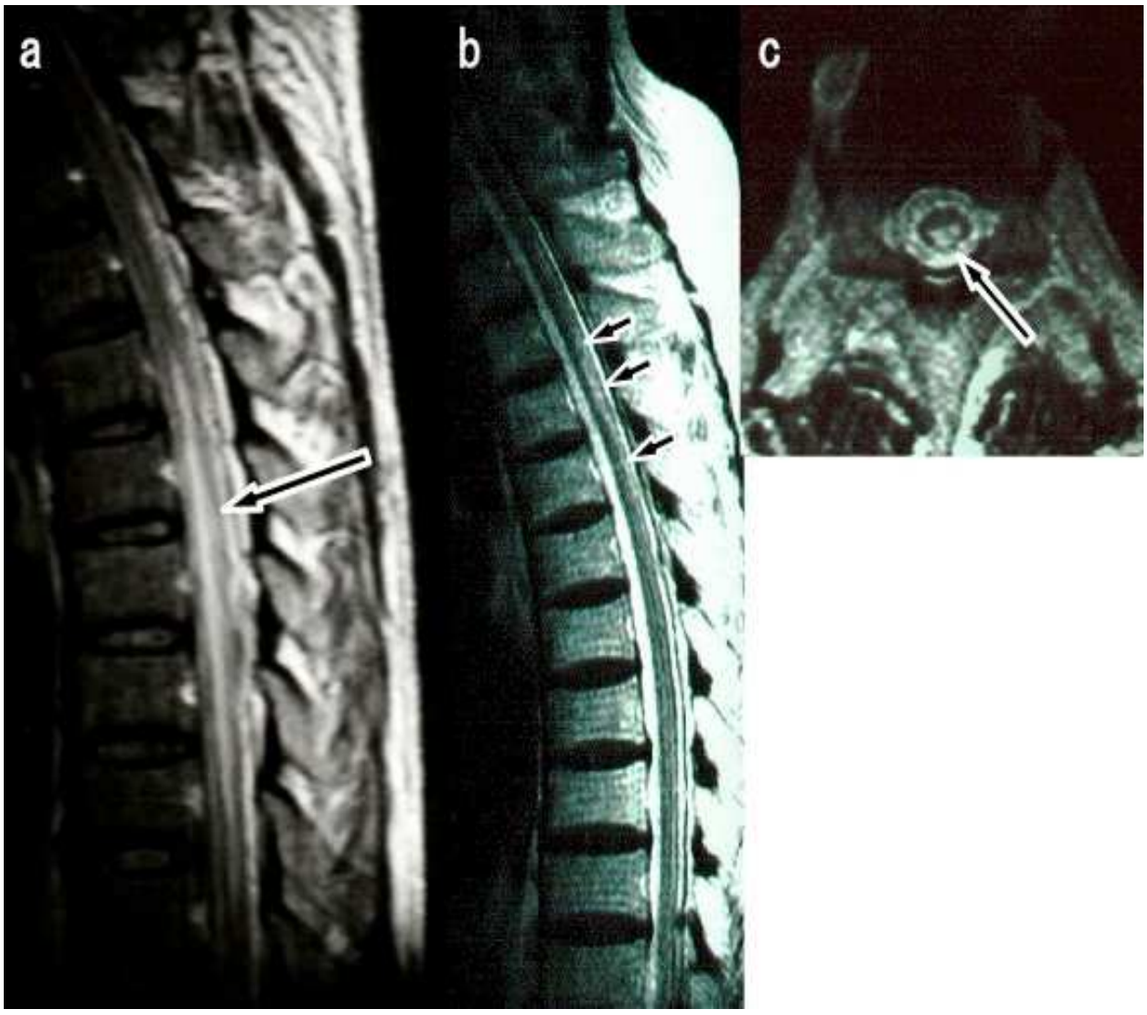


Fig. 1. MRI findings of HSM. Sagittal T2-weighted imageing of HSM (HSV-1) showed cord swellineg and a hyperintense signal in thoracic spinal cord (a). Sagital (b) and axial (c) T2-weighted imageing of HSM (HSV-2) showed hyperintense signal lesion in the posterior portion of thoracic spinal cord.

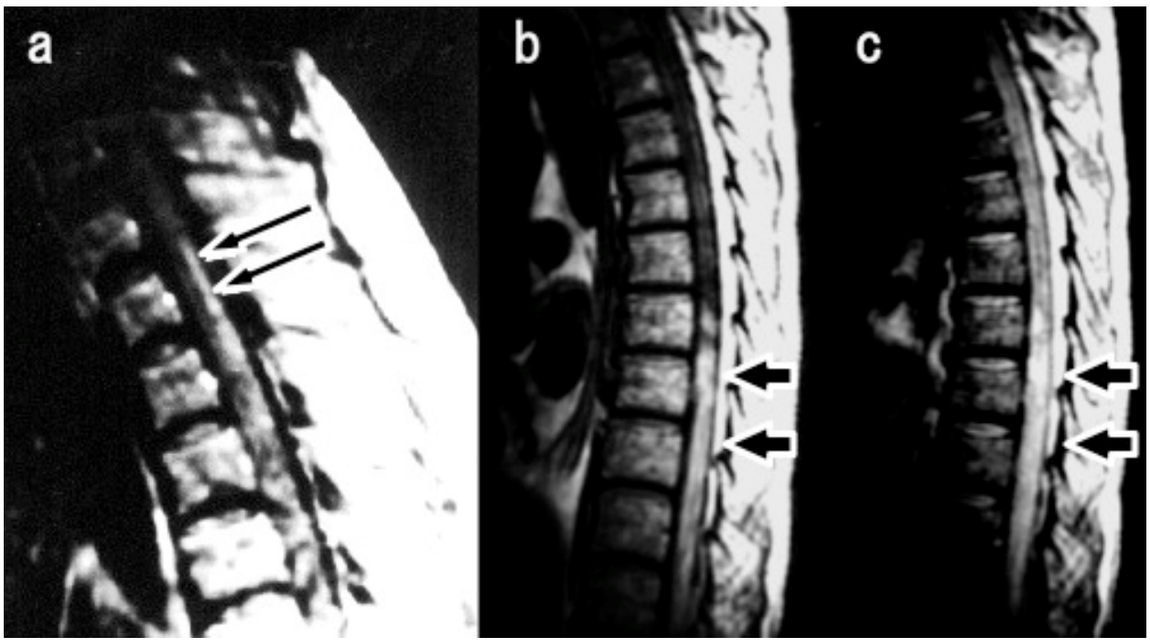


Fig. 2. MRI findings of HSM. Enhanced T1-weighted imageing of HSM (HSV-2) showed an increased signal intensity in the posterior portion of spinal cord at the T2-T4 level (a). T1-weighted imageing (TE600/TR20) (b) and T2-weighted imageing (TE3,000/TR60) (c) of HSM (HSV-2). Both sequences showed a hyperintense signal lesion which was continuous from the T7 level to the lumbosacral spinal cord.

5. Pathogenesis

All 8 reported cases of HSM that autopsy were performed were ascending myelitis (Klastersky et al, 1972; Koskiniemi et al., 1982; Britton et al., 1985; Tucker et al., 1985; Wiley et al., 1987; Nakagawa et al., 1991; Iwamasa et al., 1991; Shyu, et al., 1993). Myelitis was caused by HSV-1 in 1 patient and HSV-2 in 7 patients. Pathological findings showed marked necrosis of both gray and white matters, and such findings as hemorrhage, white blood cell (macrophage) infiltration, perivascular lymphocyte infiltration, and vascular necrosis were confirmed. These inflammatory changes and necrotic lesions were occasionally confirmed in dorsal spinal nerve roots and dorsal root ganglia. Furthermore, in these areas, Cowdry A inclusions, indicating HSV infection, were observed, and electronmicroscopy showed HSV particles. Based on these pathological findings, the onset mechanism for HSM could be deduced as follows: latent HSV-2 infection in the dorsal spinal nerve root of the lumbosacral spinal cord is reactivated by immunosuppression, and the virus enters the spine via the dorsal spinal nerve root. Since 1994, PCR-confirmed myelitis has exhibited various clinical features, such as non-ascending myelitis, recurrent or mild myelitis, and the incidence of HSV-1 has increased. While pathological analysis was not conducted, MRI showed gadolinium enhancement of the spinal nerve root and meninges (Kuker, et al., 1999), thus suggesting inflammation of the dorsal spinal nerve root. Regardless of clinical features and HSV type, myelitis is believed to be induced when HSV enters the spinal cord via the dorsal spinal nerve root or ganglion. It is difficult to ascertain whether HSM is caused by initial infection or reactivation due to latent infection. Most patients with HSM are older than

middle age, and in patients in whom serum antibody titer was measured, anti-HSV antibody titer was positive in the early stage, thus suggesting that reactivation of latent HSV in a dorsal spinal nerve root ganglion induces myelitis. Since recurrence for HSV-2 is more frequent than that for HSV-1 in genital herpes, recurrent myelitis may be due to HSV-2. Diagnosis cannot be based solely on serum HSV antibody tier, because there was a case of HSM in whom the progress of serum HSV antibody titer ashowed past infection, while that of CSF titier indicated initial HSV infection (see Case presentation case 1). Recently, one study reported a patient in whom HSV-2 caused genital herpes, while HSV-1 was detected in the CSF from Elsberg syndrome (Yoritaka et al., 2005). This suggests that even in the same host, different HSV types and strains repeat infection and reactivation.

6. Immunological regulation in HSM (animal model)

T-cell-mediated immunities have been shown to be involved in the pathology of HSM. Th1-cell-associated cellular responses (Th1 responses) are known to be essential in the host defence against systemic infections of HSV. Th1 responses are manifested by the increased production of Th1 cytokines (IL-2, IFN-1, etc.) from Th1 cells, while Th2-cell-associated cellular responses (Th2 responses) are manifested by the production of Th2 cytokines (IL-4, L-10, L-13, etc.) released from Th2 cells. Th2 cytokines are known to be inhibitors for the differentiation and expression of Th1 cells. Previously, the pathogenic role of Th2 responses on the severity of HSM was investigated in mice exposed to footpad injection of HSV-2 (Nakajima et al., 2000). Morbidity and mortality of mice with HSM increased when they were treated with a mixture of Th2 (IL-4/IL-10) cytokines (Fig. 3a). The mortality rates of HSM mice were significantly influenced by the IL-4/IL-10 mixture at doses ranging from 1 to 100 pg per mouse. High doses (more than 1000 pg per mouse) of the IL-4/IL-10 mixture did not show significant effects on the mortality of HSM mice. There is an optimum effective dose of the cytokines in these experimental methods (Fig. 3b). Patients with AIDS, malignancy, diabetes mellitus or psychosomatic stress reaction commonly carry dysfunction in T cell functions, and a shift from Th1 responses to Th2 responses has been reported in these patients. Thus, The regulation of Th1/Th2 balance may be a key on the immunological control of HSM.

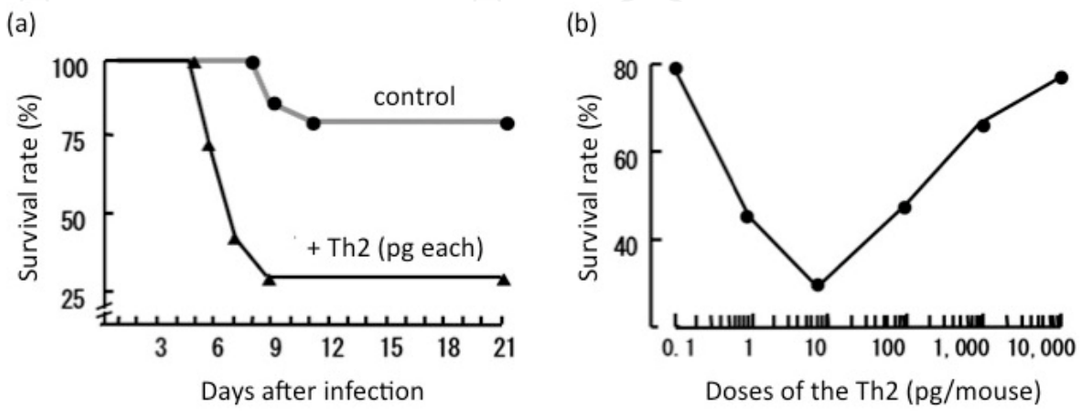


Fig. 3. a: Effect of the IL-4/IL-10 mixture on the morbidity and mortality of HSM mice. b: Effect of various doses of the IL-4/IL-10 mixture.

7. HSV subtyping

Retrospective clinical data was collected on 10 cases of herpes simplex myelitis from 5 institutions in Japan. All cases were diagnosed by using PCR. We assessed differences in clinical presentation, laboratory findings, MRI images, and treatment outcome between HSV-1 and HSV-2 (Table 3).

In most cases, herpes simplex myelitis occurred in immunocompetent persons. In 7 cases, disease onset was marked by sensorimotor disturbances of the lower extremities and urinary disturbances, with the transverse myelopathy ascending to the cervicothoracic

		Number
Age / Sex	51 (29 - 69) / male : female = 8 : 2	
Clinical symptoms		
(Initial Symptoms)	Leg numbness, weakness, urinary disturbance	8
	Unilateral hypesthesia	1
	Unilateral limb sensorimotor disturbances	1
(Maximum)	Transverse myelopathy	10
(Progression)		
	Ascending myelopathy	7
	Non-ascending pattern	3
Associated condition	HIV	1
	Diabetes mellitus	2
Herpes eruption	Genital herpes	3
Therapy	Acyclovir + Steroids	8
	Acyclovir	1
	Steroids	1
Clinical course	Acute	8 (recurrence 1)
	Subacute	2 (recurrence 1)
Prognosis	No lethal cases	
	Severe sequelae (paraplegia)	7
	Recovery	3
CSF findings	Cell	11-226 mm3
	Protein	51-276 mg/dl
	Positive oligoclonal band	1
MRI findings	Hyperintense lesions in T2WI	7
	Hyperintense lesions in T1/T2	1
	Gd-enhancement	3
HSV antibodies	IgG elevation	10
	IgM elevation	1
PCR (HSV type)	HSV-1	7
	HSV-2	3

Table 3. Clinical features of 10 patients with HSM

spinal cord level. The other 3 cases demonstrated transverse myelopathy without an ascending pattern. Two cases exhibited recurrent episodes. Nine out of 10 patients had CSF pleocytosis, and MRI demonstrated single lesions that were longitudinal or limited. HSV-2 was detected by PCR at an early stage in all 7 patients with ascending myelitis. HSV-1 DNA was detected in 2 and HSV-2 DNA was detected in 1 of the 3 cases with a non-ascending pattern. All recurrent cases were caused by HSV-2. Anti-herpetic drugs, notably acyclovir, improved mortality. However, because severe sequelae, such as paraplegia, persisted in 7 out of 10 patients, early introduction of acyclovir is necessary. These results demonstrate diverse clinical manifestations of herpes simplex myelitis. The cases of herpes simplex myelitis with an ascending pattern or recurrent course are caused by HSV-2, while those with a non-ascending pattern are mainly caused by HSV-1. Furthermore, a recurrent course is considered to be characteristic of HSV-2 infection (Table 4).

		HSV-1	HSV-2
Case number		n=2	n=8
Clinical symptoms			
Ascending myelopathy	(7 cases)	0	7
Non-ascending pattern	(3 cases)	2	1
Clinical course			
Acute	(8 cases)	1	7
Subacute	(2 cases)	1	1
Recurrence	(2 cases)	0	2
Associated condition			
HIV	(1 case)	1	0
Diabetes mellitus	(2 cases)	0	0
Cases with genital herpes	(3 cases)	0	3
Prognosis			
Severe sequelae	(7 cases)	0	7
Recovery/mild sequelae	(3 cases)	2	1

Table 4. Distinction between HSV-1 and HSV-2

8. PCR methods

PCR represents an important technique for diagnosis and therapeutic planning. PCR is widely utilized by medical institutions and private testing companies. However, because CSF samples collected by lumbar puncture from patients with encephalitis contain very small amounts of HSV DNA, the sensitivity of PCR, nested PCR or real-time PCR must be improved.

8.1 Nested PCR assay

Retrospectively PCR assays were performed as described (Nakajima et al., 1998). The CSF samples were stored at -80°C until PCR. Template DNA was extracted from 300 µl of CSF and HSV DNA was amplified by nested PCR assay. The primer sequences were chosen to

flank a conserved region in the HSV-I and HSV-2 DNA polymerase gene. The primers used in the first PCR were as follows: 5'-CAGTACGGCCCCGAGTTCGTGACCGGG-3' and 5'-TACTCGCCGATCACCCCGCG-3'. For the nested PCR, 5'- CATCATCAACTTCGACTGGC-CC-3' and 5'-GGCGTAGTAGGCGGGGATGTCGCG-3' were constructed as the inner primer pair. On the first round of PCR, the profile consisted of DNA denaturation at 94°C for I min, primer annealing at 60°C for 1 min and primer extension at 72°C for I min for 35 cycles. On the second round of PCR, l µl aliquots of the first PCR products were added to 49 pl ofa freshly prepared reaction mixture, followed by 30 cycles of amplihcation at 95°C for 30s, 60°C for 30s, and 72°C for 30s. When HSV-I or HSV-2 DNA was present in the mixture, a 298-bp sequence was amplified by nested PCR. Within this sequence, individual virus strains have unique restriction sites so that amplification product could be typed by digestion with Bgl II and Xho I. As shown in Fig 4, agarose gel electrophoresis showed nested PCR amplified products of HSV-I and HSV-2 strains at a sequence of 298 bp (a). The specific recognition sequence for Xho I is only present in HSV- I DNA, and that for Bgl II is only present in HSV-2 DNA. Xho I yields 209- and 89-bp fragments for HSV-I, whereas Bgl II yields 45- and 253-bp fragments for HSV-2 (b). 1 = HSV-I strain; 2 = HSV-2 strain; 3 = CSF sample from HSM case; 4 = CSF sample from negative control; M = molecular weight makers (Hae III digest of ΦX174).

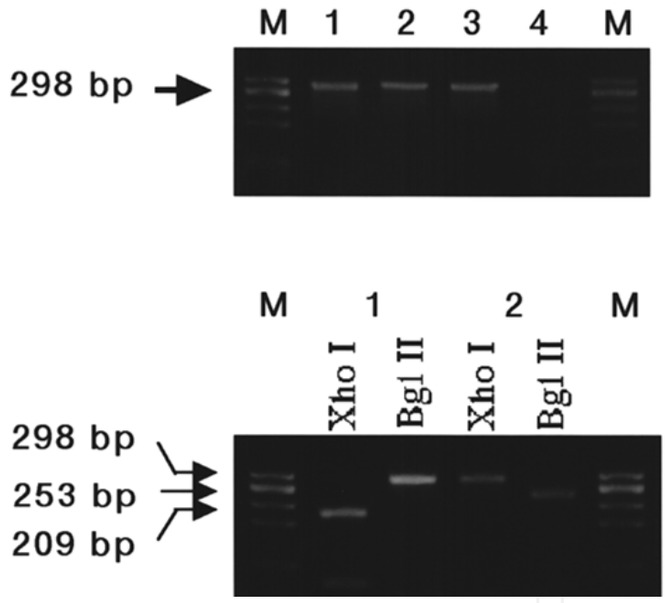


Fig. 4. Defferenciation of HSV-1 and HSV-2 by restriction pattern of PCR pruducts.

8.2 LightCycler PCR

Real-time PCR was performed using a LightCycler (Roche Diagnostics, Mannheim, Germany) and a LightCycler HSV 1/2 Detection Kit (Roche Diagnostics, Mannheim, Germany), which contains the necessary primers, fluorescent-labeled probes, Taq DNA polymerase and reaction buffers for Hybri-probe PCR. Using 20 µl reaction solutions, each containing 2 µl of CSF sample that had been boiled at 100°C. PCR was performed with preprocessing, temperature cycle (amplification) and melting curve analysis. Cycling conditions were as follows: initial denaturation /FastStart Taq DNA polymerase activation

at 95°C/10 min, 45 cycles of denaturation at 95°C/10 sec, annealing at 55°C/15 sec and extension at 72°C/15 sec. After amplification was complete, melting curve analysis was performed as follows: starting at 40°C followed by a gradual increase in temperature (transition rate of 0.1°C/sec) to 80°C with continuous fluorescence acquisition. The fragment selected for amplification and detection using the HSV 1/2 Detection Kit includes areas specific to HSV-1 and HSV-2 subtypes and an area common to the two subtypes (the primer region of the DNA polymerase gene is highly conserved for both HSV-1 and HSV-2). Sequence differences between the PCR product and hybridization probes resulted in shifts in the melting temperatures Analysis of the PCR amplification and probe melting curves was accomplished through the use of LightCycler software.

8.3 Sensitivity and selectivity (Comparison with nested PCR)

Using plasmid DNA carrying the HSV DNA polymerase gene that was included in the LightCycler HSV 1/2 Detection Kit as a positive control, serially diluted samples were analyzed. Results showed that fluorescent signals can be detected even at a concentration of 1 copy/tube, and determination was possible up to 10^3 copies/tube (Fig. 5a). Melting analysis was conducted by measuring fluorescent intensity at different melting temperatures after amplifying HSV-1- and HSV-2-positive samples. Fig. 5b shows cumulative fluorescent intensity per unit temperature. Peak melting temperature was 54 °C for HSV-1 and 67 °C for HSV-2, and HSV subtypes could be differentiated based on this difference in melting temperature. By the analysis of CSF samples, detection sensitivity of LightCycler PCR is comparable to that of nested PCR. In addition, subtype differentiation based on melting curve analysis matched that based on restriction band patterns of nested PCR products (Table 5). The process of LightCycler PCR including melting curve analysis took about 50 min to complete.

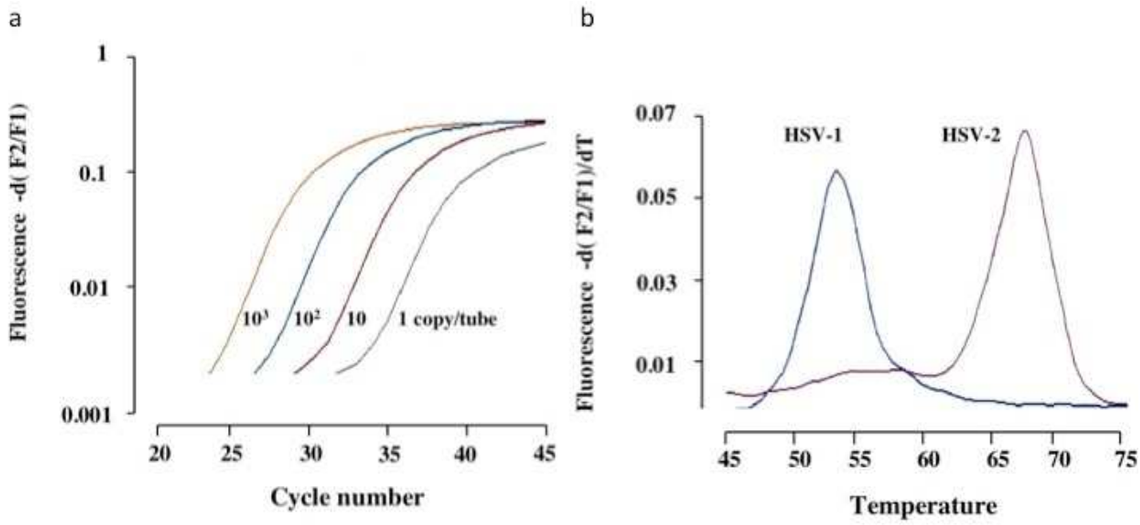


Fig. 5. a: Amplification of HSV DNA by LightCycler PCR. b: Defferenciation of HSV-1 and HSV-2 by melting curve analysis matched.

No.	Diagnosis	Conventional PCR			LightCycler
		Direct	Nested	Subtype*	
1	Encephalitis	–	+	HSV-1	HSV-1
2	Encephalitis	–	+	HSV-1	HSV-1
3	Encephalitis	–	+	HSV-1	HSV-1
4	Encephalitis	–	+	HSV-1	HSV-1
5	Encephalitis	–	+	HSV-1	HSV-1
6	Myelitis	–	+	HSV-2	HSV-2
7	Myelitis	–	+	HSV-2	HSV-2
8	Myelitis	–	+	HSV-1	HSV-1

*Differentiation of HSV types 1 and 2 was made by restriction fragment length of nested PCR productions

Table 5. Comparison of conventional PCR and LightCycler PCR

9. Diagnosis

Table 6 shows the guide for the diagnosis of HSM. In terms of clinical symptoms, ascending myelitis, back pain, and fever can indicate HSV, but it is essential to conduct neurological and virological tests. In the past, eczema herpeticum was identified during disease onset or progression, and as a result, eczema herpeticum is considered a diagnostic clue for HSM. However, according to recent reports, the frequency of eczema herpeticum is not been high, and there has been a report of a patient with Elsberg syndrome in which different HSV types were found in genital rash and cerebrospinal fluid (Yoritaka et al., 2005). Thus, it is considered essential to diagnose HSV infection using a cerebrospinal fluid sample. Table 7 lists diseases that must be differentiated from HSM. If clinical symptoms and MRI findings indicate myelitis and a cerebrospinal fluid test suggests an infection, it is important to conduct a virological test using a cerebrospinal fluid sample in order to rule out HSM. As HSM is rare disease, HSM were previously reported as case presentation, and there is no large-scale epidemiological survey. In Japan, There was a regional questionary survey of the central nervous infection caused by HSV (Kyushu/Okinawa area (the southwestern Japan): 1993-1996). That questionary survey was performed among approximately 100 pediatric

1. Clinical manifestation: neurological symptoms associated with acute or subacute myelitis/myelopathy.
2. Neurodiagnostic tests:
   - MRI shows spinal cord lesions with oedeam or necrosis.
   - CSF analysis demonstrates elevated mononuclear cells and protein.
3. Virologic analysis:
   - PCR of the CSF is the most beneficial diagnostic method.
   - CSF antibody measurements may be useful in retrospective diagnosis
   - Virus cultures are of little value for the diagnosis of HSM

Table 6. Diagnosis of HSM

and/or neurology institutions in Kyushu/Okinawa District between 1993-1996. There were 4 HSM cases among 39 HSV–induced central nerve infections diagnosed by PCR and HSV antibody titers (Table 8). HSM have been increasingly recognized by the diagnostic use of the PCR and the accumulation of the case report. In patients with spinal lesions suggesting the myelitis, virologic examination using CSF specimen should be performed.

- Viral disease
  Varicella-zoster virus, Cytomegalovirus, EB virus, HHV-6, enterovirus (poliomyelitis, Coxsackie, echo), HTLV-1, HIV
- Bacterial disease
  Mycoplasma, Lyme's disease, Syphilis
- Demyelinating disease
  Acute disseminated encephalomyelitis, Neuromyelitis optica, Multiple sclerosis
- Systemic autoimmune disease
  Sjogren's syndrome, Systemic lupus erythematosus, Anti-phospholipid syndrome, Sarcoidosis
- Allergic condition
  Atopic myelitis
- Paraneoplastic syndrome
- Vascular disorder
  Spinal cord infarction, Arteriovenous malformation, Vasculitis
- Spinal tumor
  Malignant lymphoma

Table 7. Differential diagnosis

	Number (n=39)
Temporal lobe/ limbic encephalitis	24
Brainstem encephalitis	1
Diffuse cerebrum	2
Acute disseminated encephalomyelitis	3
Myelitis	4
Meningitis	2
Others	3

Table 8. The clinical form of the central nervous infection caused by HSV: Questionary survey of Kyushu/Okinawa area (the southwestern Japan) (1993-1996)

10. Therapy

HSM was once considered a lethal disease, but in recent years, there have been reports of survival and recovery, primarily due to the use of anti-herpes agents. If HSM is suspected

based on clinical findings, diagnostic imaging, and cerebrospinal fluid findings, then acyclovir should be administered. If acyclovir is not effective, even when HSM is confirmed or strongly suggested, concurrent use of vidarabine should be considered. In the past, an antiviral agent and a steroidal agent were concurrently administered in many patients. Of the 9 patients in the above mentioned study, a steroidal agent was administered concurrently: methylprednisolone pulse therapy in 4 patients, betamethasone in 2 patients and predonine in 1 patient (Nakajima et al., 1998). Regarding the use of steroids, while steroids were used after confirming HSM in some patients, they were also used in combination with antiviral agents because of suspected demyelinating disease, such as ADEM or multiple sclerosis; autoimmune/inflammatory diseases, such as, vasculitis; or cryptogenic transverse myelopathy. Due to their anti-edema action, steroids can be used to treat HSM and herpes encephalitis, however, the immunosuppressive effects of steroids may enhance HSV proliferation. In studies using an animal model of herpes encephalitis, steroid administration did not increase the amount of HSV in the brain or exacerbate encephalitis (Thompson et al., 2000; Meyding-Lamade et al., 2003), but because there has not been a controlled clinical study on the concurrent use of steroids for the treatment of herpes encephalitis, no clear therapeutic guidelines for the treatment of herpes encephalitis using steroids have been established. However, one recent study investigated the use of steroids in herpes encephalitis in clinical settings (Kamei et al., 2005). In order to clarify the prognosticators for herpes encephalitis, the study examined age, background factors, clinical symptoms, and treatment, and the results showed that prognosis was more favorable with antiviral and steroid combination therapy than with antiviral monotherapy. Therefore, steroids can improve prognosis by suppressing edema formation, inflammatory cytokine production, and secondary autoimmune mechanisms. As a result, we believe that steroids should be actively combined with antiviral agents for the treatment of HSM.

11. Conclusions

Initially, HSM was reported to be lethal acute ascending myelitis that was likely to occur in immunocompromised hosts; however, with the recent improvements in viral diagnosis, including PCR and diagnostic imaging, it has been shown that the clinical features and onset of HSM greatly vary. With the use of antiviral agents, there have been more reports of survivors, but many patients have experienced severe aftereffects, such as paraplegia and quadriplegia, thus suggesting the importance of early diagnosis and therapy. Immunological mechanisms are involved in the onset and pathogenesis of HSM (Nakajima et al., 2000; Nakajima et al., 2001), and establishing a treatment that suppresses viral proliferation using an antiviral agent and reduces inflammation through immunological control is useful for alleviating organic damage to the central nerve, improving survival, and reducing aftereffects.

12. References

Ahmed I. (1988). Survival after herpes simplex type II myelitis. *Neurology*, Vol. 38, No. 9, (Sep.1988), pp. 1500.

Aurelius, E., Johansson, B., Skoldenberg, B., Staland, A. & Forsgren, M. (1991). Rapid diagnosis of herpes simplex encephalitis by nested polymerase chain reaction assay of cerebrospinal fluid. *Lancet*, Vol. 337, No. 8735, (Jan. 1991), pp. 189-192.

Azuma, K., Yoshimoto, M., Nishimura, Y., Fujimoto, H., Ayabe, M., Shoji, H. & Eizuru, Y. (2001). Herpes simplex virus type 1 myelitis with a favorable outcome. *Intern Med*, Vol. 40, No. 10, (Oct. 2001), pp. 1068-1069.

Britton, CB., Mesa-Tejada, R., Fenoglio CM., Hays, AP., Garvey, GG. & Miller, JR. (1985). A new complication of AIDS: thoracic myelitis caused by herpes simplex virus. *Neurology*, Vol. 35, No. 7, (Jul. 1985), pp.1071-1074.

Eberhardt, O., Kuker, W., Dichgans, J. & Weller, M. (2004). HSV-2 sacral radiculitis (Elsberg syndrome). *Neurology*, Vol. 63, NO. 4, (Aug. 2004), pp. 758-759.

Ellie, E., Rozenberg, F., Dousset, V. & Beylot-Barry, M. (1994). Herpes simplex virus type 2 ascending myeloradiculitis: MRI findings and rapid diagnosis by the polymerase chain method. *J Neurol Neurosurg Psychiatry*, Vol. 57, No. 7, (Jul. 1994), pp. 869-870.

Folpe, A., Lapham, LW. & Smith, HC. (1994). Herpes simplex myelitis as a cause of acute necrotizing myelitis syndrome. *Neurology*, Vol. 44, No. 10, (Oct. 1994), pp. 1955-1957.

Galanakis, E., Bikouvarakis, S., Mamoulakis, D., Karampekios, S. & Sbyrakis, S. (2001). Transverse myelitis associated with herpes simplex virus infection. *J Child Neurol*, Vol. 16, No. 11, (Nov. 2001), pp. 866-867.

Gobbi C., Tosi C., Stadler C., Merenda C. & Bernasconi E. (2001). Recurrent myelitis associated with herpes simplex virus type 2. *Eur Neurol*, Vol. 46, No. 4, (2001), pp. 215-218.

Iwamasa, T., Yoshitake, H., Sakuda, H., Kamada, Y., Miyazato, M., Utsumi, Y. & Nakamura, A. (1991). Acute ascending necrotizing myelitis in Okinawa caused by herpes simplex virus type 2. *Virchows Arch A Pathol Anat Histopathol*, Vol. 418, No. 1, (1991), pp. 71-75.

Kamei, S., Sekizawa, T., Shiota, H., Mizutani, T., Itoyama, Y., Takasu, T., Morishima, T. & Hirayanagi, K. (2005). Evaluation of combination therapy using aciclovir and corticosteroid in adult patients with herpes simplex virus encephalitis. *J Neurol Neurosurg Psychiatry*, Vol. 76, N0. 11, (Nov. 2005), pp. 1544-1549.

Klastersky, J., Cappel, R., Snoeck, JM., Flament, J. & Thiry, L. (1972). Ascending myelitis in association with herpes-simplex virus. *N Engl J Med*, Vol. 287, No. 4, (Jul. 1972), pp. 182-184.

Koskiniemi, ML., Vaheri, A., Manninen, V. & Nikki, P. (1982). Ascending myelitis with high antibody titer to herpes simplex virus in the cerebrospinal fluid. *J Neurol*, Vol. 227, No. 3, (1982), pp. 187-191.

Kuker, W., Schaade, L., Ritter, K. & Nacimiento, W. (1999). MRI follow-up of herpes simplex virus (type 1) radiculomyelitis. *Neurology*, Vol. 52, No. %, (Mar. 1999), pp. 1102-1103.

Kusuhara, T., Nakajima, M., Inoue, H., Takahashi, M. & Yamada, T. (2002). Parainfectious encephalomyeloradiculitis associated with herpes simplex virus 1 DNA in cerebrospinal fluid. *Clin Infect Dis*, Vol. 34, No. 9, (May. 2002), pp. 1199-1205.

Kyllerman, MG., Herner, S., Bergstrom, TB. & Ekholm, SE. (1993). PCR diagnosis of primary herpesvirus type I in poliomyelitis-like paralysis and respiratory tract disease. *Pediatr Neurol*, Vol. 9, No. 3, (May-Jun. 1993), pp. 227-229.

Meyding-Lamade, UK., Oberlinner, C., Rau, PR., Seyfer, S., Heiland, S., Sellner, J., Wildemann, BT. & Lamade, WR. (2003). Experimental herpes simplex virus encephalitis: a combination therapy of acyclovir and glucocorticoids reduces long-

term magnetic resonance imaging abnormalities. *J Neurovirol*, Vol. 9, No. 1, (Feb. 2003), pp. 118-125.

Nakagawa, M., Nakamura, A., Kubota, R., Kakazu, T., Kuba, M., Nakasone, K. & Iwamasa, T. (1991). Necrotizing myelopathy associated with malignancy caused by herpes simplex virus type 2: clinical report of two cases and literature review. *Jpn J Med*, Vol. 30, No. 2, (Mar.-Apr. 1991), pp. 182-188.

Nakajima, H., Furutama, D., Kimura, F., Shinoda, K., Ohsawa, N., Nakagawa, T., Shimizu, A. & Shoji, H. (1998). Herpes simplex virus myelitis: clinical manifestations and diagnosis by the polymerase chain reaction method. *Eur Neurol*, Vol. 39, No. 3, (1998), pp. 163-167.

Nakajima, H., Kobayashi, M., Pollard, RB. & Suzuki, F. (2000). A pathogenic role of Th2 responses on the severity of encephalomyelitis induced in mice by herpes simplex virus type 2 infection. *J Neuroimmunol*, Vol. 110, No. 1-2, (Oct. 2000), pp. 106-13.

Nakajima, H., Kobayashi, M., Pollard, RB. & Suzuki, F. (2001). Monocyte chemoattractant protein-1 enhances HSV-induced encephalomyelitiss by stimulating Th2 responses. *J Leukocyte Biol*, Vol. 70, No. 3, (Sep. 2001), pp. 374-380.

Nakajima, H., Hanafusa, T., Nakagawa, T. & Shimizu, A. (2005). Rapid detection and subtyping of herpes simplex virus DNA in CSF by means of LightCycler PCR. *Current Trends in Neurology*, Vol. 1, pp. 134-135.

Petereit, HF., Bamborschke, S. & Lanfermann, H. (1996). Acute transverse myelitis caused by herpes simplex virus. *Eur Neurol*, Vol. 36, No. 1, (1996), pp. 52-53.

Radhakrishnan, VV., Saraswathy, A., Mohan, PK. & Narayanan, SK. (1994). Necrotizing myelopathy--a report of two cases with review of literature. *Indian J Pathol Microbiol*, Vol. 37, No. 4, (Oct. 1994), pp. 439-445.

Shyu, WC., Lin, JC., Chang, BC., Harn, HJ., Lee, CC. & Tsao, WL. (1993). Recurrent ascending myelitis: an unusual presentation of herpes simplex virus type 1 infection. *Ann Neurol*, Vol. 34, No. 4, (Oct. 1993), pp. 625-627.

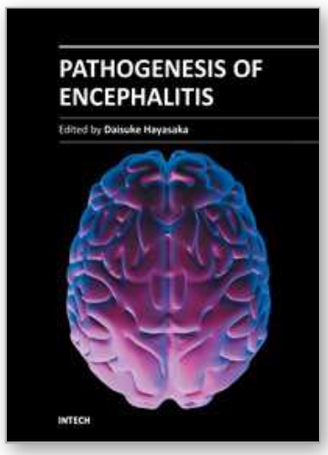
Thompson, KA., Blessing, WW. & Wesselingh, SL. (2000). Herpes simplex replication and dissemination is not increased by corticosteroid treatment in a rat model of focal Herpes encephalitis. *J Neurovirol,* Vol. 6, No. 1, (Feb. 2000), pp. 25-32.

Tucker, T., Dix, RD., Katzen, C., Davis, RL. & Schmidley, JW. (1985). Cytomegalovirus and herpes simplex virus ascending myelitis in a patient with acquired immune deficiency syndrome. *Ann Neurol*, Vol. 18, No. 1, (Jul. 1985), pp. 74-79.

Tyler, KL. (2004). Herpes simplex virus infections of the central nervous system: encephalitis and meningitis, including Mollaret's. *Herpes*, Vol. 11, Suppl 2, (Jun. 2004), pp. A57-A64.

Wiley, CA., VanPatten, PD., Carpenter, PM., Powell, HC. & Thal, LJ. (1987). Acute ascending necrotizing myelopathy caused by herpes simplex virus type 2. *Neurology*, Vol. 37, No. 11, (Nov. 1987), pp. 1791-1794.

Yoritaka, A., Ohta, K. & Kishida, S. (2005). Herpetic lumbosacral radiculoneuropathy in patients with human immunodeficiency virus infection. *Eur Neurol*, Vol. 53, No. 4, (20005), pp. 179-181.



Pathogenesis of Encephalitis
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Many infectious agents, such as viruses, bacteria, and parasites, can cause inflammation of the central nervous system (CNS). Encephalitis is an inflammation of the brain parenchyma, which may result in a more advanced and serious disease meningoencephalitis. To establish accurate diagnosis and develop effective vaccines and drugs to overcome this disease, it is important to understand and elucidate the mechanism of its pathogenesis. This book, which is divided into four sections, provides comprehensive commentaries on encephalitis. The first section (6 chapters) covers diagnosis and clinical symptoms of encephalitis with some neurological disorders. The second section (5 chapters) reviews some virus infections with the outlines of inflammatory and chemokine responses. The third section (7 chapters) deals with the non-viral causative agents of encephalitis. The last section (4 chapters) discusses the experimental model of encephalitis. The different chapters of this book provide valuable and important information not only to the researchers, but also to the physician and health care workers.

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