Ⅲ　研究成果の刊行物・別刷

Antibodies in patients with neuropsychiatric systemic lupus erythematosus

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ABSTRACT

Objective: To investigate a target for antibodies in patients with neuropsychiatric systemic lupus erythematosus (NPSLE).

Background: Pathogenesis of NPSLE may be related to autoantibody-mediated neural dysfunction, vasculopathy, and coagulopathy. However, very few autoantibodies are sensitive and specific to NPSLE because the neuropsychiatric syndromes associated with SLE are diverse in cause and presentation.

Methods: We identified antibodies against brain antigens in the sera of 7 patients with NPSLE and 12 healthy controls by 2-dimensional electrophoresis, followed by Western blotting and liquid chromatography-tandem mass spectrometry (LC-MS/MS), using rat brain proteins as the antigen source.

Results: Six antibodies were detected in patients with NPSLE. One of these 6 antibodies was found in antibodies against Rab guanosine diphosphate dissociation inhibitor α (αGDI) (which is specifically abundant in neurons and regulates synaptic vesicle exocytosis) in patients with NPSLE with psychosis. We tested more samples by 1-dimensional immunoblotting of human recombinant αGDI. Positivity of the anti-αGDI antibody was significantly higher in patients with NPSLE with psychosis (80%, 4 of 5) than in patients with NPSLE without psychosis (0%, 0 of 13), patients with systemic lupus erythematosus without neuropsychiatric symptoms (5.3%, 1 of 19), patients with multiple sclerosis (0%, 0 of 12), patients with infectious meningoencephalitis (0%, 0 of 13), patients with polyneuropathy (0%, 0 of 10), patients with psychotic syndromes (0%, 0 of 10), and healthy controls (0%, 0 of 12).

Conclusions: We propose that the anti-Rab guanosine diphosphate dissociation inhibitor α antibody is a candidate for further exploration as diagnostic marker of psychosis associated with neuropsychiatric systemic lupus erythematosus. ***Neurology*** **2010;74:1372-1379**

GLOSSARY

1D = 1-dimensional; **2-DE** = 2-dimensional electrophoresis; **αGDI** = Rab guanosine diphosphate dissociation inhibitor α; **ACR** = American College of Rheumatology; **BRAA** = brain-reactive antibodies; **CBB** = Coomassie Brilliant Blue; **LC** = liquid chromatography; **LC-MS/MS** = liquid chromatography-tandem mass spectrometry; **NPSLE** = neuropsychiatric systemic lupus erythematosus; **PAGE** = polyacrylamide gel electrophoresis; **PVDF** = polyvinylidene difluoride; **SDS** = sodium dodecyl sulfate; **SLE** = systemic lupus erythematosus; **TBS** = Tris-buffered saline; **TBST** = Tris-buffered saline Tween-20.

Neuropsychiatric systemic lupus erythematosus (NPSLE) is one of the most significant manifestations of systemic lupus erythematosus (SLE). The pathogenesis of NPSLE may be related to autoantibody-mediated neural dysfunction, vasculopathy, and coagulopathy.[1,2] Previous studies have demonstrated the association of autoantibodies in serum and CSF with CNS involvement in patients with NPSLE.[3-5] Twenty antibodies associated with NPSLE were identified by a thorough MEDLINE search.[5] However, the authors of these studies concluded that specificity was lacking among these 20 antibodies for any single neuropsychiatric manifestation.

In 1999, the American College of Rheumatology (ACR) nomenclature for NPSLE provided case definitions for 19 neuropsychiatric syndromes observed in SLE.[6] Lupus psychosis (which is 1 of the

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19 neuropsychiatric syndromes) is rare, with a reported prevalence that varies from 0% to 11%. The occurrence of psychosis in SLE is not easy to determine reliably, because the literature often fails to distinguish psychosis associated with NPSLE from that associated with other causes, such as steroid psychosis.[7] In this study, we used proteomic analysis to investigate a novel brain antigen against antibodies in sera of persons with NPSLE with psychosis.

METHODS Patients and serum samples. Serum samples were collected from 18 patients with NPSLE; 19 patients with SLE but without neuropsychiatric symptoms; 12 patients with multiple sclerosis; 13 patients with infectious meningoencephalitis (4 with bacterial meningoencephalitis, 3 with cryptococcal meningoencephalitis, 1 with herpes simplex meningoencephalitis, and 5 with viral meningitis); 10 patients with polyneuropathy (5 with chronic inflammatory demyelinating polyneuropathy and 5 with Guillain-Barré syndrome); 10 patients with psychotic syndromes (5 with schizophrenia and 5 with depression); and 12 healthy controls. Diagnosis for patients with SLE was consistent with the revised ACR criteria of 1997.[8] We examined the neuropsychiatric syndromes of patients with SLE in our hospital and selected those patients whose neuropsychiatric syndromes might be caused by SLE, and not by an opportunistic infection, another mental disorder, other abnormal metabolic conditions, or a drug-induced disorder. The neuropsychiatric syndromes of patients with SLE were classified using an ACR consensus document published in 1999.[6]

For screening antibodies, comparatively specific for NPSLE patients, we investigated all the target spots corresponding to proteins that reacted with antibodies in the sera of 7 of the 18 patients with NPSLE and the 12 healthy controls by 2-dimensional electrophoresis (2-DE), followed by Western blotting. All target spots that reacted with antibodies in the sera of the 12 healthy controls were subtracted from the spots that reacted with antibodies in the sera of the 7 NPSLE patients. After subtraction, the remaining target spots were analyzed by liquid chromatography–tandem mass spectrometry (LC-MS/MS). This study is explorative and was approved by the institutional review board of the Gifu University Graduate School of Medicine, Gifu City, Japan (20-16).

Preparation of tissue proteins. Under ether anesthesia, 56-day-old Wister rats were killed. Their cerebrums were immediately removed and frozen in dry-ice powder. The frozen brain tissue was homogenized with a tissue homogenizer, and enriched membrane proteins were extracted using a proteoExtract native membrane protein extraction kit (Calbiochem, San Diego, CA). The protein concentration was determined by Bio-Rad protein assay, based on the Bradford method (Life Science [Research, Education, Process Separations, Food/Animal/Environment Testing], Hercules, CA).

Two-dimensional electrophoresis and immunoblotting. The samples were dissolved in DeStreak rehydration solution (GE Healthcare Bio-Sciences, Piscataway, NJ), and loaded onto an immobilized and rehydrated dry strip (pH 4–7, 13 cm long, GE Healthcare). Up to 100 μg of the extracted proteins was applied to a dry strip for Western blotting. Isoelectric focusing was conducted at 20°C for 85,000 Vh, at a maximum of 8,000 V, using a horizontal electrophoresis system (Multiphor III, GE Healthcare). Before separation in the second dimension, the isoelectric polyacrylamide gel strips were equilibrated for 15 minutes in a buffer containing 2% sodium dodecyl sulfate (SDS), 6 M urea, 30% volume by volume (v/v) glycerol, 0.001% BPB, 50 mM Tris-HCl (pH 8.8) under reducing conditions, with 65 mM DTT, followed by further incubation for 15 minutes in the same buffer under alkylating conditions with 140 mM iodoacetamide. Equilibrated isoelectric polyacrylamide gel strips were transferred to a 12.5% polyacrylamide gel.

The run in the second dimension was done vertically, using an electrophoresis apparatus (ERICA-S, DRC) at a constant voltage of 300 V for 2 hours. After the electrophoresis, the SDS-polyacrylamide gel electrophoresis (PAGE) gels were stained with Coomassie Brilliant Blue (CBB) (GelCode Blue Stain Reagent, Pierce) or used for protein transfer onto polyvinylidene difluoride (PVDF) membranes. The separated proteins were electrophoretically transferred to a PVDF membrane at 0.8 mA/cm^2 for 1 hour, using a semidry blotting apparatus (TE77 PWR Semi-Dry Transfer Unit, GE Healthcare). The PVDF membrane was stained with a fluorescent total protein stain (Deep Purple Total Protein Stain, GE Healthcare) and was scanned using a variable mode imager (Typhoon 9400, GE Healthcare). Subsequently, this membrane was incubated in blocking solution (5% skim milk in 1 × Tris-buffered saline Tween-20 [TBST]; 1 × Tris-buffered saline [TBS] containing 0.1% Tween 20) overnight in a cold room and reacted with the patient's serum, diluted to 1:1,500 with 1% skim milk in 1 × TBST for 1 hour at room temperature. The PVDF membrane was washed 5 times with 1 × TBST, and reacted with peroxidase-conjugated goat antihuman Ig (A+G+M) antibodies (P.A.R.I.S.) diluted to 1:2,000 with 1% skim milk in 1 × TBST for 1 hour at room temperature. After 6 washes, the membrane was incubated with the WB detection reagent (ECL Plus, GE Healthcare) for 5 minutes, and was scanned using Typhoon 9400. The antibody-reactive protein spots were matched with the fluorescent stained total protein spots, using image analysis software (Adobe Photoshop 6.0, Adobe Systems).

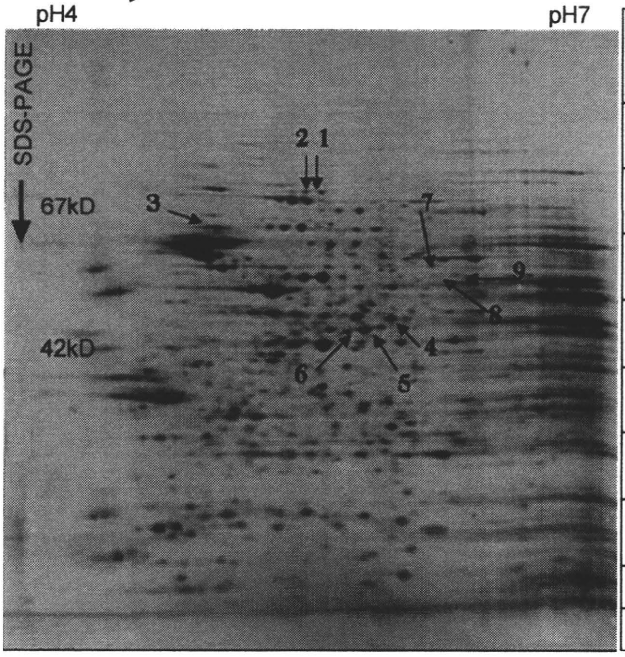
In-gel digestion and mass spectrometry. The identified spots were excised from the gel and subjected to trypsin digestion. Peptide fragments were analyzed using a nanoscale capillary liquid chromatography (LC) system (LC-VP, Shimadzu) and an ion trap tandem mass spectrometer (LCQ Advantage Max, Thermo electron). Proteins were identified from MS/MS spectra using protein identification software (X Caliber TM, Thermo Finnigan, and MASCOT Search, Matrix Science).

One-dimensional electrophoresis and immunoblotting using human recombinant Rab GDP dissociation inhibitor α. For 1-dimensional (1D) immunoblotting analysis, the commercially available Rab GDP dissociation inhibitor α (αGDI), full-length, human recombinant protein (Abnova, molecular weight: 75.28 kDa with its N-terminal GST-tag), produced by the method based on the wheat germ cell-free expression system, was separated by 4%–20% SDS-PAGE. Immunoblotting was the same as described previously. We tested the serum samples from 18 patients with NPSLE, 19 patients with SLE without neuropsychiatric symptoms, 12 patients with multiple sclerosis, 13 patients with infectious meningoencephalitis, 10 patients with polyneuropathy, 10 patients with psychotic syndromes, and 12 healthy controls.

Immunocytochemistry of human neuroblastoma culture cells. Human neuroblastoma SH-SY5Y cells on coverslips

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**

A



B

Spot No.	Protein name (SWISS-PROT accession No.)	Mascot Score / Coverage (%)	Observed M.W. (kDa) / pI [Calculated]
1	Stress-70 protein (P48721)	616 / 22	75 / 5.41 [74.097 / 5.97]
2	Stress-70 protein (P48721)	30 / 2	75 / 5.36 [74.097 / 5.97]
3	Rab GDP dissociation inhibitor alpha (αGDI) (P50398)	130 / 10	57 / 5.04 [51.074 / 5.00]
4	Isocitrate dehydrogenase [NAD] subunit alpha (Q99NA5)	363 / 19	45 / 5.8 [40.044 / 6.47]
5	L-lactate dehydrogenase B chain (P42123)	302 /29	43 / 5.68 [36.874 / 5.70]
6	F-actin-capping protein subunit alpha-2 (Q3T1K5)	83 / 6	43 / 5.6 [33.118 / 5.57]
7	Rab GDP dissociation inhibitor beta (P50399)	157 / 10	50 / 5.88 [51.018 / 5.93]
8	Not detected	--	48 / 6.0
9	Not detected	--	48 / 6.16

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-αGDI antibody, then washed, fixed, permeabilized, and single-immunolabeled or double-immunolabeled with an anti-αGDI monoclonal antibody, followed by the appropriate secondary fluorescent antibodies (see appendix e-1: Methods on the *Neurology*® Web site at www.neurology.org).

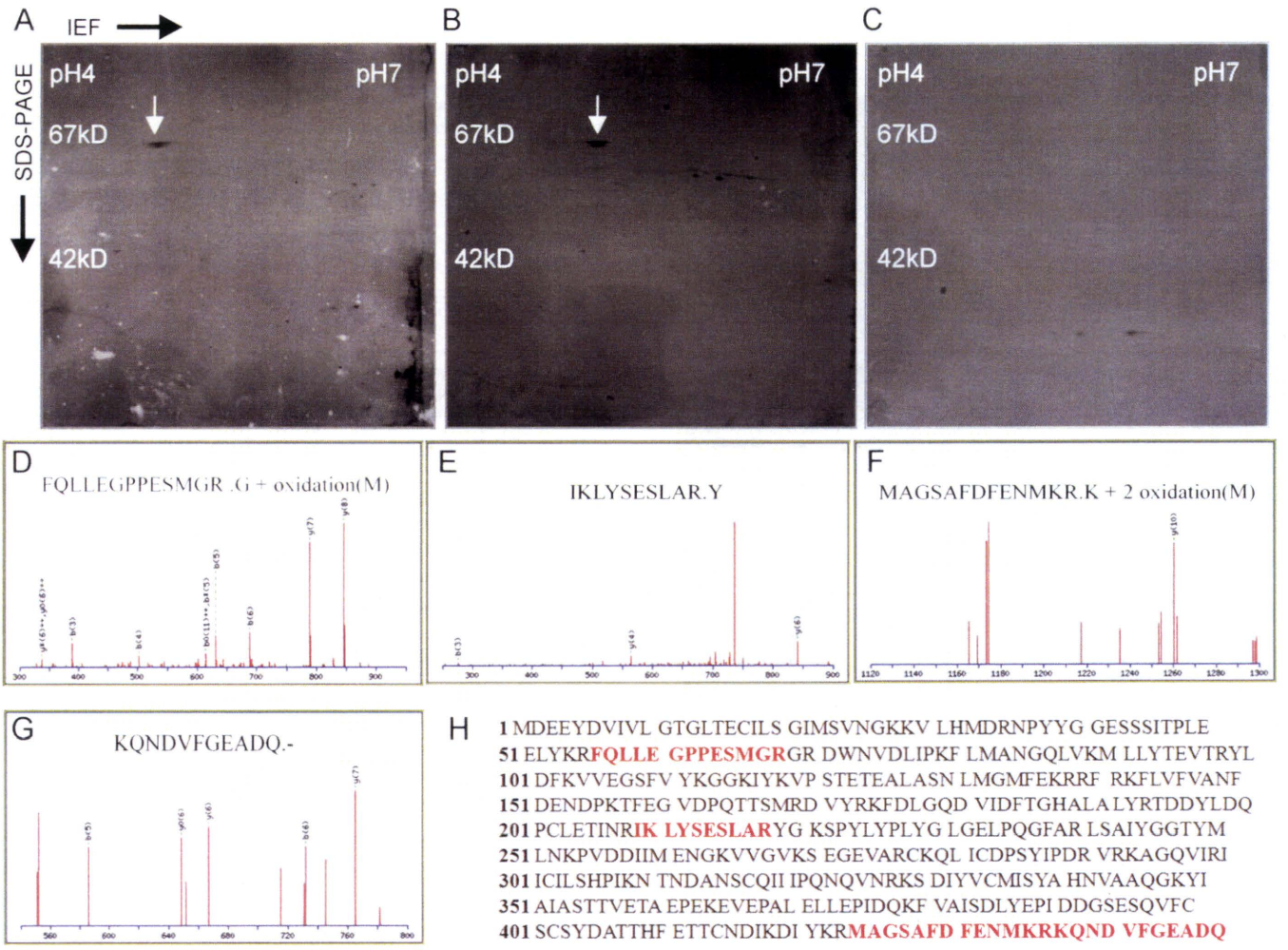
Statistical analyses. We used the Fisher exact probability test to assess differences in positivity of anti-αGDI antibodies between groups. The p values were considered statistically significant when less than 0.05.

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); αGDI (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, αGDI 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 αGDI 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 (αGDI) by mass spectrometry**



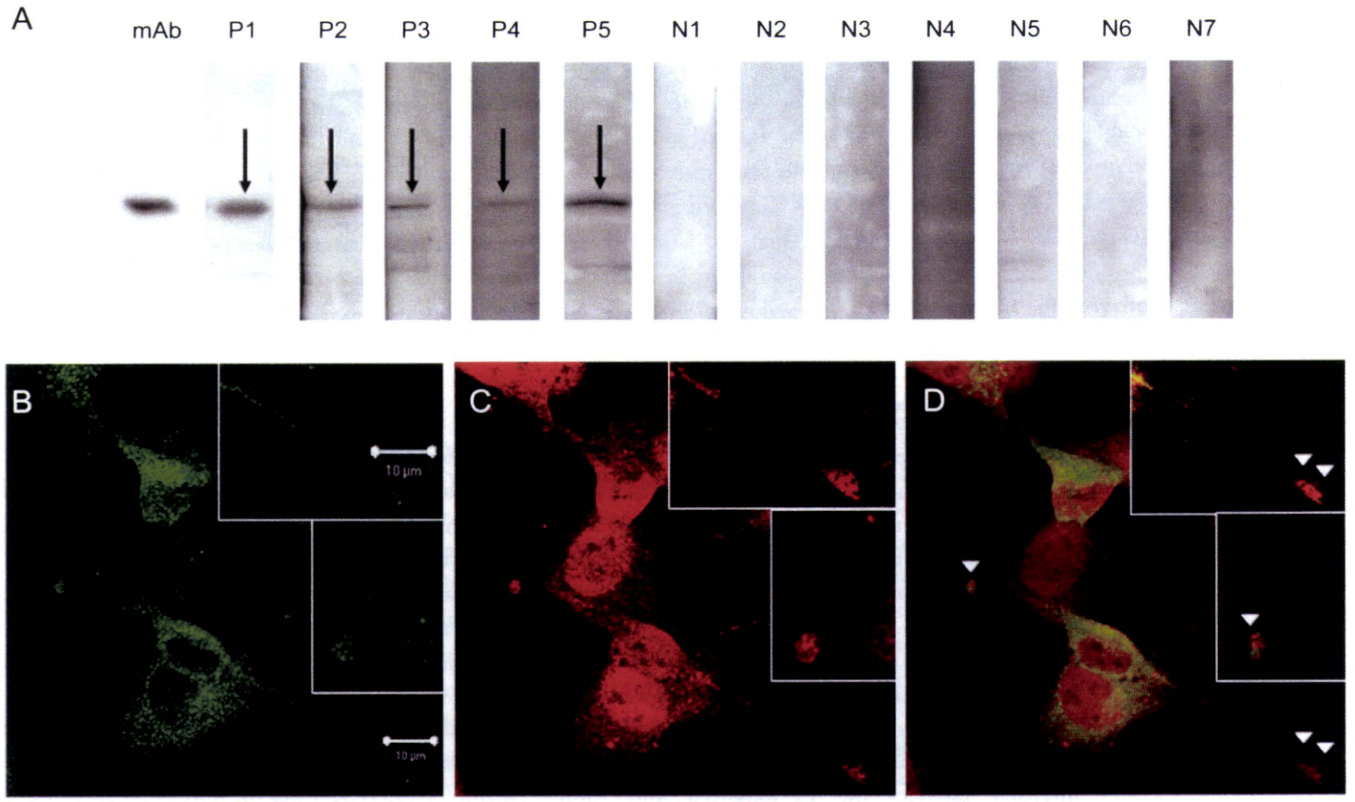
The polyvinylidene difluoride membrane reacted with sera of patients with neuropsychiatric systemic lupus erythematosus with psychosis (A: patient 2, B: patient 1 in table 1) and healthy control (C). Arrows indicate the αGDI spot. Tandem mass spectrometry spectra of 4 peptides of αGDI (D–G) and total amino acid sequences of αGDI (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 αGDI using protein identification software (H).

Immunoreactivity of sera from patients with NPSLE, patients with other conditions, and healthy controls against human αGDI 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-αGDI 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-αGDI 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-αGDI 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 (αGDI), full-length, recombinant protein, and immunocytochemistry using human neuroblastoma culture cells**



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

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

Characteristics of patients with anti-αGDI antibodies. Characteristics of patients with anti-αGDI 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-αGDI 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-αGDI 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: αGDI = 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-αGDI 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: αGDI = 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-αGDI 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-αGDI 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-αGDI antibodies showed complete remission of their psychiatric symptoms within 1 month after onset. The neuropsychiatric and nonneuropsychiatric symptoms of all 5 patients with anti-αGDI antibodies were stable with corticosteroid administration.

Immunocytochemistry of human neuroblastoma culture cells. Anti-αGDI 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-αGDI 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-αGDI 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, αGDI was the only brain-specific antigen and was localized in neurons.[9,10] It has been reported that αGDI 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 αGDI and neuropsychiatric symptoms of NPSLE in this study. We tested more samples by 1D immunoblotting of human recombinant αGDI. 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-αGDI antibody showed psychosis, as diagnosed based on ACR classification. Positivity of the anti-αGDI 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-αGDI antibody and psychosis in patients with NPSLE.

Mutations in *Gdi1*, which encodes αGDI, 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 αGDI constitutive knockout mice had altered short-term synaptic plasticity by electrophysiologic analysis.[15] The pathogenicity of the anti-αGDI antibody remains unclear. The αGDI is located in neurons but not on the membrane surface. Judging from its subcellular location, the anti-αGDI 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-αGDI antibody could penetrate the living cell and react with the αGDI. Our immunocytochemical study results revealed that the immunoreactivity of the anti-αGDI 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-αGDI antibodies inhibit the function of the αGDI, 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-αGDI antibodies are induced or the antibodies are passively administered to clarify the pathogenic role of the anti-αGDI antibody.

In this study, all 4 patients with NPSLE with the anti-αGDI 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-αGDI antibody. Anti-αGDI 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-αGDI 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 αGDI.[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-αGDI antibody.

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ORIGINAL COMMUNICATION

Motor-dominant chronic inflammatory demyelinating polyneuropathy

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Abstract We reviewed the clinical, electrophysiological an 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 ± 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

Patient	Iliopsoas	Gluteus	Hamstrings	Quadriceps	Tibialis anterior	Gastrocnemius
Lower						
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

Rt. median						Lt. median				
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	***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						Lt. 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					Rt. sural	Lt. tibial				Lt. sural
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 velocity, *MCV* motor conduction velocity, *NE* not examined, *SCV* sensory conduction velocity

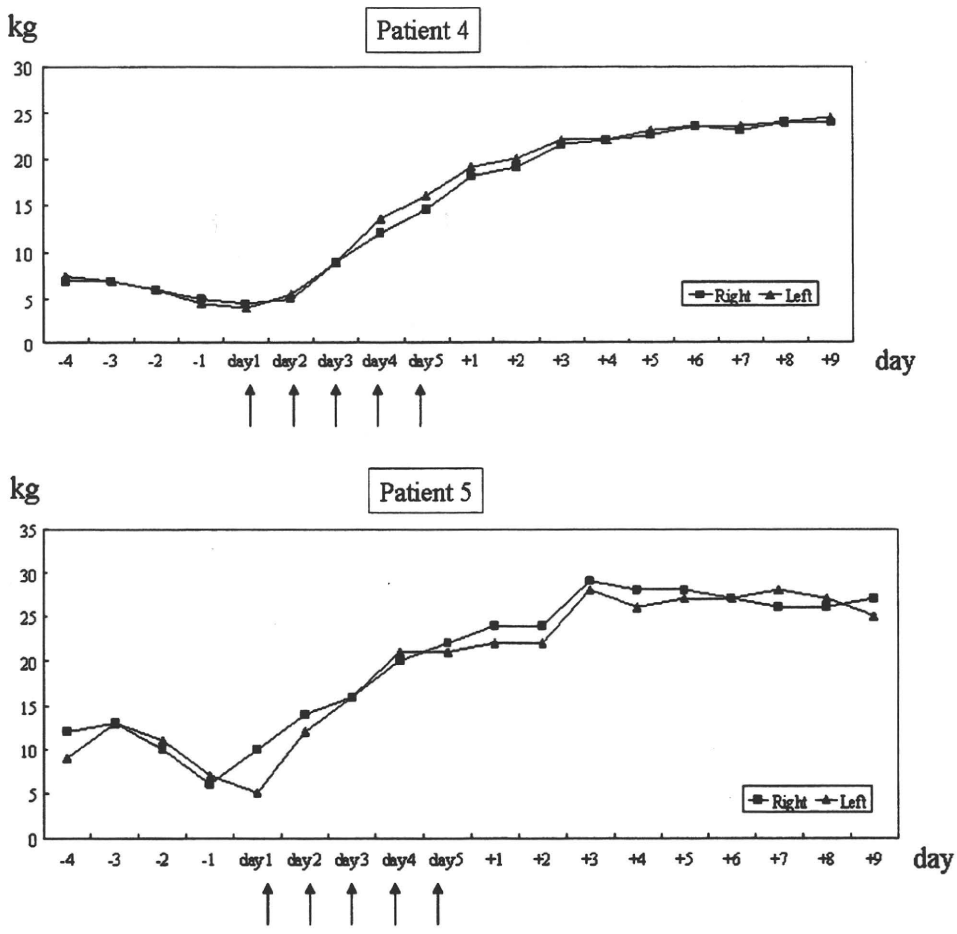

Fig. 1 Hand dynamometer values of patients with motor-dominant CIDP during IVIg therapy ↑: day of IVIg infusion

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 ± 3.2 mm^3 (mean ± SD, range 1–8 mm^3), and that of the other CIDP patients was 3.3 ± 4.8 mm^3 (range 0–14 mm^3). The CSF protein concentration in patients with motor-dominant CIDP was 97 ± 38 mg/dl (range 42–145 mg/dl), and that in the other CIDP patients was 150 ± 190 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 = 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

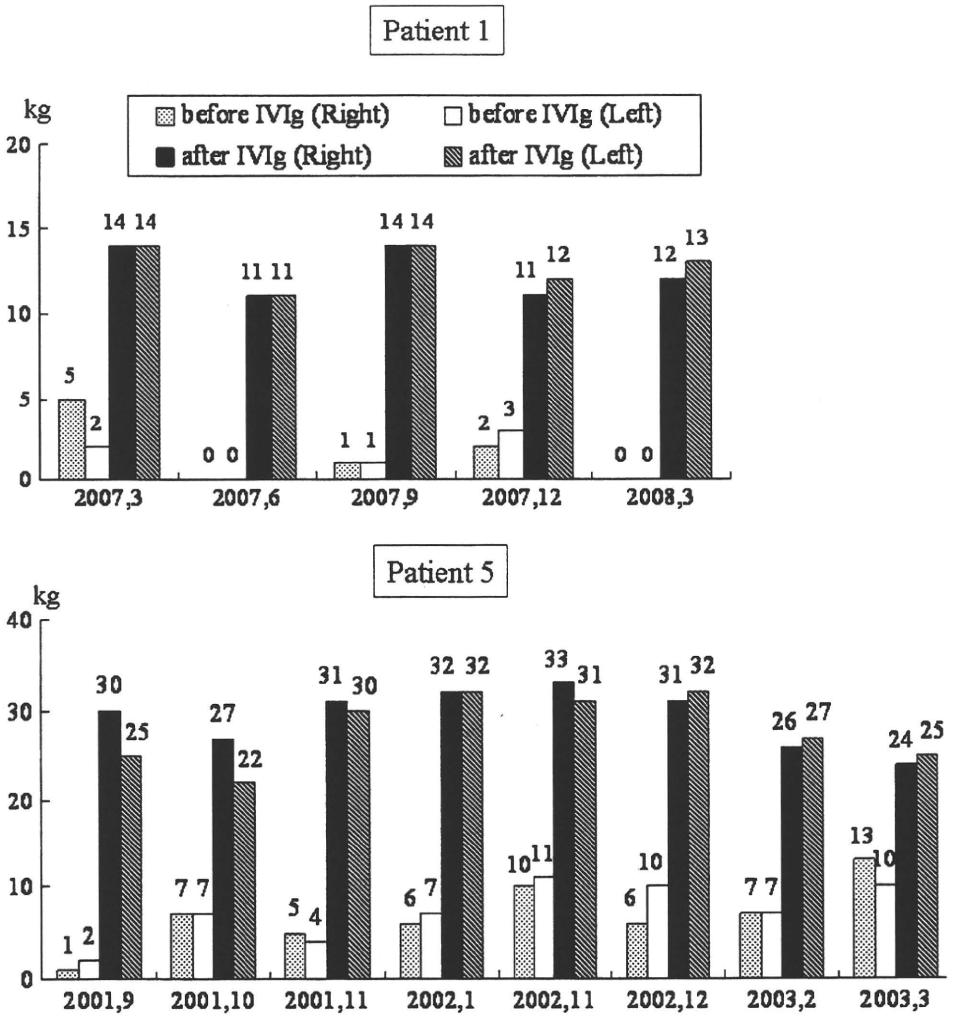

Fig. 2 Hand dynamometer values of patients with motor-dominant CIDP before and after IVIg therapy

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 ± SD;

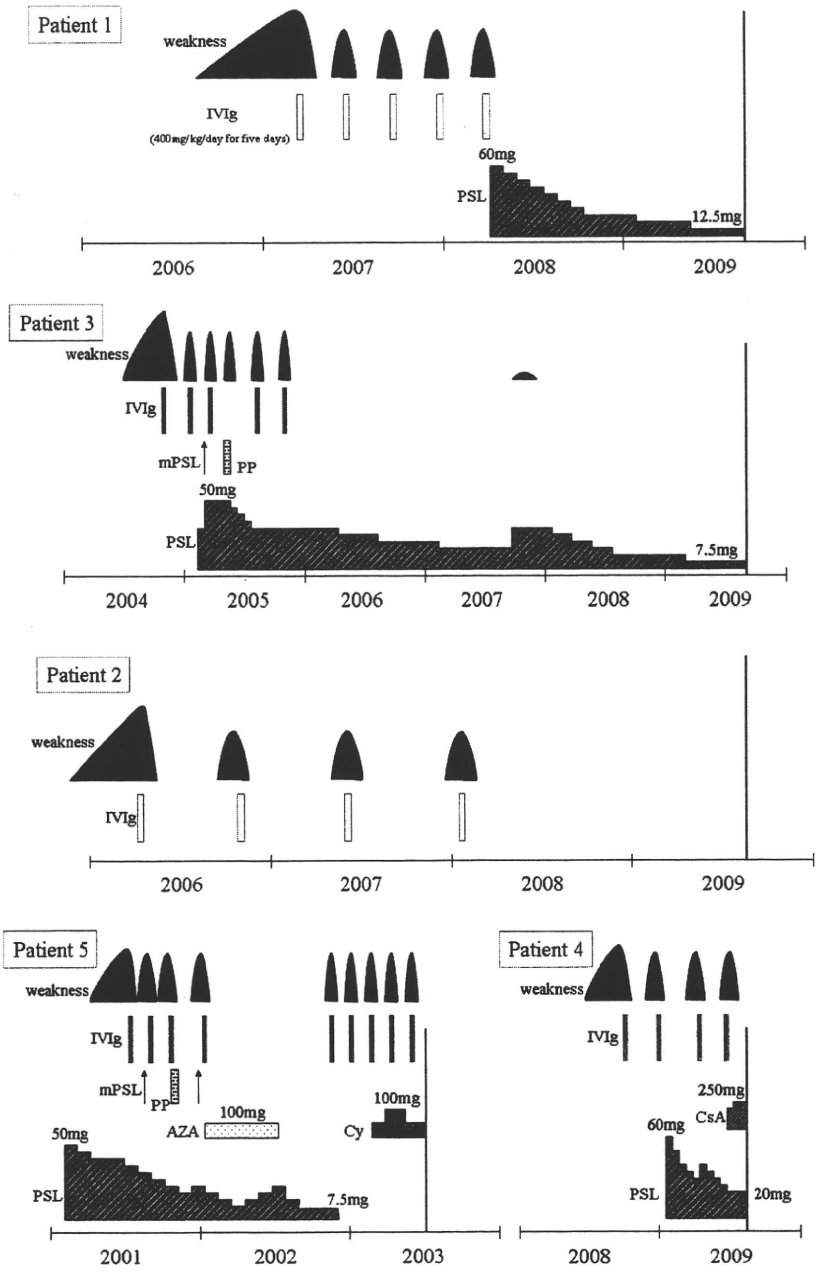

Fig. 3 Clinical course of patients with motor-dominant CIDP. *AZA* azathioprine, *CsA* cyclosporine A, *Cy* cyclophosphamide, *IVIg* intravenous immunoglobulin therapy, *mPSL* methylprednisolone pulse therapy, *PP* plasmapheresis, *PSL* prednisolone

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.

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 prevalence 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 polyvinylidine difluoride (PVDF) membranes at 0.8 mA/cm^2 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 (Coolphorestar 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 (Coolphorestar 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

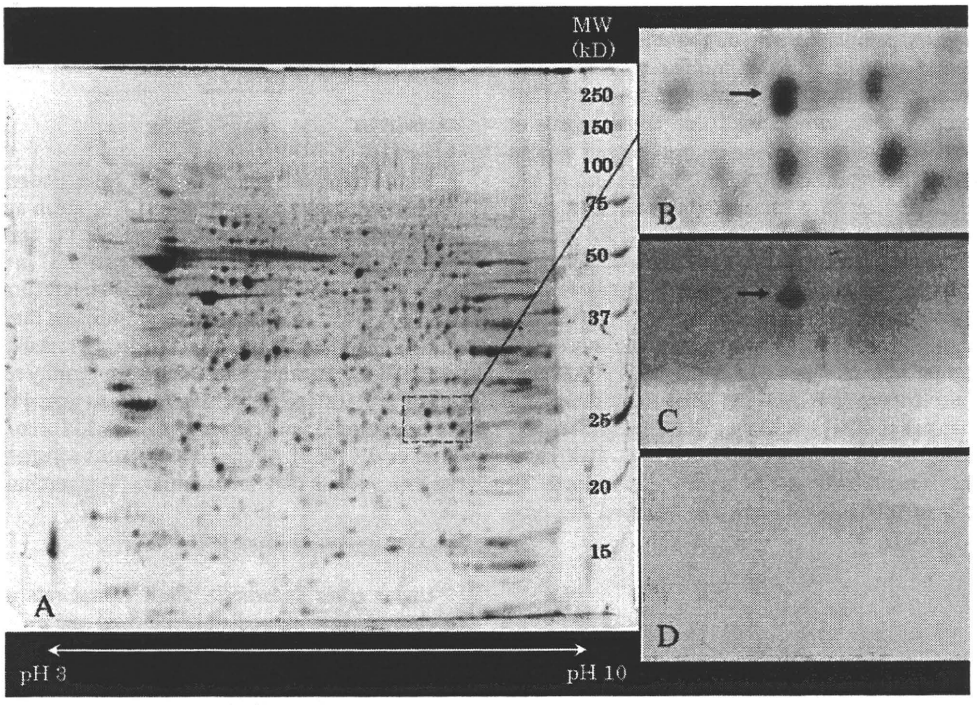

Fig. 1. Autoantibodies visualized by two-dimensional electrophoresis (2DE) and immunoblotting in sera from patients with multiple sclerosis (MS) and from healthy controls. (A, B) Total protein extracts of homogenized rat brain tissue were separated by 2DE, followed by SyproRuby staining. Arrow indicates 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.