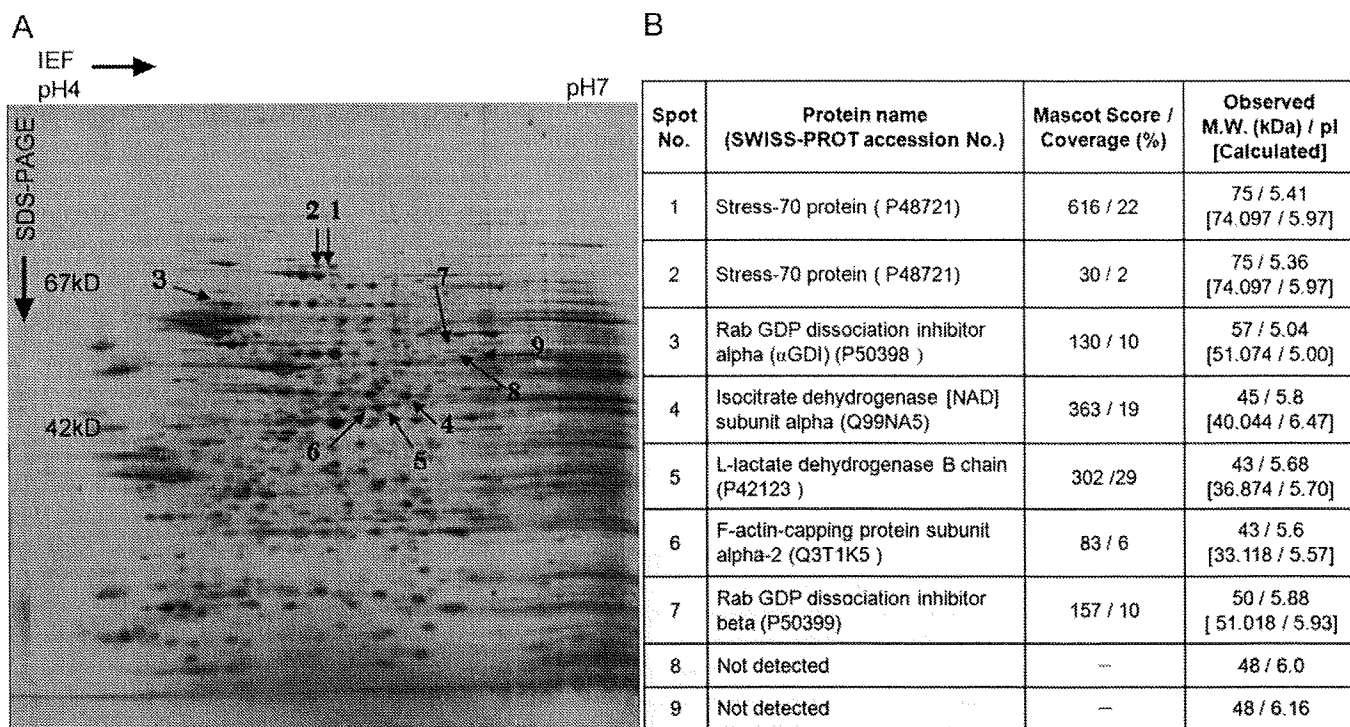


Figure 1 Polyvinylidene difluoride membrane containing proteins that were transferred and stained with the fluorescent total protein stain reagent and the target antigens, identified by mass spectrometry



The arrows indicate 9 spots that reacted with antibodies in sera from the 7 patients with neuropsychiatric systemic lupus erythematosus and did not react with antibodies in sera from the 12 healthy controls (A), as determined 2-dimensional immunoblotting (B). Spot number corresponds to the number shown in A.

were incubated overnight at 4°C with sera of the patient with anti- α GDI antibody, then washed, fixed, permeabilized, and single-immunolabeled or double-immunolabeled with an anti- α GDI monoclonal antibody, followed by the appropriate secondary fluorescent antibodies (see appendix e-1: Methods on the *Neurology*[®] Web site at www.neurology.org).

Statistical analyses. We used the Fisher exact probability test to assess differences in positivity of anti- α GDI antibodies between groups. The *p* values were considered statistically significant when less than 0.05.

RESULTS Neuropsychiatric syndromes of patients.

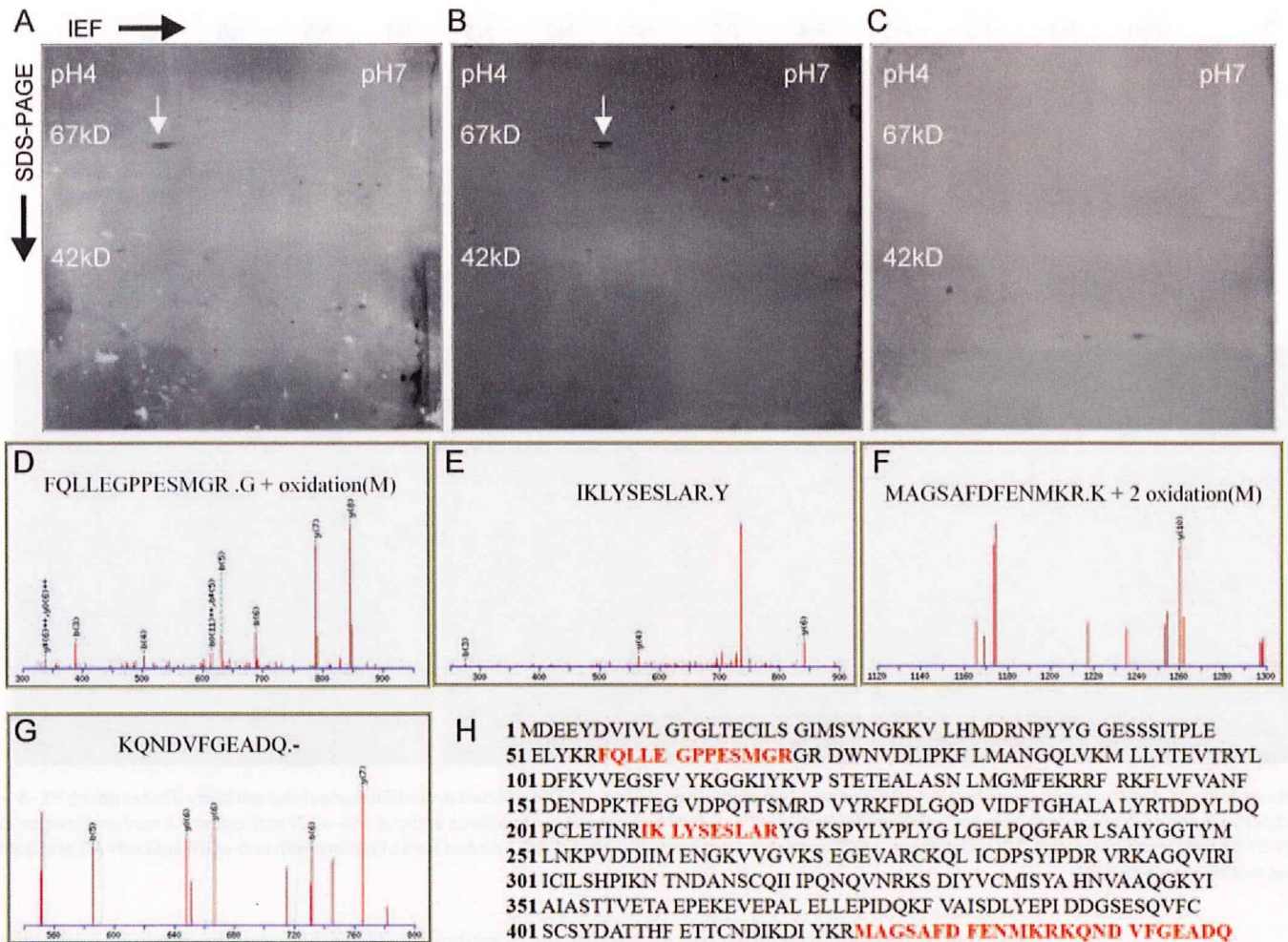
The neuropsychiatric symptoms of the 18 patients with NPSLE were classified as acute confusional state (*n* = 3), aseptic meningitis (*n* = 2), cognitive dysfunction (*n* = 2), cerebrovascular disease (*n* = 4), headache (*n* = 2), mood disorders (*n* = 5), movement disorder (chorea) (*n* = 1), myelopathy (*n* = 2), polyneuropathy (*n* = 2), psychosis (*n* = 5), and seizure disorders (*n* = 2).

Screening and identification of target proteins that reacted with antibodies in serum by 2-dimensional immunoblotting and liquid chromatography–tandem mass spectrometry. We detected 39 spots that reacted with antibodies in sera from 7 patients with NPSLE and detected 60 spots that reacted with antibodies in sera from the 12 healthy controls (figures 1 and 2). The latter 60 target spots were

subtracted from the former 39 spots. After subtraction, the remaining 9 spots only reacted with antibodies in sera of the 7 patients with NPSLE. These 9 spots that matched the proteins on the 2-DE gels were analyzed by LC-MS/MS (figure 1). Seven of these 9 immunoreactive spots were identified as stress-70 protein (spot numbers 1 and 2 in figure 1); α GDI (spot number 3); isocitrate dehydrogenase [NAD] subunit α (spot number 4); l-lactate dehydrogenase B chain (spot number 5); F-actin-capping protein subunit α -2 (spot number 6); and Rab GDP dissociation inhibitor β (GDI-2) (spot number 7). We were unable to identify the names of 2 protein spots (spot numbers 8 and 9). Among these antigens, α GDI was the only brain-specific antigen, and that was located in neurons. The other antigens are abundant in ubiquitous intracellular compartments.

Determined from location, we focused on the relation between α GDI and neuropsychiatric symptoms of NPSLE. In figure 2, we showed the 2D immunoblotting results of 2 NPSLE patients with psychosis (A, B) and a healthy control (C). Arrows corresponding to spot number 3 in figure 1 indicate the strongly immunoreactive spot in NPSLE patients with psychosis. We analyzed this

Figure 2 Polyvinylidene difluoride membrane after 2-dimensional immunoblotting and identification of Rab guanosine diphosphate dissociation inhibitor α (α GDI) by mass spectrometry



The polyvinylidene difluoride membrane reacted with sera of patients with neuropsychiatric systemic lupus erythematosus with psychosis (A: patient 2, B: patient 1 in table 1) and healthy control (C). Arrows indicate the α GDI spot. Tandem mass spectrometry spectra of 4 peptides of α GDI (D-G) and total amino acid sequences of α GDI (H). Sequences in bold red letters indicate the matched sequences of 4 peptides.

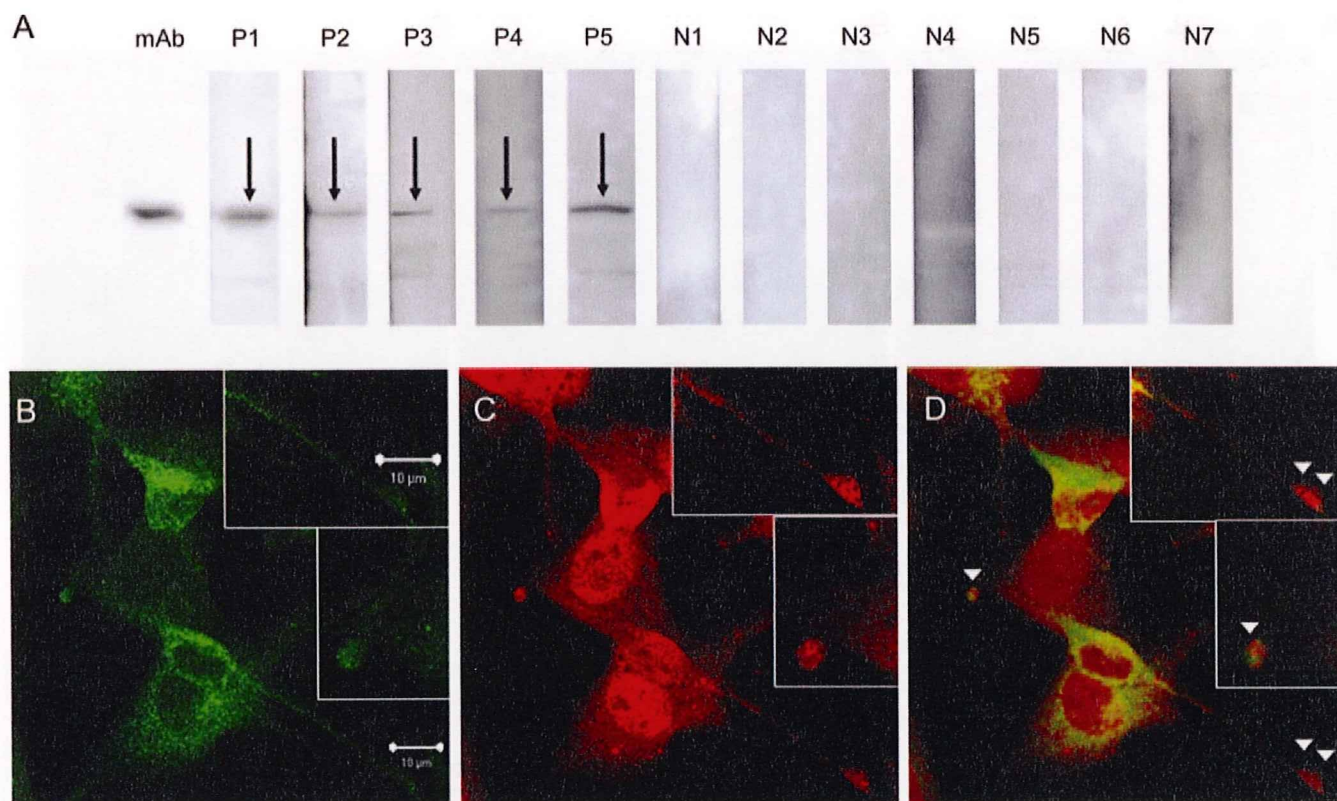
spot and obtained MS/MS spectra of 4 peptides (D-G). Subsequently, this spot was identified as α GDI using protein identification software (H).

Immunoreactivity of sera from patients with NPSLE, patients with other conditions, and healthy controls against human α GDI full-length recombinant protein. Specific positive signals were found in serum samples from 4 of the 18 patients with NPSLE and only 1 of the 19 patients with SLE without neuropsychiatric symptoms (figure 3A). The 12 patients with multiple sclerosis, 13 patients with infectious meningoencephalitis, 10 patients with polyneuropathy, 10 patients with psychotic syndromes, and 12 healthy controls showed negative signals. We summarized the clinical manifestations of 5 patients with anti- α GDI antibodies in table 1. Based on the diagnosis of ACR classification, their neuropsychiatric syndromes were psychosis in 4 of 5 patients, cognitive

dysfunction in 1 of 5 patients, myelopathy in 1 of 5 patients, and seizure disorders in 1 of 5 patients.

Given that psychosis was a commonly observed syndrome in 4 patients with NPSLE with the anti- α GDI antibody, we divided the 18 patients with NPSLE into those with psychosis ($n = 5$) and those without ($n = 13$). The number of patients with the anti- α GDI antibody was significantly higher in the patients with NPSLE with psychosis (4 of 5, 80%), compared with 0 of 13 (0%) in patients with NPSLE without psychosis ($p < 0.002$); 1 of 19 (5.3%) in patients with SLE without neuropsychiatric symptoms ($p < 0.003$); 0 of 12 (0%) in patients with multiple sclerosis ($p < 0.003$); 0 of 13 (0%) in patients with meningoencephalitis ($p < 0.002$); 0 of 10 (0%) in patients with polyneuropathy ($p < 0.004$); 0 of 10 (0%) in patients with psychotic syndromes ($p < 0.004$); and 0 of 12 (0%) in healthy controls

Figure 3 Immunoblotting of the human, Rab guanosine diphosphate dissociation inhibitor α (α GDI), full-length, recombinant protein, and immunocytochemistry using human neuroblastoma culture cells



Arrows indicate positive bands, immunoreacted with anti- α GDI antibodies. mAb = 1:1,000-diluted anti- α GDI monoclonal antibody (Proteintech); P1-5 = 1:1,500-diluted sera of patients with anti- α GDI antibodies; N1-7 = 1:1,500-diluted sera of patients without anti- α GDI antibodies (A), confocal images of SH-SY5Y cells incubated with 1:100-diluted anti- α GDI monoclonal antibody (B), with 1:1,000-diluted sera of patient with anti- α GDI antibody (C), and both reactivities are merged in D.

($p < 0.003$). We confirmed that the anti- α GDI antibodies in sera from the patients with 2 NPSLE with anti- α GDI antibodies were not present when the patients had complete remission of the psychiatric symptoms (data not shown).

Characteristics of patients with anti- α GDI antibodies. Characteristics of patients with anti- α GDI antibodies are shown in tables 1 and 2. Serologically, all patients had antinuclear antibodies and anti-dsDNA

antibodies. None had antiphospholipid antibodies (anticardiolipin and anti-beta2 glycoprotein 1 antibodies). Anti-ribosomal P antibody was not present in sera from 4 patients with NPSLE with anti- α GDI antibody (nos. 1-4 in table 2), and it was present only in the serum from 1 of the 19 patients with SLE without neuropsychiatric symptoms (no. 5 in table 2). Anti-Ro (SSA)-serum antibody and anti-Sm antibody were present in sera from 3 of 5 patients with

Table 1 Characteristics of patients with anti- α GDI antibodies (clinical manifestations)

No.	Age, y/sex	Clinical diagnosis	Neuropsychiatric syndrome	Therapy	Prognosis of psychosis
1	22/M	NPSLE	Psychosis	mPSL, PSL, Cy, PD	CR
2	32/F	NPSLE	Psychosis, myelopathy	mPSL, PSL, Cy, PD	CR
3	51/F	NPSLE	Psychosis, seizure disorders	mPSL, PSL, Cy, rituximab, PD	CR
4	85/M	NPSLE	Psychosis, cognitive dysfunction	mPSL, PSL, PD	CR
5	14/F	SLE without neuropsychiatric symptoms	—	PSL	—

Abbreviations: α GDI = Rab guanosine diphosphate dissociation inhibitor α ; CR = complete remission; Cy = cyclophosphamide; mPSL = methylprednisolone pulse therapy; NPSLE = neuropsychiatric systemic lupus erythematosus; PD = psychotropic drugs; PSL = prednisolone; SLE = systemic lupus erythematosus.

Table 2 Characteristics of patients with anti- α GDI antibodies (laboratory and neuroimaging findings)

No.	ANA	Anti-ds-DNA (IgG), IU/mL	Anti-Sm, U/mL	Anti-SS-A	Anti-SS-B	Anti-RNP, U/mL	Antiphospholipid	Anti-ribosomal P	Brain MRI	Brain SPECT
1	1,280	>400	16	130	30	—	—	—	Normal	Decrease in CBF in the bilateral frontal lobes
2	1,280	81	30	85	—	180	—	—	Cortical atrophy, SWMH of left frontal and temporal lobes	Decrease in CBF in the bilateral frontal lobes
3	1,280	249	—	—	—	190	—	—	Cortical atrophy	NE
4	>1,280	15	—	—	—	—	—	—	Cortical atrophy, hyperintensities of cerebellum and brainstem	NE
5	80	235	35.5	>500	—	68.1	—	+	Normal	NE

Abbreviations: α GDI = Rab guanosine diphosphate dissociation inhibitor α ; ANA = antinuclear antibodies; CBF = cerebral blood flow; IgG = immunoglobulin G; NE = not examined; SWMH = subcortical white matter hyperintensity.

anti- α GDI antibody (nos. 1, 2, and 5 in table 2). CSF analysis was performed in 3 patients. All showed normal cell counts, and 1 showed an elevated level of protein concentration.

We examined the antibody in 1:100-diluted CSF sample of 1 patient with anti- α GDI antibody (no. 1 in tables 1 and 2). In this CSF sample as well as the serum sample, we detected the anti- α GDI antibody. On brain MRI examination, 3 patients showed cortical atrophy predominantly in the bilateral frontal lobes and hippocampus; 2 patients showed abnormal intensity changes on T2-weighted and fluid-attenuated inversion recovery images, including 1 patient with subcortical white matter hyperintensities of the left frontal and temporal lobes and 1 patient with hyperintensities of the cerebellum and brainstem. Two patients showed no abnormal findings. We performed brain 99m Tc-ECD SPECT in 2 patients, and both patients had decreased cerebral blood flow in the bilateral frontal lobes.

Concerning therapy and outcome, 4 patients were treated with IV methylprednisolone pulse therapy, 3 patients were treated with IV cyclophosphamide, 1 patient was treated with IV rituximab, 4 patients were treated with oral psychotropic drugs, and all 5 patients were treated with oral corticosteroid. Psychosis of the 4 patients with NPSLE with anti- α GDI antibody occurred in the early stages of SLE and within the context of florid clinical and serologic disease activity. The 4 patients with NPSLE with anti- α GDI antibodies showed complete remission of their psychiatric symptoms within 1 month after onset. The neuropsychiatric and nonneuropsychiatric symptoms of all 5 patients with anti- α GDI antibodies were stable with corticosteroid administration.

Immunocytochemistry of human neuroblastoma culture cells. Anti- α GDI monoclonal antibodies showed that the immunoreactivity was specifically detected in the cytoplasm of SH-SY5Y cells (figure 3B). Conversely, the sera of the patient with anti-

α GDI antibody showed that strong immunoreactivity was detected in the nuclei, and mild immunoreactivity was detected in the cytoplasm of these cells (C). Double immunostaining showed that the immunoreactivity of the anti- α GDI monoclonal antibody and this patient's sera was partially colocalized in the cytoplasm of cell bodies and axons, including axon terminals (arrowheads in figure 3D).

DISCUSSION Of the 6 target antigens identified that reacted with antibodies in sera from 7 patients with NPSLE, α GDI was the only brain-specific antigen and was localized in neurons.^{9,10} It has been reported that α GDI functions to control the activity of the small GTPases of the Rab 3 proteins available for synaptic vesicle cycling and neurotransmitter release.^{11,12} The other 5 antigens were stress proteins, mitochondrial proteins, glycolytic enzyme, and cytoskeletal proteins. They were abundant in ubiquitous intracellular compartments.

Determined from function and location, we focused on the relation between α GDI and neuropsychiatric symptoms of NPSLE in this study. We tested more samples by 1D immunoblotting of human recombinant α GDI. Specific, positive signals were found in sera from 4 patients with NPSLE and 1 patient with SLE without neuropsychiatric symptoms. Interestingly, 4 of the 5 patients with NPSLE with the anti- α GDI antibody showed psychosis, as diagnosed based on ACR classification. Positivity of the anti- α GDI antibody in patients with NPSLE with psychosis was significantly higher than in patients with NPSLE without psychosis, patients with other diseases, and healthy controls. Further studies using a large series of patients and controls are required to clarify the relation between anti- α GDI antibody and psychosis in patients with NPSLE.

Mutations in *Gdi1*, which encodes α GDI, in families with X-linked nonspecific mental retardation (a common human disorder characterized by

mental retardation as the only clinical symptom) have been reported.¹³ In addition, *Gdi1*-deficient mice showed impairment of associative memory and alteration of social behavior without anatomic abnormality,¹⁴ and α GDI constitutive knockout mice had altered short-term synaptic plasticity by electrophysiologic analysis.¹⁵ The pathogenicity of the anti- α GDI antibody remains unclear. The α GDI is located in neurons but not on the membrane surface. Judging from its subcellular location, the anti- α GDI antibody may be generated after neuronal cell damage and may not be related to pathogenicity. However, some reports indicate that the selective autoantibody penetrates living cell membranes and binds to intracellular antigens.^{16,17}

As determined from these reports, the anti- α GDI antibody could penetrate the living cell and react with the α GDI. Our immunocytochemical study results revealed that the immunoreactivity of the anti- α GDI monoclonal antibody and sera of the patient with this antibody was partially colocalized in the cytoplasm, including the axon terminals. We considered the possibility that the anti- α GDI antibodies inhibit the function of the α GDI, and then regulate the synaptic vesicle exocytosis during neurotransmitter release associated with psychiatric symptoms in patients with NPSLE. We must perform more experiments using animal models in which the activities of anti- α GDI antibodies are induced or the antibodies are passively administered to clarify the pathogenic role of the anti- α GDI antibody.

In this study, all 4 patients with NPSLE with the anti- α GDI antibody presented with psychosis, which occurs in the early stage of SLE, and within the context of florid clinical and serologic disease activity. Their psychosis showed good response to immunosuppressive therapy, and no relapses occurred with corticosteroid administration. Neuroimaging analyses showed no common specific findings for patients with NPSLE with psychosis. These clinical features are in agreement with those of a previous report.¹⁸

It has been reported that lupus psychosis is linked with several antibodies, such as anti-brain-reactive antibodies (BRAAs), anti-microtubule-associated protein 2 antibodies, antiphospholipid antibodies, anti-ribosomal P antibodies, anti-Ro (SSA)-serum antibodies, and anti-Sm antibodies.⁵ In our analysis, antiphospholipid antibodies, anti-ribosomal P antibodies, anti-Ro (SSA)-serum antibodies, and anti-Sm antibodies showed no correlation with the anti- α GDI antibody. Anti- α GDI antibodies are different from anti-microtubule-associated protein 2 antibodies and BRAAs because they target antigens with different molecular weights (microtubule-

associated protein 2, 270 kD; BRAAs, 27.5 and 29.5 kD).^{19,20} We also could not detect antibodies with the same molecular weights on immunoblotting using sera of patients with NPSLE with the anti- α GDI antibody.

Recently, antibodies against NMDA receptor subunits (NR2a, NR2b) have been associated with neuropsychiatric lupus. They are specific to depressed mood, short-term memory, and learning.^{21,22} The molecular weight of these 2 receptor subunits is approximately 180 kD and differs from that of α GDI.²³

The diagnosis of psychosis associated with NPSLE is difficult and depends on the exclusion of other causes of CNS manifestations, such as steroid psychosis or neuroinfectious disease. It also is difficult to diagnose by neuroimaging analysis. The identification of an autoantibody associated with psychosis in patients with NPSLE is important for the accurate identification of patients who can benefit from steroid therapy. Since this is a pilot study, larger confirmatory studies regarding specificity and sensitivity are required to assess the significance of the anti- α GDI antibody.

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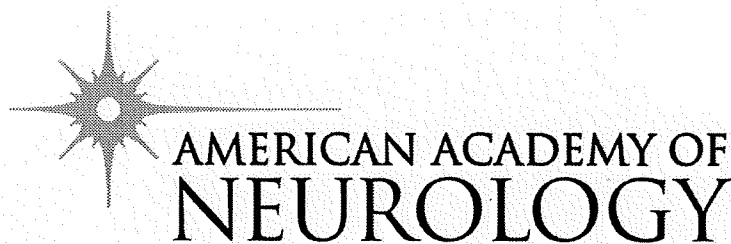
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Antibodies in patients with neuropsychiatric systemic lupus erythematosus
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Motor-dominant chronic inflammatory demyelinating polyneuropathy

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Abstract We reviewed the clinical, electrophysiological and laboratory findings, plus the therapeutics and evolution of patients with motor-dominant Chronic inflammatory demyelinating polyneuropathy (CIDP) and compared them with those of other CIDP patients. Among 12 consecutive CIDP patients, we identified five patients with motor-dominant CIDP. The five patients with motor-dominant CIDP initially presented with weakness of the upper limbs. Cervical magnetic resonance imaging (MRI) examinations of the patients with motor-dominant CIDP showed that the most affected lesions are the cervical nerve roots and brachial plexus. The clinical course of these patients was relapsing-remitting, and they improved markedly after treatment by intravenous immunoglobulin (IVIg) infusion or plasmapheresis. However, they did not improve in response to corticosteroid therapy during the acute phase of relapses. The relapses frequently occurred within 2 years, but rarely occurred after that. The score in the modified Rankin disability scale (mRDS) at the last follow-up period was statistically lower for the patients with motor-dominant CIDP than for the other CIDP

patients ($P < 0.002$). The characteristic clinical features, responsiveness to treatment, and prognosis suggest that motor-dominant CIDP is a distinct subtype of CIDP, with a specific immunological background. Repeated IVIg therapy is required to maintain the motor functions of patients with motor-dominant CIDP. We consider that treatment for recurrence prevention as an alternative to IVIg therapy is very important for patients with motor-dominant CIDP.

Keywords Corticosteroid · IVIg ·
Motor-dominant CIDP · Pure motor CIDP ·
Relapsing-remitting

Introduction

Chronic inflammatory demyelinating polyneuropathy (CIDP) is a clinically heterogeneous autoimmune disease. There are several subtypes of chronic acquired demyelinating neuropathies that are broadly classified under the umbrella term CIDP [1]. The subtypes of CIDP differ in clinical presentation, electrophysiological and laboratory features, and response to treatment [1]. There are several reports on the cases of patients with motor-dominant CIDP who showed selective motor involvement upon clinical and electrophysiological examination [2–4]. Furthermore, the clinical, pathological, electrophysiological, and prognostic features of patients with motor-dominant CIDP are poorly understood.

In this report, we reviewed the clinical, electrophysiological, laboratory findings, therapeutics and evolution of five patients with motor-dominant CIDP and compare them with those of other CIDP patients to clarify the characteristic features of motor-dominant CIDP.

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Patients and methods

Patients

Between September 2001 and July 2009, among 12 consecutive patients with definite CIDP, we identified five patients with motor-dominant CIDP. The diagnosis of CIDP was made on the basis of the criteria of the Joint Task Force of the European Federation of Neurological Societies (EFNS) and the Peripheral Nerve Society (PNS) [5]. Diagnostic criteria of motor-dominant CIDP included absence of sensory symptoms, except for mild distal paresthesia, and almost normal results in sensory conduction studies. Extensive clinical and laboratory evaluations excluded other causes of neuropathy.

Clinical assessment

Neurological examination was carried out for all 12 CIDP patients enrolled in this study. Measures of motor impairments consisted of a grip strength test and Medical Research Council (MRC) rating scale bilaterally performed on 14 muscle groups. Disability at the last follow-up period was assessed using the modified Rankin disability scale (mRDS) [6].

Electrophysiologic study

Nerve conduction studies were performed using Viking Nicolet electromyography equipment. Motor nerve conduction studies included surface recordings with stimulation of the median (wrist, elbow), ulnar (wrist, elbow), and tibial (ankle, knee) nerves. Sensory nerves were studied by antidromic recordings from the median, ulnar, and sural nerves. We defined the partial conduction block on the basis of the electrodiagnostic criteria of the Joint Task Force of EFNS and PNS [5].

Laboratory investigations

Serum and cerebrospinal fluid (CSF) samples were collected before therapy from the 12 patients with definite CIDP. We examined serum samples for anti-nuclear antibody (ANA), anti-DNA antibody, anti-Sm antibody, anti-SS A/B antibody, proteinase-3 (PR-3)-anti-neutrophil cytoplasmic antibody (ANCA), and myeloperoxidase (MPO)-anti-neutrophil cytoplasmic antibody (ANCA). We performed general CSF examination and determined the CSF IgG and CSF albumin indexes.

Other investigations

Cervical magnetic resonance imaging (MRI) was performed for two of the five patients with motor-dominant

CIDP, and thoracolumbar MRI was performed for three of the five patients with motor-dominant CIDP. A sural nerve biopsy was performed at ankle level for two of the five patients with motor-dominant CIDP. Details of the pathological analysis method were as previously reported [7, 8].

Statistical analysis

All statistical analyses were performed using the Mann-Whitney's *U* test or Fisher's exact probability test, as indicated. *P* values of < 0.05 were considered significant.

Results

Clinical features

We identified five patients (41.7%) with motor-dominant CIDP (patients 1–5) among 12 patients with definite CIDP. Patient 5 was a case we described earlier [9]. The average age of the patients with motor-dominant CIDP is 54.8 ± 8.67 years (mean \pm SD, range 46–66 years), and that of the other CIDP patients (patients 6–12) is 64.1 ± 11.2 years (range 40–72 years). The patients with motor-dominant CIDP included three males (60%) and two females, and the other CIDP patients included three males (42.9%) and four females. Two patients (patients 1 and 2) with motor-dominant CIDP also had diabetes mellitus. All the patients with motor-dominant CIDP initially presented with weakness. The initial regions affected by weakness were bilateral hands (patients 1, 2, and 5), right hand (patient 4), and left upper limb (patient 3). Besides weakness, only one of the patients had bilateral paresthesia of the hands (patient 5). The mean duration from disease onset until admission was 3.6 months (range 2–6 months). The neurological findings upon admission were symmetrical weakness of the extremities, particularly the upper limbs, and generalized areflexia. However, the only sensory deficit was mild paresthesia in the fingers (patients 1, 4, and 5) and no muscle atrophy or cranial nerve involvements were observed (Tables 1, 2).

Electrophysiological findings

All the patients with motor-dominant CIDP showed reduced motor conduction velocities (MCVs). Four of the five patients showed partial motor conduction block in at least one motor nerve (patients 1, 2, 4, and 5). Prolonged distal motor latencies were found in three of the five patients (patients 1, 2, and 3). F-wave abnormalities (prolonged F-wave latencies and decreases in the frequency of F-wave occurrence) were found in four of the four patients (patients 1, 2, 4, and 5). Reduced distal compound muscle

Table 1 Summary of clinical features and laboratory findings of 12 CIDP patients

Patient	Age/ Gender	Type	Complication	Initial symptom	Antibodies	CSF cell count (mm ³)	CSF protein content (mg/dl)	CSF IgG index	CSF albumin index
1	66/F	Motor dominant	Diabetes mellitus	Weakness (Blt. hand)	ANA	2	42	0.58	5.18
2	62/M	Motor dominant	Diabetes mellitus	Weakness (Blt. hand)	ND	1	86	0.49	12.9
3	50/F	Motor dominant	–	Weakness (Lt. upper limb)	ND	1	98	0.61	10.3
4	50/M	Motor dominant	–	Weakness (Rt. hand)	ND	6	113	0.61	17.5
5	46/M	Motor dominant	–	Weakness and parestheisa (Blt. hand)	ND	8	145	0.60	23.2
6	72/F	Sensory motor	Sjögren syndrome	Weakness (Blt. leg)	Anti-SS A	2	27	0.30	3.23
7	72/F	Sensory motor (MADSAM)	–	Dysesthesia (Lt. hand)	ANA	1	58	0.49	7.46
8	69/F	Sensory motor	–	Weakness and pain (Blt. thigh)	ND	0	54	0.52	7.87
9	69/M	Sensory motor (DADS)	–	Paresthesia (Rt. foot)	ND	2	101	0.58	15.3
10	65/M	Sensory motor (DADS)	MGUS	Hypesthesia (Rt. foot)	ND	1	53	0.60	4.00
11	62/F	Sensory motor	Multiple myeloma	Paresthesia (Blt. foot)	ND	3	183	0.44	29.7
12	40/M	Sensory motor (ataxic form)	Diabetes mellitus	Dysesthesia (Blt. hand)	ND	14	577	0.58	72.0

ANA anti-nuclear antibody, DADS distal acquired demyelinating symmetric neuropathy, MADSAM multifocal acquired demyelinating sensory and motor neuropathy, MGUS monoclonal gammopathies of unknown significance, ND not detected

Table 2 Medical Research Council (MRC) rating scale of patients with motor-dominant CIDP on admission (Right, Left)

Patient	Duration ^a (month)	Deltoid	Pectoralis major	Biceps	Triceps	Wrist extensors	Wrist flexors	Digits extensors	Digits flexors	Grip strength (kg)
Upper										
1	6	4, 4	4–, 4–	4–, 4–	4, 4	4–, 4–	3, 3	3, 3	3, 3	0, 0
2	3	5, 5	4, 4	4+, 4+	5, 5	5, 5	5, 5	4, 4	5, 5	15, 8
3	3	2, 2	NE	3, 3	3+, 3+	2–, 2–	2–, 2–	2–, 2–	2–, 2–	0, 0
4	4	2, 2	3+, 3+	3+, 3+	3+, 3+	3+, 4	3+, 3+	3+, 3+	3+, 3+	3, 3
5	2	5, 5	NE	3, 3	3, 3	2, 2	2, 2	NE	NE	4, 2
Lower										
Patient	Iliopsoas	Gluteus	Hamstrings	Quadriceps	Tibialis anterior	Gastrocnemius				
1	4, 4	4–, 4–	4–, 4–	4+, 5–	4, 4	4, 4				
2	4, 4	5, 5	5, 5	5, 5	5, 5	5, 5				
3	3+, 3+	5–, 5–	3+, 3+	2–, 2–	2–, 2–	2–, 2–				
4	4, 4	4, 4	3+, 3+	4+, 4+	5, 5	5, 5				
5	5–, 5–	NE	5–, 5–	5, 5	5, 5	5, 5				

NE not examined

^a Duration: until admission from disease onset

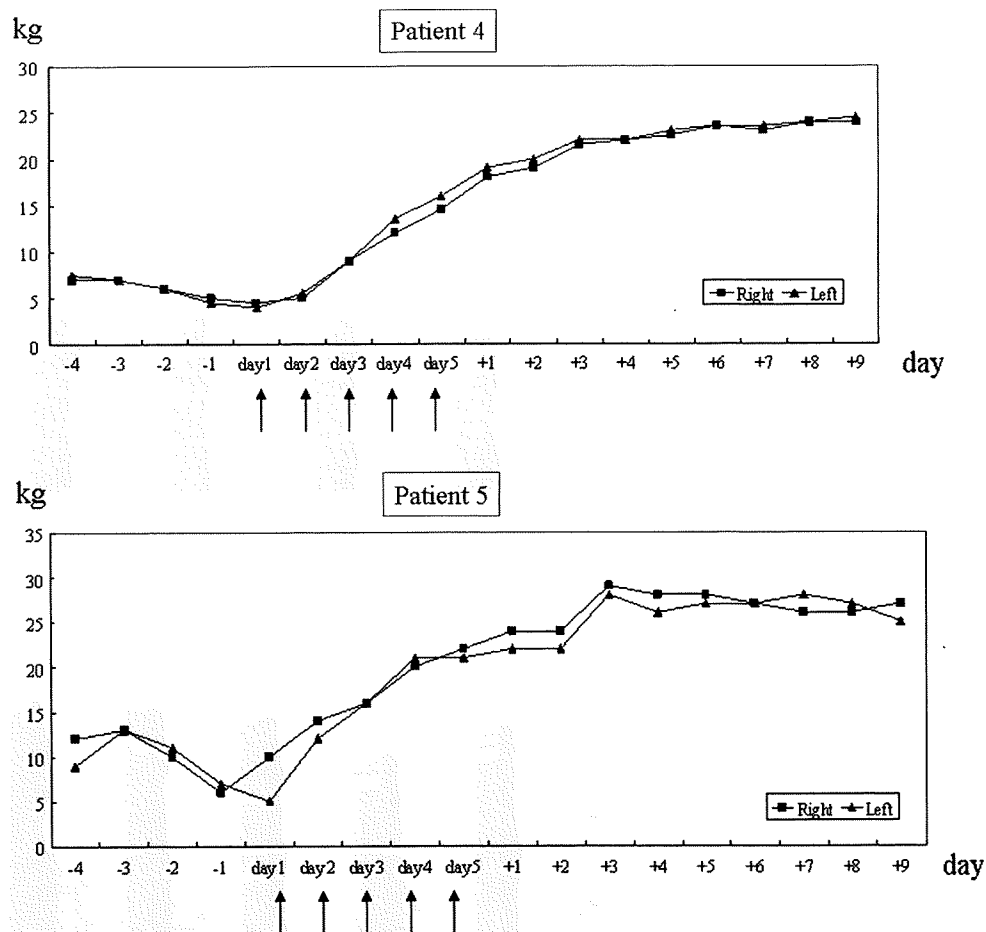
Table 3 Electrophysiological findings of patients with motor-dominant CIDP

Patient	Rt. median					Lt. median				
	Distal latency (ms)	MCV (m/s)	CMAP Amp. (mV) (dist./prox.) [CB, %]	FWCV (m/s) [occurrence, %]	SCV (m/s)	Distal latency (ms)	MCV (m/s)	CMAP Amp. (mV) (dist./prox.) [CB, %]	FWCV (m/s) [occurrence, %]	SCV (m/s)
1	4.6	33.9	10/3.1 [69]	24.0 [69]	43.3	4.4	37.1	13/7.6 [42]	42.5 [63]	45.8
2	6.0	27.3	12/8.8 [27]	22.2 [37]	44.4	5.8	30.7	9.0/3.4 [62]	23.4 [25]	42.1
3	5.1	37.7	8.0/7.2 [10]	NE	46.1	4.3	41.7	6.5/7.1 [-9.5]	NE	45.2
4	3.2	40.2	14/6.7 [52]	29.9 [6]	49.0	3.1	43.3	14/8.1 [42]	19.2 [19]	52.0
5	4.4	31.8	8.1/3.1 [62]	34.7 [68]	50.3	4.2	31.4	7.9/4.4 [44]	32.8 [31]	49.7
	Rt. ulnar									
Patient	Distal latency (ms)	MCV (m/s)	CMAP Amp. (mV) (dist./prox.) [CB, %]	FWCV (m/s) [occurrence, %]	SCV (m/s)	Distal latency (ms)	MCV (m/s)	CMAP Amp. (mV) (dist./prox.) [CB, %]	FWCV (m/s) [occurrence, %]	SCV (m/s)
1	3.6	35.4	5.5/4.3 [22]	32.7 [56]	36.0	3.8	31.7	5.6/2.9 [48]	NE	42.6
2	3.9	33.1	4.3/4.0 [21]	31.5 [93]	37.5	4.5	30.7	6.9/6.2 [10]	24.1 [87]	40.2
3	3.8	50.0	2.9/2.3 [21]	NE	55.2	3.3	37.0	4.5/4.5 [0]	NE	60.1
4	3.3	43.8	10/9.0 [10]	32.1 [38]	54.9	3.0	52.1	11/9.2 [16]	33.0 [31]	51.9
5	3.1	37.4	6.1/4.7 [23]	33.8 [68]	44.0	3.0	38.6	7.7/5.7 [26]	34.2 [93]	42.8
	Rt. tibial					Lt. tibial				
Patient	Distal latency (ms)	MCV (m/s)	CMAP Amp. (mV) (dist./prox.)	FWCV (m/s) [occurrence, %]	SCV (m/s)	Distal latency (ms)	MCV (m/s)	CMAP Amp. (mV) (dist./prox.)	FWCV (m/s) [occurrence, %]	SCV (m/s)
1	5.6	42.9	8.4/7.5	35.2 [69]	40.1	5.1	38.9	4.1/4.9	29.5 [94]	39.4
2	7.3	30.2	3.9/3.4	NE	38.5	8.7	31.5	2.7/1.1	27.4 [43]	42.3
3	5.1	37.8	7.1/4.6	NE	46.3	3.8	38.1	3.5/3.4	NE	46.2
4	3.9	41.2	18/14	40.0 [100]	50.0	3.7	41.9	23/20	40.3 [94]	52.6
5	4.3	35.8	7.9/5.7	37.6 [100]	47.8	4.6	38.2	16/8.6	34.3 [100]	42.7

Bold italic indicates abnormal value

CMAP compound muscular action potential amplitude, CB conduction block, FWCV F-wave conduction block, FWCV F-wave conduction velocity, MCV motor conduction velocity, NE not examined, SCV sensory conduction velocity

Fig. 1 Hand dynamometer values of patients with motor-dominant CIDP during IVIg therapy ↑: day of IVIg infusion



action potential (CMAP) amplitude in at least one nerve was found in three of the five patients (patients 1, 2, and 3). In three patients, the results of sensory nerve conduction studies were normal. Two patients (patient 1 and 2) had a mild sensory nerve conduction slowing in the ulnar or sural nerve. These patients have had diabetes mellitus for a long time, thus it was possible that their sensory nerve conduction slowing may have been partially affected by diabetic sensory-motor polyneuropathy. The proportion of nerves that had F-wave abnormalities (100%, 22/22 nerves) was significantly higher than that of nerves that had prolonged distal motor latencies (20%, 6/30 nerves) ($P < 0.001$, Fisher's exact probability test) (Table 3).

Laboratory findings

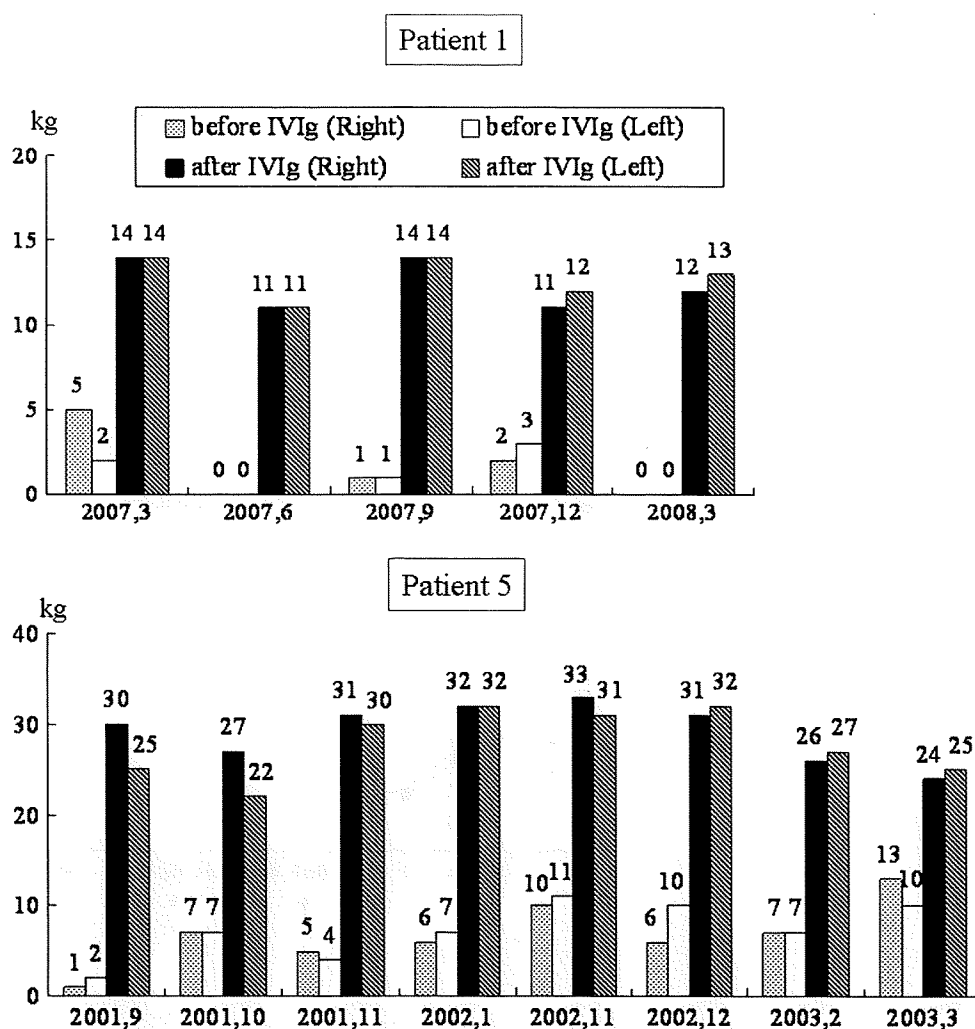
One of the five patients with motor-dominant CIDP had ANA (patient 1), but none had anti-DNA antibody, anti-Sm antibody, anti-SS A/B antibody, PR-3-ANCA, or MPO-ANCA. The CSF cell count of the patients with motor-dominant CIDP was $3.6 \pm 3.2 \text{ mm}^3$ (mean \pm SD, range 1–8 mm^3), and that of the other CIDP patients was

$3.3 \pm 4.8 \text{ mm}^3$ (range 0–14 mm^3). The CSF protein concentration in patients with motor-dominant CIDP was $97 \pm 38 \text{ mg/dl}$ (range 42–145 mg/dl), and that in the other CIDP patients was $150 \pm 190 \text{ mg/dl}$ (range 27–577 mg/dl). The CSF albumin index of the patients with motor-dominant CIDP was 14 ± 6.9 (range 5.2–23), and that of the other CIDP patients was 20 ± 25 (range 3.2–72). There were no statistically significant differences in these values and indexes between the two groups ($P = \text{n.s.}$, Mann-Whitney's U test). The CSF IgG index of the patients with motor-dominant CIDP was 0.58 ± 0.051 (range 0.49–0.61), and that of the other CIDP patients was 0.50 ± 0.11 (range 0.3–0.6). It was statistically higher in the patients with motor-dominant CIDP than in the other CIDP patients ($P < 0.05$, Mann-Whitney's U test). However, the CSF IgG indexes of the patients with motor-dominant CIDP were almost within the normal range (<0.6) (Table 1).

Other findings

The cervical MRI findings of two patients (patients 1 and 4) showed swelling and gadolinium enhancement of

Fig. 2 Hand dynamometer values of patients with motor-dominant CIDP before and after IVIg therapy



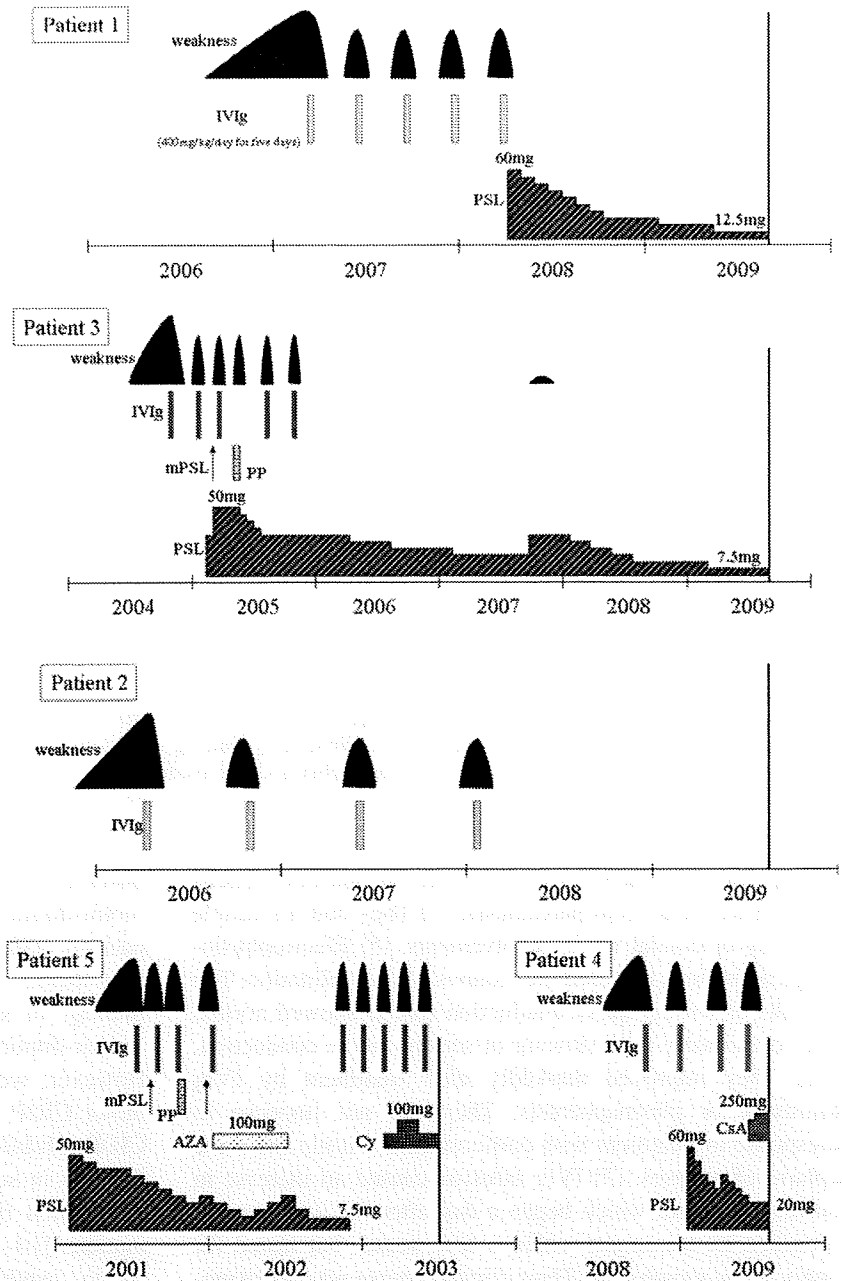
bilateral nerve roots (patient 1, in C4-7; patient 4, in C2-7) and the brachial plexus. On the other hand, the thoracolumbar MRI findings of three patients (patients 1, 3, and 4) showed no swelling or gadolinium enhancement of the nerve root or the plexus (the examinations of patients 3 and 4 were only by plain MRI). The findings of sural nerve biopsy in two patients (patients 1 and 2) were only perineurial and endoneurial edemas. There were no findings of axonal degeneration or demyelination.

Therapy and prognosis

The clinical course of all five patients with motor-dominant CIDP enrolled in this study was relapsing-remitting. Two patients (patients 3 and 5) were treated by intravenous methylprednisolone (mPSL) pulse therapy. Three patients (patients 1, 3, and 4) were treated with prednisolone (PSL) orally. One patient (patient 4) was treated with cyclosporine A (CsA) orally. One patient (patient 5) was treated with cyclophosphamide (Cy) and azathioprine (AZA) orally. Plasmapheresis was performed for two

patients (patients 3 and 5). All five patients were treated with intravenous immunoglobulin (IVIg, 400 mg/kg/day for 5 days). They improved markedly after being treated by IVIg infusion or plasmapheresis. IVIg infusion caused an increase in muscle strength, which began a day after the infusion and reached its maximum within a few weeks, but lasted for only a few months (Fig. 1). IVIg maintenance treatment has had a long-term beneficial effect on muscle strength, allowing the patients to maintain normalcy in their daily lives (Fig. 2). They did not improve in response to treatment by mPSL pulse therapy or with PSL during the acute phase of relapses. Two patients (patients 1 and 3) treated with only PSL have remained in remission for 1.8 and 1.3 years, respectively (Figs. 1, 3). Two patients (patients 4 and 5) treated with PSL did not remain in remission, and one patient (patient 2), without any treatment to prevent relapse, has remained in remission for 1.5 years from the last IVIg infusion (Figs. 2, 3). The number of relapses decreased over a long period of time, during which several relapses occurred (Table 4). During the follow-up period (mean \pm SD;

Fig. 3 Clinical course of patients with motor-dominant CIDP. AZA azathioprine, CsA cyclosporine A, Cy cyclophosphamide, IVIg intravenous immunoglobulin therapy, mPSL methylprednisolone pulse therapy, PP plasmapheresis, PSL prednisolone



2.8 ± 1.5 years, range 1–5 years), the average number of relapses was 5.8 ± 2.2 (*n* = 5, range 4–9). Within the first year after the disease onset, the average number of relapses was 3.8 ± 1.1 (*n* = 5, range 2–5). From the first to the second year after the disease onset, the average number of relapses decreased to 2.3 ± 1.9 (*n* = 4, range 1–5). After the second year, the average number of relapses decreased to 0.3 ± 0.6 (*n* = 3, range 0–1). The mRDS at the last follow-up period was 1 ± 0 (range 1) in all five patients with motor-dominant CIDP and was 3.1 ± 1.5 (range 2–6) in the other CIDP patients. Disability at the last follow-up period was statistically milder in patients with motor-dominant CIDP than in the other

CIDP patients (*P* < 0.002, Mann–Whitney’s *U* test) (Table 4; Figs. 1, 2, 3).

Discussion

In this study, we reviewed the clinical, electrophysiological and laboratory findings, plus the therapeutics and evolution of five patients with motor-dominant CIDP. Their characteristic features were as follows: (1) Within several months of disease onset, they presented with symmetrical weakness and generalized areflexia. (2) Their weakness showed upper-limb predominance, which was also the initial

Table 4 Summary of therapy and prognosis of 12 CIDP patients

Patient	Type	Clinical course	Therapy	Duration of follow-up (year)	Number of relapses (total)	Number of relapses (<1 year)	Number of relapses (1–2 years)	Number of relapses (2 years<)	mRDS
1	Motor dominant	Relapsing-remitting	IVIg, PSL	3	5	4	1	0	1
2	Motor dominant	Relapsing-remitting	IVIg	3	4	2	2	0	1
3	Motor dominant	Relapsing-remitting	IVIg, mPSL, PSL, PP	5	7	5	1	1	1
4	Motor dominant	Relapsing-remitting	IVIg, PSL, CsA	1	4	4	–	–	1
5	Motor dominant	Relapsing-remitting	IVIg, mPSL, PP, AZA, Cy	2	9	4	5	–	1
6	Sensory motor	Progressive	IVIg	5	0	0	0	0	4
7	Sensory motor (MADSAM)	Progressive	IVIg	17	0	0	0	0	3
8	Sensory motor	Progressive	IVIg, mPSL	3	0	0	0	0	3
9	Sensory motor (DADS)	Progressive	IVIg	4	0	0	0	0	2
10	Sensory motor (DADS)	Progressive	IVIg	2	0	0	0	–	2
11	Sensory motor	Progressive	IVIg, PP	5	0	0	0	0	6
12	Sensory motor (ataxic form)	Progressive	IVIg, mPSL, PSL	6	0	0	0	0	2

AZA azathioprine, CsA cyclosporine A, Cy cyclophosphamide, DADS distal acquired demyelinating symmetric neuropathy, IVIg intravenous immunoglobulin therapy, MADSAM multifocal acquired demyelinating sensory and motor neuropathy, mRDS modified Rankin disability scale, mPSL methylprednisolone pulse therapy, PP plasmapheresis, PSL prednisolone

symptom. (3) They presented with no sensory deficits except for mild distal paresthesia. (4) They had no muscle atrophy or cranial nerve involvements. (5) Electrophysiological findings revealed the features of demyelination. The results of sensory nerve conduction studies showed normal or occasionally mild slowing of sensory nerve conduction. (6) They improved markedly after treatment by IVIg infusion or plasmapheresis. They did not improve in response to treatment with corticosteroids during the acute phase of relapses. (7) IVIg infusion caused an increase in muscle strength which began a day after the infusion and reached its maximum within a few weeks, but lasted for only a few months. (8) Their clinical course was relapsing-remitting, but they maintained normalcy in their daily lives because of repeated IVIg infusions. (9) The relapses occurred frequently within 2 years, but rarely occurred after that.

There is a report of four patients with pure motor CIDP who showed bilateral selective involvement of motor nerve fibers and the absence of sensory symptoms, normal sensation at neurological examination and normal findings upon electrophysiological testing of sensory nerve fibers and sural nerve biopsy [2]. It was also reported that their clinical course was relapsing-remitting and they were steroid-unresponsive, whereas they considerably improved after treatment with immunoglobulin. The clinical features of the patients with pure motor CIDP in this

previous report resembled those of our five patients with motor-dominant CIDP. In this study, three of our five patients with motor-dominant CIDP showed mild distal paresthesia, and two patients showed mild abnormal findings of sural nerve biopsy. Thus, we used the term 'motor-dominant CIDP', rather than 'pure motor CIDP'. However, we considered that the pathogenicity of pure motor CIDP may be the same as that of motor-dominant CIDP. Multifocal motor neuropathy (MMN) has common features in some respects with motor-dominant CIDP, as determined from clinical and electrophysiological examinations [10–12]. However, MMN is an uncommon idiopathic syndrome characterized by asymmetric lower motor neuron weakness. The clinical course is usually slowly progressive, although it may occasionally have a stepwise progression [10, 11]. Some of the patients with MMN show muscle atrophy and their reflexes are usually preserved [10, 11]. We consider that motor-dominant CIDP differs from MMN.

Interestingly, all the patients with motor-dominant CIDP showed marked improvement soon after the IVIg infusion and complete remission within a few weeks. In this study, the electrophysiological and MRI findings of the patients with motor-dominant CIDP showed that the most affected lesions are the cervical nerve roots and brachial plexus. In this study, we measured the CSF albumin index to determine the disruption of the blood-nerve barrier at the site of

the ventral root. There was no statistically significant difference in this index between motor-dominant CIDP and other CIDP types. We also measured the CSF IgG index to determine the presence of intrathecal IgG production. It was statistically higher in the patients with motor-dominant CIDP than in the other CIDP patients. However, the CSF IgG indexes of both groups were almost within the normal range. In this study, we could not identify the serum and CSF markers for distinguishing motor-dominant CIDP from the other CIDP types.

The characteristic clinical features, responsiveness to treatment, and prognosis suggest that motor-dominant CIDP is a distinct subtype of CIDP, and that it has a specific immunological background. IVIg may contain numerous anti-idiotypes to neutralize pathogenic autoantibodies [13, 14]. It was reported that the titer of circulating autoantibodies rapidly decreases within hours after IVIg infusion [15]. We speculated on the presence of unknown pathogenic autoantibodies, which block the conduction of peripheral nerves, in the patients with motor-dominant CIDP. However, there are many questions, such as why the motor nerve fiber is specifically involved, that should be addressed to validate our hypothesis.

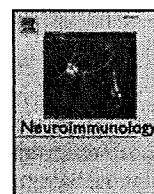
In this study, we showed that the prognosis of motor-dominant CIDP is significantly better than that of other CIDP types. It may be said that this is the result of repeated IVIg therapy within 2 years of disease onset. If we had made a misdiagnosis, such as motor neuron disease, the disabilities of these patients may have been more severe. However, the repeated IVIg therapy is very expensive. We consider treatment for recurrence prevention to be very important. In this study, two patients treated with only PSL have remained in remission for a long period. However, two other patients treated with PSL did not remain in remission and one patient without any treatment to prevent relapse has remained in remission for a long period. We consider that a large-scale control study is necessary to clarify the relapse prevention effects of steroids and other immunosuppressants in the future.

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Conflict of interest statement None.

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High prevalence of autoantibodies against phosphoglycerate mutase 1 in patients with autoimmune central nervous system diseases

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ABSTRACT

We identified the autoantibody against phosphoglycerate mutase 1 (PGAM1), which is a glycolytic enzyme, in sera from multiple sclerosis (MS) patients by proteomics-based analysis. We further searched this autoantibody in sera from patients with other neurological diseases. The prevalence of the anti-PGAM1 antibody is much higher in patients with MS and neuromyelitis optica (NMO) than in those with other neurological diseases and in healthy controls. It was reported that the anti-PGAM1 antibody is frequently detected in patients with autoimmune hepatitis (AIH). Results of our study suggest that the anti-PGAM1 antibody is not only a marker of AIH but also a nonspecific marker of central nervous system autoimmune diseases.

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1. Introduction

Over the past few years, compelling data on the roles of B cells as sensors, coordinators and regulators of the immune response have strengthened the view that B cells and autoantibodies are fundamental factors for activating T cells and/or mediating tissue injury in several autoimmune-mediated diseases of the central nervous system (CNS) (Dalakas, 2008; Hasler and Zouali, 2006). In this study, we identified the autoantibody against phosphoglycerate mutase 1 (PGAM1) in sera from multiple sclerosis (MS) patients by proteomics-based analysis. Phosphoglycerate mutase is a glycolytic enzyme that catalyzes the interconversion of 3- and 2-phosphoglycerate with 2, 3-bisphosphoglycerate as the primer of the reaction (Fothergill-Gilmore and Watson, 1989). In mammalian tissues, there are two types of phosphoglycerate mutase: type M (also known as PGAM2) in muscles and type B (also known as PGAM1) in other tissues (Omenn and Cheung, 1974; Zhang et al., 2001). However, there are few reports on the anti-PGAM1 antibody (Lu et al., 2008; Zephir et al., 2006) and the specificity of this autoantibody is not clearly understood. To evaluate the specificity of this autoantibody, we assessed the pre-

valence of this autoantibody in sera from patients with various neurological diseases and healthy controls.

2. Patients and methods

2.1. Patients

Serum samples were obtained from patients with MS [$n=21$; male:female=9:12; age range, 31–75; mean age, 49], neuromyelitis optica (NMO) [$n=13$; male:female=2:11; age range, 26–79; mean age, 49], multiple cerebral infarctions (MCI) [$n=20$; male:female=9:11; age range, 53–83; mean age, 71], infectious meningoencephalitis (IME) [$n=19$; male:female=14:5; age range, 15–71; mean age, 45], and Parkinson's disease (PD) [$n=21$; male:female=11:10; age range, 50–85; mean age, 68], and from healthy controls [$n=17$; male:female=7:10; age range, 25–74; mean age, 44]. All the MS patients were diagnosed with clinically definite MS according to the criteria of Poser et al. (1983). All the NMO patients satisfied the 2006 revision to the Wingerchuk diagnostic criteria (Wingerchuk et al., 2006).

2.2. Preparation of tissue proteins

Under ether anesthesia, adult Wistar rats were sacrificed. Their cerebrums were immediately removed and frozen in dry-ice powder. The frozen brain tissue was homogenized in lysis buffer (7 M urea, 2 M thiourea, 0.4% CHAPS, 0.1% DTT, 0.5% Triton X-100, and 0.2% SDS) and centrifuged at 100,000×g for 40 min. The obtained supernatant was used in all experiments.

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2.3. Screening for autoantibodies against protein sample in sera from MS patients

We examined autoantibodies against a prepared protein sample in sera from five MS patients and five healthy controls by one-dimensional electrophoresis (1DE) and immunoblotting. The extracted proteins were applied at 20 µg/well to 4–20% polyacrylamide gel for western blotting. The proteins were separated by SDS-PAGE and separated proteins were blotted onto polyvinylidene difluoride (PVDF) membranes at 0.8 mA/cm² for 1 h using a semidry blotting apparatus (Trans-Blot SD semidry transfer cell, Bio-Rad Laboratories). Subsequently, the membranes were incubated in blocking solution overnight in a cold room, and then reacted with the sera from MS patients and healthy controls (diluted at 1:1500) for 1 h at room temperature, followed by washing. Then the membranes were incubated with HRP-conjugated anti-human Ig (A + G + M) antibodies (Zymed) (diluted at 1:2000) for 1 h at room temperature and reacted with the ECL-Plus Western blotting detection system (GE Healthcare).

2.4. Two-dimensional electrophoresis (2DE) and immunoblotting

A sample was loaded onto an immobilized and rehydrated dry strip (pH 3–10, nonlinear 18 cm long, GE Healthcare). Up to 100 µg of extracted proteins was applied to the dry strip for western blotting. Isoelectric focusing was carried out at 20 °C for 85,000 Vh at a maximum of 8000 V using a horizontal electrophoresis system (Coolphorstar IPG-IEF Type-PX, Anatech). This IPG strip was transferred to 12.5% polyacrylamide gel. The second-dimension run was carried out vertically using an electrophoresis apparatus (Coolphorstar SDS-PAGE Dual-200 K, Anatech) at 30 mA/gel. After the electrophoresis, the SDS-PAGE gels were stained with SyproRuby (Bio-Rad Laboratories) or used for protein transfer onto PVDF membranes (Toda and Kimura, 1997). Separated proteins were electrophoretically transferred to a PVDF membrane at a constant voltage of 32 V for 3 h using a buffer transfer tank with cool equipment (Toda et al., 2000).

Subsequently, this membrane was incubated in a blocking solution (5% skim milk in 1× TBST and 1× TBS containing 0.1% Tween 20) overnight in a cold room, and then reacted with serum from a patient diluted (1:1500) with 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 (Zymed) diluted (1:2000) with 1% skim milk in 1× TBST for 1 h at room temperature. After six washes, the membrane was incubated with the WB detection reagent (ECL-Plus, GE Healthcare) for 5 min and then scanned using a variable-mode imager (Typhoon 9400, GE Healthcare). The antibody-reactive protein spots were matched with the protein spots stained with SyproRuby (Bio-Rad Laboratories) using image analysis software (Adobe Photoshop 6.0).

2.5. In-gel digestion and mass spectrometry

Proteins were detected by staining with SyproRuby (Bio-Rad Laboratories). For mass spectrometric identification, the target protein spot on the SyproRuby-stained 2D electrophoresis gel was excised using FluoroPhoreStar 3000 (Anatech). In-gel digestion was performed in according with a standard protocol (Toda and Kimura, 1997) with minor modifications. Briefly, gel pieces were dehydrated and the dried gel pieces were rehydrated in 5 µl of 100 mM ammonium bicarbonate containing 10 µg/ml trypsin (Promega) for 3 h at 37 °C. After digestion, tryptic peptides were extracted twice with 50 µl of 66% acetonitrile in 0.1% trifluoroacetic acid (TFA) in a sonicator. The extracted peptides were dried, redissolved in 0.1% TFA, and injected onto a MonoCap 0.1 mm × 250 mm monolithic C18 column (Kyoto Monotech) with Prominence Nano (Shimadzu). The column eluent was spotted every 15 s onto a µFocus MALDI plate (Shimadzu GLC) with α-cyano-4-hydroxycinnamic acid (Sigma Aldrich) as a matrix using AccuSpot (Shimadzu). Buffer A consisted of 5% acetonitrile and 0.1% (v/v) TFA and buffer B consisted of 90% acetonitrile and 0.1% (v/v) TFA. The separation gradient was 5–60% buffer B over 30 min at a flow rate of 1 µl/min. The digests were

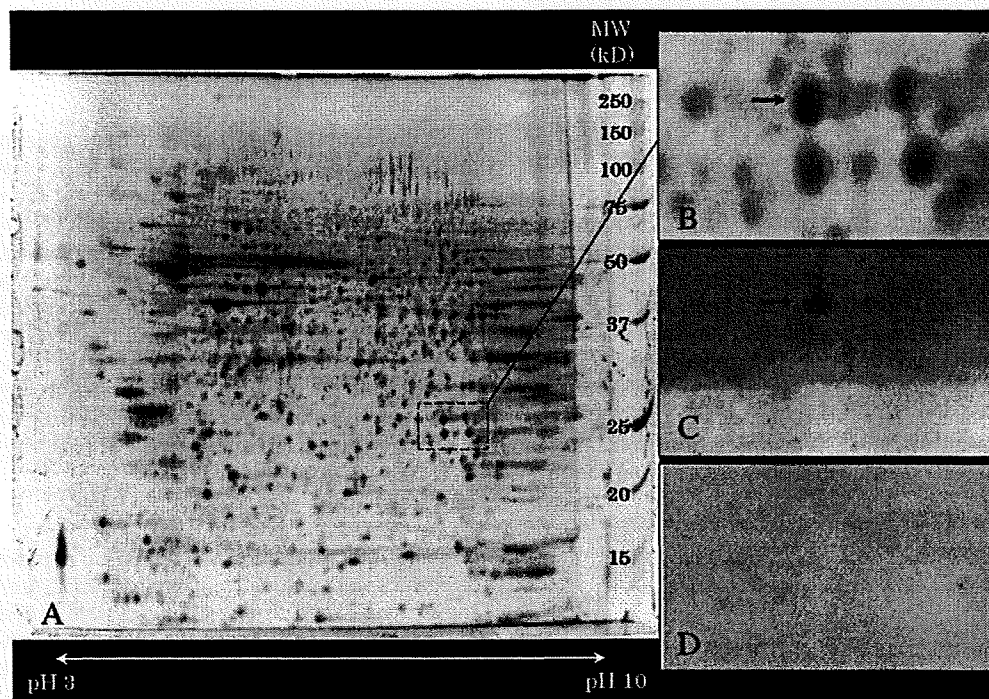


Fig. 1. Autoantibodies visualized by two-dimensional electrophoresis (2DE) and immunoblotting in sera from patients with multiple sclerosis (MS) and from healthy controls. (A, B) Total protein extracts of homogenized rat brain tissue were separated by 2DE, followed by SyproRuby staining. Arrow indicates the protein spot matching the immunoreactive spot detected by western blotting of MS patients' sera. Subsequently, this spot was identified as phosphoglycerate mutase 1 (PGAM1) by MALDI TOF-MS; (C) 1:1500-diluted MS patient's sera; arrow indicates the protein spot (PGAM1) recognized in sera from MS patients; and (D) 1:1500-diluted sera from healthy controls.

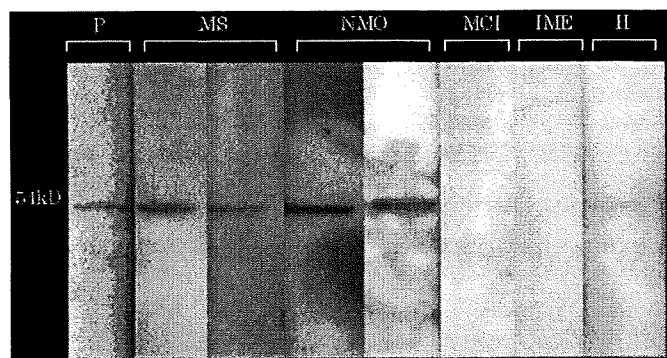


Fig. 2. Immunoblotting of human phosphoglycerate mutase 1 (PGAM1) full-length recombinant protein with GST. The recombinant protein (0.28 μ g) was applied to each well. P, 1:300-diluted anti-PGAM1 monoclonal antibody (Abnova); MS, 1:1500-diluted sera from patients with multiple sclerosis; NMO, 1:1500-diluted sera from patients with neuromyelitis optica; MCI, 1:1500-diluted sera from patients with multiple cerebral infarctions; IME, 1:1500-diluted sera from patients with infectious meningoencephalitis; H, 1:1500-diluted sera from healthy controls.

analyzed on a MALDI TOF/TOF instrument, AXIMA Performance (Shimadzu). By utilizing information on the x - y positions of spotted samples on AccuSpot, autoexperiments using AXIMA Performance were performed to analyze the samples on the plates. Every autoexperiment and protein identification were performed using an integrated software, Kompact Ver.2.8. Protein identification was carried out using the MS/MS ion search database, Mascot (<http://www.matrixscience.com/>; Matrix Science Ltd.).

2.6. Immunoreactivity of sera from patients with various neurological diseases against human PGAM1 full-length recombinant protein

We examined the anti-PGAM1 antibodies in sera from 21 MS, 13 NMO, 21 PD, 20 MCI, to 19 IME patients, and 17 healthy controls by 1DE immunoblotting using the commercially available human PGAM1 full-length recombinant protein with GST (Abnova). Immunoblotting was carried out as described in Section 2.3. The screening dilution of sera from all patients and healthy controls was 1:1500.

2.7. Statistical analysis

We used the chi-square test with Yates' continuity correction to assess the difference in the prevalence of the anti-PGAM1 antibody between groups. Differences were considered significant at $P < 0.05$.

3. Results

3.1. Screening and identification of target antigen of MS patients' autoantibodies (Fig. 1)

We examined the target antigen that reacted selectively with MS patients' sera. We detected by 1DE immunoblotting an approximately 30 kDa band corresponding to a protein that reacted with antibodies in sera from two out of five MS patients, but not with sera from five healthy controls. The same sample was subjected to 2DE, and one spot (observed MW/pI: 26,000/6.9) with a similar molecular weight reacted with the sera from these two MS patients but not with the sera from the healthy controls. We analyzed this spot by MALDI TOF-MS. This protein spot was identified as PGAM1 (accession number, P25113; score/coverage identification (%), 660/40; number of matched peptides, 11; theoretical MW/pI, 28,948/6.67).

3.2. Immunoreactivity of sera from patients with various neurological diseases against human PGAM1 full-length recombinant protein (Figs. 2 and 3)

To investigate whether the anti-PGAM 1 antibody is specific for MS, we examined this autoantibody in sera from patients with various neurological diseases (21 MS patients, 13 NMO patients, 21 PD patients, 20 MCI patients, and 19 IME patients) and 17 healthy controls by 1DE immunoblotting using the human PGAM1 full-length recombinant protein with GST (Figs. 2 and 3). As a result, the positivity rates were 81% (17 of 21) in MS patients, 77% (10 of 13) in NMO patients, 24% (5 of 21) in PD patients, 30% (6 of 20) in MCI patients, 32% (6 of 19) in IME patients, and 24% (4 of 17) in healthy controls. Statistically, the prevalence of the anti-PGAM1 antibody was significantly higher in patients with MS than in patients with PD ($P < 0.001$), MCI ($P < 0.003$), and IME ($P < 0.005$), and in healthy controls ($P < 0.002$). The prevalence of the anti-PGAM1 antibody was also significantly higher in patients with NMO than in patients with PD ($P < 0.008$), MCI ($P < 0.03$), and IME ($P < 0.04$), and in healthy controls ($P < 0.02$). These findings indicate that the anti-PGAM1 antibody has a stronger correlation with MS and NMO than with PD, MCI, IME, and being healthy.

4. Discussion

We identified PGAM1 as the target antigen of autoantibodies in sera from the MS patients by proteomics-based analysis. Western blotting analysis using the human PGAM1 recombinant protein

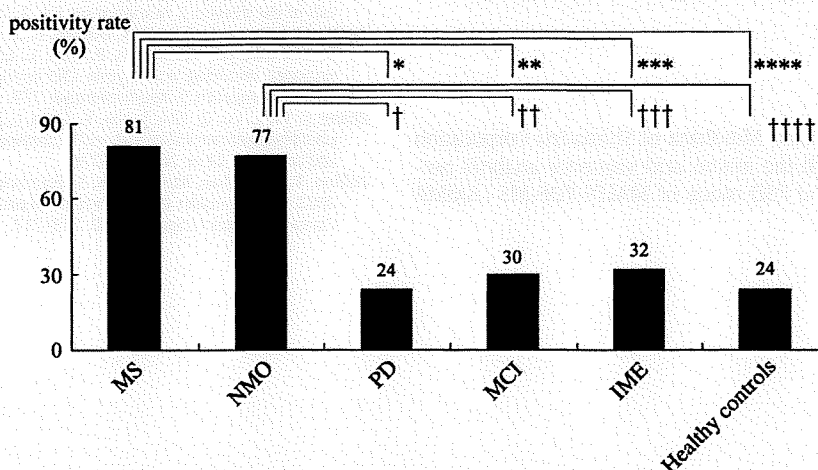


Fig. 3. Prevalence of antibodies against human phosphoglycerate mutase 1 (PGAM1) full-length recombinant protein. MS, patients with multiple sclerosis; NMO, patients with neuromyelitis optica; PD, patients with Parkinson's disease; MCI, patients with multiple cerebral infarctions; IME, patients with infectious meningoencephalitis. * $P < 0.001$, ** $P < 0.003$, *** $P < 0.005$, **** $P < 0.002$, † $P < 0.008$, †† $P < 0.03$, ††† $P < 0.04$, and †††† $P < 0.02$.

showed that the prevalence of the anti-PGAM1 antibody is much higher in not only patients with MS, but also those with patients with NMO, than in those with other neurological diseases and in healthy controls. To the best of our knowledge, this is the first study that elucidated the relationships between the anti-PGAM1 antibody and CNS autoimmune diseases. Lu et al. (2008) reported that the prevalence of the anti-PGAM1 antibody is much higher in patients with autoimmune hepatitis (AIH) than in those with other hepatic diseases and in healthy subjects. AIH is a rare liver disease and is characterized by hypergammaglobulinemia even in the absence of cirrhosis, characteristic autoantibodies, and a favorable response to immunosuppressive treatment (Zachou et al., 2004; Zolfino et al., 2002). Although the etiology of this disease is as yet unknown, the presence of several circulating autoantibodies such as the anti-nuclear antibody, anti-smooth muscle antibody, anti-liver kidney microsome type 1 antibody, and anti-liver cytosol type 1 antibody, which are serological markers for diagnostic criteria (Alvarez et al., 1999), suggests the important role of humoral mechanisms in AIH. There are several reports on MS patients with the complication of AIH (Pulicken et al., 2006; Takahashi et al., 2008; Ferrò et al., 2008). de Seze et al. (2005) reported that the prevalence of AIH seems to be about tenfold higher in patients with MS than in the general population. The anti-PGAM1 antibody can be generated in an immunological background common to both autoimmune CNS diseases and AIH.

Phosphoglycerate mutase is a glycolytic enzyme that catalyzes the interconversion of 3- and 2-phosphoglycerate with 2, 3-bisphosphoglycerate as the primer of the reaction (Fothergill-Gilmore and Watson, 1989). In mammalian tissues, PGAM exists in three isozymes, composed of homodimeric and heterodimeric combinations of two different subunits, type M (muscle form, PGAM2) and type B (brain form, PGAM1). The homodimer MM form is mainly expressed in the muscle; the BB form in the brain, kidney and liver; and the heterodimer MB form in the heart (Omenn and Cheung, 1974; Zhang et al., 2001). A previous study showed that PGAM1 is induced after hypoxia, which would occur in patients with cerebral infarction (Takahashi et al., 1998). In this study, the positivity rate of the anti-PGAM1 antibody in patients with MCI is not significantly higher than those in patients with other neurological diseases and in healthy controls. This finding suggests that an immunological background is important for production of the anti-PGAM 1 antibody.

In conclusion, the results of this study suggest that the anti-PGAM1 antibody is not only a marker of AIH but also a nonspecific marker of CNS autoimmune diseases. However, further studies are required to assess the presence of the anti-PGAM1 antibody in a large cohort of patients, including those with other autoimmune-mediated diseases, and controls.

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