Lupus (2008) 17, 16–20
http://lup.sagepub.com

PAPER

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

A Kimura[1]*, T Sakurai[1], Y Tanaka[1], I Hozumi[1], K Takahashi[2], M Takemura[2], K Saito[2], M Seishima[2] and T Inuzuka[1]

[1]Department of Neurology and Geriatrics, Gifu University Graduate School of Medicine, Gifu City, Gifu, Japan; and
[2]Department of Informative Clinical Medicine, Gifu University Graduate School of Medicine, Gifu City, Gifu, Japan

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; 60 kDa 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

*Correspondence: Dr Akio Kimura, Department of Neurology and Geriatrics, Gifu University Graduate School of Medicine, Gifu, 1-1 Yanagido, Gifu City, Gifu 501-1194, Japan. E-mail: kimura1@gifu-u.ac.jp
Received 28 July 2007; accepted 18 September 2007

10.1177/0961203307085112

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/μl, normal 3400–9200/μl) and lymphocyte cell count (1340/μl, normal 646–4177/μl); and coagulation-fibrinolysis abnormalities [increases in the levels of fibrinogen/fibrin degradation products (FDP) (11.3 μg/mL, normal ≤4.0 μg/mL), D-dimer (1.5 μg/mL, normal ≤1.0 μg/mL) and alpha2-plasmin inhibitor-plasmin complex (PIC) (1.4 μg/mL, normal ≤0.8 μg/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, such as hypertension, hyperlipidemia and diabetes mellitus.

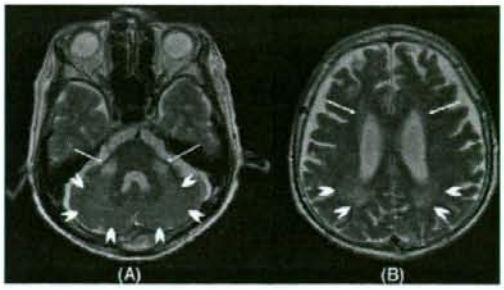


Figure 1 T2-weighted brain MR images (SE, TR/TE: 4080/100 ms). Brain MRI showed symmetrical hyperintensity lesions in bilateral cerebellar hemispheres (arrowheads), middle cerebellar peduncles (arrows) (A), periventricular white matter (arrows) and (B), deep white matter (arrowheads).

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 × 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 μg 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 polyvinylidine 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 tetrametylbenzidine 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).

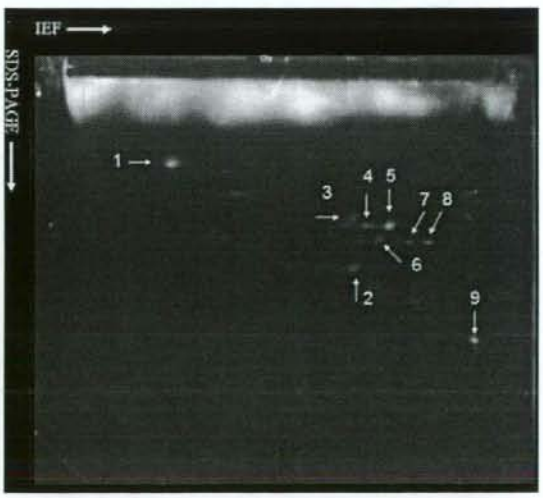


Figure 2 Two-dimensional electrophoresis (2DE) and western blotting (WB). Nine spots strongly reacted with autoantibodies in patient's serum on 2DE-WB. Five spots (No. 2–6) were analysed using mass spectrometry. No. 2: Beta actin; No. 3: Alpha-internexin; No. 4 and 5: 60 kD heat-shock protein (Hsp60); 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

[a]Spot number corresponds to the number shown in Figure 2.
[b]Alpha-Inx, Alpha-internexin.
[c]Hsp 60, 60 kD heat-shock protein.
[d]GFAP, 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 severity[19] 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.

Acknowledgements

This research was partially supported by a Japanese Health and Labour Sciences Research Grant for Research on Psychiatry and Neurological Diseases and Mental Health (H18-026, H17-017) and a Grant-in-Aid for Young Scientists (B), 1690486 from the Japanese Ministry of Education, Culture, Sports, Science and Technology and a Grant-in-Aid for Scientific Research No. 17591133.

References

1 Bell CL, Partington C, Robbins M, Graziano F, Turski P, Kornguth S. Magnetic resonance imaging of central nervous system lesions in patients with lupus erythematosus. Correlation with clinical remission and antineurofilament and anticardiolipin antibody titers. *Arthritis Rheum* 1991; **34**: 432–441.

2 Sanna G, Piga M, Terryberry JW *et al.* Central nervous system involvement in systemic lupus erythematosus: cerebral imaging and serological profile in patients with and without overt neuropsychiatric manifestations. *Lupus* 2000; **9**: 573–583.

3 Hachulla E, Michon-Pasturel U, Leys D *et al.* Cerebral magnetic resonance imaging in patients with or without antiphospholipid antibodies. *Lupus* 1998; **7**: 124–131.

4 Markus HS, Hunt B, Palmer K, Enzinger C, Schmidt H, Schmidt R. Markers of endothelial and hemostatic activation and progression of cerebral white matter hyperintensities. Longitudinal results of the Austrian stroke prevention study. *Stroke* 2005; **36**: 1410–1414.

5 Gunning-Dixon FM, Raz N. The cognitive correlates of white matter abnormalities in normal aging: a quantitative review. *Neuropsychology* 2000; **14**: 224–232.

6 Sachdev PS, Wen W, Christensen H, Jorm AF. White matter hyperintensities are related to physical disability and poor motor function. *J Neurol Neurosurg Psychiatry* 2005; **76**: 362–367.

7 Mosley TH, Knopman DS, Catelier DJ *et al.* Cerebral MRI findings and cognitive functioning. The atherosclerosis risk in communities study. *Neurology* 2005; **64**: 2056–2062.

8 ACR Ad Hoc Committee On Neuropsychiatric Lupus Nomenclature. The American College of Rheumatology nomenclature and case definitions for neuropsychiatric lupus syndromes. *Arthritis Rheum* 1999; **42**: 599–608.

9 Hochbreg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1997; **40**: 1725.

10 Bluestein HG, Zvaifler NJ. Brain-reactive lymphocytotoxic antibodies in the serum of patients with systemic lupus erythematosus. *J Clin Invest* 1976; **57**: 509–516.

11 Bonfa E, Golombek SJ, Kaufman LD *et al.* Association between lupus psychosis and anti-ribosomal P protein antibodies. *N Engl J Med* 1987; **317**: 265–271.

12 Isshi K, Hirohata S. Differential roles of the anti-ribosomal P antibody and antineuronal antibody in the pathogenesis of central nervous system involvement in systemic lupus erythematosus. *Arthritis Rheum* 1982; **25**: 1271–1277.

13 Song J, Park YB, Lee WK, Lee KH, Lee SK. Clinical associations of anti-endothelial cell antibodies in patients with systemic lupus erythematosus. *Rheumatol Int* 2000; **20**: 1–7.

14 Meroni PL, Tincani A, Sepp N *et al.* Endothelium and the brain in CNS lupus. *Lupus* 2003; **12**: 919–928.

15 Dieudé M, Senécal JL, Raymond Y. Induction of endothelial cell apoptosis by heat-shock protein 60-reactive antibodies from anti-endothelial cell autoantibody-positive systemic lupus erythematosus patients. *Arthritis Rheum* 2004; **50**: 3221–3231.

16 Martin J, Horwich AL, Hartl FU. Prevention of protein denaturation under heat stress by the chaperonin Hsp60. *Science* 1992; **258**: 995–998.

17 Jamin C, Dugué C, Alard JE *et al.* Induction of endothelial cell apoptosis by the binding of anti-endothelial cell antibodies to Hsp60 in vasculitis-associated systemic autoimmune diseases. *Arthritis Rheum* 2005; **52**: 4028–4038.

18 Schett G, Xu Q, Amberger A *et al.* Autoantibodies against HSP60 mediate endothelial cytotoxicity. *J Clin Invest* 1995; **96**: 2569–2577.

19 Zhu J, Katz RJ, Quyyumi AA *et al.* Antibodies to HSP60 are associated with the presence and severity of coronary artery disease: evidence for an autoimmune component of atherogenesis. *Circulation* 2001; **103**: 1071–1075.

20 Bason C, Corrocher R, Lunardi C *et al.* Interaction of antibodies against cytomegalovirus with heat-shock protein 60 in pathogenesis of atherosclerosis. *Lancet* 2003; **362**: 1971–1977.

21 Trysberg E, Nylen K, Rosengren LE, Tarkowski A. Neuronal and astrocytic damage in systemic lupus erythematosus patients with central nervous system involvement. *Arthritis Rheum* 2003; **48**: 2881–2887.

22 Valesini G, Alessandri C, Celestino D, Conti F. Anti-endothelial antibodies and neuropshychiatric systemic lupus erythematosus. *Ann NY Acad Sci* 2006; **1069**: 118–128.

23 Boulassel MR, Tomasi JP, Deggouj N, Gersdorff M. Identification of beta-actin as a candidate autoantigen in autoimmune inner ear disease. *Clin Otolaryngol* 2000; **25**: 535–541.

24 Rajasalu T, Teesalu K, Janmey PA, Uibo R. Demonstration of natural autoantibodies against the neurofilament protein α-internexin in sera of patients with endocrine autoimmunity and healthy individuals. *Immunol Lett* 2004; **94**: 153–160.

INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

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

Akio Kimura[1], Takeo Sakurai[1], Akihiro Koumura[1], Yoshihiro Suzuki[1], Yuji Tanaka[1], Isao Hozumi[1], Hideto Nakajima[2], Takashi Ichiyama[3] and Takashi Inuzuka[1]

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 encephalomeningitis 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 encephalomeningitis.

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

(DOI: 10.2169/internalmedicine.47.0370)

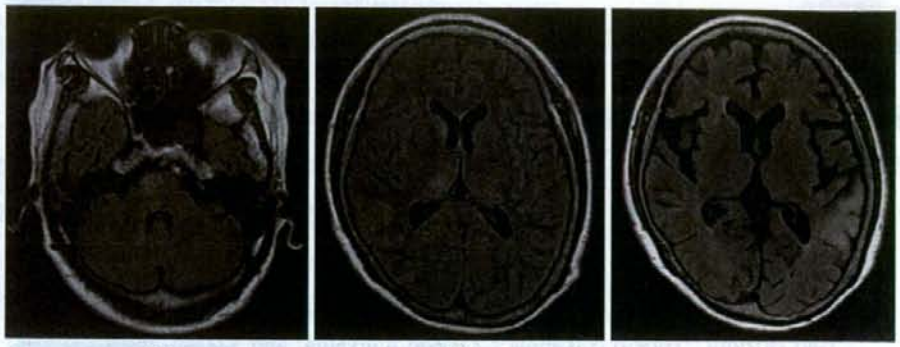
Introduction

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 patients with NSD also typically show high levels of HLAs B 54 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

[1]Department of Neurology and Geriatrics, Gifu University Graduate School of Medicine, Gifu, [2]Division of Neurology, First Department of Internal Medicine, Osaka Medical College, Takatsuki and [3]Department of Pediatrics, Yamaguchi University School of Medicine, Yamaguchi
Received for publication June 6, 2007; Accepted for publication October 3, 2007
Correspondence to Dr. Akio Kimura, kimura1@gifu-u.ac.jp

DOI: 10.2169/internalmedicine.47.0370



1-1 1-2 1-3

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 tonsilitis, 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℃, 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 semicoma 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, $15.1\times10^3/\mu l$ (normal range: $3.3\times10^3\sim7.9\times10^3/\mu l$) and neutrophil cell count, $13.9\times10^3/\mu l$ (normal range $1.5\times10^3\sim5.9\times10^3/\mu l$). Her serum C reactive protein (CRP) level was 18.8 mg/dl (normal <0.20 mg/dl). CSF examination showed 341 cells/mm^3 (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 electroencephalogram 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 tonsilitis. 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℃, 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

DOI: 10.2169/internalmedicine.47.0370

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, $13.7\times10^3/\mu l$ and neutrophil cell count, $11.5\times10^3/\mu l$. 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). ^{99m}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 obserbed. 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 administrated 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 Feburary 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-1α, 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 degenetration, 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-1α, 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

DOI: 10.2169/internalmedicine.47.0370

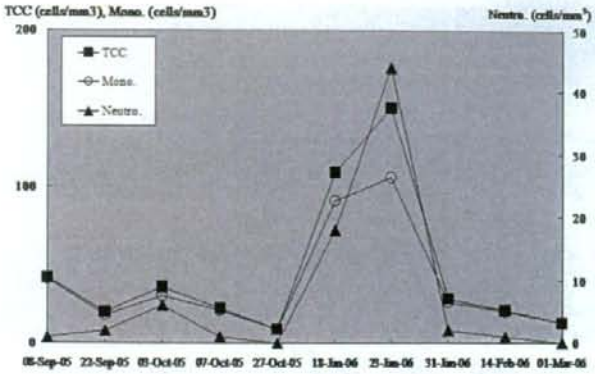


Figure 2. **Cell counts [total cell count (TCC), mononuclear cell count (Mono.), neutrophilic cell count (Neutro.), cells/mm^3] in CSF.**

Table 1. **The Levels (pg/mL) of Cytokines (1-1), Chemokines (1-2) and Total Cell Count [(TCC), Cells/mm^3] 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	*P 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	*P 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-1α (<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-

DOI: 10.2169/internalmedicine.47.0370

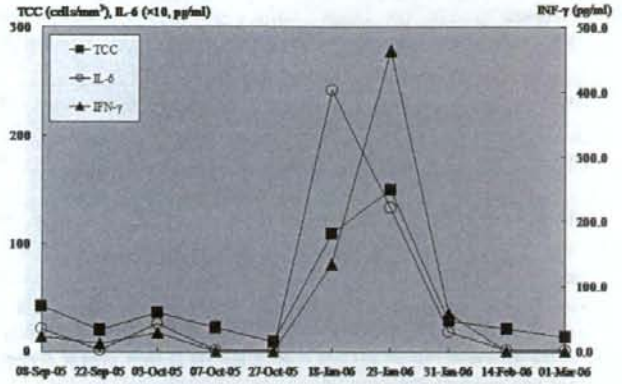


Figure 3. Levels of IL-6, IFN-γ (pg/mL) and total cell count [(TCC), cells/mm³] in CSF.

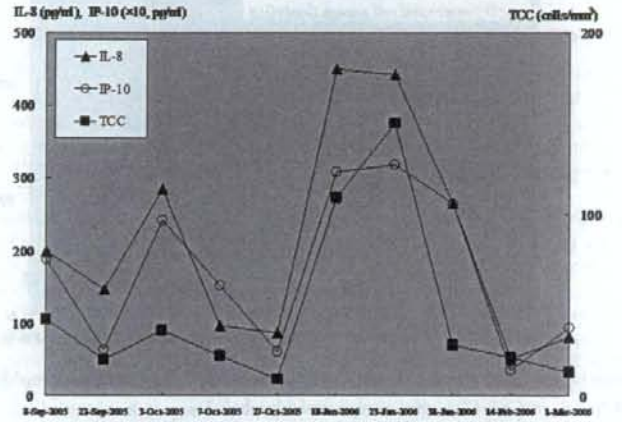


Figure 4. Levels of IL-8, IP 10 (pg/mL) and total cell count [(TCC), cells/mm³] in CSF.

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 tonsilitis. 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

DOI: 10.2169/internalmedicine.47.0370

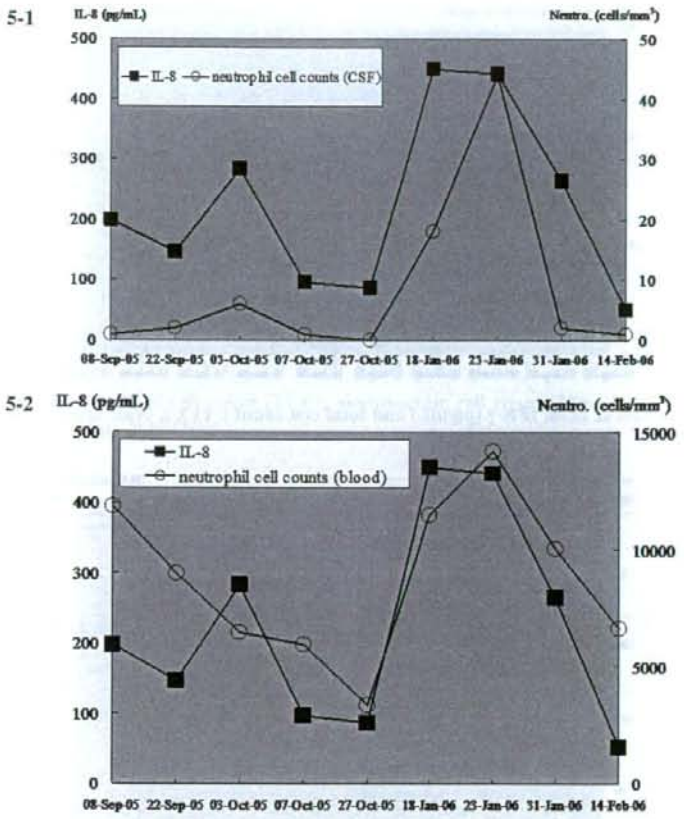


Figure 5. Correlation between level of IL-8 (pg/mL) in CSF and neutrophilic cell counts [(Neutro.), cells/mm³] in CSF (5-1) and peripheral blood (5-2).

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 reduces 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 reccurence 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.

This research was partially supported by a Grant-in-Aid for Young Scientists (B), 1690486 from the Japanese Ministry of Education, Culture, Sports, Science and Technology, a Grant-in-Aid for Scientific Research INo. 17591133 and a Health and Labor Sciences Research Grant for Research on Psychiatry and Neurological Diseases and Mental Health (H18-026).

References

1. Hisanaga K, Hosokawa M, Sato N, Mochizuki H, Itoyama Y, Iwasaki Y. "Neuro-Sweet disease": Benign recurrent encephalitis with neutrophilic dermatosis. Arch Neurol **56**: 1010-1013, 1999.
2. Hisanaga K, Iwasaki Y, Itoyama Y, Neuro-Sweet Disease; Study Group. Neuro-Sweet disease: Clinical manifestations and criteria for diagnosis. Neurology **64**: 1756-1761, 2005.
3. Mizoguchi M, Matsuki K, Mochizuki M, et al. Human leukocyte antigen in Sweet's syndrome and its relationship to Behçet's disease. Arch Dermatol **124**: 1069-1073, 1998.
4. Noda K, Okuma Y, Fukae J, et al. Sweet's syndrome associated with encephalitis. J Neurol Sci **188**: 95-97, 2001.
5. Druschky A, von den Dreisch P, Anders M, Claus D, Neundorfer B. Sweet's sndrome (acute febrile neutrophilic dermatosis) affecting the central nervous system. J Neurol **243**: 556-557, 1996.
6. Chen R, Lowe L, Wilson JD, et al. Simultaneous quantification of six human cytokines in a single sample using microparticle-based flow cytometric technology. Clin Chem **45**: 1693-1694, 1999.
7. Cook EB, Stahl JL, Lowe L, et al. Simultaneous measurement of six cytokines in a single sample of human tears using microparticle-based flow cytometry: allergics vs. non-allergics. J Immunol Methods **254**: 109-118, 2001.
8. Metelitsa LS, Naidenko OV, Kant A, et al. Human NKT cells mediate antitumor cytotoxicity directly by recognizing target cell CD1d with bound ligand or indirectly by producing IL-2 to activate NK cells. J Immunol **167**: 3114-3122, 2001.
9. Nakajima H, Fukuda K, Doi Y, et al. Expression of TH1/TH2-related chemokine receptors on peripheral T cells and correlation with clinical disease activity in patients with multiple sclerosis. Eur Neurol **52**: 162-168, 2004.
10. Romagnani S. The Th1/Th2 paradigm. Immunol Today **18**: 263-266, 1997.
11. Mosmann TR, Coffman RL. Th1 and Th2 cells: different patterns of lymphokine secretion lead to different functional properties. Annu Rev Immunol **7**: 145-173, 1989.
12. Abbas AK, Murphy KM, Sher A. Functional diversity of helper T lymphocytes. Nature **383**: 787-793, 1996.
13. Giasuddin AS, El-Orfi AH, Ziu MM, El-Barnawi NY. Sweet's syndrome: Is the pathogenesis mediated by helper T cell type 1 cytokines? J Am Acad Dermatol **39**: 940-943, 1998.
14. Nifosi G. Sweet syndrome: personal experience and review of the literature. Minerva Med **92**: 49-55, 2001.
15. Park JW, Mehrotra B, Barnett BO, Baron AD, Venook AP. The Sweet syndrome during therapy with granulocyte colony-stimulating factor. Ann Intern Med **116**: 996-998, 1992.
16. Kumar G, Bernstein JM, Waibel JS, Baumann MA. Sweet's syndrome associated with sargramostim (granulocyte-macrophage colony stimulating factor) treatment. Am J Hematol **76**: 283-285, 2004.
17. Watarai K, Tojo A, Nagamura-Inoue T, et al. Hyperfunction of neutrophils in a patient with BCR/ABL negative chronic myeloid leukemia: a case report with in vitro studies. Cancer **89**: 551-560, 2000.
18. Iwatsuki K, Deguchi K, Narai H, et al. A case of neuro-Behçet's disease with HLA B54 and predominant cerebral white matter lesions. Rinsho Shinkeigaku **40**: 921-925, 2000 (in Japanese).
19. Uysal H, Vahaboglu H, Inan L, Vahaboglu G. Acute febrile neutrophilic dermatosis (Sweet's syndrome) in neuro-Behçet's disease. Clin Neurol Neurosurg **95**: 319-322, 1993.
20. International Study, Group for Behçet's Disease. Criteria for diagnosis of Behçet disease. Lancet **335**: 1078-1080, 1990.
21. Frassanito MA, Dammacco R, Cafforio P, Dammacco F. Th1 polarization of the immune response in Behçet's disease. A putative pathogenetic role of interleukin-12. Arthritis Rheum **42**: 1967-1974, 1999.
22. Raziuddin S, Al-Dalaan A, Bahabri S, Siraj AK, al-Sedairy S. Divergent cytokine production profile in Behçet's disease. Altered Th1/Th2 cell cytokine pattern. J Rheumatol **25**: 329-333, 1998.
23. al-Dalaan A, al-Sedairy S, al-Balaa S, et al. Enhanced interleukin 8 secretion in circulation of patients with Behçet's disease. J Rheumatol **22**: 904-907, 1995.
24. Gur-Toy G, Lenk N, Yalcin B, Aksaray S, Alli N. Serum interleukin-8 as a serologic marker of activity in Behçet's disease. Int J Dermatol **44**: 657-660, 2005.
25. Hirohata S, Isshi K, Oguchi H, et al. Cerebrospinal fluid interleukin-6 in progressive neuro-Behçet's syndrome. Clin Immunol Immunopathol **82**: 12-17, 1997.
26. Hirayama M, Kiyosawa K, Nakazaki S, Fujiki N, Iida M. Measurement of gamma-interferon in sera and CSF in patients with multiple sclerosis and inflammatory neurological diseases. Rinsho Shinkeigaku **30**: 557-559, 1990 (in Japanese).
27. Markowiitz CE. Interferon-beta: Mechanism of action and dosing issues. Neurology **68**: 8-11, 2007.

ELSEVIER

Journal of the Neurological Sciences 268 (2008) 195–198

Journal of the Neurological Sciences

www.elsevier.com/locate/jns

Short communication

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

Yuichi Hayashi [a,*], Akio Kimura [a], Shimon Kato [a], Akihiro Koumura [a], Takeo Sakurai [a], Yuji Tanaka [a], Isao Hozumi [a], Yuji Sunden [b,c], Yasuko Orba [b], Hirofumi Sawa [b,c], Hitoshi Takahashi [d], Takashi Inuzuka [a]

[a] *Department of Neurology and Geriatrics, Gifu University Graduate School of Medicine, 1-1 Yanagido, Gifu, Gifu 501-1194, Japan*
[b] *Department of Molecular Pathobiology, Hokkaido University Research Center for Zoonosis Control, Sapporo, Japan*
[c] *21st Century COE Program for Zoonosis Control, Sapporo, Japan*
[d] *Department of Pathology, Brain Research Institute, Niigata University, Niigata, Japan*

Received 17 September 2007; received in revised form 3 December 2007; accepted 4 December 2007
Available online 29 January 2008

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.

Keywords: JC virus; Progressive multifocal leukoencephalopathy; Sjögren syndrome; CD4+ T-lymphocytopenia; ^{123}I-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

* Corresponding author. Tel.: +81 58 230 6254; fax: +81 58 230 6252.
E-mail address: hayashiy@gifu-u.ac.jp (Y. Hayashi).

0022-510X/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jns.2007.12.015

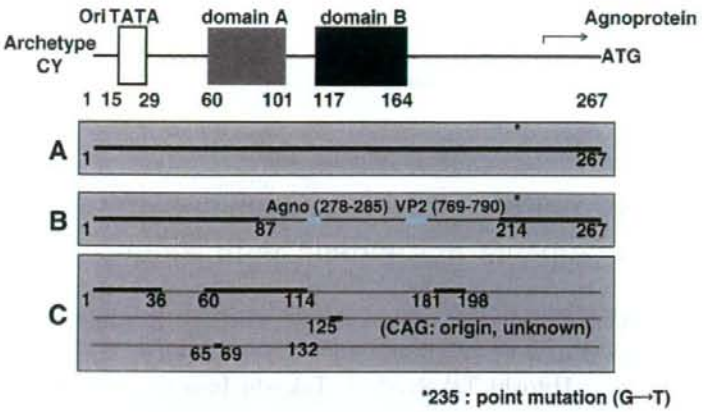


Fig. 1. The nested PCR for transcriptional control region (TCR) of the JCV test detected three types of TCR in the CSF. A: archetype with point mutation (235 G→T) virus. B and C: two different types of rearrangement of TCR virus.

chronic ischemic heart disease, he was not administrated 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/μl (%CD4; 16.1% (normal, 25–60%)) with a decline to 217 cells/μl (%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× 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 /μl, protein concentration; 24 mg/dl, glucose concentration; 56 mg/dl). Concentration of soluble IL2-receptor, β2 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

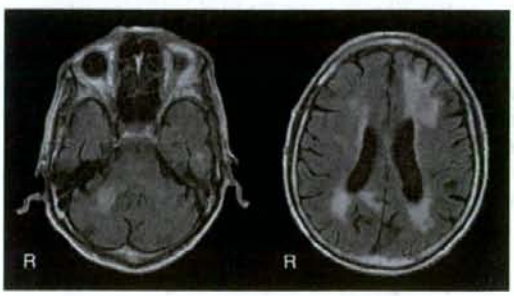


Fig. 2. Axial brain MRI on 1.5-tesla demonstrating multifocal high-intensity areas in left frontal and bilateral periventricular white matters, and right middle cerebellar peduncle on FLAIR (TE 144, TR 8002, TI 2000) images.

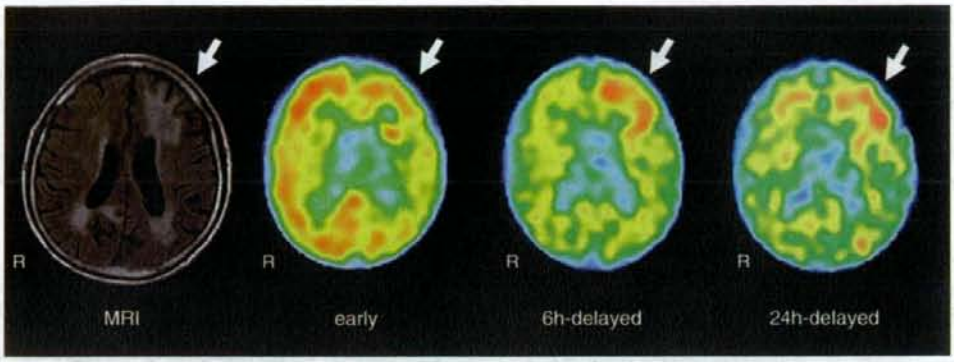


Fig. 3. Axial FLAIR MRI and Serial images of ^{123}I-IMP SPECT: early image, 6-hour delayed image and 24-hour delayed image. These serial images shows a delayed increase of radioactivity, a gradual increase and long term retention of the tracer on the left frontal white matter.

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.

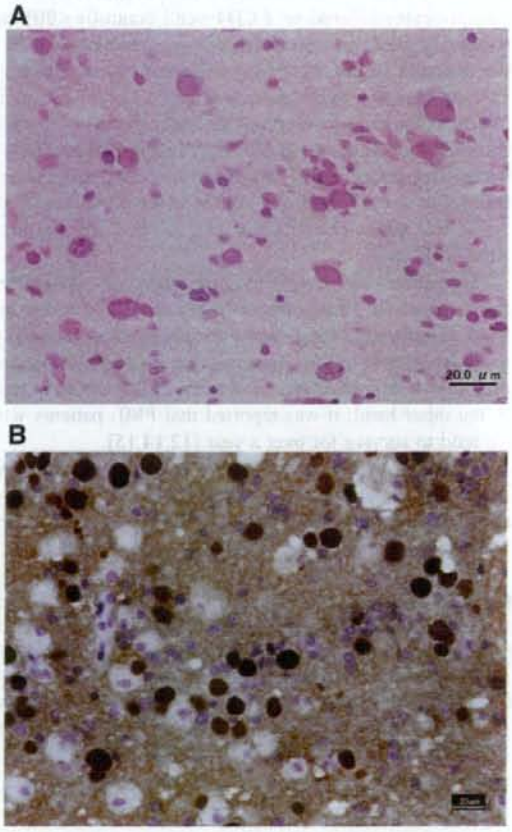


Fig. 4. (A) Brain section stained with hematoxylin-eosin, showing enlarged oligodendrocytic nuclei filled with somewhat basophilic substance in the white matter. Bar 20 μm. (B) Brain section immunostained with antibody against JCV-VP1, demonstrating that such enlarged oligodendrocytic nuclei are clearly 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 $<300/\mu l$ 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.

References

[1] Aksamit AJ. Review of progressive multifocal leukoencephalopathy and natalizumab. Neurologist 2006;12:293–8.
[2] Suzuki S, Sawa H, Komagome R, Orba Y, Yamada M, Okada Y, et al. Broad distribution of the JC virus receptor contrasts with a marked cellular restriction of virus replication. Virology 2001;286:100–12.
[3] Nakano S, Kinoshita K, Jinnouchi S, Hoshi H, Watanabe K. Unusual uptake and retention of I-123 IMP in brain tumors. Clin Nucl Med 1988;13:742–7.
[4] Goto H, Usui M, Ishii I. Efficacy of ^{123}N-isopropyl-p-[^{123}I]-iodoamphetamine single photon emission computed tomography for the diagnosis of uveal malignant melanoma. Am J Ophthalmol 2001;132:937–9.
[5] Shinoda J, Yano H, Murase S, Yoshimura S, Sakai N, Asano T. High ^{123}I-IMP retention on SPECT image in primary central nervous system lymphoma. J Neuro-Oncol 2003;61:261–5.
[6] Sugo N. Evaluating brain tumors with SPECT. J Med Soc Toho 2005;52:197–205.
[7] Sagiuchi T, Oka H, Utsuki S, Sato K, Kobayashi I, Asano Y, et al. Increased accumulations of N-isopropyl-p-[^{123}I]-iodoamphetamine related to tumefactive multiple sclerosis. Ann Nucl Med 2005;19: 603–6.
[8] Vandvik B, Norrby E, Nordal HJ, Degré M. Oligoclonal measles virus-specific antibodies isolated from cerebrospinal fluids, brain extracts, and sera from patients with subacute sclerosing panencephalitis and multiple sclerosis. Scand J Immunol 1976;5:979–92.
[9] Knowles WA, Luxton RW, Hand JF, Gardner SD, Brown DW. The JC virus antibody in serum and cerebrospinal fluid in progressive multifocal leucoencephalopathy. Clin Diagn Virol 1995;4:183–94.
[10] Wakita H, Asai T, Nakamura M, Endoh N, Hiruma K, Igarashi T, et al. Development of acute myelocytic leukemia in the course of Sjögren's syndrome. Rinsho Ketsueki 1989;30:56–60 [Japanese].
[11] Chikezie PU, Greenberg AL. Idiopathic CD4$^+$ T lymphocytopenia presenting as progressive multifocal leukoencephalopathy: case report. Clin Infect Dis 1997;24:526–7.
[12] Iwase T, Ojika K, Katada E, Mitake S, Nakazawa H, Matsukawa N, et al. An unusual course of progressive multifocal leukoencephalopathy in a patient with idiopathic CD4+ T lymphocytopenia. J Neurol Neurosurg Psychiatry 1998;64:788–91.
[13] Haider S, Nafziger D, Gutierrez JA, Brar I, Mateo N, Fogle J. Progressive multifocal leukoencephalopathy and idiopathic CD4$^+$ lymphocytopenia: a case report and review of reported cases. Clin Infect Dis 2000;31:e20–2.
[14] Kokubun N, Ishihara T, Nishibayashi M, Ikeda S, Nagashima K, Hirata K. Progressive multifocal leukoencephalopathy with idiopathic CD4 positive T-lymphocytopenia mimicking a low grade glioma on proton MR spectroscopy. A case report. Rinsho Shinekigaku 2005;45:663–8 [Japanese].
[15] Rueger MA, Miletic H, Dorries K, Wyen C, Eggers C, Deckert M, et al. Long-term remission in progressive leukoencephalopathy caused by idiopathic CD4$^+$ T lymphocytopenia: a case report. Clin Infect Dis 2006;42:e53–62.
[16] Inhoff O, Doerries K, Doerries R, Scharf J, Groden C, Goerdt S, et al. Disseminated cutaneous Kaposi sarcoma and progressive multifocal leukoencephalopathy in a patient with idiopathic CD4+ T lymphocytopenia. Arch Dermatol 2007;143:673–5.
[17] Smith DK, Neal JJ, Holmberg SD. Unexplained opportunistic infections and CD4+ T-lymphocytopenia without HIV infection. An investigation of cases in the United States. The Centers for Disease Control Idiopathic CD4+ T-lymphocytopenia Task Force. N Engl J Med 1993;328:373–9.
[18] Kirtava Z, Blomberg J, Bredberg A, Henriksson G, Jacobsson L, Manthorpe R. CD4+ T-lymphocytopenia without HIV infection: increased prevalence among patients with primary Sjögren's syndrome. Clin Exp Reumatol 1995;13:609–16.

ABSTRACT: A 20-year-old woman with selective cauda equina hypertrophy presented with muscle weakness and severe pain in the lower extremities. Serial immunotherapy was not effective. We performed biopsy of the cauda equina, and laminectomy and duraplasty of the thoracolumbar region. Biopsy revealed marked infiltration of small lymphocytes and foamy macrophages in the endoneurium. Three years after decompression surgery, her symptoms have improved slightly without progression or relapse. This is the first case of selective cauda equina hypertrophy with idiopathic inflammation. We propose that this is a new disease entity.

Muscle Nerve **38**: 1065–1069, 2008

SELECTIVE CAUDA EQUINA HYPERTROPHY WITH IDIOPATHIC INFLAMMATION

YUICHI HAYASHI, MD, PhD,[1] TAKEO SAKURAI, MD,[1] AKIO KIMURA, MD,[1] TAKAHIDE IKEDA, MD,[1] ZENJIRO MATSUYAMA, MD, PhD,[1] YOSHIHIRO SUZUKI, MD,[1] YUJI TANAKA, MD, PhD,[1] ISAO HOZUMI, MD, PhD,[1] HIDEO HOSOE, MD, PhD,[2] HITOSHI TAKAHASHI, MD, PhD,[3] and TAKASHI INUZUKA, MD, PhD[1]

[1] Department of Neurology and Geriatrics, Gifu University Graduate School of Medicine, 1-1 Yanagido, Gifu 501-1194, Japan
[2] Department of Orthopaedic Surgery, Gifu University Graduate School of Medicine, Gifu, Japan
[3] Department of Pathology, Brain Research Institute, Niigata University, Niigata, Japan

Accepted 25 March 2008

Selective cauda equina hypertrophy has been reported to be associated with Guillain–Barré syndrome,[5] chronic inflammatory demyelinating polyradiculoneuropathy,[3,8] malignant lymphoma,[9] metastatic tumor,[10] paraneoplastic syndrome,[11,12] sarcoidosis,[4] hereditary motor sensory neuropathies,[2,6] postirradiation neuropathy,[4] and infectious diseases [human immunodeficiency virus (HIV), human T-cell leukemia virus-1 (HTLV-1), cytomegalovirus, *Varicella zoster* virus, cryptococcus, and tuberculosis].[4] In most of these cases, peripheral nerve conduction abnormalities were evident.

Burton et al. first reported two cases of isolated hypertrophic radiculopathy of the cauda equina without any evidence of peripheral nerve conduction abnormalities. A paraneoplastic cause was suspected in one case because of improvement after removal of a bronchial carcinoid tumor. The other case was characterized by delayed-onset external opthalmoplegia, but the etiology remained unclear.[1] Herein we describe a case with selective cauda equina hypertrophy with idiopathic inflammation. In this patient, although opthalmoplegia was not noted, all the clinical features are similar to those described in Burton's idiopathic case.[1]

CASE REPORT

A 20-year-old woman presented with a 4-week history of progressive muscle weakness and severe pain in the lower extremities. The pain worsened in the recumbent position. Severe back pain on coughing commenced in mid-November 2003. At the beginning of December, she experienced severe pain in the left fibular region and at her hips in the recumbent position. On December 24, severe muscle weakness developed in both legs.

The patient became wheelchair-bound and was admitted for further evaluation in mid-January 2004. At admission, her general physical and mental status

Abbreviations: ANA, antinuclear antibody; c-ANCA, cytoplasmic antineutrophil cytoplasmic antibody; CA19-9, carbohydrate antigen; CEA, carcinoembryonic antigen; CSF, cerebrospinal fluid; FDG-PET, ^{18}F-deoxyglucose positron emission tomography; HBs, hepatitis B surface; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HTLV-1, human T-cell leukemia virus-1; IgE, immunoglobulin E; Met-PET, ^{11}C-methionine positron emission tomography; *MPZ*, myelin protein zero gene; MRC, Medical Research Council; MRI, magnetic resonance imaging; p-ANCA, perinuclear antineutrophil cytoplasmic antibody; *PMP22*, peripheral myelin protein gene; RNP, ribonucleoprotein antibody; SCC, squamous cell carcinoma; SUV, standardized uptake value; SS-A or -B, Sjögren's syndrome A or B; UCHL-1, ubiquitin C-terminus hydrase-L1

Key words: cauda equina biopsy; cauda equina hypertrophy; FDG-PET; inflammation; lumbar MRI

Correspondence to: Y. Hayashi; e-mail: hayashiy@gifu-u.ac.jp

Published online 5 May 2008 in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/mus.21044

Table 1. Nerve conduction studies.

Nerve	MCV	d.CMAP	p.CMAP	F wave occ. (%)	min F lat.	SCV	SNAP
Rt. Median	69.5	20.84	17.91	81.3	21.80	67.4	29.95
Rt. Ulnar	70.7	10.14	9.11			63.3	30.52
Rt. Tibial	43.1	16.35	11.74	18.8	42.75		
Lt. Tibial	55.2	27.19	22.45	37.5	40.75		
Lt. Sural						50.8	28.66

MCV: motor conduction velocity; d.CMAP: distal compound muscle action potential; p.CMAP: proximal compound muscle action potential; F wave occ.: F wave occurrence; min F lat: minimum F latency; SCV: sensory conduction velocity; SNAP: sensory nerve action potential.

was normal. Neurological examination revealed that cognitive functions and cranial nerves were intact. She had symmetrical muscle weakness in both legs without muscle atrophy [Medical Research Council (MRC) scale findings: iliopsoas, right 4/5, left 4/5; quadriceps, right 5/5, left 5/5; gluteus maximus, right 2/5, left 2/5; hamstrings, right 4/5, left 4/5; tibialis anterior, right 4/5, left 4/5; gastrocnemius, right 4/5, left 4/5; peroneus, right 4/5, left 4/5; toe extensors, right 4/5, left 4/5; and toe flexors, right 4/5, left 4/5]. Deep tendon reflexes in the arms were normal; however, patellar and Achilles tendon reflexes were absent. Mild bladder dysfunction was evident. Except for mild dysesthesia over her soles, no sensory abnormalities were detected. She complained of pain in the hips and lower extremities that was more severe in the recumbent position or with motion and urinary retention.

Hematological and biological tests, including tests for autoantibodies associated with collagen dis-

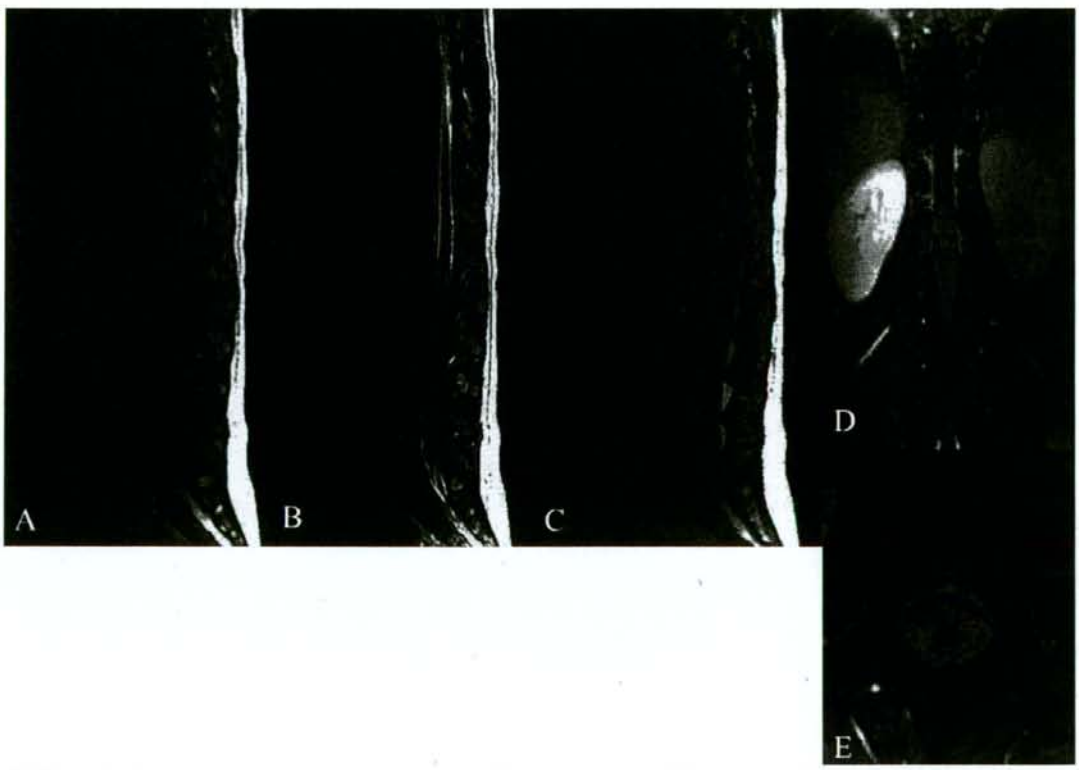


FIGURE 1. Cauda equina nerve roots were enlarged and slightly enhanced by gadolinium according to sagittal **(A–C)**, coronal **(D)**, and axial **(E)** MRI images. **(A)** T1-weighted image, **(B)** T2-weighted image, and **(C–E)** T1-weighted image with enhancement by gadolinium.

ease [antinuclear antibody (ANA), rheumatoid factor, Sjögren's syndrome A antibody (SS-A), Sjögren's syndrome B antibody (SS-B), ribonucleoprotein antibody (RNP), perinuclear antineutrophil cytoplasmic antibody (p-ANCA), and cytoplasmic antineutrophil cytoplasmic antibody (c-ANCA) levels] and autoantibodies to gangliosides revealed no abnormality except for high levels of immunoglobulin E (IgE) and tick-specific IgE. Serological tests for antibodies against HTLV-1, HIV-1/2, hepatitis B surface (HBs), and hepatitis C virus (HCV) were negative. The results of the DNA analyses for myelin protein zero (*MPZ*) and peripheral myelin protein 22 (*PMP22*) genes were normal.

In the upper and lower extremities, sensory nerve action potential and compound muscle action potential amplitudes and nerve conduction velocities were within normal ranges. The velocities and occurrence of the F-waves were decreased in the lower limbs (Table 1). In summary, nerve conduction abnormalities were only found in proximal nerve roots. Needle EMG studies of the legs were not performed.

Only a few drops of highly viscous cerebrospinal fluid (CSF) were collected. The CSF sample was dark yellow and contained 1605 lymphocytes. The lymphocytes appeared to be reactive lymphocytes, and flow-cytometric analysis of surface antigens of these cells showed no monoclonality. The CSF also revealed markedly elevated protein levels (3200 mg/dl) and β_2-microglobulin (8.88 mg/dl) levels and decreased glucose levels (25 mg/dl). Tumor marker levels (CEA, CA19-9, and SCC) were not elevated in the serum or CSF. No malignancies were detected on whole-body computed tomography (CT).

Spinal magnetic resonance imaging (MRI) revealed the cauda equina to be swollen with slight enhancement by gadolinium. The intradural CSF space was completely occupied by the swollen cauda equina (Fig. 1). ^{18}F-deoxyglucose positron emission tomography (FDG-PET) and ^{11}C-methionine positron emission tomography (Met-PET) were performed twice. FDG-PET revealed an extremely high standardized uptake value (SUV) (maximum 10.3 in March 2004) in the cauda equina, and no abnormal uptake in other organs (Fig. 2). Met-PET did not exhibit any accumulation in the body.

No change was noted after three series of steroid pulse therapy (methylprednisolone 1000 mg/day, 3 days), two series of intravenous immunoglobulin therapy (0.4 g/kg/day, 5 days), and two series of simple plasma exchange (50 ml/kg/day, on 4 alternate days). The patient's symptoms progressed gradually. She developed increased leg weakness and vesicorectal dysfunction.

FIGURE 2. According to FDG-PET, this lesion has a high uptake of glucose, with a maximum SUV of 10.3; however, there was no uptake of methionine (data not shown).

To obtain a pathological diagnosis, biopsy of the cauda equina was performed twice (in February and September 2004 the right and left S2 nerve roots were sampled). During surgery, the swollen cauda