chronic progressive EPC, even though they may have the pathology of Rasmussen's encephalitis.

Rogers et al. (6) reported glutamate receptor 3 (GluR3) as an autoantigen in Rasmussen's encephalitis, speculated to be one cause of Rasmussen's encephalitis. After their report, epilepsies causally related to autoantibodies against GluRs were highlighted. GluRs in the central nervous system (CNS) play important roles in brain function, and they are divided into ionotropic and metabotropic receptors. The ionotropic GluRs are subdivided into three major subtypes: the *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA), and kainate receptors. The NMDA-GluRs are heterodimers composed of GluRε (ε1-ε4) (NMDAR2A-2D), GluRζ(NMDAR1) subunits, and NR3 subunits (7,8). The molecular diversity of NMDA-GluRs contributes not only to their important physiological roles in development, synaptic plasticity, learning, and memory, but also to the pathological processes in ischemic brain injury, neurodegenerative diseases, and epilepsy (7–11).

GluRε2 is the essential molecule for synaptic plasticity and development (12). In Rasmussen's encephalitis, not only epileptic seizures, but also impairment of higher brain function, such as mental retardation and visual disturbance, occur frequently. Therefore, we examined autoantibodies against NMDA-GluRε2 and their epitopes in the serum and cerebrospinal fluid (CSF) in patients with chronic EPC (13). We detected NMDA-type GluRε2 autoantibodies in histologically proven Rasmussen's encephalitis (3/3 patients), clinical Rasmussen's encephalitis (6/7 patients), acute encephalitis/encephalopathy (2/3 patients), and nonprogressive EPC (2/2 patients). The presence of autoantibodies against NMDA-GluRε2 might suggest autoimmune pathological mechanisms, but is not a hallmark of Rasmussen's encephalitis. Epitope analyses showed that the autoantibodies were predominantly against C-terminal epitopes and rarely against N-terminal epitope, with inconsistency in the profile during the course of the disease. The epitope recognition spectrum of autoantibodies was broader in CSF than in serum, and the serum or CSF profile showed an increase in number of epitopes as the disease progressed in some patients. Therefore, we speculated that the autoantibodies are produced in the CNS after cytotoxic T cell–mediated neuronal damages (13).

In this study, as an extension to our previous study in Rasmussen's encephalitis patients with EPC (13), we examined autoantibodies against NMDA-GluRε2 in 10 new Rasmussen's encephalitis patients. These new patients included four histologically proven Rasmussen's encephalitis patients without EPC (this category of Rasmussen's encephalitis was not reported in our previous paper), two histologically proven Rasmussen's encephalitis patients with EPC, and four clinical Rasmussen's encephalitis patients with EPC. Consequently, we could evaluate the autoantibodies in 20 patients with Rasmussen's encephalitis. Furthermore, as an original study, we examined lymphocyte stimulation by GluRs to estimate the cellular autoimmune pathophysiology of Rasmussen's encephalitis.

PATIENTS AND METHODS

Patients

We examined the samples from 11 clinical Rasmussen's encephalitis patients with chronic progressive EPC (Patients 1–4, 11–16, and 18), and nine histologically proven Rasmussen's encephalitis patients with EPC (Patients 5, and 7–10) or without EPC (6,17,19, and 20) (Table 1). Eleven clinical patients with chronic progressive EPC manifested intractable EPC for >1 month, and all showed neuropsychological impairment. They had progressive EPC, neuroimage findings characteristic of Rasmussen's encephalitis (14), and neuropsychological impairment (clinical Rasmussen type). Detailed clinical manifestations of Patients 1–3, 13, 14, 16, and 18 were reported in our previous article (13), and those of Patient 10 were also reported (15). Patient 14 had negative tests for anti-GluR3 autoantibodies in another hospital. Nine patients were histologically diagnosed as Rasmussen's encephalitis (definitive Rasmussen type) after functional hemispherectomy (Patient 7–10) and focal resection surgeries (Patients 5, 6, 17, 19, and 20).

Informed consent was obtained from each patient or guardian after oral or written explanation, according to the ethical principle of Declaration of Helsinki.

Establishment of stable transformant cells expressing GluRε2

Using the tetracycline-induction system (16), cDNA of GluRε2 (17), pSTneoB (18), and G418 selection (19), we established stable NIH3T3 transformant cell lines expressing full-length GluRε2 (B18) and GluRδ2 subunits (D33) (13). A1 is a control cell line without GluR expression, B18 is a cell line expressing recombinant GluRε2, and D33 is a cell line expressing recombinant GluRδ2.

Detection of autoantibodies against GluRε2 in sera and CSF

Supernatants of cell extracts prepared from B18 and A1 cultured for 48 h with doxycycline (1 μg/ml) were subjected to SDS-PAGE, and the gels were transferred to nitrocellulose membranes. Each membrane was cut into 20 strips after overnight blocking with the blocking buffer (0.02 *M* Tris HCl, 0.16 *M* NaCl, 0.05% bovine serum albumin). The strips of B18 and A1 were reacted with patients' sera (diluted 20-fold with blocking buffer) or CSF (diluted 15-fold with blocking buffer) for 48 h at 4 °C, and were stained by alkaline phosphatase-labeled second antibodies (IgG, IgA, or IgM) (Jackson ImmunoResearch, West Grove, Philadelphia, PA, U.S.A.). The presence of autoantibodies against GluRε2 was judged by a positively

TABLE 1. *Clinical characteristics of patients*

	Onset age					Ope	Outcome			
Patient	Epilepsy	EPC	Sex	Causative or underlying factors	Seizure type	Age	Neurological	Mental	Seizure	At age
1	2 days	1.1 yr	M	DPT vaccination, status	CPS, EPC		QP	MR+++	Daily	4.5 yr
2	2 mo	7 mo	M	Viral meningitis	CPS, EPC		QP	MR+++	Daily	4 yr
3	2 mo	4 mo	M	Polio vaccination, status	CPS, EPC		QP	MR+++	Disappeared	8 yr
4	8 mo	8 mo	M	Respiratory infection	CPS, EPC		HP	MR+++	Daily	4 yr
5	2 yr	9 yr	M		CPS, EPC	8 yr	-	MR++	Daily	9 yr
6	2.6 yr	-	M		CPS	14 yr	-	Normal	Disappeared	15 yr
7	3.6 yr	3.7 yr	M	Infection	CPS, EPC	4 yr	HP, HA	Normal	Disappeared	5yr
8	3.8 yr	3.8 yr	M	Head trauma	EPC, CPS	9 yr	HP, HA	MR+	Disappeared	11 yr
9	3.9 yr	5.3 yr	M	Influenza	CPS, EPC	5.8 yr	HP	Normal	Disappeared	5.9 yr
10	5.2 yr	6.1 yr	F	Status	CPS, EPC	6 yr	HP, HA	Normal	Daily	6 yr
11	5.8 yr	6 yr	F		CPS, EPC		MP	Normal	Daily	20 yr
12	5.9 yr	6 yr	F		CPS, EPC		HP	MR++	Daily	6.2 yr
13	6.1 yr	6.9 yr	M		EPC, CPS		HP	MR+	Daily	10 yr
14	8.9 yr	9.5 yr	F	Infection, status	CPS, EPC		HP	MR++	Daily	15 yr
15	12 yr	15 yr	M		CPS, EPC		QP	MR+++	Daily	16 yr
16	12.5 yr	12.9 yr	M		CPS, EPC		QP	MR++	Weekly	20 yr
17	16 yr	–	M	Head trauma	CPS	34 yr	MP	MR++	Weekly	34 yr
18	23 yr	31yr	F		EPC, CPS		Normal	MR+	Daily	38 yr
19	25 yr	–	F	–	CPS	29 yr	Normal	Normal	Weekly	31 yr
20	28 yr	–	F	–	CPS	34 yr	HP	MR++	Weekly	38 yr

EPC, epilepsia partialis continua; M, male; F, female; NMD, neuronal migration disorder; DPT, diphtheria-pertussis-tetanus; Status, status epilepticus; SPS, simple partial seizure; CPS, complex partial seizure; QP, quadriplegia; HP, hemiplegia; MP, monoplegia; HA, hemianopsia; MR, mental retardation.

stained band with molecular size ~180 kDa, which was found only on the B18 strip and not on the A1 strip (13).

Preparation of bacterial fusion proteins containing peptides from the GluRε2 subunit

DNA fragments encoding amino acid residues 1–48 (an amino-terminal peptide; N), 998–1074 (a carboxyl-terminal peptide; C1), 1053–1153 (a carboxyl-terminal peptide; C2), and 1353–1432 (a carboxyl-terminal peptide; C3) (17) were amplified by PCR using appropriate synthetic oligonucleotides. The positions of candidate epitopes were determined considering molecular interactions. The fragments were cloned to the bacterial fusion protein expression plasmid vector pGEX-4T-2 (Pharmacia Biotech AB, Uppsala, Sweden) or pMAL (New England Biolabs, MA, U.S.A.) for the production of fusion protein with glutathione S-transferase (GST) or maltose binding protein (MBP) (13,20). The expression plasmids were transformed into *Escherichia coli* (BL21 or TB1), and subsequent induction with isopropyl-beta-D-thiogalactopyranoside (IPTG) was performed by routine methods (21). The transformed *Escherichia coli* were sonicated to obtain supernatants of the bacterial homogenates.

Epitope analyses of autoantibodies against GluRε2 in sera and CSF

Supernatants of bacterial homogenates containing induced GluRε2 polypeptide fusion proteins synthesized by the methods described previously were subjected to SDS-PAGE, and the proteins in gels were transferred to nitrocellulose membranes. Each membrane was reacted with serum or CSF (diluted 20- or 15-fold with blocking buffer) for 48 h at 4°C, and stained by alkaline phosphatase-labeled second antibodies (IgG or IgM). A positive reaction with an epitope was judged by obtaining a stained band at the appropriate molecular weight (13).

Lymphocyte stimulation test by GluRs

Peripheral venous blood was collected in heparinized sterile vacutainer tubes (Becton Dickinson, Oxford, U.K.). Blood cells and serum were separated by centrifugation at 1,800 rpm for 5 min. Blood cells were diluted with PBS (-) and applied on Ficoll-Conray (d = 1.077), and centrifuged at 1800 rpm for 20 min to separate the lymphocytes. The lymphocytes were washed twice in PBS by centrifugation at 2,000 rpm for 5 min. Then lymphocytes were diluted to 1×10^6 cells/ml in complete RPMI-1640 supplemented with 20% autologous serum. These cells were cultured in triplicate in 96-well microplates for 3 days with the corresponding antigens. Then ^{3}H-thymidine (0.25μCi/well) was added to each well. After incubation for 16 h, the cells were harvested using an automatic cell harvester and the incorporated radioactivity was measured by liquid scintillation counter. The supernatants of homogenates from A1, D33, and B18, and phytohemagglutinin (PHA) were used as antigens.

Statistical analysis

For the comparison of LST data, we used Mann–Whitney's U test or Wilcoxon signed-ranks test, using statcel software. The numbers indicate mean ± SD (standard deviation).

TABLE 2. *Autoantibodies against glutamate receptors*

			EPC				Whole GluRε2			Epitope analysis			
Pt	Age at exam	Latency	Ext	Face	CPS	Sample	IgG	IgM	IgA	N	C1	C2	C3
1	0.8 yr	0.8 yr	–	–	D	Serum	–	–					
	0.9 yr	0.9 yr	–	–	D	Serum	+	+	–	–	–	+	–
2	1.1 yr	0.9 yr	+	–	D	Serum	+	+	–	–	–	–	–
	1.1 yr	0.9 yr	+	–	D	CSF	+	+	–	+	+	+	+
	4.0 yr	3.8 yr	+	–	W	Serum	+	–	–	–	–	+	–
3	3.8 yr	3.7 yr	–	–	C	Serum	–	–	–	–	–	–	–
4	3.5 yr	2.8 yr	+	+	–	CSF	–	–					
	4.0 yr	3.3 yr	+	+	–	CSF	+	–					
5	9 yr	7 yr	–	+	D	CSF	+	–					
6	11 yr	7 yr	–	–	D	Serum	+	+		–	–	+	–
	15 yr	12 Y	–	–	C	Serum	+	+					
7	3.9 yr	0.2 yr	+	–	M	Serum	+	–		–	+	+	+
	3.9 yr	0.2 yr	+	–	M	CSF	+	–		–	+	+	+
8	3.9 yr	0.1 yr	+	–	M	Serum	–	–	–				
	4.7 yr	0.9 yr	+	–	M	Serum	+	+		–	–	–	–
	5.2 yr	1.4yr	–	–	Y	Serum	–	–					
	8.1 yr	4.3 yr	+	+	M	Serum	+	–		–	–	–	–
	10 yr	6.2 yr	–	–	C	Serum	+	+					
9	5.4 yr	2.5 yr	+	–	W	Serum	+	–					
	5.4 yr	2.5 yr	+	–	W	CSF	+	–					
10	6.1 yr	0.9 yr	+	–	W	Serum	+	–		–	+	–	+
	6.7 yr	1.5 yr	+	–	D	Serum	+	–		–	+	–	+
11	20 yr	14 yr	+	–	C	Serum	+	+					
12	6.1 yr	0.2 yr	–	+	D	Serum	+	–					
	6.1 yr	0.2 yr	–	+	D	CSF	+	–					
13	7.1 yr	1 yr	+	+	C	Serum	–	–		–	–	+	–
	7.1 yr	1 yr	+	+	C	CSF	–	+		–	–	+	–
	11.3 yr	5.2 yr	–	+	Y	Serum	+	+					
14	12 yr	3 yr	+	–	D	Serum	+	–		–	–	+	–
15	15 yr	3 yr	+	–	D	Serum	+	+					
	16 yr	4 yr	+	–	D	Serum	–	–					
16	18.5 yr	6 yr	–	–	W	Serum	+	–		+	–	+	+
	19.5 yr	7 yr	–	–	W	CSF	+						
17	34 yr	18 yr	–	–	W	Serum	+	+					
18	38 yr		–	–	D	Serum	+	+		–	+	–	+
19	29 yr	4 yr	–	–	D	Serum	+	+		+	+	–	+
20	36 yr	8 yr	–	–	W	Serum	–	–					
	38yr	10 yr	–	–	W	Serum	+	+					

Latency, latency from onset to examination; EPC, epilepsia partialis continua; Age at exam, age at examination of autoantibodies; Ext, EPC involving extremities; Face, EPC involving facial muscles, tongue, or throat; CSF, cerebrospinal fluid; GluR, glutamate receptor; NT, not tested. In the column of CPS, D indicates daily frequency of CPS, W indicates weekly, M indicates monthly, Y indicates yearly, C indicates controlled. In the column of whole GluRε2 and epitope analysis, + indicates presence of autoantibodies, and – indicates absence of autoantibodies.

RESULTS

Autoantibodies against GluRε2 molecules in sera and CSF

Autoantibodies against full-length GluRε2 molecule were studied in the sera and or CSF of 20 patients with definitive or clinical Rasmussen encephalitis (Table 2). All nine patients with definitive Rasmussen's encephalitis (five with EPC and four without EPC), and 10 of 11 patients (except Patient 3) with clinical Rasmussen's encephalitis had the autoantibodies in serum or CSF. Although Patient 3 had no autoantibodies against GluRε2, the test was done at the residual stage (22) when EPC and CPS had both remitted. Nineteen patients had IgG autoantibodies against the GluRε2 molecule during the course of disease, and 11 patients had IgM autoantibodies. None of the patients we examined had IgA autoantibodies against GluRε2. In Patients 1, 4, 8, and 13, the IgG and IgM autoantibodies against GluRε2 were absent in the early stage when epileptic seizures already became frequent, and appeared subsequently. In Patient 8, after intravenous high-dose gamma-globulin infusion therapy (200 mg/kg weight, 5 days), EPC remitted and CPSs decreased to a yearly occurrence, accompanied by negative autoantibodies at the age of 5.2 years. At age 7.3 years, EPC recurred and the autoantibodies again became positive. Thus, Patient 8 showed fluctuation of autoantibodies in relation to the severity of clinical symptoms modulated by immunotherapy. In Patient 15, the autoantibodies became negative after steroid pulse therapy, but epileptic seizures persisted. In Patients 6 and 8, the autoantibodies persisted in the serum after frontal lobe resection or functional hemispherectomy, although epileptic seizures were controlled.

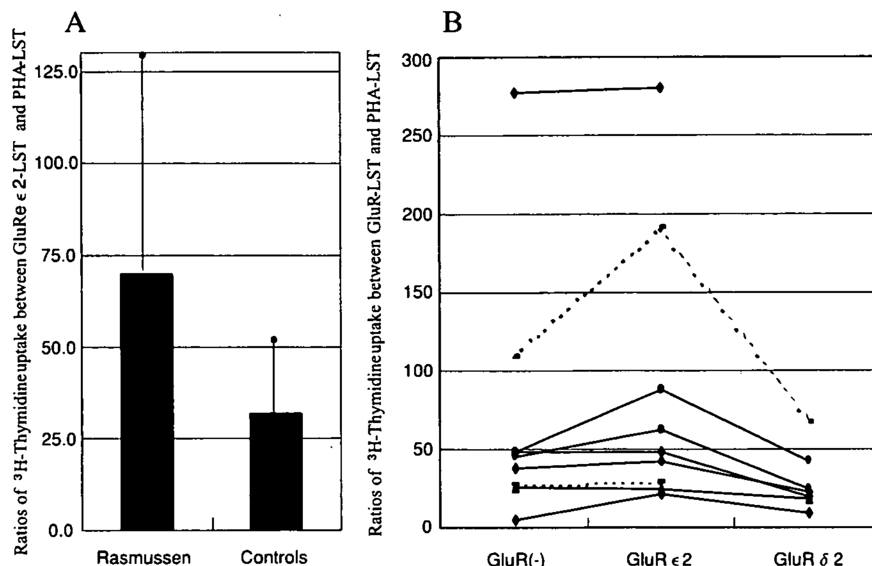

FIG. 1. A: Mean ^{3}H-thymidine ratios of uptake stimulated by GluRε2 relative to PHA in lymphocyte stimulation test (LST) using lymphocytes from patients with Rasmussen syndrome and healthy controls. GluRε2-LST and PHA-LST were conducted using supernatants of cell culture homogenates containing recombinant GluRε2 (B18) and PHA. Vertical axis indicates ratios of uptake stimulated by supernatant of B18 homogenate containing recombinant GluRε2 to that stimulated by PHA (GluRε2/PHA ×1000). **B:** Ratios of uptake stimulated by GluRε2 relative to PHA, those stimulated by GluRδ2 relative to PHA, and those stimulated by control homogenate [GluR (-)] relative to PHA for each patient. GluR (-): Uptake stimulated by control homogenates (A1)/uptake stimulated by PHA ×1000. GluRε2: Uptake stimulated by B18 homogenate containing recombinant GluRε2/uptake stimulated by PHA ×1000. GluRδ2: Uptake stimulated by D33 homogenate containing recombinant GluRδ2/uptake stimulated by ×1000 PHA.

Epitope analyses of autoantibodies against GluRε2 in sera and CSF

IgG autoantibodies against the putative epitopes (N, C1, C2, and C3) of the GluRε2 molecule were examined using bacterial fusion proteins containing the corresponding GluRε2 polypeptides (13). Of 12 patients with IgG autoantibodies against the GluRε2 molecule, three patients had serum autoantibodies to N epitope, four had autoantibodies to C1, seven had autoantibodies to C2, and six had autoantibodies to C3 (Table 2). Autoantibodies to C2 epitope predominated, while autoantibodies to the extracellular N epitope were rare. The epitope pattern in patients without EPC was not different from that in patients with EPC. The spectra of epitope recognition of the autoantibodies were broader in the CSF than in the serum in almost all patients, judging from simultaneously collected samples.

Lymphocyte stimulation by GluRs

In a preliminary study, we examined ^{3}H-thymidine uptake after stimulation with B18 homogenates at different protein concentrations in six definitive patients, and the uptake was highest at the concentration of 40 μg/ml. Subsequently, we conducted lymphocyte stimulation tests (LSTs) using homogenates of D33 (cell line expressing GluRδ2 subunits), B18 (cell line expressing GluRε2), and A1 (control cell line without GluR expression) at protein concentrations of 40 and 400 μg/ml. The mean ^{3}H-thymidine uptake ratios for B18 stimulation (uptake stimulated by B18 divided by uptake stimulated by PHA × 1000) were 69.3 ± 60.7 (n = 23) in patients with definitive and clinical Rasmussen encephalitis and 23.1 ± 19.7 (n = 7) in controls ($p < 0.05$, Mann–Whitney's U test) (Fig. 1A). In patients with definitive Rasmussen encephalitis, the mean ^{3}H-thymidine uptake ratios were 69.3 ± 82.8 (n = 9) for A1 stimulation (uptake stimulated by A1 divided by uptake stimulated by PHA ×1000), 87.6 ± 89.4 (n = 9) for B18 stimulation (uptake stimulated by B18 stimulation divided by uptake stimulated by PHA ×1000), and 29.0 ± 19.7 (n = 7) for D33 stimulation (uptake stimulated by D33 divided by uptake stimulated by PHA ×1000). The uptake ratios for B18 stimulation were significantly higher than those for A1 ($p < 0.05$, Wilcoxon signed-ranks test) and D33 stimulation ($p < 0.05$, Wilcoxon signed-ranks test). The uptake ratios of GluRε2 to PHA were relatively low in Patients 6 and 8, after seizures were controlled after epilepsy surgeries, comparing with those in patients with seizures.

DISCUSSION

Rasmussen's encephalitis is divided into two clinical subtypes by the existence of EPC. In our previous article (13), we reported that autoantibodies against NMDA-type GluRε2 were detected in Rasmussen's encephalitis patients with EPC (definitive Rasmussen's encephalitis patients, 3/3 patients, and clinical Rasmussen's encephalitis patients, 6/7 patients). Subsequently, the present study demonstrated the autoantibodies also in patients with definitive Rasmussen's encephalitis without EPC

(4/4 patients). This suggests that autoantibodies against GluRε2 are important for the diagnosis of both subtypes of Rasmussen's encephalitis, independent of EPC. Because EPC is a typical clinical diagnostic marker of Rasmussen's encephalitis, presurgical diagnoses of Rasmussen's encephalitis are usually difficult in patients without EPC, compared with those with EPC. Detection of autoantibodies against GluRε2 can contribute to the presurgical diagnosis and may facilitate immunological treatment in patients with dominant side involvement.

In these 19 patients with Rasmussen's encephalitis and autoantibodies against the NMDA GluRε2 subunit, the epitope pattern in patients with EPC was not different from that in patients without EPC. Clinical subtypes might be determined by some factors other than difference of epitopes of the autoantibodies. We need to investige further into the distribution of immunological lesions to decide the factors.

In this study, we could reveal the existence of peripheral lymphocytes selectively stimulated by GluRε2 in patients with Rasmussen's encephalitis using LST. SD in patients' data of the mean ^{3}H-thymidine uptake ratios is slightly large. This might be attributed to the effect of treatments and the stage of Rasmussen's encephalitis. The ^{3}H-thymidine uptake ratios were lower mainly in patients with steroid therapy, and the ratios tended to become lower in the progressed stage (data not shown). Lymphocytes stimulated by LST are usually T cells. Although we could not confirm a subset of stimulated T cells ($CD4^{+}$ or $CD8^{+}$) by GluRε2, activated effector T cells that could invade the CNS beyond the blood brain barrier definitively exist in peripheral blood circulation. We estimate that these activated T cells are produced by cross-reaction using molecular mimicry after an infection and play an important role in the subsequent onset of Rasmussen's encephalitis.

In previous reports, lymphocytic infiltration containing predominantly T cells and sparsely B cells were reported in resected tissues from Rasmussen's encephalitis patients (23), and local CNS immune responses in Rasmussen's encephalitis included local clonal expansion of T cells responding to discrete antigen epitopes (24). Our proof about the existence of peripheral lymphocytes stimulated by GluRε2 can explain the infiltration of T cells in the CNS. $CD8^{+}$ T cell cytotoxicity was reported to contribute to the pathogenesis of Rasmussen's encephalitis, and neuronal loss resulting from cytotoxic T cell attack and concomitant release of antigens such as GluR3 were speculated to generate secondary antibody-mediated damage (25,26). The probable heterogeneity of autoantibodies in Rasmussen's encephalitis, epitope spreading and autoantibodies directed predominantly against intracellular epitopes of NMDA GluRε2, also suggests an important role of T cell-mediated autoimmunity (13). Mice immunized with the GluR3B peptide produced autoantibodies but exhibited no epilepsy, even by facilitated entry of the autoantibodies into the brain (27). These data have suggested that the primary cause of Rasmussen's encephalitis is not autoantibodies, but T cell–mediated autoimmunity. In four patients, the autoantibodies against GluRε2 were absent in the early stage, even when epileptic seizures had already become frequent, and appeared later. This delayed appearance of autoantibodies against GluRε2 also supports the hypothesis about primary cause of Rasmussen's encephalitis.

After epilepsy surgeries, Patients 6 and 8 became seizure free, but the autoantibodies persisted in the serum. However, the uptake ratios for GluRε2 to PHA in LST were relatively low in Patients 6 and 8, when seizures were controlled after epilepsy surgeries. These data suggest that autoantibodies against the GluRε2 in sera are not causally related to active pathophysiological processes, and is not a marker of activity of Rasmussen's encephalitis, while the uptake ratio of GluRε2 to PHA in LST might be a marker of disease activity.

International collaborating studies on the contribution of multiple autoantibodies and cellular immunity to the pathophysiology of Rasmussen's encephalitis are expected to elucidate the pathophysiological mechanisms mediated by autoimmunity in Rasmussen's encephalitis and other neurological diseases.

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□ CASE REPORT □

Encephalopathy with Isolated Reversible Splenial Lesion of the Corpus Callosum

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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.
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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

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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.

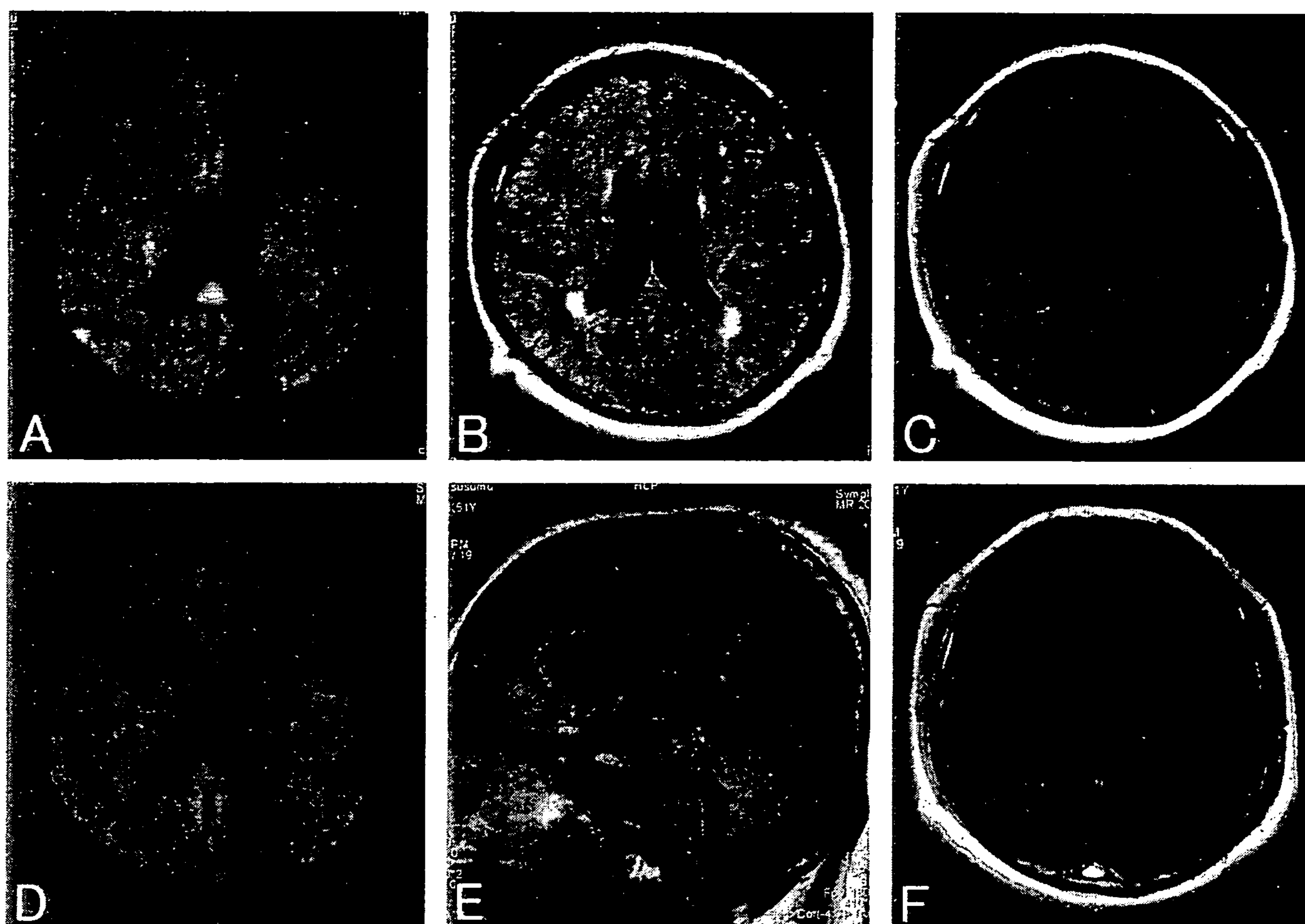

Figure 1. Magnetic resonance (MR) imaging on admission, showing an isolated small lesion in the splenium of the corpus callosum on diffusion-weighted image (DWI) (A), fluid-attenuated inversion recovery (FLAIR) image (B) and T1-weighted image (C). MR imaging on day 5, showing resolutions of the lesion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

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HHV-6 and Seizures

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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,[13] 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.[19] 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 focal 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[15] 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.[8] 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,[16] 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 immunocompromised 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.

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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.

グルタミン酸受容体の分子生物学
──自己免疫性脳炎との関連

Molecular biology of glutamate receptor (GluR) ──Antibodies against GluR in autoimmune encephalitis

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◎中枢神経系において興奮性の速い神経伝達を担うグルタミン酸受容体(GluR)チャネルは，グルタミン酸の結合により陽イオンを透過するとともに，神経細胞内にシグナル伝達を行う分子である．GluR チャネルは神経回路発達，記憶・学習などの生理機能にかかわるのみならず，その異常な活性化は，急性・慢性の脳疾患で観察される神経細胞死に関与する．遺伝子クローニングにより GluR チャネルの分子実体が解明され，GluR チャネルサブユニットの分子的多様性，構造，機能が明らかにされた．一方，急性脳炎や難治性のてんかんの病態に GluR 自己抗体が関与していることが示唆され，その発症における役割が検討されている．抗 GluR 抗体の存在を検討し，その抗体認識部位の構造や抗体の機能，ならびに抗体産生機構を明らかにすることが，これらの病態の解明と治療につながると考えられる．

Key word：グルタミン酸受容体，シナプス可塑性，神経細胞死，自己免疫性脳炎

グルタミン酸受容体(GluR)チャネルは，中枢神経系での主要な興奮性神経伝達を担うのみならず，発生過程での神経回路形成やシナプス伝達の可塑性にかかわる重要な分子である．一方で，GluR チャネルの異常な活性化は，細胞内への過剰な陽イオンの流入を介して神経細胞障害やいわゆる興奮毒性を導くと考えられている．GluR チャネルは，特異性の高い薬物を用いた薬理学的研究により AMPA 型，カイニン酸型，NMDA 型に分類されていたが，1989 年に Hollmann らが，分子生物学的方法とアフリカツメガエル卵母細胞を用いた電気生理学的手法を組み合わせ AMPA 型 GluR1 サブユニットの cDNA クローニングを報告し[1]，現在までに 7 つのサブファミリーに分類される 18 種類のサブユニットの存在が明らかにされている(表 1)．

一方，GluR に対する自己免疫性疾患が示唆されたのは，AMPA 型 GluR3 サブユニットに対する抗体作成過程で，免疫を施したウサギにてんかん様症状と神経細胞死が観察され，難治性てんかんを伴う脳炎である Rasmussen 症候群と相似していたことに端を発している[2]．その後，NMDA 型 GluRε1(NR2A)，GluRε2(NR2B)サブユニット，さらには小脳 Purkinje 細胞に特異的に発現する GluRδ2 サブユニットに対する自己抗体の存在が示唆されている．

本稿では分子生物学的観点から，GluR チャネルサブユニットの構造と機能を中心に解説し，自己抗体が自己免疫性脳炎において果たしている役割について考察する．

GluRチャネルサブユニットの構造と機能

GluR チャネルサブユニットはアミノ(N)末端側のシグナルペプチドと推定される部分および分子のカルボキシル(C)末端寄りに(GluRε では分子の中央付近に)，4 カ所の疎水性領域(M1-M4)

表 1　GluRチャネルサブユニットファミリー

サブタイプ	サブファミリー	サブユニット
AMPA 型	GluR1-4(GluRα)	GluR1(GluRA, GluRα1) GluR2(GluRB, GluRα2) GluR3(GluRC) GluR4(GluRD)
カイニン酸型	GluR5-7(GluRβ)	GluR5 GluR6(GluRβ2) GluR7
	KA1, 2	KA1 KA2(GluRγ2)
δ型	GluRδ	GluRδ1 GluRδ2
NMDA 型	GluRε	GluRε1 GluRε2 GluRε3 GluRε4
	NR1(GluRζ1)	NMDAR1(GluRζ1, NR1)
	GluRχ	GluRχ1(NR3A) NR3B

をもつ膜蛋白質である．このうち M2 は細胞膜を貫通しないループ構造をとっていると考えられており，したがって，N 末端から M1 まで，および M3-M4 間が細胞外に存在し，M4 以降が細胞内に存在すると考えられている(図 1)．GluR チャネルサブユニットの領域は大きく，①N 末端(*N*-terminal domain：NTD)領域，②グルタミン酸結合領域(S1, S2)，③イオンチャネル領域(M2)，④細胞内 C 末端領域からなる．これらの領域の機能は以下のように考えられている．

① **NTD領域**……N 末端から約 400 アミノ酸残基に相当するこの領域の機能は，生化学的実験からサブユニット間の会合やサブユニット分子の安定性に寄与すると考えられている．最近，AMPA 型 GluR2 サブユニットの NTD 領域が樹状突起のスパイン形成に重要であることが示され，細胞接着分子 N-cadherin との相互作用を介してシナプス構造の制御にかかわることが示唆されている[3]．後述するように細胞障害性の自己抗体のエピトープがこの領域内に存在する．

② **グルタミン酸結合領域**……リガンド結合領域は，N 末端細胞外領域の M1 に近い部分(S1)と M3-M4 間の細胞外領域(S2)により構成される[4]．

③ **チャネル領域**……AMPA 型 GluR は通常

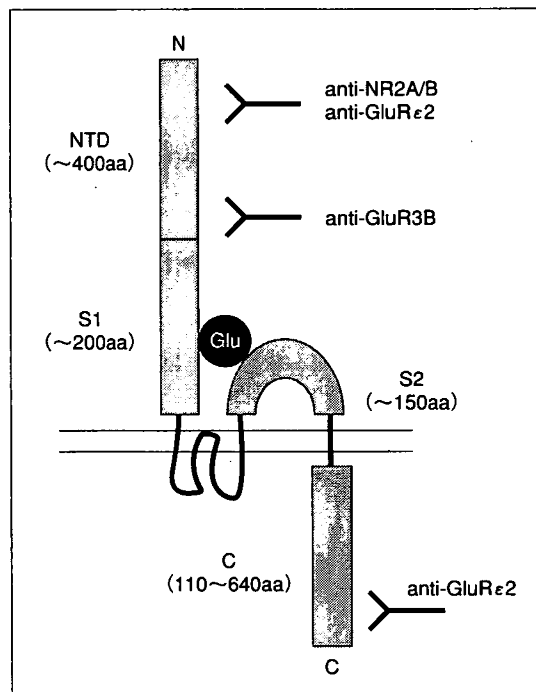

図 1　GluRチャネルサブユニットの構造とエピトープ
GluR チャネルはサブユニットの四量体から構成されるが，ここではひとつの GluR サブユニットを模式的に示した．GluR チャネルサブユニットは，N 末端側から NTD, S1, M1-M3, S2, M4, C の領域からなる．自己抗体のエピトープとして，NTD 領域と C 領域が同定されている．

Ca^{2+}を透過しない．このイオン選択性は GluRα2(GluR2)サブユニットの M2 領域配列内のアルギニン(R)残基で決定されている．AMPA 型 GluR のチャネル領域内の R，Q に対応する位置に NMDA 受容体サブユニットである GluRε，GluRζ1 サブユニットではアスパラギン(N)が存在し，この Asn が NMDA 型 GluR の重要な制御機構である Mg^{2+}による阻害と Ca^{2+}の透過性を制御している[5,6]．

④ **細胞内C末端領域**……AMPA 型 GluR1 サブユニットの細胞内 C 末端は，いくつかのリン酸化酵素でリン酸化される．そして，これらの部位のリン酸化が AMPA 受容体の機能を直接亢進する[7,8]．また，AMPA 型 GluR サブユニットの C 末端領域には post synaptic density(PSD)蛋白群が結合し，GluR サブユニットのシナプス局在を制御している[9]．

抗GluR抗体が結合するエピトープと抗体の作用

1. AMPA型GluR3サブユニット

GluR に対する自己免疫性疾患が示唆されたのは，AMPA 型 GluR3 サブユニットに対する抗体作成過程で，免疫を施したウサギに Rasmussen 症候群と相似した症状が観察されたことに端を発している[2]．実際に Rasmussen 症候群の患者血清に GluR3 に対する自己抗体の存在が示され，血漿交換治療法の有効性が報告された．このような作用を引き起こす GluR3 上のエピトープとして，GluR3 の 372〜395 番目の領域(GluR3B，アミノ酸配列は NEYERFVPFSDQQISNDSSSSENR)が同定された[10]．この領域は他の AMPA 型 GluR サブユニット(GluR1，GluR2，GluR4)とは相同性の低い領域で，GluR3 サブユニットに特有の配列である．また，GluR3B 領域は NTD の C 末端側に位置し，直接的にグルタミン酸の結合にかかわるとは考えにくいが，この領域に対する抗体は神経細胞に GluR の活性化による電流応答を引き起こす．この抗体による電流応答は AMPA 型 GluR の特異的阻害薬 CNQX により抑制される．したがって，GluR3B に対する抗体がアゴニストとして作用し興奮毒性を引き起こすと考えられる．さらに，GluR3B ペプチドを用いてマウスやラットを免疫し，Rasmussen 脳炎の動物モデルが作製されている．マウスに GluR3B ペプチドで免疫して得られた抗体が培養神経細胞を補体非依存的に障害し，アポトーシスによる神経細胞死を引き起こすことが報告されている[11]．

さらに，いくつかの近交系マウスを用いて GluR3B ペプチドで免疫すると，系統ごとに抗 GluR3B 抗体の産生量が異なり，主要組織適合性抗原をはじめとする遺伝的背景が GluR3B に対する免疫反応に影響を与えることが示唆された[12]．また，これらのマウス血清中には GluR3B 抗体のみならず，全身性エリテマトーデス(SLE)でみられるような抗 DNA 抗体も産生されていた．

これらの液性免疫反応のみならず，免疫されたマウスでは，GluR3B に反応し増殖する T 細胞が存在し，細胞性免疫の関与も示唆された．免疫誘導マウスの脳では Rasmussen 脳炎で観察されるのと同様の髄膜の肥厚，リンパ球や単球の脳内への浸潤，グリオーシスなどがみられているが，このマウスでは重篤な脳炎やてんかん発作を示さない．さらに，GluR3B 免疫ラットも報告されている．このラットモデルでも脳組織障害が観察されているが，GABA 受容体の拮抗薬である PTZ 誘発てんかん発作に対して抵抗性を示した[13]．この抵抗性の機構は現時点では不明である．

GluR3B 抗体が内在性につくられる機構に関し，T 細胞の活性化がかかわる可能性が最近示唆されている[14]．T 細胞には機能的な GluR3 サブユニットが存在し，10 nM 程度のグルタミン酸が T 細胞の接着や走性の制御にかかわる．一方，T 細胞受容体が活性化されて放出されるセリンプロテアーゼ Granzyme B が GluR3 サブユニットを切断し，GluR3B ペプチドがあらたな抗原として産生される可能性がある．このようにして末梢で産生された GluR3B に対する抗体が自己抗体として神経細胞障害にかかわる可能性が示唆されている．これらの報告の一方，かならずしも抗 GluR3 抗体の存在と Rasmussen 脳炎発症に相関がない症例の報告もある[15]．

2. NMDA受容体サブユニット

SLE 患者の血清中に存在する自己抗体が NMDA 型

GluRε1, GluRε2(NR2A, NR2B)サブユニットの283番目から287番目のアミノ酸配列(D/E-W-D-Y-S/G)を認識する可能性が示唆され，この抗体が培養神経細胞死を誘導するのみならず，マウス脳内に直接注入すると神経細胞死を誘導する．さらに，神経細胞死がNMDA受容体のチャネルブロッカーであるMK-801の処置により抑制される[16]．以上のことからNMDA受容体のこの領域に対する抗体が，脳血管関門(BBB)が破綻した際の神経細胞死の誘導にかかわる可能性が示唆されている．この配列はNTD領域内にあり，NMDA受容体サブユニット上での機能は不明である．

高橋らは，限局性脳炎の急性期にIgM-GluRε2抗体が髄液中に認められ，IgGおよびIgM自己抗体はN末端エピトープを含んでいることから，GluRε2抗体が発病に関与している可能性を指摘している[17]．また，Rasmussen脳炎の患者において血清中の抗GluRε2抗体のエピトープの広がりが髄液中のエピトープの広がりより狭いことから，髄液中の抗体は髄液内でつくられていること，つまり細胞性免疫による障害の後に自己抗体ができ，その抗体がてんかん発作，さらなる細胞死などを引き起こしている可能性も示唆されている[18]．

3. GluRδ2サブユニット

GluRδ2サブユニットは，薬理学的性質は不明のままであるが，小脳Purkinje細胞に特異的に発現し，このサブユニットのノックアウトマウスの解析から神経回路形成，シナプス可塑性，協調運動や運動学習に必須の分子であることが示されている[19]．GluRδ2サブユニットの機能を明らかにする目的で，グルタミン酸が結合する可能性のあるS1領域内の505番目から514番目のペプチド(GISALTITPDRENV)に対する抗体が作製され，マウス小脳に直接注入すると一過的な協調運動障害が生じることが報告されている[20]．最近，急性の小脳失調症例でGluRδ2に対する自己抗体の存在が報告されているが，エピトープは不明である[21]．

おわりに

以上のように，GluRサブユニットは神経伝達などの生理機能とともに，神経細胞死の誘導においても鍵分子のひとつであることが明らかとなってきた．現時点ではGluRチャネルサブユニットに対し作動性を示す自己抗体が過剰なグルタミン酸と同様の興奮毒性を引き起こしている可能性があり，自己免疫性脳炎の発症と進展に直接かかわる可能性が示唆される．しかし，そのエピトープはリガンド結合領域外に存在することから，抗体の結合がどのようなGluRチャネルサブユニットの構造的変化を引き起こして活性化しているのか明らかにする必要がある．また，GluRチャネルサブユニットのさまざまな領域がそれぞれ重要な機能を担っている．したがって，エピトープ解析が十分になされていないGluRに対する自己抗体は，その出現が脳炎に直接かかわるものなのか，あるいは脳炎による細胞破壊の結果として生じた抗原領域に対するものなのか，見極める必要がある．自己抗体のエピトープを網羅的に迅速に検索するシステムの開発が，自己免疫性脳炎の的確な診断と治療方針の決定に重要になると考えられる．

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＝症例報告＝

グルタミン酸受容体（GluR）抗体が陽性であった髄膜脳炎の16歳男児例

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要旨 全般性けいれんの後に発熱，頭痛，項部硬直が持続した16歳男児．ごく軽度の意識低下，脳波で前頭葉に連続性棘徐波および髄液細胞数上昇，IgG indexの上昇とoligoclonal IgG band陽性を認めた．頭部MRIのFLAIR像で両側半球に散在する部分的灰白質の信号亢進が疑われた．髄膜脳炎と判断し，methylprednisolone pulse療法を実施したところ，臨床症状と脳波異常は軽快した．髄液中の抗グルタミン酸受容体（以下GluR）は入院時ε2・δ2に対するIgG・IgM抗体がともに陽性であり，軽快時は両抗体がともに陰性となった．抗GluR抗体が陽性になる髄膜脳炎の中に，Rasmussen脳炎とは明らかに異なる経過をとり，治療に反応する予後良好な一群が存在する可能性が強く示唆された．

見出し語 自己免疫性髄膜脳炎，IgG index，グルタミン酸受容体抗体，methylprednisolone pulse療法

はじめに

ステロイド治療に良好に反応する脳炎と反応に乏しい脳炎があることは以前から報告されている．しかし，その機序は現在も明らかにはされていない．

一方，大脳の神経細胞に対する抗体が検出され，発症や症状増悪に自己免疫的機序の関与が疑われる脳炎がある．このうちRasmussen脳炎，辺縁系脳炎は，グルタミン酸受容体（以下GluR）に対する抗体が時に髄液中に検出されることが報告されている[1～3]．今回我々は，発熱，項部硬直とごく軽度の意識低下，髄液細胞数上昇とGluR抗体が陽性で，前頭葉に脳波で連続性棘徐波複合を認めた自己免疫性髄膜脳炎と思われる患児を経験した．同症例にmethylprednisolone（以下m-PSL）pulse療法を施行し，著効したので経過を報告する．

I 症例

症例 16歳，男児．

主訴 4週間以上持続する発熱，頭痛，項部硬直．

既往歴 1歳時に嘔吐下痢症，3歳時に無菌性髄膜炎で入院歴あり，13歳時に*Campylobacter*腸炎に罹患した．

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現病歴 生来健康な男児．平成14年12月5日から37度台の微熱，5分間の全般性けいれんが認められ，近医に入院した．髄液一般検査に異常なく，細菌培養は陰性であった．初回けいれん後の脳波を図1aに示す．左前頭部に散発性の棘徐波を認め，局在関連性てんかんの診断でcarbamazepine 400 mgを開始した．一旦解熱したが，1週間後より発熱し，12月12日と27日に数分の全般性強直けいれんを再度起こし，valproate sodium 1,200 mgの内服を併用した．項部硬直を認めたため髄液検査を再施行し，細胞数30/mm³（単核球90%），蛋白50 mg/d*l*，圧300 mm H_2Oと上昇を認めた．細菌性髄膜炎を疑い，cefotaxime, panipenem, betamipron, amikacin, sulfateclindamycinの単剤もしくは2剤の抗生物質の静脈投与とclarithromycin内服を実施し，また，ガンマグロブリンを1月3日より3日間連続で合計200 mg/kg投与した．髄液細菌培養は2度目も陰性であった．発熱が持続し精査加療目的で1月7日に当院小児科に紹介入院した．

入院時現症 見当識，記憶力，計算力に問題はなかったが，笑顔表出がなく表情に乏しかった．項部硬直と眼底の鬱血乳頭，歩行時に軽度のふらつきがあり，指-鼻-指試験等の協調運動は可能であった．知覚，深部腱反射に異常を認めなかった．

入院時検査所見 血算でWBC 16,100/μ*l*，分葉核球88%と増加を認めた．生化学検査ではCRP 0.7 mg/d*l*，赤沈は30 mm/hrと軽度亢進していた．血清免疫グロブリン・尿一般検査に異常を認めなかった．髄液検査（表1）では前医同様に細胞数40/mm³（単核球37/mm³），蛋白58 mg/d*l*と増加があり，糖は正常であった．髄液中のoligoclonal IgG bandは陽性，IgG index 0.71，myelin basic protein（以下MBP）207