Japan. No To Hattatsu 35 : 321—326. 2003.

6) Ayukawa R, Fujimoto H, Ayabe M, et al : An unexpected outbreak of Japanese encephalitis in the Chugoku district of Japan, 2002. Jpn J Infect Dis 57 : 63—66, 2004.

7) Ikeda T, Hamaguchi T, Iwasa K, et al : A case of Japanese encephalitis an interval of 17 years after the previous outbreak in Ishikawa prefecture, Japan. Shinkeinaika 61 : 459—462, 2004 (in Japanese).

8) Shoji H, Hiraki Y, Kuwasaki N, et al : Japanese encephalitis in the Kurume region of Japan. J Neurol 236 : 255—259, 1989.

9) Rosas H, Wippold II FJ : West Nile virus : case report with MR imaging findings. Am J Neuroradiol 24 : 1376—1378, 2003.

10) Cerna F, Mehrad B, Luby JP, et al : St. Louis encephalitis and the substantia nigra : MR imaging evaluation. Am J Neuroradiol 20 : 1281—1283, 1999.

SHORT REPORT

Anti-Ma2 associated paraneoplastic neurological syndrome presenting as encephalitis and progressive muscular atrophy

M Waragai, A Chiba, A Uchibori, T Fukushima, M Anno, K Tanaka

J Neurol Neurosurg Psychiatry 2006;**77**:111–113. doi: 10.1136/jnnp.2005.068775

A 36 year old man with a history of testicular germ cell tumour presented six months after bilateral orchidectomy with progressive amnesia, irritability, vertical gaze palsy, and generalised seizures. Eight months after initial onset of symptoms, he demonstrated a head drop with muscular atrophy of the upper limbs, shoulder girdle, and posterior neck. He reported no sensory disturbances and his sensory examination was normal. The overall clinical presentation was consistent with motor neurone disease. Cerebrospinal fluid analysis revealed mild pleocytosis and increased protein concentration. Serum and cerebrospinal fluid were positive for the anti-Ma2 antibody by western blot analysis and immunostaining. Abnormal high signal in the grey matter was noted in the cervical spinal cord and brain by T2 weighted magnetic resonance imaging (MRI). The patient was treated with corticosteroids, intravenous immunoglobulin, and antiepileptic medication. The patient improved clinically and symptom progression ceased after initiation of treatment. There was complete resolution of the abnormal brain MRI lesions; however, the cervical spinal cord MRI lesion and muscular atrophy remained unchanged. It is suggested that the anti-Ma2 antibody is involved not only in encephalitis, but may also play a role in the cervical spinal cord lesions resulting in a motor neurone disease-like presentation.

Paraneoplastic neurological syndromes including paraneoplastic limbic encephalitis are neurological disorders that occur in patients with cancer.[1–6] Paraneoplastic limbic encephalitis is often associated with small cell lung carcinoma and testicular germ cell tumours.[1–6]

Recently, it was suggested that anti-Ma2 associated encephalitis differs from classic paraneoplastic limbic encephalitis because of its variable clinical features.[6]

Here, we report the clinical and neuroradiological findings in a patient with an anti-Ma2 associated paraneoplastic neurological syndrome who presented with encephalitis and progressive muscular atrophy.

To our knowledge, this is the first report describing the unique clinical features of a cervical spinal cord lesion confirmed by magnetic resonance imaging (MRI) in a patient with anti-Ma2 associated paraneoplastic neurological syndrome.

CASE REPORT

A 36 year old man was admitted to our hospital for progressive amnesia, hypersomnia, diplopia, and generalised convulsions in September 2003.

He had undergone a bilateral orchidectomy for testicular germ cell tumour six months before presentation. There was no recurrence of the tumour noted at the time of presentation.

On admission, the patient presented with confusion and seizures. There was no cerebellar ataxia or sensory disturbance noted on examination. Progressive muscular atrophy, weakness, and fasciculations of the upper extremities, shoulder girdle, and neck began eight months after initial presentation. Thereafter, he developed flaccid paralysis of the upper extremities and a head drop. A Babinski's sign was present bilaterally.

Cerebrospinal fluid (CSF) contained 5×10^3 lymphocytes/litre and 830 mg protein/litre. There were no atypical lymphocytes or tumour-like cells noted in the CSF. IgM titres for herpes simplex were negative. Muscle computed tomography at the level of C5 demonstrated severe muscular atrophy of the limb, shoulder girdle, paraspinal, and thoracic muscles. Electromyogram studies showed neurogenic changes in the muscles of the upper extremities and shoulder girdle, including the deltoid, biceps, triceps, scapular, and upper paraspinal muscles, and no changes to the muscles of the lower extremities and pelvic girdle. The results of a nerve conduction study, including sensory and motor evoked potentials, were within the normal ranges for all extremities.

Brain MRI revealed high signal intensity in the grey matter of the right frontal and bilateral mesial temporal lobes (fig 1A, B). T2 weighted MRI revealed high signal intensity in the grey matter of the cervical spinal cord (fig 1C, D). Serum and CSF were positive for the anti-Ma2 antibody confirmed by the binding to the recombinant Ma-2 protein, but were negative for anti-Ma1, anti-Hu, and anti-Yo antibodies (fig 2A, B). Thus, the patient was diagnosed as having an anti-Ma2-associated paraneoplastic neurological syndrome. The patient was treated with two courses of intravenous immunoglobulin (2.5 g/day for five days), followed by intravenous methylprednisolone (1 g/day for three days) during which the patient showed clinical improvement. The patient stabilised and progression of the muscular atrophy ceased, although he continued to have severe flaccid paralysis of the upper extremities and a head drop. The symptoms of amnesia, irritability, overall cognitive decline, hypersomnia, and vertical gaze palsy also remained unchanged. The patient was subsequently treated with antiepileptic medication to prevent further seizure activity. Brain MRI abnormalities resolved after the course of treatment, but the MRI cervical cord lesion remained.

Abbreviations: CSF, cerebrospinal fluid; MRI, magnetic resonance imaging

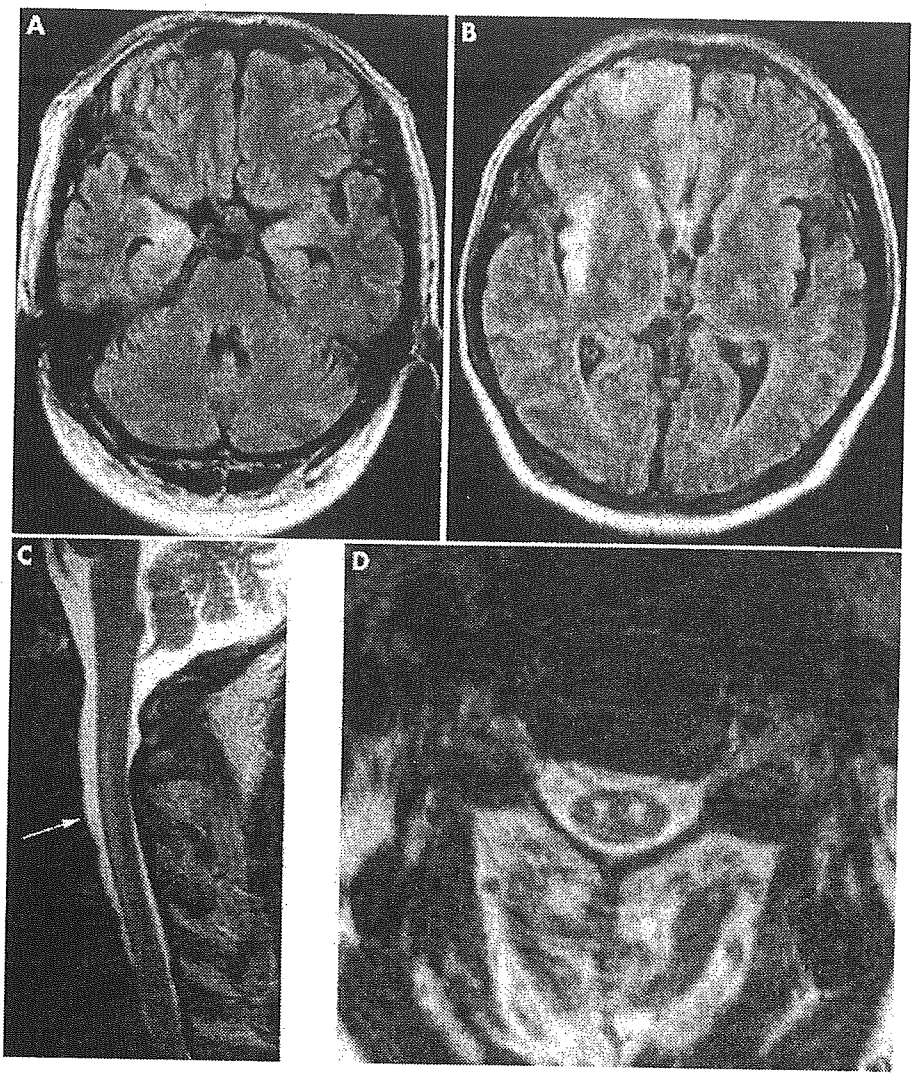

Figure1 (A, B) The magnetic resonance imaging (MRI) fluid attenuated inversion recovery image on admission demonstrated a high signal intensity in the bilateral mesial temporal lobe, right frontal cortex, and right insula. There was no enhancement by gadolinium-DTPA (diethylene triamine pentaacetic acid). These abnormal signal lesions disappeared following treatment with intravenous immunoglobulin and steroids. (C, D) T2 weighted MRI demonstrated a symmetrical high signal lesion (arrow), which was relatively confined to the grey matter of the cervical spinal cord. The lesion was not enhanced by gadolinium-DTPA.

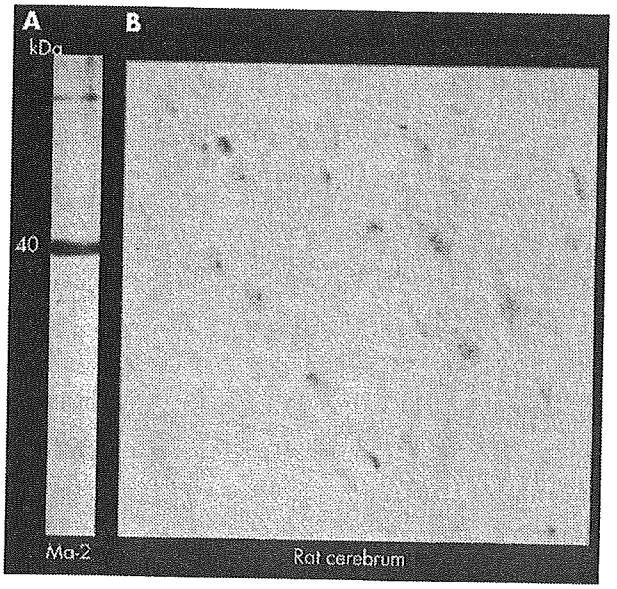

Figure 2 (A) Western blotting analysis showed that the patient's serum reacts with the 40 kDa protein band representing recombinant Ma-2 protein. (B) Immunostaining using the patient's serum and rat cerebrum shows nucleolar rather than nuclear staining.

DISCUSSION

We report the case of an anti-Ma2 associated paraneoplastic neurological syndrome in a patient with a history of testicular germ cell tumour who presented with encephalitis and motor neurone disease-like features. T2 weighted MRI of the cervical spinal cord showed a well confined high signal intensity lesion, which correlated with the extensive muscular atrophy and weakness.

Dalmau *et al* studied the clinical findings of 38 patients with anti-Ma2-associated encephalitis.[6] They reported that eye movement abnormalities were prominent in 92% of the patients with brainstem dysfunction, and 60% of these patients had vertical gaze paresis. Among 34 patients with cancer, 18 had testicular germ cell tumours. They concluded that anti-Ma2 encephalitis should be suspected in patients with limbic, diencephalic, or brainstem dysfunction who have MRI abnormalities in these regions and CSF inflammatory changes. In young male patients who present with paraneoplastic neurological syndromes, the primary tumour is usually located in the testis.[6] Dalmau *et al* also described a 58 year old man with adenocarcinoma of the lung, who developed proximal weakness, muscular atrophy, and fasciculations of the upper extremities without evidence of MRI abnormalities in the brain or spinal cord.[6] Our patient's presentation was characterised not only by encephalitis, but

also by motor neurone disease-like clinical features, which are probably attributable to the cervical spinal cord lesion.

Although it remains unclear whether the anti-Ma2 antibody associated with testicular germ cell tumours is directly involved in the pathogenesis of encephalitis and cervical spinal cord lesions, the response to immunomodulatory treatments such as intravenous immunoglobulin and steroids supports the idea that anti-Ma2 has anti-tumour immune activity.

Our case supports the unique clinical diversity of the anti-Ma2-associated paraneoplastic neurological disorder.

Authors' affiliations
M Waragai, T Fukushima, Division of Neurology, JR Tokyo General Hospital, 2-1-3, Yoyogi, Shibuya-ku, Tokyo 151-8528, Japan
A Chiba, A Uchibori, Department of Neurology, Kyorin University School of Medicine, Mitaka-shi, Tokyo 181-8611, Japan
M Anno, Department of Neurology, Tokyo Metropolitan Matsuzawa Hospital, Tokyo 156-0057, Japan
K Tanaka, Department of Neurology, Brain Research Institute, Niigata University, Niigata 951-8585, Japan

Competing interests: none declared

The patient gave full consent for this report to be published

Correspondence to: Dr M Waragai, Division of Neurology, JR Tokyo General Hospital, 2-1-3, Yoyogi, Shibuya-ku, Tokyo 151-8528, Japan; warawaram2002@aol.com

Received 29 March 2005
Revised form received 25 April 2005
Accepted 3 May 2005

REFERENCES

1 **Voltz R**, Gultekin SH, Rosenfeld MR, *et al.* A serologic marker of paraneoplastic limbic and brain-stem encephalitis in patients with testicular cancer. *N Engl J Med* 1999;**340**:1788–95.
2 **Barnett M**, Prosser J, Sutton I, *et al.* Paraneoplastic brain stem encephalitis in a woman with anti-Ma2 antibody. *J Neurol Neurosurg Psychiatry* 2001;**70**:222–5.
3 **Rosenfeld MR**, Eichen JG, Wade DF, *et al.* Molecular and clinical diversity in paraneoplastic immunity to Ma proteins. *Ann Neurol* 2001;**50**:339–48.
4 **Sahashi K**, Sakai K, Mano K, *et al.* Anti-Ma2 antibody related paraneoplastic limbic/brain stem encephalitis associated with breast cancer expressing Ma1, Ma2, and Ma3 mRNAs. *J Neurol Neurosurg Psychiatry* 2003;**74**:1332–5.
5 **Scheid R**, Lincke T, Voltz R, *et al.* Serial 18F-fluoro-2-deoxy-D-glucose positron emission tomography and magnetic resonance imaging of paraneoplastic limbic encephalitis. *Arch Neurol* 2004;**61**:1785–9.
6 **Dalmau J**, Graus F, Villarejo A, *et al.* Clinical analysis of anti-Ma2-associated encephalitis. *Brain* 2004;**127**:1831–44.

Encephalopathy with Isolated Reversible Splenial Lesion of the Corpus Callosum

Masamitsu YAGUCHI, Hisa YAGUCHI, Tetsuro ITOH* and Koichi OKAMOTO**

Abstract

We report a 51-year-old Japanese man with chronic alcoholism who complained of mental confusion following respiratory and intestinal infections. The splenium of the corpus callosum showed hyperintensity on both diffusion-weighted MR images and fluid-attenuated inversion recovery images and hypointensity on T1-weighted images. These findings were resolved on MR images obtained 3 days later. He showed complete neurological recovery within 2 months. We suspected that he had mild encephalopathy with a reversible splenial lesion after systemic viral or bacterial infection.
(Internal Medicine 44: 1291–1294, 2005)

Key words: encephalopathy, splenium of the corpus callosum, infection, MRI, diffusion-weighted image

Introduction

The prevalence of acquired lesions of the corpus callosum was estimated to be about 3% in a MR study of 450 patients and the differential diagnosis of corpus callosum abnormalities is considered to be difficult (1). Splenial lesions in the corpus callosum include Marchiafava-Bignami disease, ischemia, diffuse axonal injury, multiple sclerosis, hydrocephalus, tumors, epilepsy, antiepileptic drugs, hypoglycemia, cerebral malaria and encephalitis/encephalopathy (1–7).

We report a man with chronic alcoholism, who complained of acute confusion following a one-day illness with cough and diarrhea, and showed an isolated reversible splenial lesion of the corpus callosum on MR images.

Case Report

A 51-year-old Japanese man was admitted to our hospital because of high fever (39.8°C) and mental confusion following a one-day illness with cough and diarrhea. He had a long history of alcoholism, drinking over 400 ml of rough distilled spirits (proof 20%) every day. He was confused and disoriented, but there were no signs of meningeal irritation. Oculomotor and cranial nerve functions were normal. Neither ataxia, paresis nor asterixis were observed. Deep tendon reflexes on the extremities were normal, and plantar responses were flexor. Chest X ray disclosed pneumonia in the right lower lung field. Laboratory tests showed elevations of CK, AST, ALT, BUN, CRE and CRP (Table 1). Serum vitamins were not measured. *Mycoplasma pneumoniae* antibody and *Chlamydia psittaci* antibody were negative. Cerebrospinal fluid (CSF) studies were normal. Rapid antigen detection assay from a nasopharyngeal swab did not demonstrate influenza. There were no pathogenic organisms isolated from blood or sputum. Cultures of the stool were not performed. Electroencephalography (EEG) showed slow basic activities with no paroxysmal discharges. Abdominal ultrasonography was normal. MR images on admission (Fig. 1A–C) demonstrated an isolated small lesion in the splenium of the corpus callosum that was markedly hyperintense on diffusion-weighted images (DWI), hyperintense on fluid-attenuated inversion recovery (FLAIR) images and slight hypointense on T1-weighted images (T1WI). A few small areas showing high signals were seen in the cerebral white matter. Steady clinical recovery was noted following treatment with intravenous administration of vitamin B complex and antibiotics. On follow-up MR studies (Fig. 1D–F) obtained 3 days after the initial study, resolution of the abnormality without contrast enhancement was noted. Neuropsychological examinations 3 weeks after admission demonstrated mild impairment of memory and cognitive functions but there was no sign of callosal disconnection. His score on the mini-mental state

From the Department of Neurology, *the Department of Internal Medicine, Shinoda General Hospital, Yamagata, and **the Department of Neurology, Gunma University Graduate School of Medicine, Maebashi

Received for publication May 24, 2005; Accepted for publication August 25, 2005

Reprint requests should be addressed to Dr. Masamitsu Yaguchi, the Department of Neurology, Shinoda General Hospital, 2-68 Sakura-machi, Yamagata 990-0045

Table 1.

Blood Cell Count		Glucose	176 mg/dl (70–110)
WBC	8,600/mm^3 (4,100–9,500)	NH_3	63 μg/dl (12–66)
RBC	455×10^4/mm^3 (420–560)	CRP	47.4 mg/dl (<0.47)
Hb	15.3 g/dl (13–17)	TSH	1.12 μIU/ml (0.05–5.00)
Hct	44.3% (40–51)	FT_3	2.96 pg/ml (2.30–4.30)
Plt	9.1×10^4/mm^3 (15.4–33.0)	FT_4	1.42 ng/dl (0.90–1.70)
Blood Chemistry		**Urinalysis**	
T-Bil	0.8 mg/dl (0.2–1.2)	protein	(2+)
TP	6.4 g/dl (6.5–8.3)	occult blood	(3+)
AST	278 IU/l (8–38)	glucose	(–)
ALT	129 IU/l (4–44)	ketone	(–)
ALP	170 IU/l (104–338)	**Blood gas analysis**	
γ-GTP	40 IU/l (16–73)	pH	7.51 (7.35–7.45)
CHE	208 IU/l (203–460)	$PaCO_2$	30.1 mmHg (35–45)
CK	5,990 IU/l (56–244)	PaO_2	60.0 mmHg (80–90)
T-Cho	171 mg/dl (120–220)	HCO_3^-	23.7 mmol/l (22–26)
BUN	48.5 mg/dl (8.0–20.0)	Base Excess	0.9 mmol/l (0±2)
CRE	2.3 mg/dl (0.6–1.1)	**CSF study**	
Na	134 mEq/l (135–150)	cell count	3/3 mm^3
K	2.6 mEq/l (3.5–5.0)	protein	26 mg/dl
Cl	99 mEq/l (98–110)	glucose	63 mg/dl
Ca	0.97 mmol/l (8.5–10.5)	IgG	2.0 mg/dl

(MMS) was 23/30 points at that time. MMS on day 53 after admission showed a full score.

Discussion

We report a Japanese man with encephalopathy demonstrating an isolated reversible splenial lesion of the corpus callosum on MR images. The splenial lesion resolved rapidly within a few days, and the complete neurological recovery was obtained within 2 months. A history of long-term chronic alcoholism suggested that he had Marchiafava-Bignami disease; however, we suspected another disorder due to the existence of systemic infection preceding central nervous system manifestations.

Recently, Tada et al reported 15 patients with clinically mild encephalitis/encephalopathy with reversible lesions in the splenuim of the corpus callosum on MR images (7). They were relatively young (12 patients were below 20 years old), and fever and/or diarrhea preceded manifestations of the central nervous system including impairments of consciousness, seizures and vertigo. In their report, CSF studies demonstrated normal cell counts or pleocytosis with normal glucose and protein levels. EEG showed slow basic activities. The splenial lesions were hyperintense on T2WI and DWI, and isointense to slightly hypointense on T1WI. There was no enhancement of the lesion after gadolinium administration. Resolution of splenial abnormalities was usually noted within one week on follow-up MR studies, and a complete recovery was obtained within one month. The associated pathogens were varied, including O-157 *Escherichia coli* (8), measles virus (9), rotavirus (10), *Salmonella enteritidis* (11), influenza virus (7, 12), *Legionella pneumophila* (13), adenovirus, mumps virus, varicella-zoster virus and an unknown pathogen, however the pathogen was not clarified in 10 of 15 patients (7). It is unknown why the splenium is involved as an isolated site. The pathogenesis is speculated to involve pathogens or antibodies induced by them that directly damage axons or the myelin sheath in the splenium of the corpus callosum (7, 8, 10), and the participation of elevated inflammatory cytokines such as interleukin-6 is also postulated in the pathogenesis (7, 12). However, these hypotheses remain controversial. Except for the history of long-term chronic alcoholism, the clinical and radiological course of the present case is compatible with that of the syndrome. Although the pathogens causing diarrhea and pneumonia were not clarified, there was a possibility that the pathogenesis of encephalopathy in our case was similar to that in the syndrome.

Similar reversible and isolated symmetrical lesions in the splenium of the corpus callosum, which are hyperintense on DWI and T2WI and hypointense on T1WI, could be seen in diffuse axonal injury (14), epilepsy or an influence of antiepileptic drugs (4), hypoglycemia (5) and Marchiafava-Bignami disease (15). These diseases are usually differentiated from the encephalitis/encephalopathy reported by Tada et al (7) by their clinical courses and laboratory findings. Minor splenial lesions may be asymptomatic, and MR examinations are not performed routinely in the clinical setting. The incidence of the encephalopathy/encephalitis with reversible splenial lesion after systemic infection might be higher than conceived. It is important for clinicians to note that an isolated reversible splenial lesion is observed in a variety of disorders.

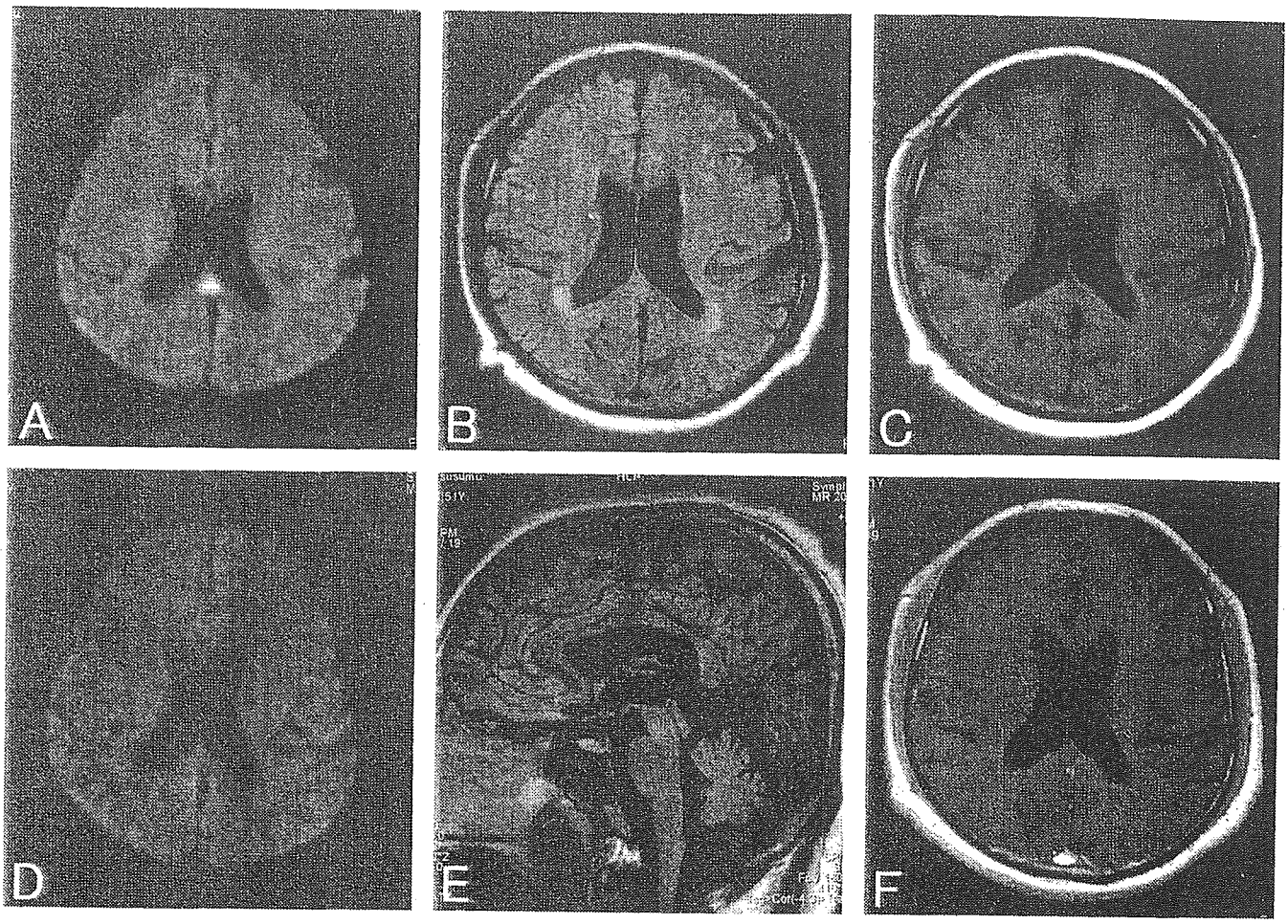

Figure 1. Magnetic resonance (MR) imaging on admission, showing an isolated small lesion in the splenium of the corpus callosum on diffusion-weighted image (DWI) (A), fluid-attenuated inversion recovery (FLAIR) image (B) and T1-weighted image (C). MR imaging on day 5, showing resolutions of the lesion on DWI (D), FLAIR image (E) and enhanced image after gadolinium administration (F).

References

1) McLeod NA, Williams JP, Machen B, Lum GB. Normal and abnormal morphology of the corpus callosum. Neurology **37**: 1240–1242, 1987.
2) Friese SA, Bitzer M, Freudenstein D, Voigt K, Kuker W. Classification of acquired lesions of the corpus callosum with MRI. Neuroradiology **42**: 795–802, 2000.
3) Bourekas EC, Varakis K, Bruns D, et al. Lesions of the corpus callosum: MR Imaging and differential considerations in adults and children. AJR Am J Roentgenol **179**: 251–257, 2002.
4) Maeda M, Shiroyama T, Tsukahara H, Shimono T, Aoki S, Takeda K. Transient splenial lesion of the corpus callosum associated with antiepileptic drugs: evaluation by diffusion-weighted MR imaging. Eur Radiol **13**: 1902–1906, 2003.
5) Takeuchi M, Kawaguchi T, Nakatani M. Reversible increased signal intensities in the splenium on diffusion-weighted imaging caused by transient hypoglycemia. No To Shinkei (in Japanese) **57**: 420–421, 2005.
6) Cordoliani YS, Sarrazin JL, Felten D, Caumes E, Leveque C, Fisch A. MR of cerebral malaria. AJNR Am J Neuroradiol **19**: 871–874, 1998.
7) Tada H, Takanashi J, Barkovich AJ, et al. Clinically mild encephalitis/encephalopathy with a reversible splenial lesion. Neurology **63**: 1854–1858, 2004.
8) Ogura H, Takaoka M, Kishi M, et al. Reversible MR findings of hemolytic uremic syndrome with mild encephalopathy. AJNR Am J Neuroradiol **19**: 1144–1145, 1998.
9) Mito Y, Yoshida K, Kikuchi S. Measles encephalitis with peculiar MRI findings. Report of two adult cases. Neurol Med **56**: 251–256, 2002 (in Japanese, Abstract in English).
10) Kobata R, Tsukahara H, Nakai A, et al. Transient MR signal changes in the splenium of the corpus callosum in rotavirus encephalopathy: value of diffusion-weighted imaging. J Comput Assist Tomogr **26**: 825–828, 2002.
11) Kobuchi N, Tsukahara H, Kawamura Y, et al. Reversible diffusion-weighted MR findings of Salmonella enteritidis-associated encephalopathy. Eur Neurol **49**: 182–184, 2003.
12) Takanashi J, Barkovich AJ, Yamaguchi K, Kohno Y. Influenza-associated encephalitis/encephalopathy with a reversible lesion in the splenium of the corpus callosum: a case report and literature review. AJNR Am J Neuroradiol **25**: 798–802, 2004.
13) Morgan JC, Cavaliere R, Juel VC. Reversible corpus callosum lesion in Legionnaires' disease. J Neurol Neurosurg Psychiatry **75**: 651–654, 2004.
14) Liu AY, Maldjian JA, Bagley LJ, Sinson GP, Grossman RI. Traumatic

brain injury: diffusion-weighted MR imaging findings. AJNR Am J Neuroradiol **20**: 1636–1641, 1999.
15) Inagaki T, Saito K. A case of Marchiafava-Bignami disease demonstrated by MR diffusion-weighted image. No To Shinkei **52**: 633–637, 2000 (in Japanese, Abstract in English).

HHV-6 and Seizures

Nobuko Yamashita and **Tsuneo Morishima**, Department of Pediatrics, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan

KEYWORDS

HUMAN HERPES VIRUS-6 ▮ *SEIZURE* ▮ *NEONATAL SEIZURE* ▮ *FEBRILE SEIZURE* ▮ *TEMPORAL LOBE EPILEPSY* ▮ *ENCEPHALITIS* ▮ *ENCEPHALOPATHY*

SUMMARY

Human herpes virus-6 (HHV-6) is a ubiquitous virus, but one that can induce various neurological diseases. Recently, several seizures have been reported as new HHV-6-associated diseases based on virological analysis. Neonates who are perinatally infected with HHV-6 can develop afebrile seizures, which are considered to be exanthem subitum (ES) in the neonatal period. Infants with ES also tend to develop atypical febrile seizures. After primary infection, HHV-6 commonly establishes latency in the central nervous system (CNS) and sometimes reactivates in the hippocampus, causing limbic encephalitis and temporal lobe epilepsy. These HHV-6-associated CNS diseases due to virus reactivation can occur in both immunocompromised and immunocompetent hosts. This article summarizes HHV-6-associated seizures during childhood.

Introduction

HUMAN HERPES VIRUS-6 (HHV-6), a ubiquitous human β-herpesvirus, is acquired primarily in approximately 70% of infants by 24 months of age.[1–4] Primary HHV-6 infections are usually symptomatically associated with nonspecific febrile illness, although some infants present with the classic manifestations of exanthem subitum (ES) (roseola infantum).[1,5] Febrile seizures (FS), encephalitis and encephalopathy have been recognized as major neurological complications of primary HHV-6 infection,[2,6–8] but these neurological complications may also be associated with reactivation of the virus in the central nervous system (CNS).

Two variants of HHV-6 can be classified on the basis of immunological and molecular biological analyses: HHV-6A and HHV-6B. These variants are very closely related and display >90% nucleotide identity, but pathogenesis and clinical manifestations differ substantially.[9] Primary HHV-6B infection usually appears as ES, a common acute febrile illness during early childhood. Clinical findings in ES are a 3–4-day history of high fever, bulging fontanelles and maculopapular skin rash after the fever subsides. Neurological complications such as FS and encephalitis/encephalopathy can also occur. Contrasting with HHV-6B, HHV-6A has predominantly been isolated from immunocompromised hosts, such as HIV/AIDS patients. In association with HIV/AIDS infection, geographical variations exist between HHV-6A and -6B. HHV-6A has been identified more frequently in an AIDS epidemic region in Africa, accounting for 44% of HHV-6 cases of nonspecific febrile illness among infants.[10]

After primary infection, HHV-6 remains latent not only in saliva, but also in the CNS.[11–13] HHV-6 DNA can be detected by polymerase chain reaction (PCR) in the cerebrospinal fluid (CSF) of children during and after primary infection,[11] and in adult brain tissue of patients who died from other causes not associated with HHV-6 infection.[11] Both primary infection and virus reactivation in the CNS have recently been shown to cause various neurological complications. HHV-6 can occasionally reactivate systematically under immunodeficient status. In immunocompetent hosts, HHV-6 can reactivate during pregnancy,[14] or with HHV-7,[15] measles[16] or dengue infections.[17] The present article reviews primary HHV-6 infection- or virus reactivation-associated seizures based on data including case reports of individuals and small groups of patients, as the aetiopathogenesis of these seizures and encephalitis/encephalopathy has remained largely unknown.

HHV-6 and Neonatal Seizure

In recent years, attention has been focused on seizures in neonates younger than 1 month old who are infected with HHV-6 in the fetal period or after birth. Horizontal transmission through saliva is thought to represent the most common route of transmission for HHV-6. However, vertical transmission has been considered as another route of transmission. Reactivation of HHV-6 is not uncommon during pregnancy, and HHV-6 DNA is detectable in about 20% of cervical swabs from pregnant woman.[18] Congenital HHV-6 infection through vertical transmission may not be rare.[19,20] Hall *et al.*[19] detected HHV-6 DNA in 57 of 5638 cord blood samples, and reactivation at birth was observed in five samples (HHV-6B positive in all five samples). HHV-6 DNA persisted more frequently in neonates who were HHV-6 DNA-positive in blood at birth than in infants with primary infection after 1 month old.[18] Eliminating HHV-6 may be difficult in neonates with congenital infection, but the clinical significance of HHV-6 DNA persistence remains unclear.

Human herpes virus-6 infection through vertical transmission is usually asymptomatic, and few reports have described symptomatic neonatal HHV-6 infection.[2,21–25] Sugimoto *et al.*[23] reported two neonates at 14 and 27 days old who presented with high fever and skin rash after fever subsided. HHV-6 DNA was detected in peripheral blood mononuclear cells (PBMC) using PCR, despite the presence of maternal antibody. Immunoglobin (Ig)G antibody titres to HHV-6 were 1:40 on neutralization assay. Zerr *et al.*[21] identified another neonate with HHV-6 antibodies both transferred from the mother and acquired independently, using Western blot analysis. The patient was a 3-week-old baby with afebrile clonic seizures and maculopapular rash. HHV-6B DNA was detected in serum and CSF. From these reports, maternal antibody titres may be low and insufficient to protect against HHV-6 infection after birth, but HHV-6 infection might not be able to be prevented by humoral immunity alone.[23] Severe neurological complications were not observed in any of these cases, and infection was considered to have occurred after birth, as ES during the neonatal period. The incubation period for HHV-6 is about 10 days.[23,25]

Neurodevelopmental retardation may occur as a sequela of congenital HHV-6 infection. Another report described a baby who developed symptoms in the first few hours of life.[22] Initial symptoms comprised hypotonus, hypoactivity, bradycardia and dermographia. Shortly thereafter, opisthotonos and prolonged local clonic seizures were identified. HHV-6B DNA was positive in CSF, although leucocyte counts in CSF were not elevated. This case was considered to represent congenital and symptomatic HHV-6 infection with neurological involvement.

Primary HHV-6 Infection and Febrile Seizures

Febrile seizures are an age-dependent condition occurring in children from 9 months to 5 years old. Family histories from siblings and parents indicate a strong genetic predisposition toward FS. No specific gene responsible for simple FS has yet been identified, but putative FS genes, on chromosomes 8 and 19, have been mapped in several large families.[26] Incidence of FS differs between races, reportedly occurring in 2–5% of infants in Europe and North America, and 6–9% of infants in Japan.[27]

An association between HHV-6 and FS has long been suspected, as: both primary HHV-6 infection and first FS occur in similar age groups; secondary HHV-6 displays neurotrophic properties; and bulging fontanelles are often observed in ES.[13,28,29] Frequency of FS in HHV-6 infection has also been considered high compared to the incidence of FS. Asano *et al.*[3] reported that 8% of infants in Japan with ES experienced convulsions. Hall *et al.*[2] noted that 9.7% of infants and children under the age of 3 years in the USA with acute febrile illness displayed primary HHV-6 infection, and 13% had seizures with primary HHV-6 infections. However, whether HHV-6 infection triggers FS has become controversial. Zerr *et al.*[4] prospectively studied patterns of HHV-6 acquisition in 277 children using HHV-6 DNA PCR of saliva samples. In that study, most children with primary HHV-6 infection displayed symptoms such as fussiness (70%), rhinorrhoea (66%) and fever (58%), but not seizures.[4] Hukin *et al.*[30] examined the incidence of primary HHV-6 infection in a case-control study that compared patients with FS between 6 months and 2 years of age, and age-matched controls with only fever. In that study, acute HHV-6 infection was identified in 15 of the 35 FS patients and 15 of the 33 controls, indicating no significant differences in frequency. In addition, neither HHV-6 nor HHV-7 RNA was detected in CSF of 23 control-matched patients with FS.[31]

As clinical characteristics of FS in primary HHV-6 infection, atypical seizures such as partial, prolonged and repeated seizures appear more frequently than simple FS.[6] Suga *et al.*[6] also reported that HHV-6 infection might be observed more frequently in infants <1 year old with FS.

As suggested above, age-related factors and genetic predispositions to FS may be more closely involved in the development of simple FS than HHV-6 infection. Moreover, FS in HHV-6 infection tend to be atypical seizures, rather than simple FS.[6,32] Interestingly, abnormal CSF findings are not usually observed in patients with both FS and HHV-6 infection, despite positive findings of HHV-6 DNA using PCR. Further studies are needed regarding the mechanisms of HHV-6-associated atypical FS, as this may be associated with the development of epilepsy during childhood.

Recurrent Seizures with HHV-6 Reactivation

HHV-6 is easily reactivated by other viral infections, such as HHV-7[33] and measles.[16] HHV-6 reactivation/re-infection is detectable in 1% of healthy children using reverse transcriptase PCR of PBMCs.[33] It is important to know whether HHV-6 reactivation causes neurological diseases.

Kondo *et al.*[28] reported that HHV-6 DNA was more frequently detected in patients with ≥3 occurrences of FS, rather than single FS (80% vs 14%). In contrast, Jee *et al.*[34] conducted a prospective comparison of HHV-6 culture-positive and culture-negative patients at first FS, regarding subsequent seizure recurrence. As a result, 20% of HHV-6-positive patients and 40% of HHV-6-negative patients experienced recurrent seizures ≤1 year after first FS. Primary HHV-6 infection was thus not considered to result in increased risk of recurrent FS.[34] Genetic predisposition strongly affects FS, including recurrent FS, so the role of HHV-6 infection in recurrent FS remains controversial.[29]

HHV-6 and Encephalitis/Encephalopathy

Seizures, particularly for status epilepticus and recurrent seizure, sometimes represent the first neurological symptoms of HHV-6-associated encephalitis/encephalopathy.[6] Fetal encephalitis with primary HHV-6 infection has been well documented, and HHV-6 infection of pontine tissue was recently confirmed in an immunocompetent 23-month-old girl who died following haemorrhagic encephalitis in the pontine tegmentum and medial thalamic areas.[35] As for HHV-6-reactivation-associated encephalitis, four immunocompetent patients were reported showing seizures, tremors, ataxia and blurred vision at disease onset.[36]

Patients with primary HHV-6 infection can develop acute encephalopathy associated with CNS hypoperfusion.[37,38] One patient with left hemiparesis displayed diffuse hypoperfusion in the right hemisphere.[38] In addition, HHV-6-associated acute necrotizing encephalopathy (ANE) was also reported in a patient with status epilepticus, quadriplegia and abducens nerve palsies.[37] ANE is a type of acute encephalopathy characterized by multiple, symmetrical brain lesions affecting bilateral thalami and cerebral white matter. It has been well recognized in Asia in relation to influenza-associated encephalopathy. Sugaya *et al.*[39] suggested that HHV-6/HHV-7 may play a role in influenza-associated encephalopathy by dual infection with influenza virus and HHV-6/HHV-7, or reactivation of HHV-6/HHV-7 caused by influenza infection. They showed that HHV-6 DNA was positive in two of eight CSF supernatant samples from patients with CNS complication of influenza, using nested PCR. However, in our investigation of 25 CSF samples (18 patients with encephalopathy, seven patients with FS), neither HHV-6 DNA nor HHV-7 DNA was detected by real-time or nested PCR. No association between HHV-6/HHV-7 and influenza-associated encephalopathy was identified in our study.[40]

Regarding HHV-6-associated chronic encephalopathy, a case involving a patient suffering from frequent epileptic seizures due to HHV-6 reactivation has been reported.[41] Such HHV-6-associated encephalitis/encephalopathy could induce secondary epileptic seizures.

HHV-6 Reactivation and Temporal Lobe Epilepsy

As with FS, epilepsy is a symptom of brain dysfunction, which, in some cases, may be induced by the interaction of genetic predispositions and environmental factors. The human brain may represent an important reservoir of latent HHV-6.[11] HHV-6 DNA is commonly detected by PCR in human brain tissues. Chan *et al.*[11] examined HHV-6 DNA in adult brain tissues from post-mortem cases. HHV-6 DNA was detected in 34 of 40 patients, with no variation according to gender, age or anatomical position of sampled tissue. Both HHV-6A and -6B were detected, although HHV-6B was more frequent (75%) and displayed a wider distribution within the brain.

Human herpes virus-6 sometimes causes limbic encephalitis and temporal lobe epilepsy (TLE).[12,42,43] Wainwright *et al.*[42] reported HHV-6-associated hippocampal encephalitis in patients after bone marrow transplantation. These patients presented with limbic encephalitis ≤1 month after bone marrow transplantation, and symptoms included insomnia and short-term memory loss, but not seizures. PCR examination revealed HHV-6B in CSF samples,

increased hippocampal T2-weighted signals on magnetic resonance imaging, and temporal spikes and slowed background on electroencephalography.[42]

Human herpes virus-6 DNA was demonstrated in surgical tissue from some TLE patients.[12,43] Uesugi *et al.*[43] detected HHV-6 DNA in six of 17 TLE patients who developed TLE at a mean of 11 years old and received temporal lobectomy at a mean of 24 years old. Of the six patients who were positive for HHV-6, three had no past history of encephalitis or meningitis. Histopathologically, inflammatory findings were apparent in only one of the six patients. In addition, one patient who had been diagnosed with measles encephalitis showed positive results for HHV-6 and negative results for measles. HHV-6 could reactivate after primary measles infection,[26] and measles-associated HHV-6 reactivation may have occurred in this TLE patient. Donati *et al.*[12] also demonstrated positive HHV-6B DNA findings in four of five patients with mesial TLE, and used real-time PCR to demonstrate that levels of HHV-6 DNA were highest in samples from hippocampus. They also suggested that HHV-6 was localized to astrocytes.[12] As mentioned above, HHV-6 is usually latent in the CNS after primary infection. Reactivation can occur for various reasons, particularly in the hippocampus.[44]

Conclusions

Human herpesvirus-6 was first isolated from patients with lymphoproliferative syndromes in 1986.[45] After this discovery, numerous aspects of the virus have been clarified very rapidly using the tools of molecular biological analysis. HHV-6 infections are very common and usually mild, but several CNS complications can result owing to both primary infection and reactivation.

In primary infection, FS have been considered the most common HHV-6-associated CNS complication. However, recent reports have indicated the possibility that simple FS are not provoked solely by HHV-6 infection. As other HHV-6 primary infection-associated seizures, some recent reports have described congenital HHV-6 infection, atypical FS in ES, encephalitis in immunocompetent hosts and encephalopathy due to CNS hypoperfusion. Encephalitis in immuno-compromised hosts and TLE have been reported as HHV-6 reactivation-associated CNS complications. The roles of HHV-6 in CNS pathogenesis may vary. Human immunity to HHV-6 reactivation in the CNS has remained unclear. Genetic factors predisposing toward the development of these CNS complications also remain to be clarified. Further analyses are needed regarding the mechanisms of HHV-6 infection in relation to host immune status.

Conflicts of Interest

No conflicts of interest were declared in relation to this article.

Address for correspondence:
Tsuneo Morishima, MD, Department of Pediatrics, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1 Shikata-cho, Okayama, 700-8558, Japan.

E-mail: morishim@md.okayama-u.ac.jp

Received for publication: 3 May 2005
Accepted for publication: 18 July 2005

REFERENCES

1. Prukszapanonda P, Hall CB, Insel RA, McIntyre K, Pollett PE, Long CE *et al.* Primary human herpesvirus 6 infection in young children. *N Engl J Med* 1992;**326:** 1445–1450.
2. Hall CB, Long CE, Schnabel KC, Caserta MT, McIntyre KM, Costanzo MA *et al.* Human herpesvirus-6 infection in children. A prospective study of complications and reactivation. *N Engl J Med* 1994;**331:**432–438.
3. Asano Y, Yoshikawa T, Suga S, Kobayashi T, Nakashima T, Yazaki T *et al.* Clinical features of infants with primary human herpesvirus 6 infection (exanthem subitum, roseola infantum). *Pediatrics* 1994;**93:**104–108.
4. Zerr DM, Meier AS, Selke SS, Frenkel LM, Huang ML, Wald A *et al.* A population-based study of primary human herpesvirus 6 infection. *N Engl J Med* 2005;**352:** 768–776.
5. Yamanishi K, Okuno T, Shiraki K, Takahashi M, Kondo T, Asano Y *et al.* Identification of human herpesvirus-6 as a causal agent for exanthem subitum. *Lancet* 1988;**1:**1065–1067.
6. Suga S, Suzuki K, Ihira M, Yoshikawa T, Kajita Y, Ozaki T *et al.* Clinical characteristics of febrile convulsions during primary HHV-6 infection. *Arch Dis Child* 2000;**82:**62–66.
7. Ishiguro N, Yamada S, Takahashi T, Takahashi Y, Togashi T, Okuno T *et al.* Meningo-encephalitis associated with HHV-6 related exanthem subitum. *Acta Paediatr Scand* 1990; **79:**987–989.
8. Jones CM, Dunn HG, Thomas EE, Cone RW, Weber JM. Acute encephalopathy and status epilepticus associated with human herpes virus 6 infection. *Dev Med Child Neurol* 1994;**36:**646–650.
9. Clark DA. Human herpesvirus 6. *Rev Med Virol* 2000;**10:**155–173.
10. Kasolo FC, Mpabalwani E, Gompels UA. Infection with AIDS-related herpesviruses in human immunodeficiency virus-negative infants and endemic childhood Kaposi's sarcoma in Africa. *J Gen Virol* 1997;**78:**847–855.
11. Chan PK, Ng HK, Hui M, Cheng AF. Prevalence and distribution of human herpesvirus 6 variants A and B in adult human brain. *J Med Virol* 2001;**64:**42–46.
12. Donati D, Akhyani N, Fogdell-Hahn A, Cermelli C, Cassiani-Ingoni R, Vortmeyer A *et al.* Detection of human herpesvirus-6 in mesial temporal lobe epilepsy surgical brain resections. *Neurology* 2003;**61:** 1405–1411.
13. Caserta MT, Hall CB, Schnabel K, McIntyre K, Long C, Costanzo M *et al.* Neuroinvasion and persistence of human herpesvirus 6 in children. *J Infect Dis* 1994;**170:** 1586–1589.
14. Dahl H, Fjaertoft G, Noested T, Wang FZ, Mousavi-Jazi M, Linde A. Reactivation of human herpesvirus 6 during pregnancy. *J Infect Dis* 1999; **180:**2035–2038.
15. Tanaka-Taya K, Kondo T, Nakagawa N, Inagi R, Miyoshi H, Suzugawa T *et al.* Reactivation of human herpesvirus 6 by infection of human herpesvirus 7. *J Med Virol* 2000;**60:**284–289.
16. Suga S, Yoshikawa T, Asano Y, Nakashima T, Kobayashi I, Yazaki T. Activation of human herpesvirus-6 in children with acute measles. *J Med Virol* 1992;**38:**278–282.
17. Balachandra K, Chimalwatra K, Suprumajake P, Wasi C, Yamamoto T, Mukai T *et al.* High rate of reactivation of human herpesvirus 6 in children with dengue hemorrhagic fever. *J Infect Dis* 1994;**170:**746–748.
18. Okuno T, Oishi H, Hayashi K, Nonogaki M, Tanaka K, Yamanishi K. Human herpesviruses 6 and 7 in cervixes of pregnant women. *J Clin Microbiol* 1995;**33:** 1968–1970.
19. Hall CB, Caserta MT, Schnabel KC, Boettrich C, McDermott MP, Lofthus GK *et al.* Congenital infections with human herpesvirus 6 (HHV6) and human herpesvirus 7 (HHV7). *J Pediatr* 2004;**145:**472–477.
20. Pass RF. HHV6 and HHV7: persistence and vertical transmission. *J Pediatr* 2004;**145:**432–435.
21. Zerr DM, Young LC, Obringewitch KM, Huang ML, Frenkel LM, Corey L. Case report: primary human herpesvirus-6 associated with an afebrile seizure in a 3-week-old infant. *J Med Virol* 2002;**66:**384–387.
22. Lanari M, Papa I, Venturi V, Lazzarotto T, Faldella G, Gabrielli L *et al.* Congenital infection with human herpesvirus 6 variant B associated with neonatal seizures and poor neurological outcome. *J Med Virol* 2003;**70:**628–632.
23. Sugimoto T, Tanaka-Taya K, Ono T, Miyoshi H, Okada S, Yamanishi K. Human herpesvirus-6 infection in neonates: Not protected by only humoral immunity. *Pediatr Int* 2002;**44:**281–285.
24. Kawaguchi S, Suga S, Kozawa T, Nakashima T, Yoshikawa T, Asano Y. Primary human herpesvirus-6 infection (exanthem subitum) in the newborn. *Pediatrics* 1992;**90:**628–630.
25. Yoshikawa T, Suzuki K, Umemura K, Akimoto S, Miyake F, Usui G *et al.* Atypical clinical features of a human herpesvirus-6 infection in a neonate. *J Med Virol* 2004;**74:**463–466.
26. Kaneko S, Okada M, Iwase H, Yamakawa K, Hirose S. Genetics of epilepsy: current status and perspectives. *Neurosci Res* 2002;**44:**11–30.
27. Kugler SL, Johnson WG. Genetics of the febrile seizure susceptibility trait. *Brain Dev* 1998;**20:**265–274.
28. Kondo K, Nagafuji H, Hata A, Tomomori C, Yamanishi K. Association of human herpesvirus 6 infection of the central nervous system with recurrence of febrile convulsions. *J Infect Dis* 1993;**167:**1197–1200.
29. Osman H, Human

Herpesvirus 6 and febrile convulsions. *Herpes* 2000;**7**:33–37.
30. Hukin J, Farrell K, MacWilliam LM, Colbourne M, Waida E, Tan R *et al.* Case-control study of primary human herpesvirus 6 infection in children with febrile seizures. *Pediatrics* 1998;**101**:E3.
31. Teach SJ, Wallace HL, Evans MI, Duffner PK, Hay J, Faden HS. Human herpesviruses types 6 and 7 and febrile seizures. *Pediatr Neurol* 1999;**21**:699–703.
32. Yoshikawa T, Asano Y. Central nervous system complications in human herpesvirus-6 infection. *Brain Dev* 2000;**22**:307–314.
33. Caserta MT, McDermott MP, Dewhurst S, Schnabel K, Carnahan JA, Gilbert L *et al.* Human herpesvirus 6 (HHV6) DNA persistence and reactivation in healthy children. *J Pediatr* 2004;**145**:478–484.
34. Joe SH, Long CE, Schnabel KC, Sehgal N, Epstein LG, Hall CB. Risk of recurrent seizures after a primary human herpesvirus 6-induced febrile seizure. *Pediatr Infect Dis J* 1998;**17**:43–46.
35. Ahtiluoto S, Mannonen L, Paetau A, Vaheri A, Koskiniemi M, Rantalaiho P *et al. In situ* hybridization detection of human herpesvirus 6 in brain tissue from fatal encephalitis. *Pediatrics* 2000;**105**:431–433.
36. Isaacson E, Glaser CA, Forghani B, Amad Z, Wallace M, Armstrong RW *et al.* Evidence of human herpesvirus 6 infection in 4 immunocompetent patients with encephalitis. *Clin Infect Dis* 2005;**40**:890–893.
37. Oki I, Yoshida H, Tokunaitsu A, Takahashi S, Miyamoto A, Yoda M *et al.* Serial neuroimages of acute necrotizing encephalopathy associated with human herpesvirus 6 infection. *Brain Dev* 1995;**17**:366–369.
38. Ohta H, Watanabe Y, Sumimoto S, Kojima N, Ishigaki T, Todo G *et al.* Hypoperfusion of right hemisphere on brain SPECT in a patient with exanthem subitum and left hemiparesis. *Ann Nucl Med* 2000;**14**:223–225.
39. Sugaya N, Yoshikawa T, Miura M, Ishizuka T, Kawakami C, Asano Y. Influenza encephalopathy associated with infection with human herpesvirus 6 and/or human herpesvirus 7. *Clin Infect Dis* 2002;**34**:461–466.
40. Kawada J, Kimura H, Hara S, Ito Y, Kawashima H, Okuno T *et al.* Absence of associations between influenza-associated encephalopathy and human herpesvirus 6 or human herpesvirus 7. *Pediatr Infect Dis J* 2003;**22**:115–119.
41. Arpino C, Lopez L, Anzidei G, Camportondo MP, Poveromo D, Curatolo P. Reactivation of human herpesvirus 6 infection in an immunocompetent host with chronic encephalopathy. *J Infect Dis* 2002;**185**:1843; author reply 1843–1844.
42. Wainwright MS, Martin PL, Morse RP, Lacaze M, Provenzale JM, Coleman RE *et al.* Human herpesvirus 6 limbic encephalitis after stem cell transplantation. *Ann Neurol* 2001;**50**:612–616.
43. Uesugi H, Shimizu H, Maehara T, Arai N, Nakayama H. Presence of human herpesvirus 6 and herpes simplex virus detected by polymerase chain reaction in surgical tissue from temporal lobe epileptic patients. *Psychiatry Clin Neurosci* 2000;**54**:589–593.
44. Belford A, Myles O, Magill A, Wang J, Myhand RC, Wasebenko JK. Thrombotic microangiopathy (TMA) and stroke due to human herpesvirus-6 (HHV-6) reactivation in an adult receiving high-dose melphalan with autologous peripheral stem cell transplantation. *Am J Hematol* 2004;**76**:B156–B162.
45. Salahuddin SZ, Ablashi DV, Markham PD, Josephs SF, Sturzenegger S, Kaplan M *et al.* Isolation of a new virus, HBLV, in patients with lymphoproliferative disorders. *Science* 1986;**234**:596–601.

Key Papers

CMV

Congenital cytomegalovirus infection: association between virus burden in infancy and hearing loss.
Boppana SB, Fowler KB, Pass RF, Rivera LB, Bradford RD, Lakeman FD *et al. J Pediatr* 2005;**146**:817–823.

Objective: To determine the relationship between the virus burden in infancy and hearing loss in congenital cytomegalovirus (CMV) infection. **Study design:** A cohort of 76 infants with congenital CMV infection identified by means of newborn virological screening was monitored for outcome. The amount of infectious CMV was analysed in urine specimens obtained during early infancy. Peripheral blood (PB) samples obtained during early infancy were available from 75 children and CMV DNA was quantitated with a real-time quantitative polymerase chain reaction. **Results:** Infants with clinical abnormalities at birth (symptomatic congenital CMV infection) had higher amounts of CMV in urine (P=0.005) and CMV DNA in PB (P=0.001) than infants with no symptoms. Eight children with and four children without symptoms had hearing loss. Among children without symptoms, those with hearing loss had a significantly greater amount of CMV in urine (P=0.03) and PB virus burden (P=0.02) during infancy than those with normal hearing. Infants with $<5 \times 10^3$ pfu/ml of urine CMV and infants with $<1 \times 10^4$ copies/ml of viral DNA in PB were at a lower risk for hearing loss. **Conclusion:** In children with asymptomatic congenital CMV infection, hearing loss was associated with increased amounts of urine CMV and PB CMV DNA during early infancy.

CMV

Repertoire, diversity and differentiation of specific CD8 T-cells are associated with immune protection against human cytomegalovirus disease.
Sacre K, Carcelain G, Cassoux N, Fillet AM, Costagliola D, Vittecoq D *et al. J Exp Med* 2005;**201**:1999–2010.

To determine the correlates of immune recovery from active human cytomegalovirus (HCMV) disease, we compared the antigenic repertoire, diversity, magnitude and differentiation of HCMV-specific CD8(+) T-cells in HIV–HCMV co-infected subjects with no, cured or active HCMV disease and in healthy HIV-negative HCMV-positive controls. ELISPOT-IFN-γ assays using peptide pools spanning the pp65 and immediate early 1 (IE1) HCMV proteins showed that HCMV-specific CD8(+) T-cells had a significantly broader antigenic repertoire and greater diversity in HIV-positive patients controlling HCMV replication than in those with active HCMV disease, but the magnitude of the CD8 T-cell response did not differ between the different groups. HCMV-specific T-cells mainly were focused against IE1 during the short-term recovery from retinitis, and switched toward pp65 during long-term recovery. HCMV-specific T cells displaying an 'early' (CD8[+]CD27[+]CD28[+]) and 'intermediate' (CD8[+]CD27[-]CD28[+]) differentiation phenotype were increased significantly during long-term recovery compared with other HIV-positive patients and were nearly undetectable during active HCMV disease. HCMV-specific T-cells with a 'late' (CD8[+]CD27[-]28[-]) differentiation phenotype predominated in all cases. Therefore, restoration of immune protection against HCMV after active HCMV disease in immunodeficient individuals is associated with enlarged repertoire and diversity, and with early differentiation of virus-specific CD8(+) T-cells, thus defining immune correlates of protection against diseases caused by persistent viruses.

MAJOR ARTICLE

Analysis of Shedding of 3 β-Herpesviruses in Saliva from Patients with Connective Tissue Diseases

Tetsushi Yoshikawa,[1] Masaru Ihira,[3] Hiroaki Taguchi,[2] Shunji Yoshida,[2] and Yoshizo Asano[1]

Departments of [1]Pediatrics and [2]Internal Medicine, Fujita Health University School of Medicine, and [3]Department of Medical Information Technology, Fujita Health University College, Toyoake, Aichi, Japan

Background. Whether an association exists between infection with β-herpesviruses and connective tissue diseases remains unclear, as are the mechanisms for the regulation of these infections in the salivary glands.

Methods. Human herpesvirus (HHV)–7 was isolated and viral DNA was quantified by real-time polymerase chain reaction (PCR) in serially collected saliva samples, to determine whether viral load correlated with infectivity. Then, to examine the role played by β-herpesviruses in connective tissue diseases, cytomegalovirus, HHV-6, and HHV-7 DNA loads were examined by real-time PCR in serially collected saliva samples from 21 patients with connective tissue diseases.

Results. Although subjects with frequent HHV-7 shedding were more likely to have a high viral load than were other subjects, high viral loads were detected in saliva samples from a portion of the subjects with low viral shedding rates. No significant difference between the quantity of HHV-7 DNA in saliva samples from which active virus was isolated and that amplified from samples without detectable virus was observed. Patients with adult-onset Still disease consistently had high HHV-7 DNA loads, in contrast to patients with other connective tissue diseases ($P = .0003$) and healthy adults ($P = .0224$). The mean HHV-6 ($P = .012$) and HHV-7 ($P < .0001$) DNA loads in patients with connective tissue diseases were lower than those in healthy adults.

Conclusion. These data suggest that a number of host factors in patients with adult-onset Still disease may function to accelerate HHV-7 replication in the salivary glands.

Human herpesvirus (HHV)–7 was originally isolated from peripheral-blood mononuclear cells (PBMCs) from healthy adults in 1990 [1]. This virus, a member of the β-herpesvirinae subfamily, is closely related to HHV-6. Primary infection with either of these 2 viruses causes exanthem subitum [2, 3], a common febrile disease of childhood [2, 3]. Because most infants and young children acquire infections with both of these viruses [4, 5], both are ubiquitous among adult populations. The epidemiologic profile of each virus is similar to that of cytomegalovirus (CMV), the original member of the β-herpesvirinae subfamily. After primary infection, herpesviruses persistently infect the host throughout its lifetime. The salivary glands have been proposed as reservoir sites for persistent infection with β-herpesviruses; HHV-7 can frequently be isolated from saliva [6, 7]. Using longitudinal viral-isolation analysis, we recently demonstrated that HHV-7 shedding in saliva varies among individuals [8]. This finding suggests that host factors may play an important role in the regulation of viral replication in the salivary glands. It will be significant to determine whether the kinetics of shedding of the other β-herpesviruses in saliva are similar to that of HHV-7, because it will help to illuminate the mechanisms that control viral replication in the salivary glands.

The role played by such viral infections in the pathogenesis of autoimmune diseases remains unclear. Herpesviruses, including Epstein-Barr virus [9] and CMV [10], interact with the immune system in a variety of ways and establish latency in either lymphocytes or monocytes/macrophages while retaining the potential for reactivation. In addition to these viruses, HHV-6

Received 18 April 2005; accepted 3 June 2005; electronically published 28 September 2005.

Presented in part: 29th International Herpesvirus Workshop, Reno, Nevada, 25–30 July 2004 (abstract 9.16).

Potential conflicts of interest: none reported.

Financial support: Ministry of Education, Culture, Sports, Science, and Technology of Japan (grant-in-aid to the 21st Century Center of Excellence Program of Medicine and the Open Research Center, Fujita Health University).

Reprints or correspondence: Dr. Tetsushi Yoshikawa, Dept. of Pediatrics, Fujita Health University School of Medicine, Toyoake, Aichi 4701192 Japan (tetsushi@fujita-hu.ac.jp).

The Journal of Infectious Diseases 2005;192:1530–6

0022-1899/2005/19209-0005$15.00

Table 1. Characteristics of the patients with connective tissue diseases.

Patient (age [in years], sex)	Disease	Disease activity	Steroids	Pulse therapy	Immunosuppressive drugs	Positivity/antibody titer		
						CMV	HHV-6	HHV-7
1 (45, F)	MCTD	Inactive	No	No	No	+	+	32
2 (46, F)	MCTD	Inactive	Yes	No	No	+	+	32
3 (52, F)	Polymyositis	Onset	Yes	Yes	No	+	+	NS
4 (59, F)	Systemic sclerosis	Inactive	No	No	No	+	NS	NS
5 (59, F)	Systemic sclerosis	Inactive	No	No	No	+	+	16
6 (55, M)	Rheumatoid arthritis	Inactive	Yes	No	MTX	+	+	32
7 (66, F)	Rheumatoid arthritis	Inactive	Yes	No	MTX	+	+	128
8 (68, M)	Rheumatoid arthritis	Inactive	Yes	No	No	+	+	16
9 (28, F)	SLE	Inactive	Yes	No	No	+	+	256
10 (43, F)	SLE	Relapse	Yes	No	No	+	+	16
11 (62, F)	SLE	Inactive	Yes	No	No	+	+	16
12 (39, F)	SLE	Inactive	Yes	No	No	+	NS	NS
13 (32, F)	SLE	Relapse	Yes	Yes	No	ND	NS	NS
14 (20, F)	SLE	Inactive	Yes	No	No	–	+	NS
15 (20, M)	AO Still disease	Relapse	Yes	Yes	MTX, CyA, AZP	ND	+	16
16 (23, F)	AO Still disease	Relapse	Yes	No	No	+	+	32
17 (33, F)	AO Still disease	Relapse	Yes	Yes	MTX, CyA	–	+	32
18 (33, M)	AO Still disease	Onset	Yes	Yes	No	+	+	8
19 (32, F)	AO Still disease	Onset	Yes	No	No	+	+	64
20 (36, M)	AO Still disease	Onset	Yes	No	No	+	+	64
21 (55, F)	AO Still disease	Onset	Yes	No	MTX	+	+	16

NOTE. AO, adult onset; AZP, azathioprine; CMV, cytomegalovirus; CyA, cyclosporin A; F, female; HHV, human herpesvirus; M, male; MCTD, mixed connective tissue disease; MTX, methotrexate; ND, not done; NS, nonspecific fluorescence; SLE, systemic lupus erythematosus.

and HHV-7 latently infect PBMCs [11–13], periodically reactivating in immunosuppressed patients [14]. It has recently been suggested that these 2 viruses may modulate the immune response [15–19]; an association between HHV-6 and multiple sclerosis, an autoimmune-mediated demyelinating disease of the central nervous system, has been intensely debated in recent years [20, 21]. Thus, it is important to determine the role played by these 2 β-herpesviruses in the pathogenesis of connective tissue diseases. Furthermore, it is well known that patients with adult-onset Still disease, which is one of the popular connective tissue diseases, often have hypercytokinemia. Because hypercytokinemia might play an important role in the control of viral replication in the salivary glands, analysis of the relationship between connective tissue diseases (Still disease, in particular) and infection with β-herpesviruses would provide us with important information that will elucidate the host factors that control viral replication.

In the present study, we sought to identify host factors involved in the regulation of 3 β-herpesviruses (HHV-7, in particular) in the salivary glands. Because isolating HHV-7 from saliva is difficult and analyzing large numbers of samples is time-consuming, we first determined whether real-time polymerase chain reaction (PCR), instead of viral isolation, could be used for monitoring viral replication in the salivary glands. In addition to exploring the host factors that control viral replication in the salivary glands, we also sought to elucidate the association between infection with β-herpesviruses and autoimmune connective tissue diseases.

SUBJECTS, MATERIALS, AND METHODS

Subjects and sample preparation. The subjects included 19 healthy adults (9 men and 10 women between 21 and 29 years old), 7 patients with adult-onset Still disease, and 14 patients with other connective tissue diseases (such as systemic lupus erythematosus [6], rheumatoid arthritis [3], systemic sclerosis [2], poliomyelitis [1], and mixed connective tissue disease [2]). The patients' characteristics, such as disease activity and treatment (including receipt of several immunosuppressive drugs), are summarized in table 1. All subjects consented to participation in this study. Saliva samples were serially collected from the healthy adults weekly for 6 weeks and from the patients with connective tissue diseases monthly for 6 months. Two milliliters of saliva from the healthy adults was used for viral isolation and measurement of HHV-7 DNA load by real-time PCR. A total of 114 saliva samples from the healthy adults were analyzed in this study. One milliliter of saliva from the patients with connective tissue diseases was used to measure the viral DNA load of each of 3 β-herpesviruses (CMV, HHV-6, and HHV-7) by real-time

PCR. A total of 126 saliva samples were prospectively collected from the patients and were used to measure viral DNA load.

Isolation of HHV-7 from saliva samples. For the healthy adults, HHV-7 shedding in saliva was evaluated by use of the viral-isolation method. HHV-7 was isolated by cocultivation of saliva with preactivated cord-blood mononuclear cells, as described elsewhere [6]. Briefly, 1 mL of saliva was diluted 1:2 in RPMI 1640 and then centrifuged at 2000 *g*. Supernatants were filtered through a 0.45-μm-pore filter, were inoculated onto preactivated cord-blood mononuclear cells, and were centrifuged at 2000 *g* for 60 min, to enhance viral absorption. After centrifugation, cells were cultured in growth medium at 37°C in 5% CO_2. Viral isolation was considered to be achieved when large, round cell formation and specific immunofluorescence staining with a monoclonal antibody to HHV-7 (KR4; provided by T. Okuno, Department of Microbiology, Hyogo College of Medicine, Hyogo, Japan) were observed.

Serological assays. Specific anti–HHV-6 and anti–HHV-7 IgG antibody titers were measured by indirect immunofluorescence, as described elsewhere [4, 5], with the FG-1 HHV-6 strain and the Sato HHV-7 strain as standard antigens, respectively. An antibody titer was defined as the reciprocal of the serum dilution necessary to obtain specific fluorescence. Anti-CMV IgG antibody titers were measured by ELISA in a commercial laboratory (SRL, Tokyo, Japan).

DNA extraction and real-time PCR. DNA samples extracted from 200 μL of saliva by use of a QIAamp Blood Kit (QIAGEN) were stored at −20°C. Real-time PCR was used to quantitate CMV, HHV-6, and HHV-7 DNA copy numbers. Details on the real-time PCR methods used to assess CMV and HHV-6 DNA loads have been described elsewhere [22]. The validity of this real-time PCR technique to quantify HHV-7 DNA was established by use of the *U31* gene, which encodes the HHV-7 tegument protein, as a positive control sequence. The forward (5′-AAAGAATGGTTTTGTTCAACTCCAA-3′) and reverse (5′-ACATTCACTTTGCGTGCATTTTC-3′) primers and the probe (5′-[FAM]-TCATCGAGAACATAGGAGAAGCTCC-AGCA-[TAMRA]-3′) sequence were designed by use of Primer Express (version 3.0; PE Applied Biosystems) [23]. PCRs were performed by use of the TaqMan PCR Kit (PE Applied Biosystems). Standard curves for each viral DNA assessment were constructed on the basis of the cycle-threshold (C_T) values obtained from serial dilution of plasmid DNA containing the target sequence. To construct plasmids containing the target sequence, viral DNAs were amplified from the AD-169 CMV strain, the Z29 HHV-6 strain, or the RK HHV-7 strain with the primers designed for real-time PCR. Amplified PCR products were then subcloned by use of the pGEM-T Vector System (Promega), in accordance with the manufacturer's protocol. The C_T values for each sample were plotted on a standard curve, allowing automatic calculation of the DNA copy number by use of Sequence Detector (version 1.6; PE Applied Biosystems). Each sample was tested in duplicate; the mean of the 2 values defined the sample copy number.

Statistical analysis. Viral loads were compared by the Mann-Whitney *U* test, by use of StatView (version J-5.0; SAS Institute).

RESULTS

Association between HHV-7 isolation and viral DNA load, as determined by real-time PCR. It is complicated and time-consuming to isolate HHV-7; for this reason, if HHV-7 DNA load, as determined by real-time PCR, correlates well with viral isolation, measurement of viral DNA load would be a valuable tool for monitoring active viral infection. Because the amount of HHV-7 DNA in saliva from healthy adults is stable in each individual, 6 saliva samples serially collected over a course of 6 weeks from each healthy adult in the present study were used to analyze the correlation between viral isolation and viral DNA load. During the observation period, 114 saliva samples were collected from 19 healthy adults, all of whom were seropositive for HHV-7. The frequency of HHV-7 shedding (calculated as the number of saliva samples from which active virus was isolated per the 6 samples collected from the subject during the observation period) in the saliva samples varied between individuals, as was expected (figure 1). Virus could not be isolated from any of the 6 saliva samples from 1 (5.2%) of the healthy adults. Two (10.5%) of the healthy adults excreted significant amounts of virus in all saliva samples. The saliva samples from the healthy adults with frequent viral shedding (5 or 6 positive results; figure 2*A*) generally had higher viral DNA loads than did the saliva samples from the healthy adults with intermediate viral shedding (3 or 4 positive results) (figure 2*B*). Several healthy adults with infrequent viral shedding (0–2 positive results), however, had high viral DNA loads (figure 2*C*). There were no statistical differences in HHV-7 DNA load between the

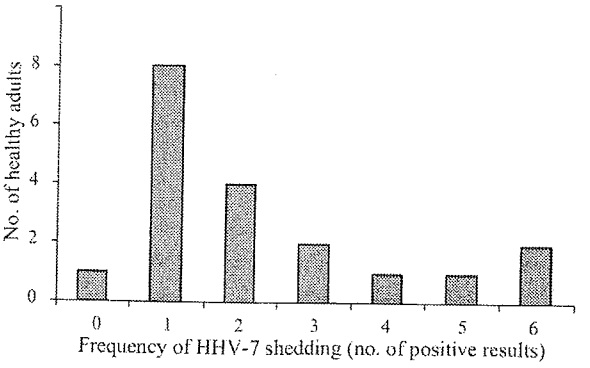

Figure 1. Frequency of human herpesvirus (HHV)–7 shedding in 6 saliva samples serially collected from healthy adults during the 6-week observation period. Virus was isolated from saliva by cocultivation with preactivated cord-blood mononuclear cells.

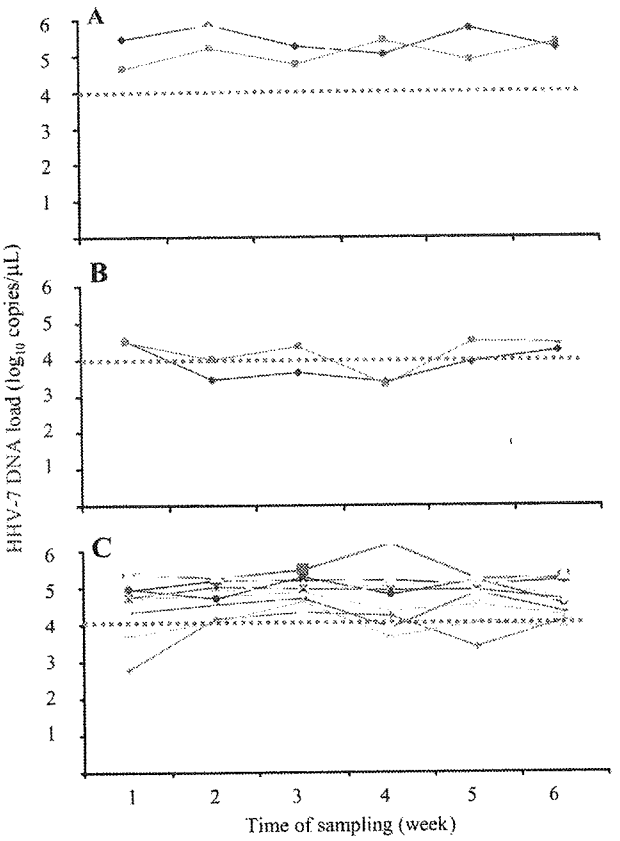

Figure 2. Association between frequency of human herpesvirus (HHV)–7 shedding and DNA load in saliva samples from healthy adults ($n = 19$). Each line denotes the HHV-7 DNA loads of 1 healthy adult. *A*, Frequent shedding (5 or 6 positive results during the 6-week observation period; $n = 3$); *B*, Intermediate shedding (3 or 4 positive results; $n = 3$); *C*, Infrequent shedding (0–2 positive results; $n = 13$). The dotted red lines are included to facilitate comparison among the groups and do not indicate data.

saliva samples from which active virus was isolated and those from which virus was not isolated ($P = .2837$) (table 2).

Viral DNA load for 3 β-herpesviruses in saliva. The kinetics of changes in viral DNA load for the 3 β-herpesviruses were compared among the healthy adults, the patients with adult-onset Still disease, and the patients with other connective tissue diseases (figure 3). It is likely that HHV-7 DNA load differed among the 3 groups. In contrast to HHV-7 DNA load, both HHV-6 and CMV DNA load varied significantly during the observation period. HHV-7 DNA load was higher than HHV-6 DNA load for all 3 groups. CMV DNA was detected in only 2 patients with other connective tissue diseases and 2 patients with adult-onset Still disease. No CMV DNA was detected in the saliva samples from the healthy adults, despite their CMV seropositivity.

To compare the viral DNA loads among the 3 groups, we calculated the mean viral DNA copy numbers in the 6 saliva samples serially collected from each subject. The mean viral DNA loads for the subjects were then compared by the Mann-Whitney *U* test (table 3). Because no CMV shedding was detected in any of the healthy adults, only the HHV-6 and HHV-7 DNA loads were analyzed. HHV-7 DNA load was significantly higher in the patients with adult-onset Still disease (mean ± SD, 649,149 ± 751,504 copies/mL) than in either the healthy adults (mean ± SD, 119,382 ± 119,878 copies/mL; $P = .0224$) or the patients with other connective tissue diseases (mean ± SD, 10,733 ± 21,896 copies/mL; $P = .0003$). There were no statistically significant differences, however, in HHV-6 DNA load between the patients with adult-onset Still disease (mean ± SD, 875 ± 1120 copies/mL) and the healthy adults (mean ± SD, 312 ± 269 copies/mL; $P = .6438$) or between the patients with adult-onset Still disease and the patients with other connective tissue diseases (mean ± SD, 489 ± 1325 copies/mL; $P = .0862$). The patients with other connective tissue diseases had significantly lower viral DNA loads than did the healthy adults for both HHV-6 (mean ± SD, 489 ± 1325 vs. 312 ± 269 copies/mL; $P = .0120$) and HHV-7 (mean ± SD, 10,733 ± 21,896 vs. 119,382 ± 119,878 copies/mL; $P < .0001$). Because the diseases of a subset of the patients with other connective tissue diseases (11/14 patients) were inactive, we compared HHV-6 and HHV-7 DNA loads between patients in an active disease period and those in an inactive disease period, to determine the effect of disease activity on viral DNA load. We could not identify a statistical difference in either HHV-6 or HHV-7 DNA load between the patients in an active disease state and those in an inactive disease state (mean ± SD for HHV-6, 521 ± 902 vs. 480 ± 1456 copies/mL; $P = .6854$) (mean ± SD for HHV-7, 25,772 ± 42,219 vs. 6631 ± 13,432 copies/mL; $P = .5858$), although the number of patients in an active disease state was small.

DISCUSSION

Clinical isolation of HHV-7 requires the cocultivation of samples with preactivated cord-blood mononuclear cells, a time-consuming and troublesome process, especially for the analysis of a large number of samples. Real-time PCR often replaces viral isolation in the analysis of HHV-7 infection. However, information on the association between viral DNA load in saliva and viral isolation has been limited. We attempted to correlate viral DNA load with viral isolation in serially collected saliva samples from healthy adults. The kinetics of changes in HHV-7 DNA load in saliva observed in this study

Table 2. Comparison of human herpesvirus (HHV)–7 DNA load in saliva samples positive or negative by the viral-isolation assay.

Isolation of HHV-7	HHV-7 DNA load in saliva, mean ± SD, copies/mL	*P*
Yes	143,766 ± 168,839	.2837
No	105,158 ± 191,571	

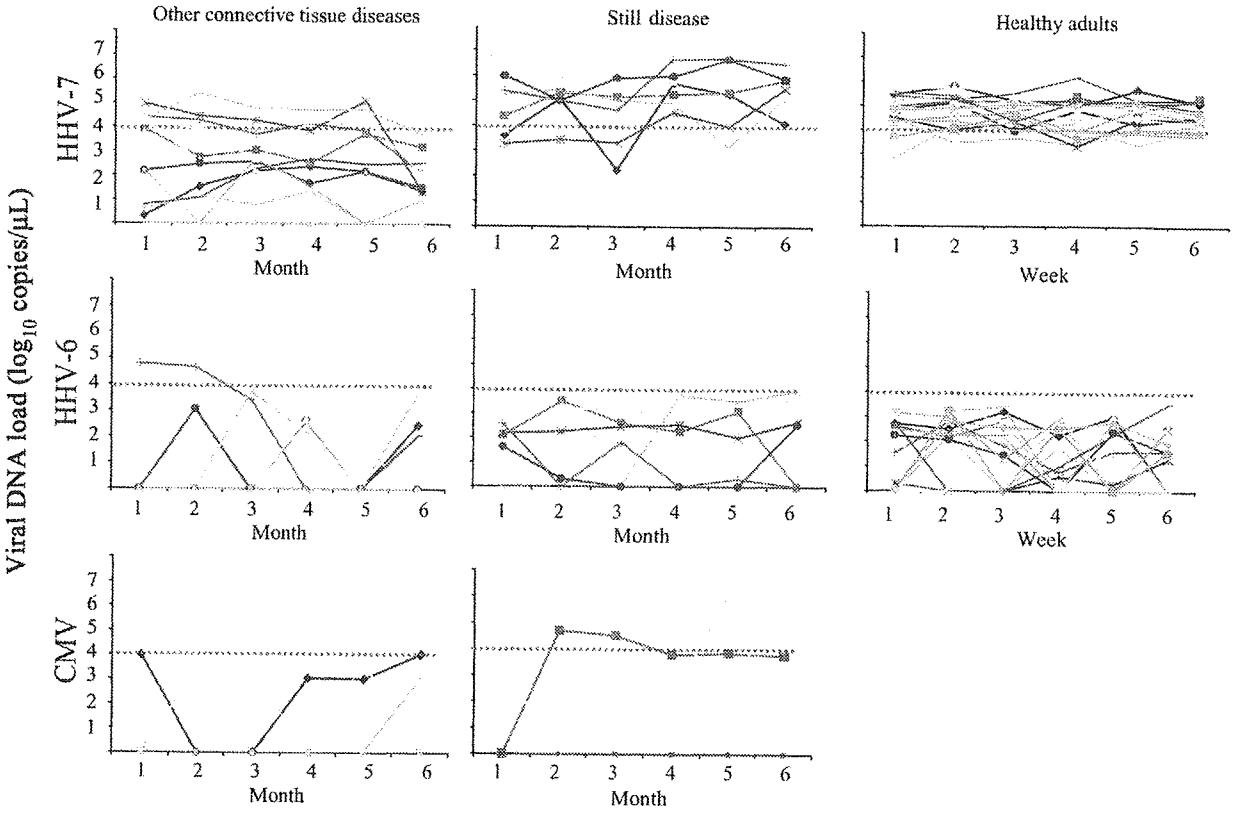

Figure 3. Comparison among cytomegalovirus (CMV), human herpesvirus (HHV)–6, and HHV-7 DNA loads in saliva samples from healthy adults, patients with adult-onset Still disease, and patients with other connective tissue diseases. The kinetics of changes in viral DNA load in serially collected saliva samples were also examined. Because no CMV DNA could be detected in any of the samples from the healthy adults, data on CMV DNA loads for this group are not shown in this figure. The dotted red lines are included to facilitate comparison among the groups and do not indicate data.

were consistent with the results of 2 previous longitudinal studies (in which samples were collected for 1 and 3 months) [24, 25], demonstrating that HHV-7 DNA load in saliva varies between individuals. Because 3 healthy adults with frequent viral shedding appeared to excrete higher levels of viral DNA than did 3 healthy adults with intermediate viral shedding (figure 2*A* and 2*B*), those with frequent viral shedding likely excrete higher copy numbers of viral DNA in their saliva than do those with lower levels of viral shedding. Several healthy adults with infrequent viral shedding, however, excreted significant quantities of viral DNA in saliva, particularly the 3 individuals. Despite this trend, there was no statistical difference in HHV-7 DNA load between the saliva samples from which active virus was isolated and those from which no virus was isolated ($P = .2837$) (table 2). Although the number of subjects was smaller than that examined in this study, similar findings have been reported by Fujiwara et al. [25]. This discrepancy may be the result of the presence of a substance that inhibits viral isolation or of large volumes of fragmented viral DNA in the saliva of certain individuals. To better understand the viral life cycle, further study is necessary to elucidate the molecular mechanisms underlying this discrepancy between viral isolation and real-time PCR analysis. These findings suggest that, although real-time PCR can be used for monitoring viral replication in the salivary glands, it might be inappropriate for an analysis of viral transmission because of the lack of a strong correlation between infectious viral particles and viral DNA load in some saliva samples.

In the present study, we measured the viral DNA loads of 3 β-herpesviruses in saliva samples from healthy adults and patients with connective tissue diseases by real-time PCR. We sought to evaluate viral replication in the salivary glands to establish an association between viral infection and connective tissue disease, potentially implicating the host factors involved in the control of tissue viral replication. We believe that real-time PCR is appropriate to address these questions.

Although these 3 β-herpesviruses share many genetic and biological similarities, the kinetics of changes in viral DNA load in saliva differed between CMV, HHV-6, and HHV-7 (figure 3). Although the quantity of HHV-7 DNA in saliva differed among subjects, the majority of the patients with connective tissue diseases and the healthy adults excreted consistent amounts of viral DNA in saliva. Both the frequency of HHV-6 DNA excretion and the HHV-6 DNA loads, however, were low in comparison

Table 3. Comparison of mean human herpesvirus (HHV) DNA loads (HHV-6 and HHV-7) in saliva samples from healthy adults, patients with adult-onset Still disease, and patients with other connective tissue diseases.

Virus, group	Viral DNA load in saliva, mean ± SD, copies/mL	*P*		
		Healthy vs. Still	Still vs. other	Healthy vs. other
HHV-6		.6438	.0862	.0120
Healthy	312 ± 269			
Adult-onset Still disease	875 ± 1120			
Other connective tissue diseases	489 ± 1325			
HHV-7		.0224	.0003	<.0001
Healthy	119,382 ± 119,878			
Adult-onset Still disease	649,149 ± 751,504			
Other connective tissue diseases	10,733 ± 21,896			

to those for HHV-7. CMV DNA was detected in saliva samples from only a few patients with connective tissue diseases, likely in samples collected after immunosuppression. Several investigators have reported frequencies of viral detection in either healthy control subjects or immunosuppressed patients, such as patients with AIDS [26, 27]. Because differences in the ethnicities of subjects or the details of PCR methods might affect viral DNA detection in saliva samples, it is difficult to compare directly all of these observations. Although our findings are similar to those reported by Lucht et al. [27], they compared healthy control subjects and patients with AIDS by qualitative PCR. In addition, although the number of subjects was limited, Fujiwara et al. [25] reported that HHV-7 copy numbers in saliva were higher than those for HHV-6. To our knowledge, ours is the first report analyzing longitudinal DNA loads in saliva for 3 β-herpesviruses. In conjunction with previous studies, our results should be helpful in examining the kinetics of changes in viral load in patients with other diseases.

In the present study, the patients with adult-onset Still disease constantly excreted a significantly higher number of copies of HHV-7 DNA in their saliva than did the patients with other connective tissue diseases and the healthy adults (figure 3 and table 3). These data suggest that a number of host factors in patients with adult-onset Still disease may function to accelerate HHV-7 replication in the salivary glands. Patients with Still disease exhibit a characteristic hypercytokinemia. Proinflammatory cytokines, such as tumor necrosis factor–α, play an important role in CMV reactivation by inducing the transcription of CMV immediate early genes via induction of NF-κB [28–31]. Although the mechanisms underlying both HHV-7 reactivation and the regulation of HHV-7 immediate early genes are not well understood, the advent of hypercytokinemia during adult-onset Still disease may control the up-regulation of HHV-7 immediate early gene expression, resulting in the reactivation of the virus. Impairment of host immune responses against HHV-7 may accelerate viral replication in the salivary glands. Although CMV-specific T cell–mediated immunity is important in the prevention of symptomatic viremia, it is not important in the local control of viral replication [32–34]. To elucidate the mechanisms underlying the up-regulation of HHV-7 replication in the salivary glands of patients with adult-onset Still disease, we explored both the virological and immunological aspects of this regulation. No activation of CMV infection was demonstrated in the patients with adult-onset Still disease in the present study. Although the sensitivity of real-time PCR for CMV might be insufficient, low rates of CMV excretion in saliva have been demonstrated by other investigators [27]. Therefore, it is possible that the salivary glands are not the main site of persistent infection of the virus. Further in-depth analysis is needed to elucidate an association between CMV infection and connective tissue disease.

Both HHV-6 and HHV-7 DNA loads in saliva were significantly lower in the patients with other connective tissue diseases than in the patients with adult-onset Still disease and the healthy adults (figure 3 and table 3). Because the majority of patients (11/14) with other connective tissue diseases were in an inactive disease period, we examined the effect of disease activity on viral DNA load in saliva and observed that disease activity did not affect either HHV-6 or HHV-7 DNA load in saliva. Thus, a portion of the host factors involved in other connective tissue diseases may prevent replication of these β-herpesviruses in the salivary glands. Because multiple factors, including age, underlying disease, and treatment protocols, may be involved in the control of viral replication in the salivary glands, a detailed multivariate analysis of a large number of patients should be undertaken in the future. The mechanisms underlying the regulation of β-herpesvirus in the salivary glands are not well understood. Future work is necessary to elucidate these mechanisms, which hopefully will provide a greater understanding of the viral life cycle and the clinical course of connective tissue diseases.

Acknowledgments

We thank Akiko Yoshikawa and Maki Sawamura, for technical support.

References

1. Frenkel N, Schirmer EC, Wyatt LS, et al. Isolation of a new herpesvirus from human CD4+ T cells. Proc Natl Acad Sci USA **1990**; 87:748–52.
2. Yamanishi K, Okuno T, Shiraki K, et al. Identification of human herpesvirus-6 as a causal agent for exanthem subitum. Lancet **1988**; 1:1065–7.
3. Tanaka K, Kondo T, Torigoe S, Okada S, Mukai T, Yamanishi K. Human herpesvirus 7: another causal agent for roseola (exanthem subitum). J Pediatr **1994**; 125:1–5.
4. Yoshikawa T, Suga S, Asano Y, Yazaki T, Kodama H, Ozaki T. Distribution of antibodies to a causative agent of exanthem subitum (human herpesvirus-6) in healthy individuals. Pediatrics **1989**; 84:675–7.
5. Yoshikawa T, Asano Y, Kobayashi I, et al. Seroepidemiology of human herpesvirus 7 in healthy children and adults in Japan. J Med Virol **1993**; 41:319–23.
6. Wyatt LS, Frenkel N. Human herpesvirus 7 is a constitutive inhabitant of adult human saliva. J Virol **1992**; 66:3206–9.
7. Hidaka Y, Liu Y, Yamamoto M, et al. Frequent isolation of human herpesvirus 7 from saliva samples. J Med Virol **1993**; 40:343–6.
8. Ihira M, Yoshikawa T, Ohashi M, et al. Variation of human herpesvirus 7 shedding in saliva. J Infect Dis **2003**; 188:1352–4.
9. Pender MP. Infection of autoreactive B lymphocytes with EBV, causing chronic autoimmune diseases. Trends Immunol **2003**; 24:584–8.
10. Sekigawa I, Nawata M, Seta N, Yamada M, Iida N, Hashimoto H. Cytomegalovirus infection in patients with systemic lupus erythematosus. Clin Exp Rheumatol **2002**; 20:559–64.
11. Kondo K, Kondo T, Okuno T, Takahashi M, Yamanishi K. Latent human herpesvirus 6 infection of human monocytes/macrophages. J Gen Virol **1991**; 72:1401–8.
12. Katsafanas GC, Schirmer EC, Wyatt LS, Frenkel N. In vitro activation of human herpesviruses 6 and 7 from latency. Proc Natl Acad Sci USA **1996**; 93:9788–92.
13. Wilborn F, Schmidt CA, Lorenz F, et al. Human herpesvirus type 7 in blood donors: detection by the polymerase chain reaction. J Med Virol **1995**; 47:65–9.
14. Yoshikawa T. Human herpesvirus 6 infection in hematopoietic stem cell transplant patients. Br J Haematol **2004**; 124:421–32.
15. Lusso P, Malnati MS, Garzino-Demo A, Crowley RW, Long EO, Gallo RC. Infection of natural killer cells by human herpesvirus 6. Nature **1993**; 362:458–62.
16. Furukawa M, Yasukawa M, Yakushijin Y, Fujita S. Distinct effects of human herpesvirus 6 and human herpesvirus 7 on surface molecule expression and function of CD4+ T cells. J Immunol **1994**; 152:5768–75.
17. Isegawa Y, Ping Z, Nakano K, Sugimoto N, Yamanishi K. Human herpesvirus 6 open reading frame U12 encodes a functional beta-chemokine receptor. J Virol **1998**; 72:6104–12.
18. Nakano K, Tadagaki K, Isegawa Y, Aye MM, Zou P, Yamanishi K. Human herpesvirus 7 open reading frame U12 encodes a functional beta-chemokine receptor. J Virol **2003**; 77:8108–15.
19. Smith AP, Paolucci C, Di Lullo G, Burastero SE, Santoro F, Lusso P. Viral replication-independent blockade of dendritic cell maturation and interleukin-12 production by human herpesvirus 6. J Virol **2005**; 79: 2807–13.
20. Cermelli C, Berti R, Soldan SS, et al. High frequency of human herpesvirus 6 DNA in multiple sclerosis plaques isolated by laser microdissection. J Infect Dis **2003**; 187:1377–87.
21. Goodman AD, Mock DJ, Powers JM, Baker JV, Blumberg BM. Human herpesvirus 6 genome and antigen in acute multiple sclerosis lesions. J Infect Dis **2003**; 187:1365–76.
22. Tanaka N, Kimura H, Hoshino Y, et al. Monitoring four herpesviruses in unrelated cord blood transplantation. Bone Marrow Transplant **2000**; 26:1193–7.
23. Yoshikawa T, Ihira M, Akimoto S, et al. Detection of human herpesvirus 7 DNA by loop-mediated isothermal amplification. J Clin Microbiol **2004**; 42:1348–52.
24. Kidd IM, Clark DA, Ait-Khaled M, Griffiths PD, Emery VC. Measurement of human herpesvirus 7 load in peripheral blood and saliva of healthy subjects by quantitative polymerase chain reaction. J Infect Dis **1996**; 174:396–401.
25. Fujiwara N, Namba H, Ohuchi R, et al. Monitoring of human herpesvirus-6 and -7 genomes in saliva samples of healthy adults by competitive quantitative PCR. J Med Virol **2000**; 61:208–13.
26. Gautheret-Dejean A, Aubin JT, Poirel L, et al. Detection of human betaherpesvirinae in saliva and urine from immunocompromised and immunocompetent subjects. J Clin Microbiol **1997**; 35:1600–3.
27. Lucht E, Brytting M, Bjerregaard L, Julander I, Linde A. Shedding of cytomegalovirus and herpesviruses 6, 7, and 8 in saliva of human immunodeficiency virus type 1–infected patients and healthy controls. Clin Infect Dis **1998**; 27:137–41.
28. Stein J, Volk HD, Liebenthal C, Kruger DH, Prosch S. Tumor necrosis factor alpha stimulates the activity of the human cytomegalovirus major immediate early enhancer/promoter in immature monocytic cells. J Gen Virol **1993**; 74:2333–8.
29. Fietze E, Prosch S, Reinke P, et al. Cytomegalovirus infection in transplant recipients: the role of tumor necrosis factor. Transplantation **1994**; 58:675–80.
30. Docke WD, Prosch S, Fietze E, et al. Cytomegalovirus reactivation and tumor necrosis factor. Lancet **1994**; 343:268–9.
31. Simon CO, Seckert CK, Dreis D, Reddehase MJ, Grzimek NK. Role for tumor necrosis factor alpha in murine cytomegalovirus transcriptional reactivation in latently infected lungs. J Virol **2005**; 79:326–40.
32. Chen SF, Tu WW, Sharp MA, et al. Antiviral CD8 T cells in the control of primary human cytomegalovirus infection in early childhood. J Infect Dis **2004**; 189:1619–27.
33. Jonjic S, Mutter W, Weiland F, Reddehase MJ, Koszinowski UH. Site-restricted persistent cytomegalovirus infection after selective long-term depletion of CD4+ T lymphocytes. J Exp Med **1989**; 169:1199–212.
34. Lucin P, Pavic I, Polic B, Jonjic S, Koszinowski UH. Gamma interferon-dependent clearance of cytomegalovirus infection in salivary glands. J Virol **1992**; 66:1977–84.

運動ニューロン疾患を伴う痴呆症

臨床症候・検査所見

岡本幸市

はじめに

運動ニューロン疾患に伴う痴呆症(D-MND)とは，前頭-側頭葉を主とした変性と運動ニューロン徴候を合わせもつ疾患であり[1~10]，病理学的には，1）運動ニューロン系には通常の筋萎縮性側索硬化症(ALS)と同じ所見，2）前頭-側頭葉皮質第2～3層の非特異的な変性所見，3）前頭-側頭葉皮質第2～3層の小型神経細胞，および海馬歯状回の顆粒細胞にubiquitin陽性tau陰性の細胞質内封入体の出現，4）中脳黒質の変性，であると要約できる[11,12]．特に3）の病理所見がD-MNDの病理学的診断マーカーとして注目されているが，この病理所見がみられながら臨床的に「痴呆」様の症状が明らかでない症例や，明らかな運動ニューロン徴候を呈さない症例(motor neuron disease-inclusion dementia：MNDID)も存在し[13]，それらの症例の位置付けがなお曖昧である．

臨床的特徴[1~10]

一部家族内発症例もあるが，多くは孤発性である．やや男性に多いとの報告もある．従来，本邦からの報告例が多かったが，最近では欧米からの報告も増加している．発症年齢は35～75歳が多い．精神症状または球麻痺症状のいずれかで発症するが，どちらから発症する例が多いかについては，報告者により差がみられる．最近，球麻痺で発症し，数ヵ月以内に精神症状がみられるようになる例が多いとの報告がある[14]．一般に進行は通常のALSより速い．

運動ニューロン徴候

運動ニューロン徴候としては，球麻痺型で発症する例が多く，錐体路徴候よりも下位運動ニューロン徴候が目立つ．下肢の症状は一般に軽く，末期まで歩行は可能である例が多い．23例の球麻痺型ALSの検索では，その中の11例(48%)がD-MNDであったという報告があり[14]，球麻痺型ALSでは半数近くに痴呆様症状が生じうるという重要な指摘である．D-MNDでは中脳黒質のメラニン含有細胞の脱落もみられるが，これに対応したパーキンソニズムは通常ほとんどみられない．

痴呆様症状

前頭側頭型痴呆(FTD)の臨床的診断基準[6]に記載されているように，1）緩徐進行性の行動異常か，2）緩徐進行性の言語機能異常で発症することが多い[1~10]．前者の症状としては，状況にそぐわない行動，脱抑制，興味の低下，口運び傾向・過食，常同的行動などがみられる．2）の言語機能異常では，単語や物品の名前が出てこないなどの言語表出が早期に障害され，言語理解は比較的保たれる．次第に音読や書字が障害され，無言状態となる．言葉の流暢性の異常も高頻度にみられるが，球麻痺症状がある場合には言葉の流暢性の異常の有無の判断が難しい．D-MNDでは末期に至っても痴呆症状は軽度から中等度に留まっていることが多い．本症に特徴的な症候はないが，行動面での脱抑制は重要な症候である．失行，失認，視空間失認は通常みられない．

このような痴呆様症状を，「痴呆」といってよいかどうかについては議論があるところである．林はALS患者では日常生活上で，「強迫的・固執的に，具体的な自己の訴えを繰り返し，周囲からの説明を受け入れない状況」が頻繁に生じていることを指摘し，これを「情動制止困難」として，ALSの高次精神運動障害の一つとして捉えている[15]．さらに，この症状がD-MNDの症状と極めて類似しており，ALSおよびD-MNDではlimbic motor system(辺縁運動系)が障害され，両者が同一の線上で捉えられる可能性も指摘している．

おかもと こういち　群馬大学教授/大学院医学系研究科 脳神経内科学

0289-0585/05/¥500/論文/JCLS