

Fig. 2. Immunohistochemistry. **A, B** Coronal sections of rat brain immunoreacted with serum samples from patient 1. **C, D** Coronal sections immunoreacted with serum samples from patient 4. The diluted sera (1:2,000) of these patients reacted with the cytoplasm of rat hippocampal (**A, C**) and cortical (**B, D**) neurons. The same pattern of reactivity was observed for the serum samples from patient 3 and the anti-NMDA ϵ_2 antibody. The sections are slightly counterstained with hematoxylin. Magnification $\times 400$.

rum. Patient 1 showed an elevated level of the antithyroxine antibody in the serum, which was not detected in CSF. We measured the titers of voltage-gated potassium channel (VGKC) antibodies by radioimmunoassay using whole rabbit brain homogenate as described previously [13]. The titers of VGKC antibodies showed normal levels in these 4 patients (patient 1: 0 pM, patient 2: 21 pM, patient 3: 0 pM, patient 4: 18 pM, normal range <100 pM).

Neuroimaging and Physiological Examination

Brain MRI at disease onset showed signal abnormalities in the bilateral mesial temporal lobes in patients 1 and 3 and on one side of the mesial temporal lobe in patient 2. In patient 4, brain MRI showed no signal abnormalities. However, several months (range, 6 months to 1 year)

after the disease onset, cerebral atrophy of various degrees, including atrophy in the mesial temporal lobe, was common (fig. 1). SPECT was performed in the acute or subacute phase. All of the patients except patient 1 showed hypoperfusion in the mesial temporal lobe. In patient 1, ^{99m}Tc -HMPAO SPECT performed the day after admission revealed hyperperfusion in the bilateral mesial temporal lobes; however, hypoperfusion was observed 17 months later. All the patients showed irregular diffuse cortical hypoperfusion. In 3 patients, EEG revealed small focal spikes. In all the patients, a mixture of diffuse θ -waves was observed. No tumors were detected in any of the patients using various methods [chest, abdomen and pelvic CTs (all patients), whole-body FDG-PET (patient 1) and gallium scintigraphy (patient 2)].

Immunohistochemical Findings

The diluted serum samples from patients 1, 3 and 4 reacted with the cytoplasm of rat hippocampal and cerebral cortical neurons (fig. 2). The most appropriate dilution of serum for immunohistochemical staining was 1:2,000–4,000. The sections incubated with the anti-NMDA ϵ_2 antibody showed the same pattern of immunoreactivity as those incubated with the patients' sera. The serum of the healthy control did not significantly immunoreact with the sections. The immunoreactivity of sera of the 3 above-mentioned patients to the anti-GluR ϵ_2 antibody was markedly decreased by prior incubation with the supernatants of extracts from stable transformant cells expressing GluR ϵ_2 . Patients 1, 2 and 3 were negative for serum paraneoplastic anti-Yo, anti-Hu, anti-Ri, anti-CV2, anti-Tr, anti-Ma-2 and anti-amphiphysin antibodies.

Discussion

We presented 4 patients with acute encephalitis who had IgM autoantibodies against GluR ϵ_2 in CSF in the early phase of the disease; however, these antibodies were not detectable after several months. Other acute encephalitis/encephalopathy patients (patients 5–12) had neither the IgG nor IgM autoantibody against GluR ϵ_2 in CSF. Four antibody-positive patients had the characteristic clinical features of reversible autoimmune limbic encephalitis such as intractable convulsion, psychiatric symptoms, recent memory disturbance and sufficient responsiveness to immunotherapy. Concerning the cause of limbic encephalitis, there are many reports in which limbic encephalitis is associated with cancer, most commonly a small-cell carcinoma of the lung [14–20]. The 4 antibody-positive patients had no findings of viral infection or cancer. Several reports have been published concerning the immunotherapy response form of nonparaneoplastic limbic encephalitis [21–26]. Recently, a VGKC antibody has been detected in patients with reversible autoimmune limbic encephalitis [27–32]. However, the titers of VGKC antibodies were normal in our 4 antibody-positive patients' sera. We speculate that reversible autoimmune limbic encephalitis is heterogeneous. Some forms of this disease may be mediated by autoantibodies against antigens such as ion channels or ionotropic receptors in the limbic system.

In our immunohistochemical analysis of the sera of patients with the IgM autoantibody against GluR ϵ_2 , immunoreactivity was detected in the cytoplasm of neurons

in the hippocampus and cerebral cortex. The immunoreactivity was specifically demonstrated using an immunoadsorption test. The NMDA receptor is one of the ionotropic glutamate receptors essential for excitatory neurotransmission and synaptic plasticity, which underlie memory and learning [10, 11]. An antibody-mediated disturbance of NMDA-type GluR function might influence synaptic plasticity in the hippocampus and cortical neuronal excitability. The clinical response to immunotherapy and the results of immunoblotting and immunohistochemistry suggest that IgM autoantibodies may be related to pathogenesis in a subgroup of patients with reversible autoimmune limbic encephalitis.

In a previous study, autoantibodies against GluR ϵ_2 were detected in patients with chronic EPC causally related to Rasmussen syndrome [7]. There are some reports that these antibodies have also been detected in patients with encephalitis other than chronic EPC [6, 7]. In this study, we detected autoantibodies against GluR ϵ_2 in patients with reversible autoimmune limbic encephalitis. We suggest that autoantibodies against GluR ϵ_2 contribute to the onset of localized encephalitis such as EPC and reversible autoimmune limbic encephalitis [7].

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PAPER

Proteomic analysis of autoantibodies in neuropsychiatric systemic lupus erythematosus patient with white matter hyperintensities on brain MRI

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The pathogenesis of neuropsychiatric systemic lupus erythematosus (NPSLE) may be related to autoantibody-mediated neural dysfunction, vasculopathy and coagulopathy. We encountered an NPSLE patient whose brain showed characteristic diffuse symmetrical hyperintensity lesions in the cerebral white matter, cerebellum and middle cerebellar peduncles on T2-weighted magnetic resonance (MR) images. In this study, we investigated all the antigens that reacted strongly with autoantibodies in this patient's serum by two-dimensional electrophoresis (2DE), followed by western blotting (WB) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) using rat brain proteins as the antigen source. As a result, we identified four antigens as beta-actin, alpha-internexin, 60 kDa heat-shock protein (Hsp60) and glial fibrillary acidic protein (GFAP). There are several reports on the detection of anti-endothelial cell antibodies (AECAs) in an SLE patients. Recently, one of the antigens reacting with AECAs in SLE patient's sera has been identified as human Hsp60. We speculated that the abnormal findings on brain MR images of our patient may be due to impairment of microcirculation associated with vascular endothelial cell injury mediated by the antibody against Hsp60. This proteomic analysis is a useful tool for identifying autoantigens in autoimmune diseases involving autoantibodies. *Lupus* (2008) 17, 16-20.

Key words: endothelial cell; 60kDa heat shock protein (Hsp60); neuropsychiatric systemic lupus erythematosus (NPSLE); proteome; white matter hyperintensity (WMH)

Introduction

Patients with neuropsychiatric systemic lupus erythematosus (NPSLE) frequently show various abnormal findings including white matter hyperintensities (WMHs) on T2-weighted brain magnetic resonance (MR) images.¹⁻³ White matter hyperintensities appear to represent asymptomatic cerebral small vessel disease (SVD).⁴ There is accumulating evidence that WMHs are associated with several impairments such as cognitive deficits.⁵⁻⁷ The pathogenesis of cerebral SVD is poorly understood, but endothelial activation and dysfunction may play a causal role.⁴

Here, we report the case of an NPSLE patient, whose brain MRI showed characteristic WMHs on T2-weighted and fluid-attenuated inversion recovery (FLAIR) images. We examined the reactivity of his serum antibodies against rat brain antigens using the two-dimensional immunoblotting method and identified the antigens that reacted with these autoantibodies by the proteomic method.

Materials and methods

Patient and serum samples

Serum samples were collected from an untreated 69-year old male patient with NPSLE. His clinical features are summarized as follows:

1. He showed slowly progressive polyneuropathy predominantly in the lower limbs and subsequent

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encephalopathy one year after the onset of polyneuropathy.

2. Proteinuria was detected by urinalysis and membranous nephropathy was demonstrated by renal biopsy.
3. Our patient presented delusion and hallucination without insight. Disorientation of time and place were noted. His recent memory was impaired, as he was unable to recall any of three objects after 5 min. He showed impairment of complex attention [disability of digit span (backward)]. Our patient's neurological symptoms were cognitive dysfunction, psychosis and polyneuropathy, as determined on the basis of American College of Rheumatology (ACR) Nomenclature on the NPSLE.⁸
4. Laboratory tests revealed the presence of several autoantibodies [anti-nuclear antibody, anti-DNA antibody, anti-Sm antibody, anti-RNP antibody and lupus anti-coagulant (dRVVT 1.31; normal <1.3)]; hyperglobulinemia [IgG (3448 mg/dL, normal 890–1850 mg/dL)]; decreases in the levels of complements [CH50 (16.9 CH50U/mL, normal 23–46 CH50U/mL) and C4 (2 mg/dL, normal 12–30 mg/dL)], white blood cell count (3620/ μ l, normal 3400–9200/ μ l) and lymphocyte cell count (1340/ μ l, normal 646–4177/ μ l); and coagulation-fibrinolysis abnormalities [increases in the levels of fibrinogen/fibrin degradation products (FDP) (11.3 μ g/mL, normal \leq 4.0 μ g/mL), D-dimer (1.5 μ g/mL, normal \leq 1.0 μ g/mL) and alpha2-plasmin inhibitor-plasmin complex (PIC) (1.4 μ g/mL, normal \leq 0.8 μ g/mL) and decreases in the value of the thrombotest (48%, normal 70–150%), fibrinogen level (150 mg/dL, normal 150–350 mg/dL), anti-thrombin III activity (71%, normal 80–130%), protein C activity (58%, normal 64–146%), protein C antigen level (61%, normal 70–150%) and protein S activity (52%, normal 60–150%)].
5. Brain MRI showed characteristic diffuse symmetrical hyperintensity lesions in the cerebral white matter, cerebellum and middle cerebellar peduncles on T2-weighted and FLAIR images (Figure 1). Diffusion-weighted images (DWIs) showed high intensities in the bilateral middle cerebellar peduncles with decreased apparent diffusion coefficient (ADC) values. These findings on DWIs and the ADC map suggest that the lesions represent cytotoxic edema caused by ischemic changes.
6. The findings of a nerve conduction study revealed sensory motor axonal degeneration predominantly in the lower limb.

The above-mentioned findings fulfilled the ACR criteria on SLE.⁹ He had no risk factors for atherosclerosis,

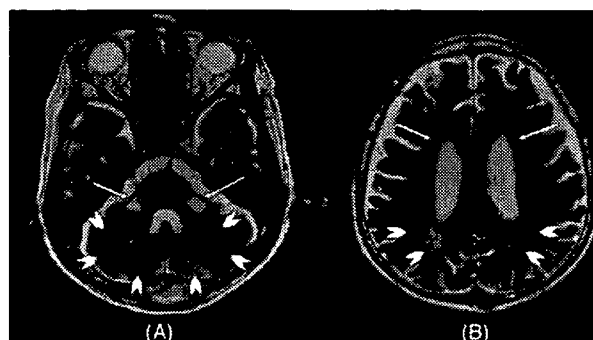


Figure 1 T2-weighted brain MR images (SE, TR/TE: 4080/100 ms). Brain MRI showed symmetrical hyperintensity lesions in bilateral cerebellar hemispheres (arrowheads), middle cerebellar peduncles (arrows) (A), periventricular white matter (arrows) and (B), deep white matter (arrowheads).

such as hypertension, hyperlipidemia and diabetes mellitus.

Preparation of tissue proteins

Under ether anesthesia, adult Sprague-Dawley rats were sacrificed. The cerebrums were immediately removed and frozen in dry-ice powder. The frozen brain tissue was homogenized with a tissue homogenizer in lysis solution, consisting of 20 mM Tris, 7M urea, 2M thiourea, 4% CHAPS, 10 mM 1,4-dithioerythritol (DTT), 1 mM EDTA and 1 mM phenylmethylsulfonyl fluoride containing a cocktail of protease inhibitors (Calbiochem, San Diego, CA, USA). The homogenate was centrifuged at $150\,000 \times g$ for 45 min and the supernatant was used in all experiments. Protein concentration was determined by Bio-Rad Protein assay based on the Bradford method (Bio-Rad Laboratories, Hercules, CA, USA).

Two-dimensional electrophoresis

The samples were dissolved in destreak rehydration solution (GE Healthcare, Buckinghamshire, UK) and loaded by in-gel rehydration into 7-cm long immobilized pH gradient dry strips (GE Healthcare, Buckinghamshire, UK). Up to 250 μ g of extracted proteins was applied to the dry strips for Western blotting (WB). Isoelectric focusing was conducted at 20°C for 24 000 Vh at a maximum of 5000 V using a horizontal electrophoresis system, Multiphor III (GE Healthcare, Buckinghamshire, UK). Before separation in the second dimension, the IPG strips were equilibrated for 15 min in a buffer containing 2% SDS, 6M Urea, 30% v/v glycerol, 0.001% BPB and 50 mM Tris-HCl (pH 8.8) under reducing conditions with 65 mM DTT, followed by incubation for 15 min in the same buffer

under alkylating conditions with 140 mM iodoacetamide. Equilibrated IPG strips were transferred to a 12.5% polyacrylamide gel and run at 15 mA/gel. After the electrophoresis, the SDS-PAGE gels were stained with Coomassie Brilliant Blue (GelCode Blue Stain Reagent, Pierce) or used for protein transfer onto polyvinylidene difluoride (PVDF) membranes.

Immunoblotting

Separated proteins were electrophoretically transferred to a PVDF membrane at 50 volts for 3 h using a buffer transfer tank with cold equipment. The PVDF membrane was incubated in blocking solution (5% skim milk in 1 × TBST; 1 × TBS containing 0.1% Tween 20) overnight in a cold room and then reacted with the patient's serum diluted (1:1000) in 1% skim milk in 1 × TBST for 1 h at room temperature. The PVDF membrane was washed five times with 1 × TBST and reacted with peroxidase-conjugated goat anti-human Ig (A + G + M) antibodies (P.A.R.I.S, France) diluted (1:1000) with 1% skim milk in 1 × TBST for 1 h at room temperature. After six washes, the membrane was incubated with the ECL reagent for 1 min and then exposed to an x-ray film for 15–300 s.

Gel digestion and mass spectrometry

The target spot was excised from the gel and subjected to trypsin digestion and peptide fragments were analysed using a nanoscale capillary LC system (LV-VP, Shimadzu) and an ion trap tandem mass spectrometer (LCQ Advantage Max, Thermo Electron). Proteins were identified from MS/MS spectra using protein identification software (X calibur TM, Thermo Finnigan and MASCOT Search, Matrix Science).

Determination of anti-60 kDa heat shock protein antibodies

We determined by enzyme-linked immunosorbent assay (ELISA) the titers of anti-Hsp60 antibodies in sera from our patient, patients with NPSLE (n = 5; age range, 22–58; mean age, 42.4) without abnormal WMHs and healthy controls without abnormal WMHs (n = 7; age range, 17–67; mean age, 43.1). We carried out ELISA to analyse the reactivities of autoantibodies against human Hsp60, which were measured using an ELISA kit (Stressgen, Ann Arbor, MI, USA). Sera diluted 1:1000 in a dilution buffer were added to a precoated ready-to-use recombinant human Hsp60 immunoassay plate and then incubated for 2 h at room temperature (RT). After four washes, peroxidase-conjugated anti-human IgG, A or M was added to each

well and then incubated for 1 h at RT. After four washes, a stabilized tetramethylbenzidine substrate was added to each well and then incubated for 15 min at RT. The reaction was stopped by adding acid stop solution and the plate was read at 450 nm on a microplate reader. The OD of control wells without Hsp60 was subtracted from the OD of Hsp60-coated wells. Serial dilutions of serum samples of healthy blood donors having high antibody levels against the tested Hsp60 were used as standards.

Results

Screening and identification of target proteins that reacted with autoantibodies in patient's serum

We detected nine spots (pI 4.2–124 kDa, pI 5.15–kDa, pI 5.3–53 kDa, pI 5.4–53 kDa, pI 5.5–53 kDa, pI 5.25–57 kDa, pI 5.4–57 kDa, pI 5.15–63 kDa, pI 8.0–35 kDa) that strongly reacted with autoantibodies in patient's serum on 2DE-WB (Figure 2). Five among the nine spots that matched proteins on 2-DE gels were analysed by LC-MS/MS. These immunoreactive proteins were identified as beta-actin (pI 5.15–46 kDa), alpha-internexin (pI 5.15–63 kDa), Hsp60 (pI 5.25–57 kDa and pI 5.4–57 kDa) and glial fibrillary acidic protein (GFAP) (pI 5.3–53 kDa) (Table 1).

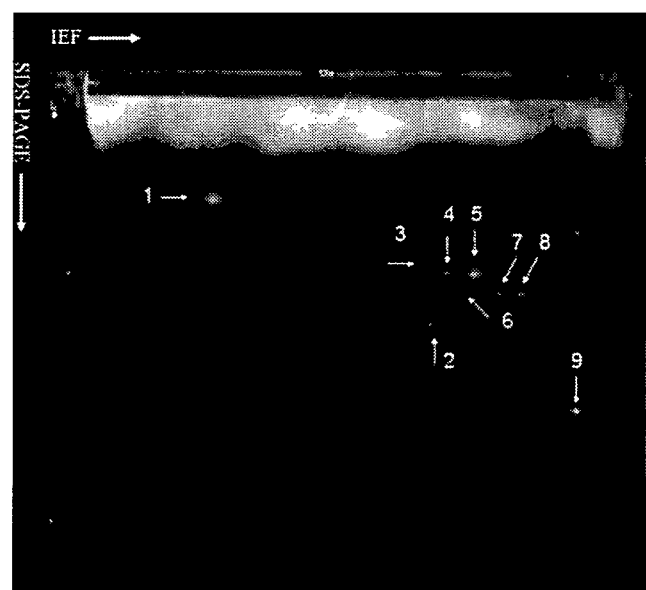


Figure 2 Two-dimensional electrophoresis (2DE) and western blotting (WB). Nine spots strongly reacted with autoantibodies in patient's serum on 2DE-WB. Five spots (No. 2–6) were analysed using mass spectrometry. No. 2: Beta actin; No. 3: Alpha-internexin; No. 4 and 5: 60 kD heat-shock protein (Hsp60); No. 6: Glial fibrillary acidic protein (GFAP); No. 1, 7, 8 and 9: no identification was made.

Table 1 Autoantigens identified using mass spectrometry

Spot Number ^a	Protein name	Mascot score	Number of peptides	Coverage%	Observed M.W.(kDa) /pI	Calculated M.W.(kDa) /pI
2	Beta-actin	421	16	41	46/5.15	42/5.29
3	Alpha-Inx ^b	203	5	12	63/5.15	56/5.20
4	Hsp 60 ^c	112	3	6	57/5.25	61/5.91
5	Hsp 60	112	3	8	57/5.4	61/5.91
6	GFAP ^d	113	2	2	53/5.3	50/5.35

^aSpot number corresponds to the number shown in Figure 2.

^bAlpha-Inx, Alpha-internexin.

^cHsp 60, 60 kD heat-shock protein.

^dGFAP, Glial fibrillary acidic protein.

Detection of anti-Hsp60 antibodies in our patient and controls

The titer of the anti-Hsp60 antibody in our patient was 133.6 ng/mL. The mean titer of this antibody in five NPSLE patients without WMHs on brain MR images was 19.72 (SD 10.65; range 9.0–32.2) ng/mL. The mean titer of this antibody in the seven healthy controls without WMHs on brain MR images was 15.36 (SD 11.85; range 5.7–39.5) ng/mL.

Discussion

In this study, we detected some autoantigenic proteins reacting with autoantibodies in a serum sample from a patient with NPSLE using the proteomic approach and we identified four autoantigens, namely, beta-actin, alpha-internexin, Hsp60 and GFAP. There are some previous studies demonstrating the association of autoantibodies in serum and cerebrospinal fluid (CSF) with central nervous system involvement in patients with NPSLE.^{10–12} Anti-endothelial cell antibodies (AECAs) have been detected in SLE patients.^{13,14} Recently, one of the antigens that reacted with AECAs in a SLE patient's sera has been identified as human Hsp60.¹⁵ Human Hsp60 is a molecular chaperone that participates in the folding of mitochondrial proteins and facilitates proteolytic degradation of misfolded or denatured proteins.¹⁶ However, it has also been reported that an enhanced expression of this protein on endothelial cells has been noted and antibodies against human Hsp60 induce endothelial cell toxicity.^{15,17,18}

Our patient's brain MR images showed characteristic cerebral WMHs, which appear to represent cerebral SVD. The pathogenesis of cerebral SVD is poorly understood, but endothelial activation and dysfunction may play a causal role.⁴ It has been reported that the anti-Hsp60 antibody is present in most patients with coronary artery disease that its titer correlates with disease severity¹⁹ and that it may contribute to the initiation or amplification of vascular endothelial cell damage in atherosclerosis, which is considered a crucial event.²⁰ Our patient's laboratory findings showed

a slightly high level of lupus anticoagulant and some coagulation-fibrinolysis abnormalities. It has been reported that the anti-Hsp60 antibody bind to endothelial cells and induce a thrombotic cascade following endothelial cell apoptosis in SLE patients with the anti-phospholipid antibody.¹⁵ In this study, we determined by ELISA the titer of the anti-Hsp60 antibody in sera from our patient and controls without WMHs on brain MR images. The titer of this antibody in serum from our patient was markedly higher than the mean +2SD of NPSLE patients without WMH or that of healthy controls without WMHs. Thus, the abnormal WMH lesions on brain MR images in our patient may be at least partially due to the impairment of microcirculation associated with vascular endothelial cell dysfunction mediated by the antibody against Hsp60. Further studies using a large series of controls are required to clarify the relationship between the anti-Hsp60 antibody and WMHs on brain MR images.

On the other hand, the clinical significance of the anti-GFAP antibody in NPSLE remains controversial. There is a report showing that the anti-GFAP antibody is specific for NPSLE.² Another report suggested that GFAP might be a useful marker in the diagnosis and monitoring of NPSLE, because GFAP level increases in the CSF of NPSLE.²¹ However, Valesini *et al.*²² have reported that the presence of the anti-GFAP antibody in sera of SLE patients showed no significant correlation with neurologic or psychiatric morbidity. Further study will be necessary to clarify the association between the anti-GFAP antibody and NPSLE.

Previously, there were several reports, which described that the autoantibodies against beta-actin and alpha-internexin were detected from non-neurological diseases or healthy controls.^{23,24} Therefore, we thought that these autoantibodies were not specifically related to NPSLE and are parts of the natural autoantibody repertoire.

In this study, we detected several autoantibodies from our NPSLE patient and identified the autoantigens that they reacted. Several autoantibodies are generated in systemic autoimmune diseases, and an understanding of the interaction among these

autoantibodies will help clarify their pathogenesis. The proteomic analysis used in our study is a very useful tool for identifying several autoantigens reacting with autoantibodies at one time.

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Longitudinal Analysis of Cytokines and Chemokines in the Cerebrospinal Fluid of a Patient with Neuro-Sweet Disease Presenting with Recurrent Encephal meningitis

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Abstract

Background Neuro-Sweet disease (NSD) has recently been identified as Sweet disease with central nervous system (CNS) involvement characterized by multisystem neutrophilic infiltration. However, the pathogenesis of this disease remains unknown. Neutrophil and other inflammatory cell activities are influenced by many cytokines and chemokines, but to date, no studies have examined the levels of these factors in patients with NSD.

Patient and Methods The patient presented with encephal meningitis twice in one year and was diagnosed with NSD. We measured the levels of cytokines (i.e., IL-2, IL-4, IL-6, IL-10, IFN- γ , and TNF- α) and chemokines (i.e., CCL2, CCL3, CCL5, CXCL8, CXCL10 and GM-CSF) in 10 CSF samples from the patient longitudinally for one year including those during two episodes of encephal meningitis.

Results The elevations of IL-6, IFN- γ , CXCL8 (IL8) and CXCL10 (IP10) were markedly higher than the levels in uninfected control subjects with neurological disorders. The levels of these cytokines and chemokines were statistically correlated with total CSF cell counts ($p < 0.01$).

Conclusion CD4+ helper T (Th) cells can be divided into the Th1 and Th2 subtypes according to their cytokine secretion patterns, and IFN- γ and IP10 are the Th1-type cytokine and chemokine indicating the involvement of Th1 cells in NSD. In addition, the level of IL8, a specific neutrophil chemoattractant, correlated well with the neutrophil cell counts in CSF. Our data suggest the important roles of Th1 cells and IL8 in the pathogenesis of NSD.

Key words: CXCL8 (IL-8), CXCL10 (IP-10), IL-6, IFN- γ , neutrophil cell, Th1 cell

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Introduction

Neuro-Sweet disease (NSD) has recently been identified as Sweet disease with central nervous system (CNS) involvement characterized by multisystem neutrophilic infiltration (1, 2). Patients present with painful erythematous plaques on their skin and histological examination of the plaques shows dense dermal infiltration of neutrophils with no signs of vasculitis. This characteristic finding, together with HLA B51 negativity, is important in distinguishing NSD from neuro-Behçet disease (NBD) (2, 3). Japanese pa-

tients with NSD also typically show high levels of HLAs B54 and CW1 (2).

Encephalitis and meningitis are common neurological manifestations of NSD (1). Systemic corticosteroid therapy is highly effective and most patients recover from their neurological deficits without sequelae (1, 2, 4, 5). Despite effective treatment, however, some patients have recurrent episodes indicating that more effective therapies are still needed. A clearly defined pathogenesis for NSD and reliable laboratory markers reflecting disease activity remain elusive. Here, we report the first longitudinal analysis of the levels of cytokines and chemokines in the cerebrospinal fluid

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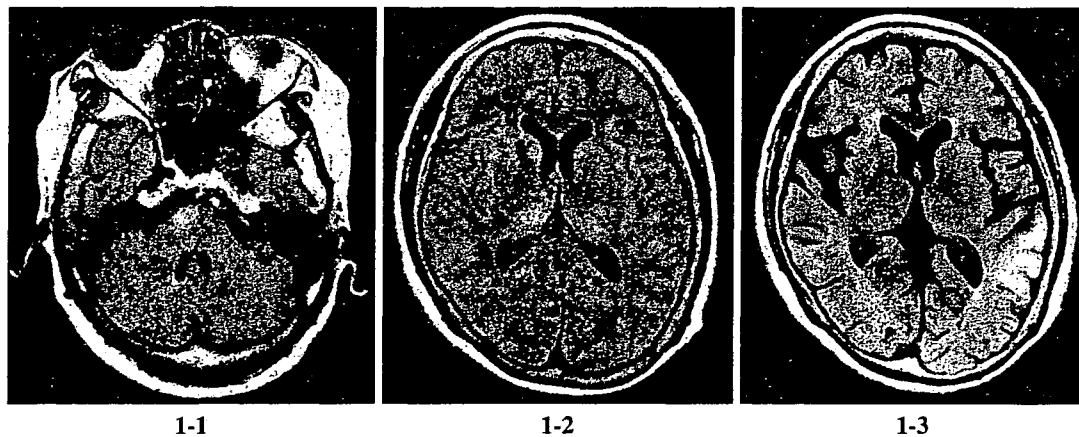


Figure 1. Magnetic resonance images on admission. FLAIR images show high-intensity lesion in the brainstem (1-1), right thalamus and caudate nucleus (1-2) at first hospitalization, and high-intensity lesion in the cortex and subcortical white matter of the left temporal lobe at second hospitalization (1-3).

(CSF) of a patient with NSD. Our results provide important clues to the pathogenesis of NSD and may contribute to the formulation of more effective preventative NSD therapies.

Patient and Methods

Patient

At first hospitalization

A 59-year-old woman had a sore throat and a fever in late August 2005. Four days later, she visited a local hospital. She was diagnosed with acute tonsillitis, admitted to a hospital and treated with antibiotic therapy in early September 2005. The day after admission, she became drowsy and she was transferred to our hospital. She had a history of acute hepatitis B viral infection. She had a temperature of 36.6°C, a pulse of 78/min, and a blood pressure of 148/65 mm Hg. She had erythematous plaques on both legs. On neurological examination, her consciousness level was semicomatose and she presented with right pupillary dilatation and delayed light reflex. The deep tendon reflexes of all four limbs were hyperactive except for the bilateral Achilles tendon reflexes. Laboratory evaluation revealed increased numbers of peripheral blood leukocytes and neutrophils: white blood cell (WBC) count, $15.1 \times 10^3/\mu\text{l}$ (normal range: $3.3 \times 10^3 \sim 7.9 \times 10^3/\mu\text{l}$) and neutrophil cell count, $13.9 \times 10^3/\mu\text{l}$ (normal range $1.5 \times 10^3 \sim 5.9 \times 10^3/\mu\text{l}$). Her serum C reactive protein (CRP) level was 18.8 mg/dl (normal <0.20 mg/dl). CSF examination showed 341 cells/mm³ (mononuclear cells, 298; neutrophilic cells, 43) and a total protein concentration of 172 mg/dl. A culture of a CSF sample was negative for bacteria, tuberculosis and fungi. Antibodies against herpes simplex virus were absent and PCR analysis also showed no herpes simplex virus. A brain MRI scan showed increased signal intensities on T2-weighted and fluid-attenuated inversion recovery (FLAIR) images in the brainstem (Fig. 1-1), right thalamus and caudate nucleus (Fig. 1-2). The electroen-

cephalogram showed slow basic rhythm and diffuse θ activity. After admission she was treated with an intravenous infusion of antibiotics and acyclovir. Subsequently, the disturbance of consciousness became progressively worse and mechanical respiratory management was required two days after admission. She suffered a generalized tonic seizure and was treated with phenytoin. The seizures were difficult to control, however, and required treatment with the anesthetic agent propofol. Because a brain MRI scan showed increased signal intensities on T2-weighted and FLAIR images in various subcortical brain structures, a diagnosis of acute disseminated encephalomyelitis (ADEM) was suspected. Thus, four days after admission intravenous dexamethasone (12 mg/day for 5 days) was administered for 4 days and then, ten days later, methylprednisolone (1,000 mg/day for 3 days) was administered for three days. Her condition gradually improved and she did not require respiratory management. However four weeks after admission a brain MRI scan showed an abnormal signal intensity lesion in the periventricular white matter of the left parietal lobe and expansion of the brainstem lesion. Then her symptoms and abnormal brain MRI findings gradually improved and she was discharged from the hospital without any sequelae in early November 2005.

At second hospitalization

The patient had a sore throat and a fever in mid-January 2006. Five days later, she consulted an otolaryngologist and was diagnosed with acute tonsillitis. She was treated with an intravenous infusion of antibiotics. Five days later she suffered a sudden, generalized tonic seizure during infusion and was referred to our department. She had a temperature of 37.8°C, a pulse of 95/min, and a blood pressure of 153/83 mm Hg. Her throat was reddish and the palatal tonsil was swelling with velaque. Erythematous plaques were apparent on her cheek, forearms and legs. On neurological examination, she was disoriented and could not remember her name

and birthday correctly. The deep tendon reflexes of all four limbs were hyperactive predominantly in left upper and lower limbs. She presented with bilateral Hoffman reflexes and spasticity of the lower limbs. Laboratory tests revealed increased numbers of peripheral blood leukocytes and neutrophils: WBC count, $13.7 \times 10^3/\mu\text{l}$ and neutrophil cell count, $11.5 \times 10^3/\mu\text{l}$. Her serum CRP level was elevated at 11.3 mg/dl (normal <0.20 mg/dl). The serum rheumatoid factor and antibodies including antinuclear, anti-SS-A, anti-SS-B, anti-DNA, anti-Sm, and anti-RNP antibodies, and the perinuclear anti-neutrophil cytoplasmic antibody (P-ANCA), and the cytoplasmic anti-neutrophil cytoplasmic antibody (C-ANCA) were all absent. Human leukocyte antigen (HLA) typing showed B-54 and CW1. CSF examination showed 108 cells/ mm^3 (mononuclear cells, 91; neutrophilic cells, 17), a total protein concentration of 41 mg/dl. A culture of the CSF sample was negative for bacteria, tuberculosis and fungi. Antibodies against herpes simplex virus, varicella zoster virus and toxoplasma were negative. PCR analysis also showed no herpes simplex virus. A brain MRI revealed increased signal intensity on T2-weighted and FLAIR images in the cortex and subcortical white matter of the left temporal lobe (Fig. 1-3). $^{99\text{m}}\text{Tc}$ -HMPAO SPECT performed on the third day of hospitalization revealed hyperperfusion in the left temporal lobe. An electroencephalogram showed diffuse slow activity with small spikes and sharp waves in the left temporal region. There were no ocular lesions such as uveitis, episcleritis and conjunctivitis. Neither oral aphthae nor genital ulcers were observed. We performed a malignancy survey including a whole-body CT, an examination by gastrointestinal endoscopy, a bone marrow aspiration study, and a gynecological consultation, all of which showed negative results. After admission she was treated with an intravenous infusion of antibiotics and acyclovir. Her consciousness was progressively disturbed and she suffered frequent generalized tonic seizures; therefore, at ten days after admission she required propofol treatment and mechanical respiratory management. A skin biopsy of the erythema on her right forearm was performed. Histological examination showed dense dermal infiltration of neutrophils with no signs of vasculitis, and as a result she was diagnosed with Sweet's disease. Corticosteroid therapy was initiated with an intravenous administration of methylprednisolone (1,000 mg/day for 3 days) from the tenth day of admission, followed by 50 mg of prednisolone administered orally. Her symptoms gradually improved by the end of January 2006 she no longer required mechanical ventilation. However, she continued suffering from a slight fever, and elevated levels of CRP and WBCs without signs of infection and presented with aphasia. As a result, she was treated with a second intravenous administration of methylprednisolone (1,000 mg/day for 3 days) in early February 2006. Subsequently, her symptoms and laboratory data improved, and she was discharged from the hospital without any sequelae about three weeks later.

Methods

Analysis of levels of cytokines and chemokines

We measured the levels of cytokines (i.e., IL-2, IL-4, IL-6, IL-10, IFN- γ , and TNF- α) and chemokines (i.e., CCL2/MCP-1, CCL3/MIP-1 α , CCL5/RANTES, CXCL8/IL-8, CXCL10/IP-10 and GM-CSF) in 10 CSF samples from the patient throughout the clinical course. We also measured the levels of those cytokines and chemokines in CSF samples from the control subjects. The control subjects for cytokines were 21 noninfected patients with neurological disorders (epilepsy, 8; psychomotor delay, 5; psychogenic response, 5; functional headache, 1; myopathy, 1; agenesis of corpus callosum, 1) and the control subjects for chemokines were 10 noninfected subjects with neurological disorders (functional headache, 3; Parkinson disease, 1; normal pressure hydrocephalus, 2; spinocerebellar degeneration, 2; amyotrophic lateral sclerosis, 2). CSF samples were obtained from them on routine analysis and they all had normal CSF cell counts. All upper values of control subjects are expressed as mean + 3SD.

Determination of cytokine levels

The levels of IFN- γ , TNF- α , IL-2, IL-4, IL-6, and IL-10 in CSF were measured with a cytometric bead array (CBA) kit (BD Pharmingen, San Diego, CA) as previously described (6-8), with the exception that data analysis was performed using GraphPad Prism software (GraphPad Prism Software, San Diego, CA). The lower detection limits for IFN- γ , TNF- α , IL-2, IL-4, IL-6, and IL-10 were 7.1 pg/mL, 2.8 pg/mL, 2.6 pg/mL, 2.6 pg/mL, 2.5 pg/mL, and 2.8 pg/mL, respectively.

Determination of chemokine levels

The levels of CCL2/MCP-1, CCL3/MIP-1 α , CCL5/RANTES, CXCL8/IL-8, and GM-CSF were measured using ELISA kits (Endogen, Woburn, MA, USA), and the concentration of CXCL10/IP-10 was measured using an ELISA kit (R&D Systems, Minneapolis, MN, USA) on the basis of the quantitative sandwich enzyme immunoassay technique, as previously described (9). The sensitivity of these assays was 10 pg/mL.

Statistical analysis

The Spearman rank correlation was calculated to assess the correlation between the levels of cytokines and total CSF cell counts, and the levels of chemokines and total CSF cell counts.

Results

Clinical course (Fig. 2)

Clinical manifestations and brain MRI findings correlated

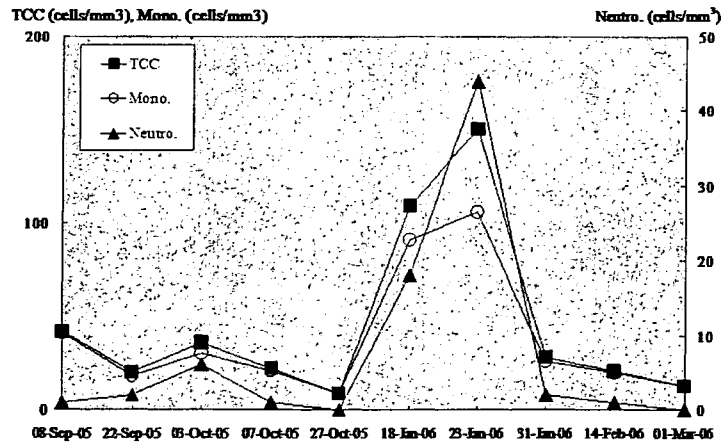


Figure 2. Cell counts [total cell count (TCC), mononuclear cell count (Mono.), neutrophilic cell count (Neuro.), cells/mm³] in CSF.

Table 1. The Levels (pg/mL) of Cytokines (1-1), Chemokines (1-2) and Total Cell Count [(TCC), Cells/mm³] in CSF

(1-1)

Date	8-Sep 2005	22-Sep 2005	3-Oct 2005	7-Oct 2005	27-Oct 2005	18-Jan 2006	23-Jan 2006	31-Jan 2006	14-Feb 2006	1-Mar 2006	<i>P</i> value
IL-6 (<12.1)*	209	14.5	255.8	12.3	9.3	2417.2	1329.4	182.7	13.6	12.6	<0.01
IL-4 (<14.3)*	7	<2.5	6	5	<2.5	17.6	13.2	4.4	<2.5	<2.5	<0.01
IL-2 (<5.5)*	2.7	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	ns
IFN- γ (<60.3)*	22.6	12.5	29.1	<7.1	<7.1	134.6	463.7	58	<7.1	<7.1	<0.01
TNF- α (<7.2)*	2.8	<2.8	<2.8	<2.8	<2.8	<2.8	<2.8	<2.8	<2.8	<2.8	ns
IL-10 (<7.2)*	4	3.3	4.6	<2.8	<2.8	5.9	5.3	2.9	<2.8	<2.8	<0.01
TCC	42	20	36	22	9	109	150	28	21	13	

(1-2)

Date	8-Sep 2005	22-Sep 2005	3-Oct 2005	7-Oct 2005	27-Oct 2005	18-Jan 2006	23-Jan 2006	31-Jan 2006	14-Feb 2006	1-Mar 2006	<i>P</i> value
MCP-1 (<1380)*	348.5	740.8	1344.6	730.6	962.5	1403.2	1217.2	689.9	1097.5	1235.1	ns
IL-8 (<55.23)*	198.5	146.2	283.6	96	86.5	449.4	441.4	264	50.9	80.7	<0.01
MIP-1 α (<10.05)*	24.4	18.5	25.2	17.5	17.5	23.7	49.6	26.7	14.5	25.9	ns
RANTES (<7.22)*	40	56	48	26.7	21.3	13.3	50.7	37.3	42.7	50.7	ns
IP-10 (<579.2)*	1880.5	632.1	2417.8	1511.8	605.8	3076.2	3176.3	2639	358.2	932.2	<0.01
TCC	42	20	36	22	9	109	150	28	21	13	

* the levels of CSF cytokines and chemokines of the control subjects (< mean + 3SD)

well with CSF cell counts. The disease activity was divided into active and inactive phases. September 8, 2005, October 3, 2005, January 18, 2006, and January 23, 2006 correspond to the active phases.

Cytokine levels (Table 1-1, Fig. 3)

The levels of IL-6 and IFN- γ in CSF were statistically correlated with total CSF cell counts ($p < 0.01$). The elevations of these cytokines were markedly higher than the lev-

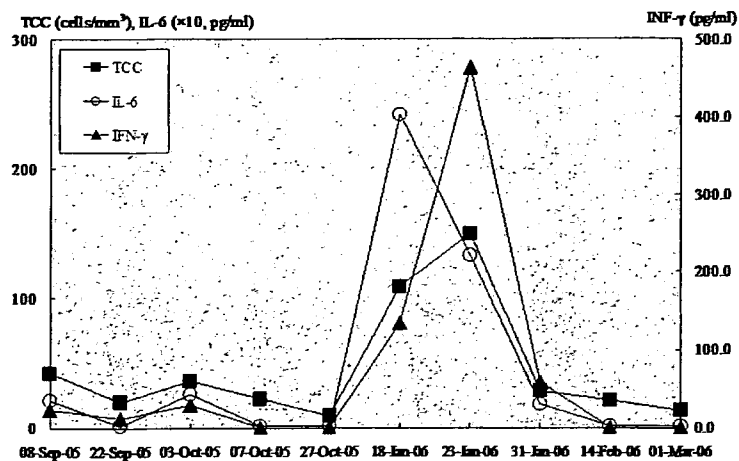


Figure 3. Levels of IL-6, IFN- γ (pg/mL) and total cell count [(TCC), cells/mm³] in CSF.

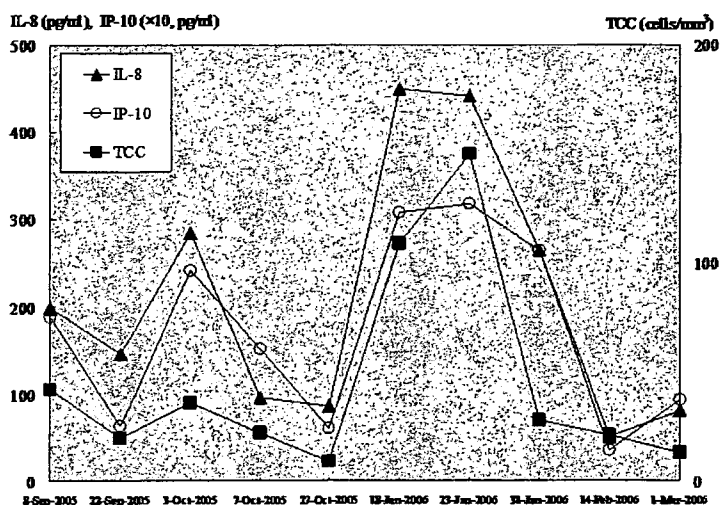


Figure 4. Levels of IL-8, IP 10 (pg/mL) and total cell count [(TCC), cells/mm³] in CSF.

els in 21 uninfected subjects with neurological disorders. The levels of IL-4 and IL-10 in CSF were also statistically correlated with total CSF cell counts. However, the elevations of these cytokines were almost within normal ranges of control subjects. The levels of IL-2 and TNF- α in CSF were equal to or below the detection limits. The levels of CSF cytokines of the control subjects are shown in Table 1-1.

Chemokine levels (Table 1-2, Fig. 4)

The levels of IL-8 and IP-10 in CSF were statistically correlated with total CSF cell counts ($p < 0.01$). The elevations of these chemokines were markedly higher than the levels in 10 uninfected subjects with neurological disorders. The levels of other chemokines in CSF also showed various changes during the follow-up period; however, there was no significant correlation between these levels and total CSF cell counts. The levels of GM-CSF in all of the CSF samples were below the detection limits. The levels of CSF chemokines of the control subjects are shown in Table 1-2.

Correlations between level of IL-8 in CSF and neutrophilic cell counts in peripheral blood and CSF

The level of IL-8 in CSF correlated with the neutrophilic cell count in CSF (Fig. 5-1). The level of IL-8 in CSF also correlated with the peripheral neutrophilic cell count except for during the active phase (October 3, 2005) at the first time hospitalization (Fig. 5-2).

Discussion

The patient's symptoms are compatible with probable NSD consistent with the criteria advocated by Hisanaga et al and the Neuro-Sweet Disease Study Group (2). The present patient's clinical features are summarized according to the following findings: 1. She presented with recurrent encephalomeningitis with subsequent acute pharyngitis and tonsillitis. 2. She had erythematous plaques on her cheek, forearms and legs. A histological examination of the skin biopsy revealed predominant neutrophilic infiltration of the dermis, spared epidermis, and the absence of leukocytoclastic vasculitis. 3. On HLA typing, B-54 and CW1 were positive, but B-51

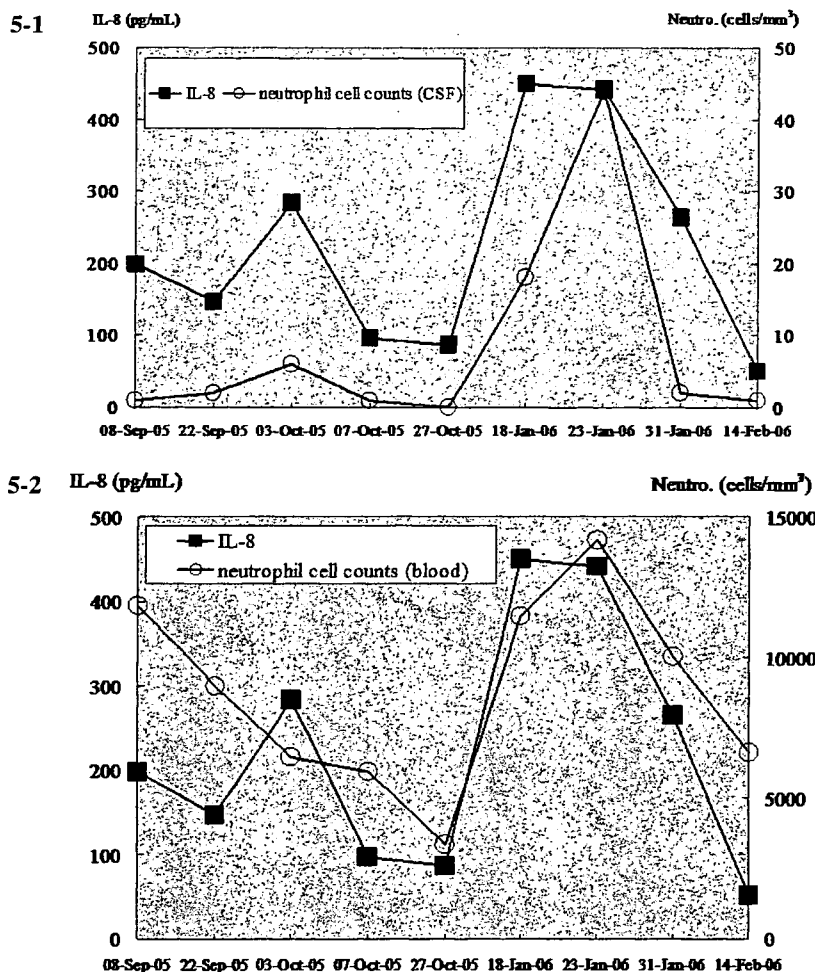


Figure 5. Correlation between level of IL-8 (pg/mL) in CSF and neutrophilic cell counts [(Neutro.), cells/mm³] in CSF (5-1) and peripheral blood (5-2).

was negative. 4. Antibiotics and antiviral therapy were not effective, but systemic glucocorticoids were so effective that the neurologic symptoms and laboratory findings markedly improved. 5. She did not display cutaneous vasculitis and thrombosis, which are seen in Behçet's disease. 6. Abnormal signal intensities on MRI were demonstrated in various CNS regions without site predilection.

The cytokines and chemokines in CSF that correlated well with the clinical state and total CSF cell counts were IL-6, IFN- γ , IL-8 and IP-10. CD4⁺ helper T (Th) cells can be divided into the Th1 and Th2 subtypes according to their cytokine secretion patterns (10-12). IFN- γ and IP-10, the levels of which increased in our patient, are Th1-type cytokines. Coincidentally, Th1-type cytokines have previously been implicated as mediators of the pathogenesis of Sweet's disease (13, 14). Our data suggest an important role of Th1 cells in the pathogenesis of NSD which is Sweet disease with CNS involvement. The levels of IL-4 and IL-10, which are Th2-type cytokines, were also statistically correlated with total CSF cell counts. However, the elevations of these cytokines were almost within normal ranges of control subjects. It is known that these cytokines in turn cause a decrease in the release of the Th1-type cytokines, thereby regulating the inflammatory response. We thought that the

elevations of these cytokines were induced by the elevations of the Th1-type cytokines. IFN- γ causes overexpression of adhesion molecules, responsible for neutrophilic adherence and diapedesis (13). There are multiple reports that suggest that neutrophil chemotactic dysfunction may be the basis of Sweet disease (15-17). In this study, the level of IL-8, a specific neutrophil chemoattractant, correlated with the neutrophil cell count in CSF indicating that NSD may also result from neutrophil chemotactic dysfunction. The level of GM-CSF, a neutrophil chemoattractant similar to IL-8, was below the detection limits. We were therefore unable to show any correlation for this chemokine. The increases in the levels of cytokines and chemokines in the CSF of the patient at the second hospitalization were generally higher than those at the first hospitalization. This finding may be attributed to the delay of the systemic glucocorticoid therapy at the second hospitalization.

Recently, the differences between NSD and NBD have been discussed (2, 18, 19). The present patient did not fulfill the criteria of BD (20) and the HLA type (Cw1 and B54) and histology of a skin biopsy from our patient corresponded to NSD, but not to NBD (2). There are several reports of BD that demonstrate an elevation in the levels of Th1-type cytokines in the serum of patients in the active

phase (21, 22) and in turn suggest that IL-8 could be a serological marker of disease activity (23, 24). In addition, elevated levels of IFN- γ and IL-6 in CSF are detectable in patients in the active phase of NBD (25, 26). Our cytokine data suggested that there are common aspect of pathogenesis between NSD and NBD.

Therapy with systemic glucocorticoids is usually effective in improving the neurologic symptoms in patients with NSD; however, like our patient, some patients occasionally experience recurrent episodes of neurological manifestations after glucocorticoid therapy is discontinued (1). Preventive therapies have not been established, but our study demonstrates that the levels of the Th1 cytokines, IL-6 and IL-8 in CSF are important markers of disease activity in patients with NSD. It is known that the treatment with IFN- β re-

duces the amount of Th1 proinflammatory cytokines and shifts the immune response toward a Th2 profile (27). Therefore, this treatment might have the potential to prevent the recurrence of NSD. We believe that these results provide useful information for clarifying the pathogenesis of NSD, which may contribute to the development of future therapeutic strategies.

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Short communication

Progressive multifocal leukoencephalopathy and CD4+ T-lymphocytopenia in a patient with Sjögren syndrome

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Abstract

We report progressive multifocal leukoencephalopathy (PML) and CD4+ T-lymphocytopenia in a 71-year-old man with Sjögren syndrome (SjS). The patient was admitted to our hospital because of progressive dementia and gait disturbance. T2-weighted MR images showed high-intensity lesions in his left frontal white matter, thalamus, cerebellum and brainstem. A pathological diagnosis of PML was made by brain biopsy. SjS is frequently accompanied with immunological complications; however, there are few reports on PML in patients with SjS. Recently, isolated CD4+ T-lymphocytopenia is reported to be one of the based immunological conditions associated with the development of PML. In the present case, CD4+ T-lymphocytopenia was also observed on admission, which is also associated with SjS. © 2007 Elsevier B.V. All rights reserved.

Keywords: JC virus; Progressive multifocal leukoencephalopathy; Sjögren syndrome; CD4+ T-lymphocytopenia; ¹²³I-IMP-SPECT; Brain biopsy

1. Introduction

Progressive multifocal leukoencephalopathy (PML), which is caused by the JC virus (JCV), usually develops as a central nervous system (CNS) opportunistic infectious disease in immunocompromised patients, such as those with acquired immunodeficiency syndrome (AIDS), a variety of collagen diseases, or those who are undergoing steroid hormone or immunosuppressive therapy [1]. Sjögren syndrome (SjS) is one of the collagen diseases and highly accompanied with immunological complications. However, to date, the disease has not been considered to be a high risk factor of PML. Here we report a rare case of PML

developing in an aged patient with SjS accompanied with CD4+ T-lymphocytopenia.

2. Case report

The patient, a 71-year-old Japanese man with a 5-year history of primary SjS, was admitted to our hospital because of progressive dementia and gait disturbance that were observed 2 months and 1 month before his visit, respectively. His first symptom of SjS was dry mouth. His diagnosis of SjS was made 5 years ago by Saxon and Shirmer tests, scintigraphy of the parotid and submandibular glands, and lip biopsy that revealed many lymphocytes and plasma cells infiltrating the small salivary glands. Several months after the diagnosis of SjS, he developed interstitial pneumonia associated with SjS and required oxygen therapy at home. Since he was suffering from myocardial infarction, and

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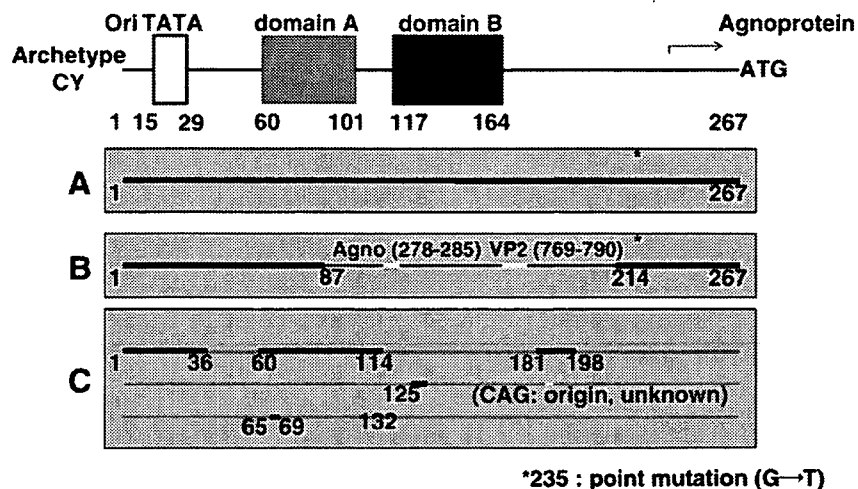


Fig. 1. The nested PCR for transcriptional control region (TCR) of the JCV test detected three types of TCR in the CSF. A: archetype with point mutation (235 G→T) virus. B and C: two different types of rearrangement of TCR virus.

chronic ischemic heart disease, he was not administered corticosteroid or immunosuppressive therapy for SjS at any time before he visited our hospital.

On admission, he was afebrile, his blood pressure was 130/64 mmHg, his heart rate was 70 bpm and regular, his heart sounds had systolic murmur (Levine 3/IV), his respiratory sounds had fine crackles in the bilateral inferior areas of the chest, and no cervical or axially lymph nodes were palpable. Neurological examination revealed dementia, right hemiparesis, truncal and limb ataxia, normal deep tendon reflex, positive Babinski and Chaddock signs on the right side, and forced grasping in both hands.

Laboratory tests of his peripheral blood showed normal WBC counts with low percentage of lymphocytes; his CD4+ T-lymphocyte count of 272 cells/ μ l (%CD4; 16.1% (normal, 25–60%)) with a decline to 217 cells/ μ l (%CD4; 13.6%) within 1 month; and C-reactive protein level was 2.68 mg/dl. The serological tests for antibodies against HIV-1/-2, HTLV-1 and HBs were negative. The serological tests for anti-HCV antibodies were weakly positive; the result of the HCV RNA-PCR was negative. The syphilis serological tests (TPHA, STS) were negative. Autoimmune serological examination demonstrated 1280 \times antinuclear antibodies. However, the serological tests for other antibodies, including those to Rheumatoid factor, ds-DNA-IgG, Sm, SS-A, SS-B, RNP, and Scl-70, were entirely negative. Other laboratory findings were unremarkable, including the tumor markers. Analysis of his CSF revealed normal pressure and normal parameters with respect to cell count and chemical analysis (cell count; 3 mononuclear cell/ μ l, protein concentration; 24 mg/dl, glucose concentration; 56 mg/dl). Concentration of soluble IL2-receptor, β 2 microglobulin and ferritin were not elevated in CSF. Oligoclonal bands (OCBs) were detected in CSF. Nested PCR revealed 3 types of JCV transcriptional genomes, including an archetype in CSF (Fig. 1). Whole body CT and 67 Ga-citrate scintigraphy revealed no malignancy in his body.

Brain MRI showed T1-weighted images with low-intensity lesions, and T2-weighted and FLAIR images with high-intensity lesions in the left frontal white matter, bilateral parietal and left occipital white matter, left thalamus, and right middle cerebellar peduncle, which were not enhanced by Gadolinium (Fig. 2). Spinal cord MRI showed no obvious abnormalities. Serial images of early, 6-hour delayed and 24-hour delayed 123 I-IMP SPECT (IMP-SPECT) showed a gradual increase in radioactivity and a gradual increase in amount and a long-term retention of the tracer in the left frontal white matter (Fig. 3). Brain biopsy from the left frontal deep white matter revealed disseminated enlarged oligodendrocytic nuclei that were immunopositive with rabbit polyclonal antibody against JCV-VP1 protein (Fig. 4) [2], JCV-agnoprotein and a large T protein (data not shown).

Treatments with steroid pulse therapy (methylprednisolone 1 g/day, 3 days) and cytarabine (AraC 2 mg/kg/day, 5 days) were not effective, and the patient developed akinetic mutism approximately 3 months after the onset of PML, and



Fig. 2. Axial brain MRI on 1.5-tesla demonstrating multifocal high-intensity areas in left frontal and bilateral periventricular white matters, and right middle cerebellar peduncle on FLAIR (TE 144, TR 8002, TI 2000) images.

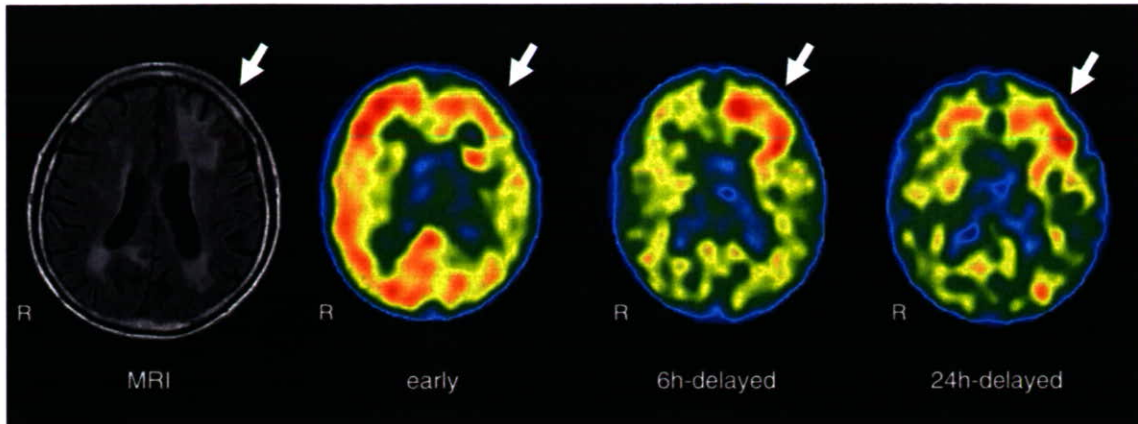


Fig. 3. Axial FLAIR MRI and Serial images of ^{123}I -IMP SPECT: early image, 6-hour delayed image and 24-hour delayed image. These serial images shows a delayed increase of radioactivity, a gradual increase and long term retention of the tracer on the left frontal white matter.

he has been alive for 18 months without any improvement in clinical state.

3. Discussion

We have described a case of PML with SjS, in which progressive dementia, right hemiparesis, and truncal and limb ataxia were the clinical features.

In this case, tumefactive multiple sclerosis (MS), cerebral vasculitis with SjS and primary CNS malignant lymphoma were initially considered as differential diagnoses from the MRI findings.

IMP-SPECT images demonstrated delayed high accumulation of IMP in the left frontal white matter. Primary CNS malignant lymphoma, malignant astrocytoma, and metastatic brain tumors, including malignant melanoma, have been reported to be the lesions to show high accumulation of IMP on delayed IMP-SPECT images [3–6]. Compared with that in normal brain tissues, a small amount of IMP is retained in tumor cells due to the differences in the number of amine receptors, fat distribution and tissue pH [6]. Recently it has been reported that IMP-SPECT shows the delayed accumulation of IMP in tumefactive MS lesions. Therefore, it appears to be a pitfall for distinguishing between MS and malignant lymphoma [7]. The mechanism underlying IMP delayed accumulation is still unclear, but we speculate a small amount of IMP is retained in JCV infected oligodendrocytes as well as in tumor cells.

OCBs were detected in our patient's CSF. OCBs are frequently detected in MS, and in some infectious diseases (meningitis, subacute sclerosing panencephalitis (SSPE), and PML), and other neurological diseases. OCBs observed in SSPE and PML are measles virus-specific [8] and JCV-specific IgG antibodies [9], respectively. Detecting OCBs in CSF is associated with intrathecal synthesis of IgG, and is not pivotal in distinguish between MS and other neurological diseases.

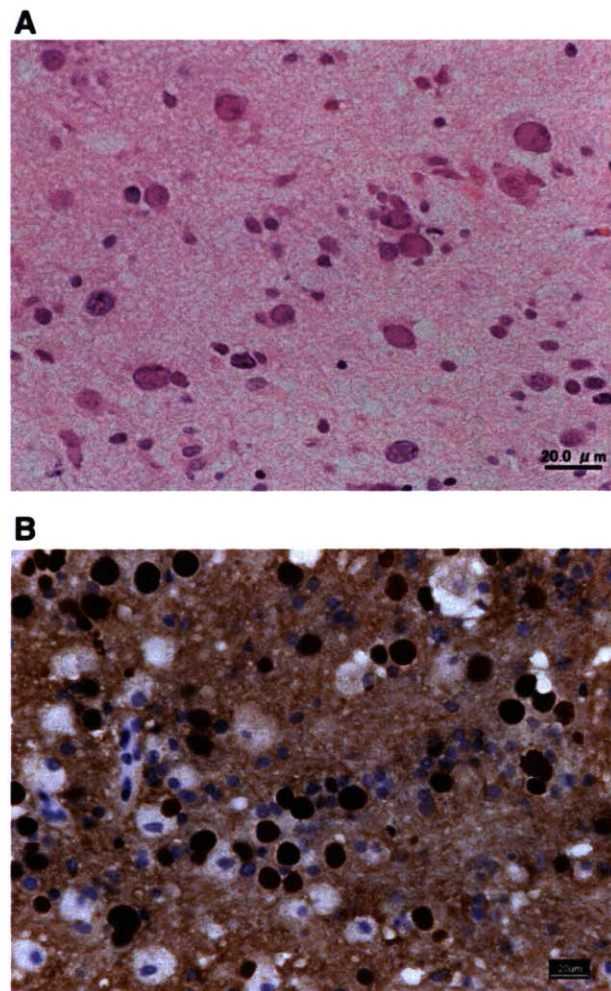


Fig. 4. (A) Brain section stained with hematoxylin-eosin, showing enlarged oligodendrocytic nuclei filled with somewhat basophilic substance in the white matter. Bar 20 μm . (B) Brain section immunostained with antibody against JCV-VP1, demonstrating that such enlarged oligodendrocytic nuclei are clearly positive for this protein. Bar 20 μm .

In the present case, we eventually considered that brain biopsy was necessary for making an accurate diagnosis of the brain lesions; a small brain tissue was collected from the left frontal white matter, where delayed accumulation of IMP was evident. The biopsy specimen showed many enlarged oligodendrocytic nuclei immunopositive for JCV-VP1 protein in demyelinated lesions. Subsequently, the presence of PML types of JCV DNA was also detected in the patient's CSF. Interestingly, the other Sjögren syndrome's patient with PML was from Japan [10]. This case was associated with acute myelocytic leukemia during the clinical course of SJS, and SJS itself was not considered to be the basic disease [10].

Recently, several reports have shown the development of PML in patients with idiopathic CD4⁺ T-lymphocytopenia (ICL) [11–16]. ICL is a recently described syndrome characterized by a marked decrease in the number of circulating CD4⁺ T-lymphocytes in the absence of any identifiable causes of immunologic abnormalities, and can be defined by the presence of a documented absolute number of CD4⁺ T-lymphocytes <300/μl or a CD4⁺ cell count of <20% of the total T cells on two occasions, no evidence of HIV infection, and the absence of any defined immunodeficiency or therapy leading to the decrease in the CD4⁺ T cell level [17]. It has also been reported that according to the definition mentioned above, 5.2% of SJS patients have CD4⁺ T-lymphocytopenia [18]. Therefore, we also studied the presence or absence of this condition in the present case. As a result, the laboratory data obtained fulfilled the definition of this syndrome.

The patient has been alive but has not shown any improvement for 18 months since the onset of the disease: this is an unusually long course for the disease. Generally, the clinical course of PML in the absence of an HIV infection is rapidly progressive, and most patients die within a year. On the other hand, it was reported that PML patients with ICL tend to survive for over a year [12,14,15].

In conclusion, it is now necessary to consider PML as one of the differential diagnoses in patients with SJS showing leukoencephalopathy and also to study the number of CD4⁺ T cells in such patients.

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抗GluR ϵ 2抗体陽性成人急性脳炎患者の臨床的特徴 ならびに免疫組織学的解析

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NMDA型グルタミン酸受容体(GluR) ϵ 2サブユニットは、辺縁系ならびに大脳皮質を含む前脳領域に発現し¹⁾、記憶・学習に重要な役割を果たしていることが知られている²⁾。著者らは、成人急性脳炎および脳症患者の血清および髄液検体を用い抗GluR ϵ 2抗体の測定をリコンビナント蛋白を用いたWestern blot法により施行し³⁾、同抗体を有する急性脳炎および脳症の臨床的特徴の検討と免疫組織学的解析を行った⁴⁾。

ⅢⅢⅢ 抗GluR ϵ 2抗体陽性脳炎

2004年より当科で経験した成人急性脳炎および脳症患者連続例計23例の血清と22例の髄液検体を用い、抗GluR ϵ 2抗体を測定した。症例の内訳は、急性散在性脳脊髄炎(ADEM)1例、細菌性髄膜炎1例、CNSループス6例、クリプトコッカス髄膜炎1例、原因不明の髄膜炎1例、ミトコンドリア脳筋症(MELAS)1例、intravascular malignant lymphoma 1例、神経Sweet

表 1 NPNHLE 6例の抗GluR ϵ 2抗体測定結果

case	age/sex	serum IgM/IgG	CSF IgM/IgG
1	45/F	+/+	+/+
2	30/M	+/+	+/-
3	53/F	+/+	±/+
4	62/M	-/-	+/+
5	82/F	+/-	-/-
6	22/F	-/-	-/-

病1例、非傍腫瘍性非ヘルペス性辺縁系脳炎(non paraneoplastic, non herpetic limbic encephalitis : NPNHLE) 6例、抗Hu抗体陽性傍腫瘍性辺縁系脳炎1例、インフルエンザ脳症1例、海綿状血管腫に伴う脳出血1例、脳幹脳炎1例であった。23例中9例の血清中に、22例中6例の髄液中にIgM型とIgG型の抗GluR ϵ 2抗体を検出した。

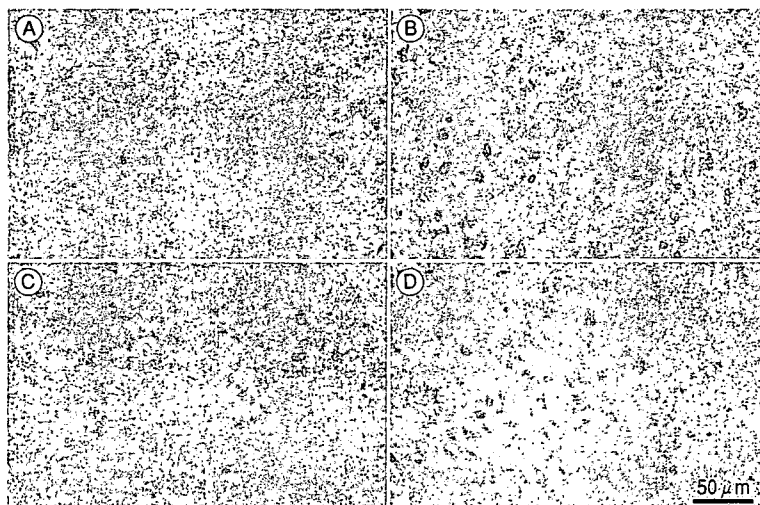


図 1 患者血清を用いた免疫組織学的解析⁴⁾

A, B : case 1 血清(1 : 2000), C, D : case 2 血清(1 : 2000).

ラット脳海馬(A, C)および大脳皮質(B, D)ニューロンの細胞質が染色される。Case 3 血清および抗GluR ϵ 2抗体を用いた免疫染色でも同様のパターンが確認された。