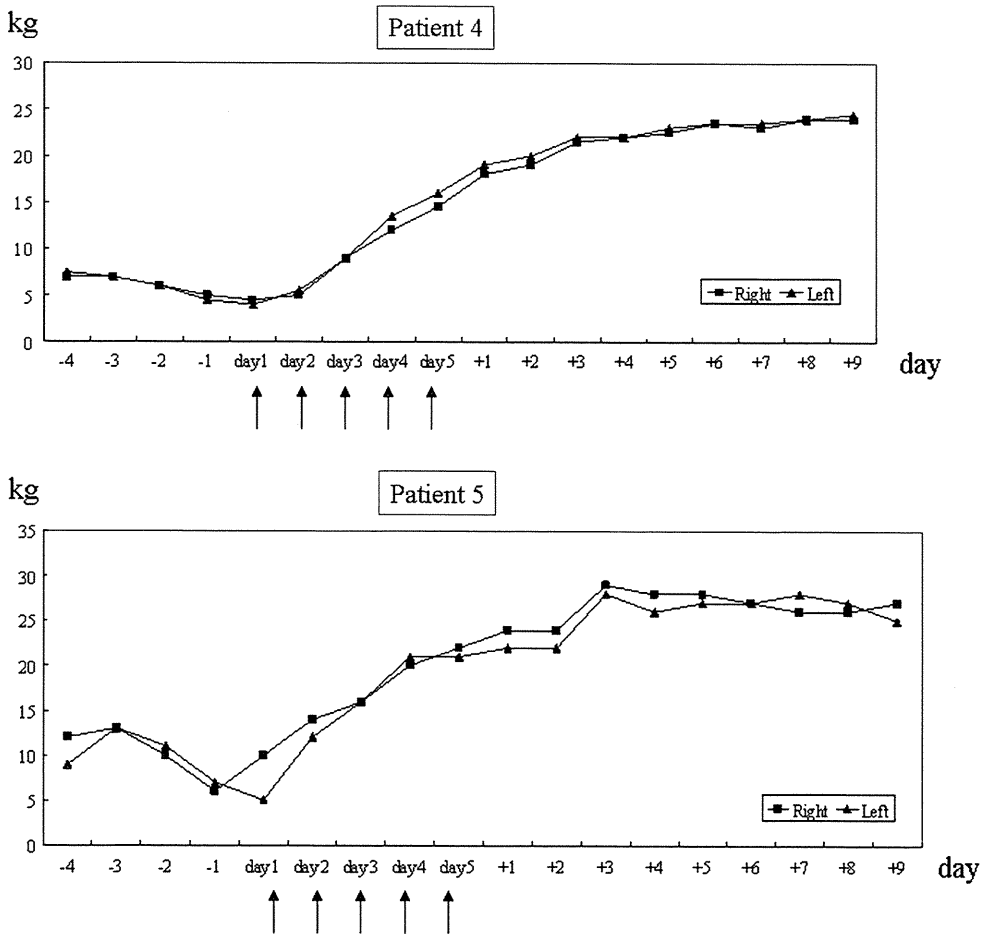

Fig. 1 Hand dynamometer values of patients with motor-dominant CIDP during IVIg therapy ↑: day of IVIg infusion

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

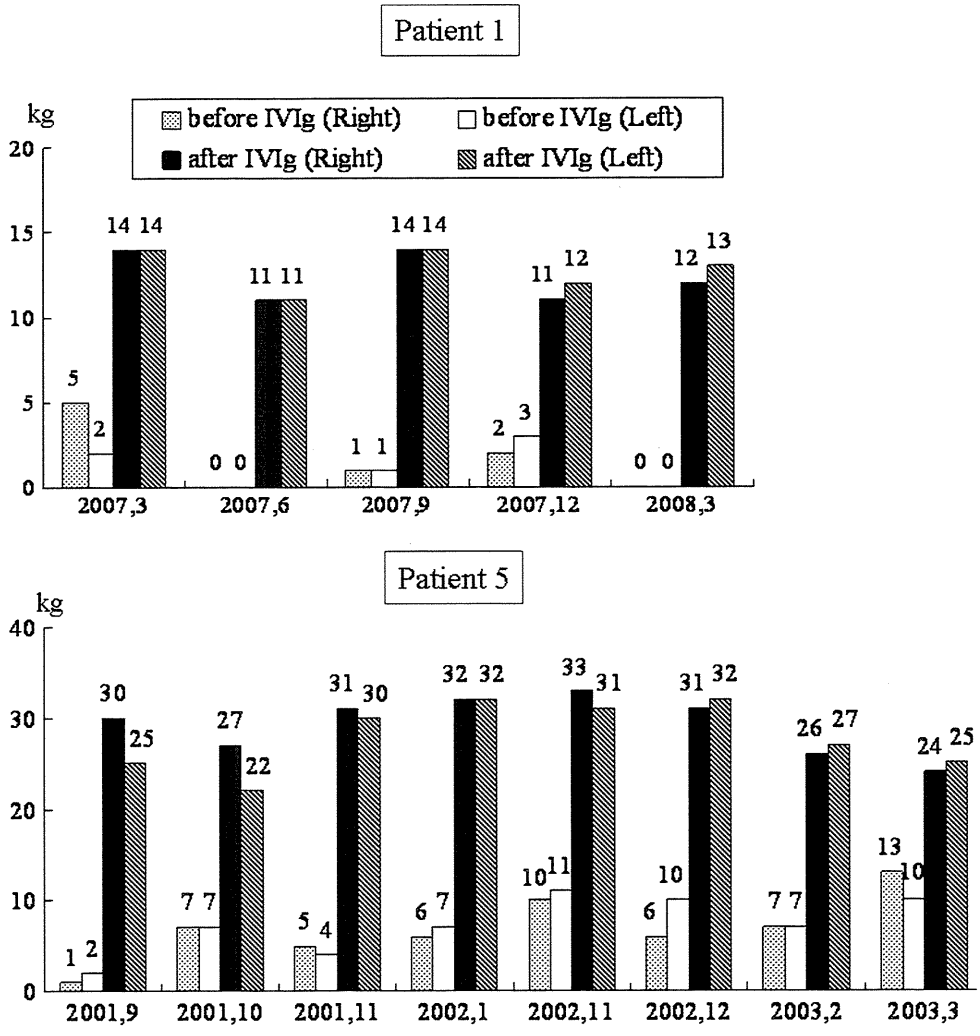

Fig. 2 Hand dynamometer values of patients with motor-dominant CIDP before and after IVIg therapy

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;

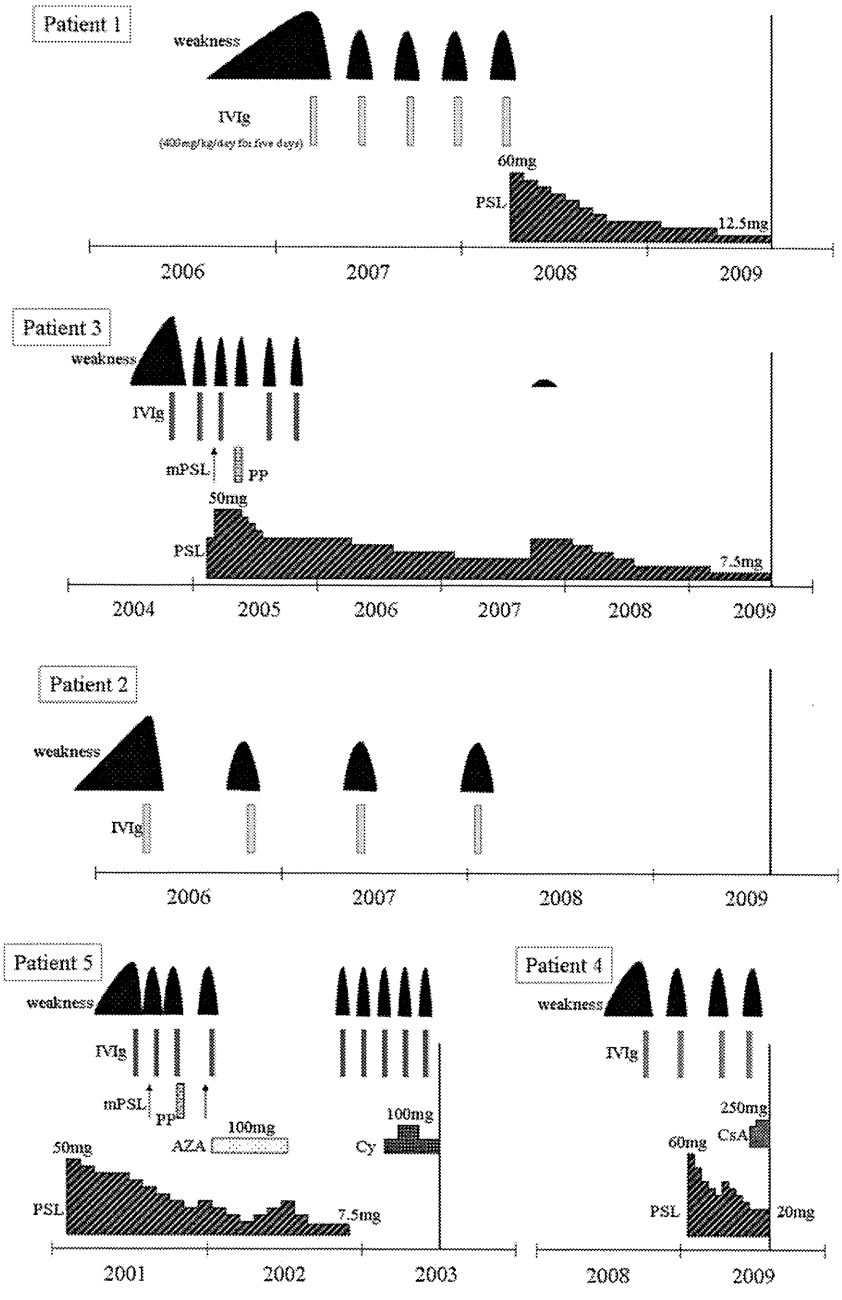

Fig. 3 Clinical course of patients with motor-dominant CIDP. *AZA* azathioprine, *CsA* cyclosporine A, *Cy* cyclophosphamide, *IVIg* intravenous immunoglobulin therapy, *mPSL* methylprednisolone pulse therapy, *PP* plasmapheresis, *PSL* prednisolone

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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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

Supplemental data at www.neurology.org

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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).

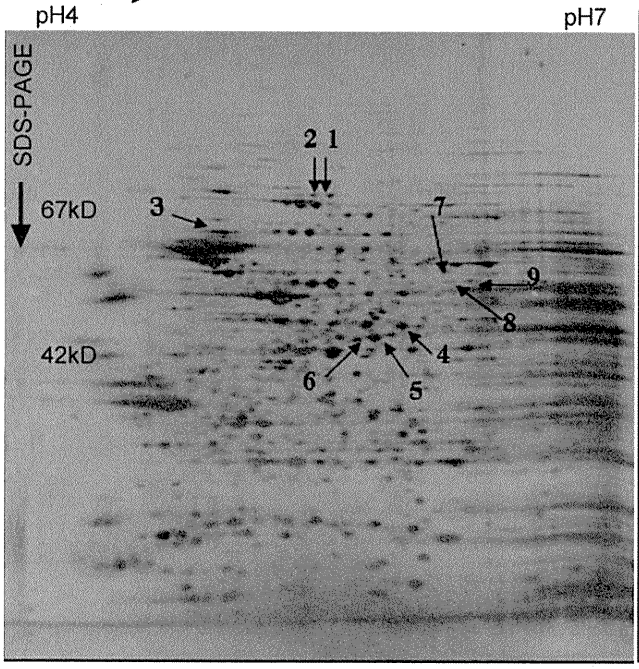
In-gel digestion and mass spectrometry. The identified spots were excised from the gel and subjected to trypsin digestion. Peptide fragments were analyzed using a nanoscale capillary liquid chromatography (LC) system (LC-VP, Shimadzu) and an ion trap tandem mass spectrometer (LCQ Advantage Max, Thermo 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.

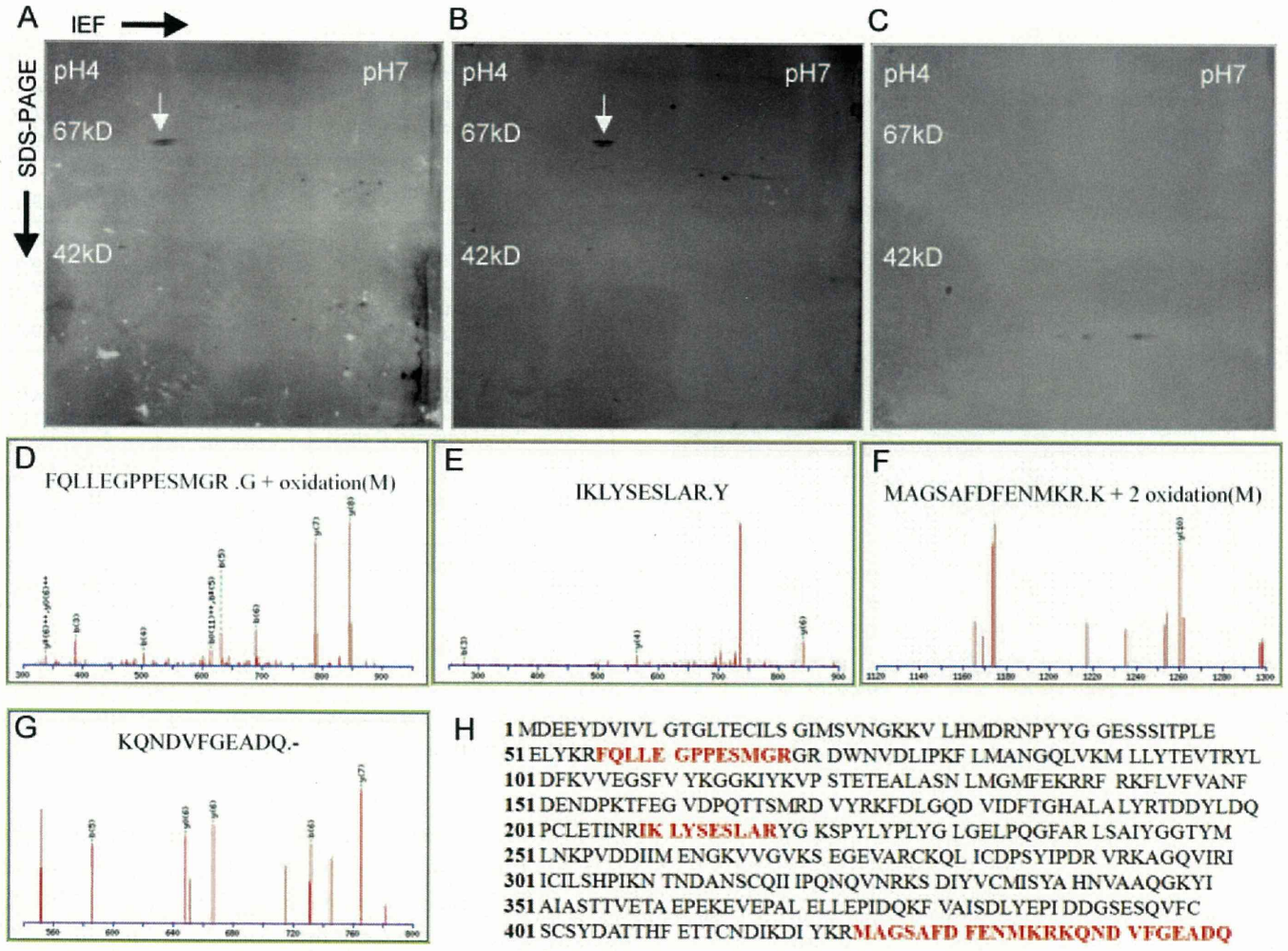
RESULTS

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



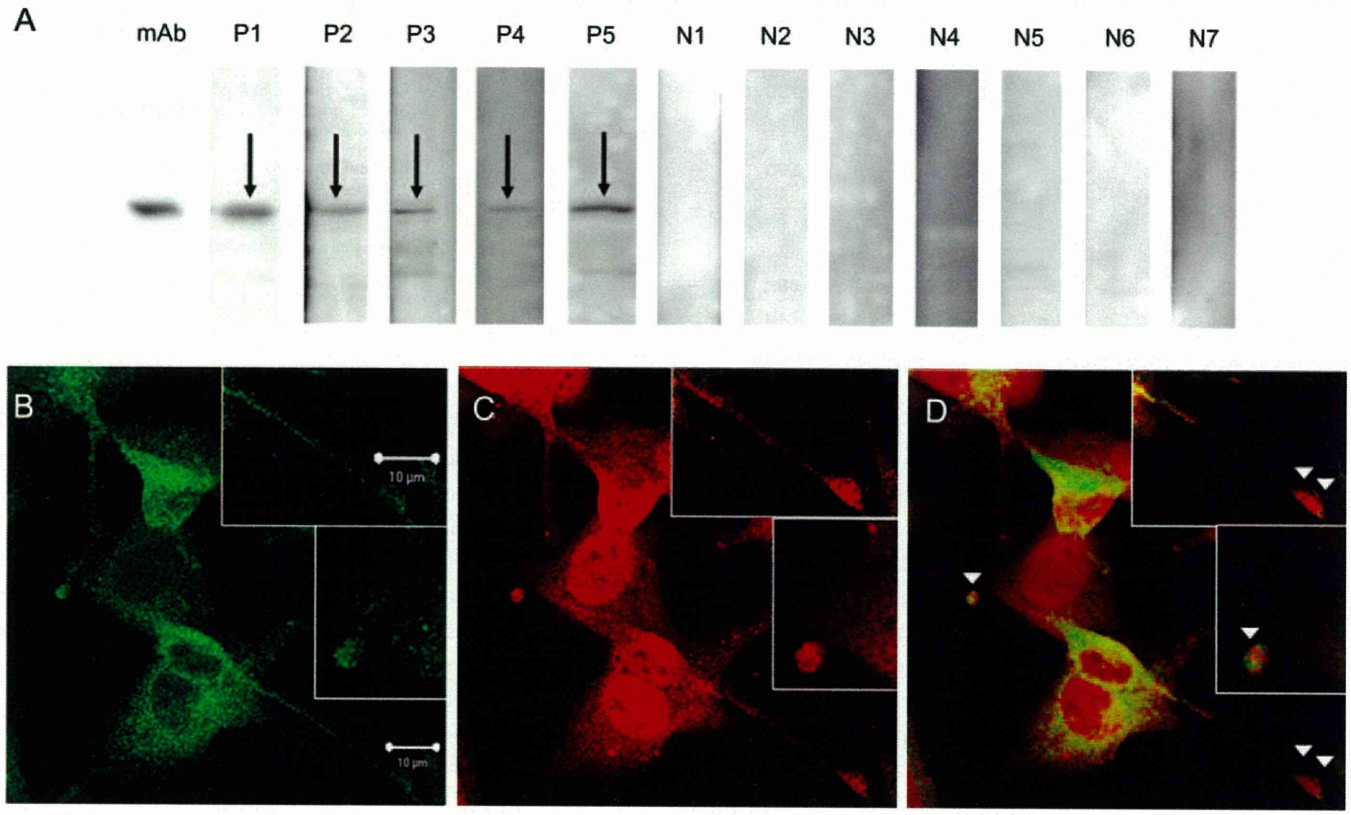
The polyvinylidene difluoride membrane reacted with sera of patients with neuropsychiatric systemic lupus erythematosus with psychosis (A: patient 2, B: patient 1 in table 1) and healthy control (C). Arrows indicate the αGDI spot. Tandem mass spectrometry spectra of 4 peptides of αGDI (D–G) and total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

胃腸炎後に発症した抗GQ1b抗体陽性の小脳失調を伴わない急性一側性外転神経麻痺の1例*

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Key Words : acute ophthalmoparesis, abducens nerve palsy, anti GQ1b antibody, gastroenteritis

50歳以下の若年成人において外転神経麻痺のみをきたすことは稀であるが，その原因疾患の中には感染後に外転神経麻痺のみを呈する症例が含まれる[1]．われわれは，胃腸炎後に急性に一側性外転神経麻痺をきたし抗GQ1b抗体陽性を示したことから，感染を契機とした免疫学的機序が原因と考えられた症例を経験したので報告する．

症　例

患者：39歳，女性．

主訴：左方注視時の複視，右手足のしびれ．

既往歴：特記すべきことはない．

家族歴：父が高血圧症，糖尿病，母が三叉神経痛，姉が橋本病に罹患．

現病歴：2007年12月下旬に1週間ほど急性胃腸炎に罹患した．12月28日に右手掌のしびれが出現し，30日の昼頃から右足底のしびれが出現した．31日，左方注視時に複視を自覚するようになった．症状が持続するため，2008年2月7日に当科に入院した．

入院時現症：身長151cm，体重37kg．体温36.2℃，血圧95/60mmHg，脈拍78/分・整．リンパ節腫大は認めなかった．心音，呼吸音に異常なく，腹部は平坦かつ軟であった．神経学的所見では意識清明で，脳神経領域では左眼の軽度外転制限を認め，左方視時に複視を自覚した．また，注視方向性のnystagmoid jerkを認めた．運動系では，筋力低下はなく，筋トーヌス，協調運動は正常であった．反射では，両上肢腱反射が亢進しており，病的反射は認めなかった．感覚系では，右手，右足底の異常感覚(ピリピリした感じ)を認めた．他覚的には表在覚，深部感覚に異常は認めなかった．立位・歩行，自律神経系に明らかな異常は認めなかった．

入院時検査所見：検血および一般生化学検査で異常は認めなかった．HbA1cは正常(4.9%，正常4.3～5.8%)で糖尿病は認めなかった．髄液検査では細胞数1/mm^3(単核球)，蛋白33mg/dl，糖56mg/dlと正常範囲であり，IgG indexは正常(0.51，正常0.34～0.85)でmyeline basic proteinの上昇はなく，oligoclonal bandは陰性であった．入院時の眼窩MRI画像(造影)では外眼筋や海綿静脈洞，脳幹部に異常信号域，造影効果は認めなかった．Hess chartでは左眼の外転制限を認めた(図1)．末梢神経伝導検査(右正中神経，右尺骨神経，右後脛骨神経，右腓腹神経で施行)，下肢SSPEで異常は認めなかった．

* Acute abducens nerve palsy following gastroenteritis without cerebellar ataxia and with positive anti GQ1b antibody. A case report.(Accepted September 4, 2009).

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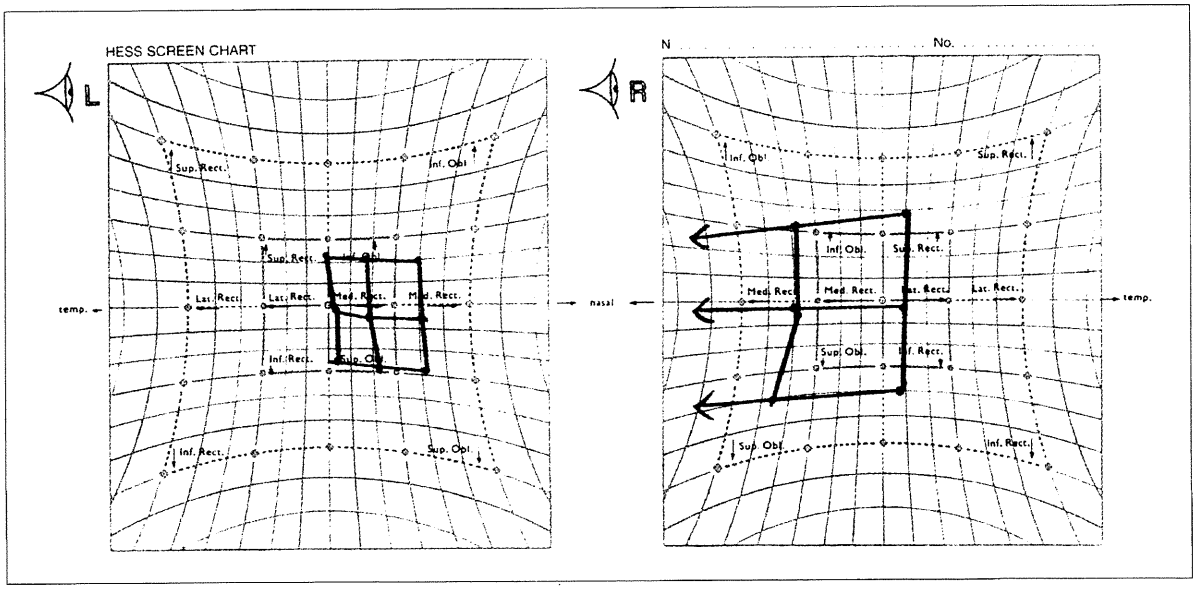

図1 Hess chart
左眼の外転制限を認める．

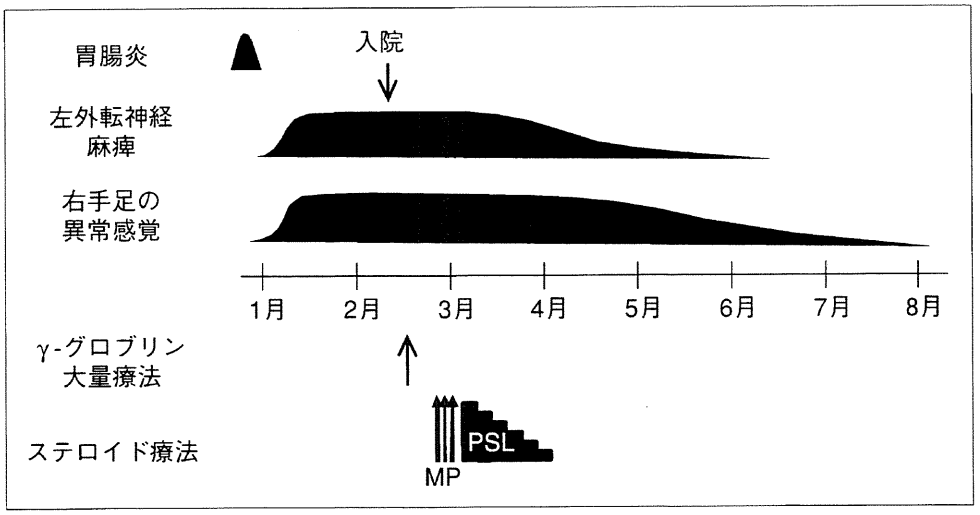

図2 臨床経過
MP：methylprednisolone 1,000mg/day×3days, PSL：prednisolone 60mg/dayから漸減．

入院後経過(図2)：入院後，左外転神経麻痺の原因として感染後の外転神経麻痺を考え，γ-グロブリン大量療法(献血グロベニン-I®)400mg/kg/day×5日間)を施行した．施行直後には明らかな治療効果を認めなかったため，さらにステロイドパルス療法(methylprednisolone 1,000mg/day×3日間)および後療法として経口ステロイド療法(prednisolone 60mg/dayから開始，1カ月で漸減)を行った．その約1週間後から徐々に左眼の外転制限，右手足のしびれは改善した．後に，当科入院時の血清で抗GQ1b-IgG抗体，抗GT1a-IgG抗体が強陽性と判明した．

考　察

本例の特徴は，①胃腸炎後に発症した急性一側性外転神経麻痺で，②小脳失調を伴わず，③後に抗GQ1b抗体陽性が判明した点である．症状，経過などから免疫学的機序の関与が想定される，抗GQ1b抗体陽性の小脳失調を伴わない急性一側性外転神経麻痺と診断した．経過や検査所見などから外転神経麻痺をきたす他疾患は否定した．

外転神経麻痺の原因は，高齢者では微小血管障害が多く，背景に高血圧症，糖尿病，高脂血症を伴うことが多い．しかし，20歳から50歳の若年成

人では外転神経麻痺のみを呈することは稀で，その原因疾患も高齢者とは異なると報告されている．本例でも高血圧症，糖尿病，高脂血症は認められていない．Georgeら[1]は，20歳から50歳の若年成人における外転神経麻痺の原因疾患について検討し，もっとも多い疾患は中枢神経系の腫瘤性病変(33%)で，次いで多発性硬化症(24%)，特発性(13%)，ウイルス感染後(9%)などと報告している．

感染後に外転神経麻痺をきたした100例のまとめ[2]では，平均年齢は42歳，性差はなく，先行感染では上気道炎がもっとも多く(38%)，下痢は14%にみられたとしている．また神経所見では，外転神経麻痺が両側にみられた症例は29%，腱反射低下～消失は27%，遠位部の異常感覚は7%に認め，抗GQ1b-IgG抗体は25%で陽性と報告されている．

感染後の外転神経麻痺の機序に関して，Miller Fisher症候群あるいはGuillain-Barré症候群の不全型として免疫学的な関与が考えられている．Chibaら[3]は，抗GQ1bモノクローナル抗体がヒトの動眼神経，滑車神経，外転神経の，脳幹外の末梢神経の部分に強く反応することを報告している．またShibataら[4]は，抗GQ1b抗体陽性で外転神経麻痺を呈した症例において，造影MRIで外転神経の脳槽内の部分が造影されたことを報告している．これらの報告から抗GQ1b抗体が核下性の外転神経障害に関与することが示唆される．しかし一方でYukiら[5]は，垂直性注視麻痺を伴わず外転障害に内転障害が加わった症例を報告し，その症例における原因病巣として中枢(とくに橋網様体傍正中部など)での障害を推察している．本例では深部腱反射の低下はなく，むしろ両上肢で亢進していたことから中枢性の障害も想定したが，電気生理学的検査，画像所見などにおいて中枢に責任病巣を示唆する変化を指摘できなかった．

本例での異常感覚に関して，電気生理学的検査で異常はなかったものの，本疾患はMiller Fisher症候群あるいはGuillain-Barré症候群の不全型と考えられており，末梢神経障害の可能性が考えられた．

ま と め

胃腸炎後に発症した小脳失調を伴わない急性一側性外転神経麻痺の39歳女性例を経験した．抗GQ1b抗体が陽性であり，免疫学的機序の関与が想定された．抗ガングリオシド抗体の測定がその発症機序の推定や治療に有用と思われた．

抗ガングリオシド抗体を測定していただいた，近畿大学神経内科・楠　進先生に深謝申し上げます．

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＜Abstract＞

Acute abducens nerve palsy following gastroenteritis without cerebellar ataxia and with postive anti GQ1b antibody. A case report.

by
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A 39-year-old female developed left acute abducens nerve palsy, and dysesthesia in her right hand and sole following gastroenteritis. We diagnosed her as having post-infection abducens nerve palsy and treated her with high-dose intravenous immunoglobulin. Since the treatment did not improve her symptoms, we added steroid therapy. One week later, her symptoms were ameliorated. Laboratory tests showed positive anti-GQ1b-IgG and anti-GT1a-IgG antibodies. The final diagnosis in this case was acute left abducens nerve palsy without cerebellar ataxia induced by anti GQ1b antibody.

トピックス

III. 最近の話題
2. 膠原病における新たな抗神経抗体の検索

木村　暁夫　　犬塚　貴

要　旨

神経障害を伴う膠原病患者において自己免疫異常を背景として出現した抗神経抗体は神経障害に密接な関りをもつ可能性がある．したがって特異的な抗神経抗体を同定することは，その病態解明に重要である．また，時に神経系の日和見感染症や精神疾患との鑑別が問題となるこれら神経症状の，適切な診断と治療効果のメルクマールの確立につながると考えられる．我々はプロテオミクス解析の手法を用いて，神経障害を伴う膠原病に特異的と考えられた新たな抗神経抗体をいくつか同定してきたので報告する．

〔日内会誌　99：1865～1870，2010〕

Key words：膠原病，神経障害，抗神経抗体，二次元免疫ブロット，プロテオミクス

1. 膠原病患者の神経障害と自己抗体

膠原病患者に合併する神経障害と抗神経抗体を含めた自己抗体との関連性を報告した論文はこれまでに多数認められるが，その多くが全身性エリテマトーデス（systemic lupus erythematosus：SLE）[1]に関するものであり，次いでSjögren症候群[2]に関するものである．最近のレビューによると，これまでに精神神経症状を合併したSLEすなわちneuropsychiatric systemic lupus erythematosus（NPSLE）患者において，その血清ないし髄液中より約20種類の自己抗体が報告されている[1]．特に以前より血清ないし髄液中の抗リボソームP抗体がループス精神病と有意な相関があることが指摘されており[3]，近年同抗体の認識抗原がneuronal surface P antigen（NSAP）と名づけられた神経細胞表面に存在する蛋白であることが証明された[4]．一方，SLE患者に存在する抗二本鎖DNA抗体がN-methyl-D-aspartate（NMDA）型抗グルタミン酸受容体の一部（NR2AおよびNR2B）と交差反応性を示し，さらにはアポトーシス経路を介して神経細胞死をもたらすとする報告もある[5]．この報告に基づき，SLE患者血清中の抗NR2A抗体の存在とうつ症状および短期記憶障害との関連性を指摘する報告や，NMDAレセプターに対する抗体を発現するマウスを作成し，lipopolysaccharide（LPS）により血液脳関門を破綻させることで抗体が海馬の神経細胞と結合し，認知機能障害の原因となる神経細胞死に至ったとする報告もある[6]．更にこの報告ではLPSを投与する以前にNMDAレセプターのアンタゴニストであるmemantineを加えることで神経細胞障害を防ぐことができたとして，今後の新たな治療法としての可能性を指摘している．しかし，これらの報告

きむら　あきお，いぬづか　たかし：岐阜大学大学院神経内科・老年学分野

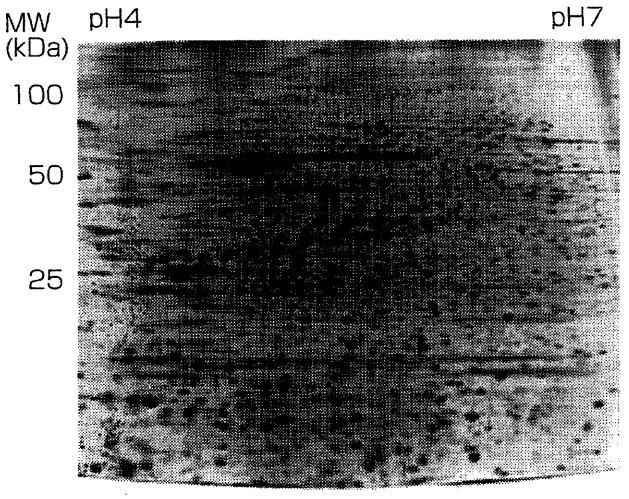

図1. ラット大脳ホモジネートをサンプルとした二次元電気泳動後のゲル画像
蛍光色素（SYPRO® Ruby : Invitrogen）により染色した全蛋白スポット画像を示す

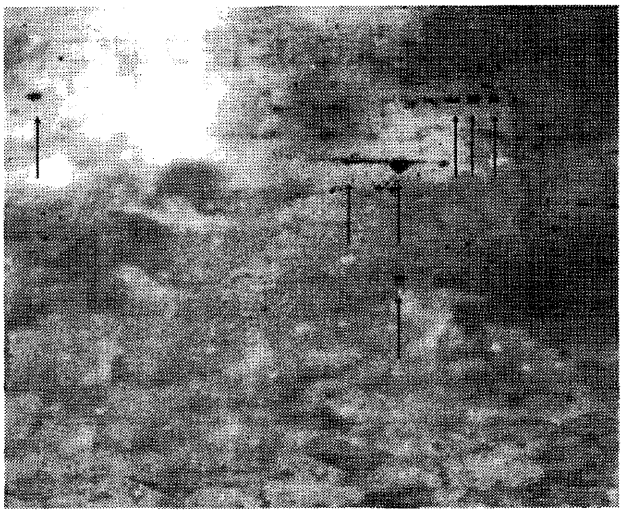

図2. 二次元免疫ブロット後のPVDFメンブレン画像
200倍に希釈した辺縁系脳炎患者の髄液を用い，二次抗体として2,000倍希釈の抗ヒトIgGAM抗体を用いて，抗原抗体反応を施行した．矢印に抗体反応スポットを示す．

も含めNPSLEに特異的といえる自己抗体は未だ確立していない．その理由の一つとしてNPSLE患者にみられる精神神経症状は多彩であることがあげられる．アメリカリウマチ学会（ACR）は1999年に，SLEの精神神経病変を議論するための共通の基盤を作るために，SLEの精神神経症状の新たな分類を提唱した．この分類では，19にわたる精神神経症状を大きく中枢神経病変と末梢神経病変に分け，さらに前者を「神経症状」（neurologic syndromes）と「精神症状」（diffuse psychiatric/neuropshychological syndromes）に大別している．このようなNPSLE患者に合併する多彩な神経障害の病態背景には，多様な機序が存在することが推測され，関与しうる自己抗体も多種類存在する可能性が予想される．今後，NPSLE患者において検出された自己抗体の特異性を議論するにあたり，ACRの分類に基づいた精神神経症状との関連性を詳細に検討する必要があると考えられる．

Sjögren症候群に関する報告では，以前より感覚優位の末梢神経障害の患者で脊髄後根神経節細胞に対する自己抗体を検出したとする報告がある[2]．また，四肢筋力低下・小脳失調を呈した症例で脊髄運動ニューロン・大脳皮質ニューロン・小脳Purkinje細胞に免疫反応性を有する約34 kDaの抗神経抗体を検出したとする報告[7]や，下位運動ニューロン徴候を呈した症例で大脳もしくは脊髄に存在する約50 kDaの蛋白に対する抗神経抗体を検出したとする報告などがある[8,9]．しかし，これらの報告はいずれも症例報告にとどまり，検出された抗神経抗体の病的意義は不明である．今後の症例の蓄積と抗原蛋白の同定ならびに特異性の検討が重要であると思われる．

2. 抗神経抗体の検索

我々は抗神経抗体の検出方法として，二次元電気泳動後にウエスタンブロットを施行する二次元免疫ブロット法を用いている．二次元電気泳動とは一次元目として固定化pH勾配ストリップゲルを使用した等電点電気泳動を行い，さらに二次元目として等電点電気泳動終了後のストリップゲルをポリアクリルアミドゲルにのせて，sodium dodecyl sulfate-polyacrylamide gel electrophoresis（SDS-PAGE）を行うものである．二次元電気泳動を行うことでサンプル中に含まれ

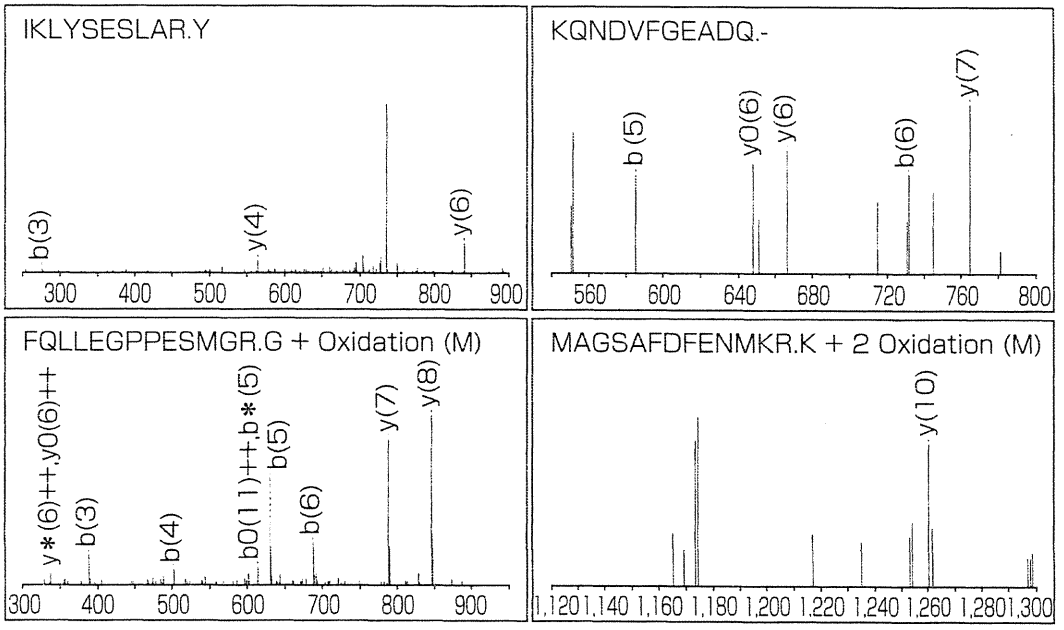

図3. 質量分析の結果得られたペプチドのMS/MSスペクトルとアミノ酸配列
αGDIの4つのペプチドに相当するスペクトルが検出された.

る蛋白質は個々のもつ電荷と分子量の違いによりゲル上に多数のスポットとして展開される. 我々の施設では抗神経抗体の検出のためラット大脳ホモジネートを抗原サンプルとして用いているが, この二次元電気泳動によりラット大脳サンプル中に含まれる最大約1,400個の蛋白スポットを再現性よく分離することが可能である(図1). この方法の長所として抗原となる蛋白質の糖鎖などの側鎖構造がそのまま保たれる点があげられる. 一方, 短所としてSDS-PAGEにより蛋白質の立体構造が失われること, 高分子量蛋白や塩基性蛋白の分離が比較的困難であることがあげられる. 次に二次元電気泳動終了後のポリアクリルアミドゲル内の蛋白をpolyvinylidene difluoride (PVDF) メンブレンにブロットし, ブロット後のメンブレンを用いて抗原抗体反応を行うことにより網羅的に患者血清・髄液中に存在する抗神経抗体を検出している (図2).

3. 抗神経抗体認識抗原蛋白の同定の実際

初めにラット大脳サンプルを同時に2枚のゲルを用いて二次元電気泳動で展開し, 蛍光色素を用いて全蛋白染色する. 一枚のゲルはスポット切り出し用に保存し, 他方のゲルはスキャナーで全蛋白染色後の画像を取りこんだ後, 免疫ブロットを行う. この時, 抗原抗体反応を施行する前のメンブレンの全蛋白染色画像 (全蛋白染色したゲル上の蛋白は色素を保持したままメンブレンに移行する) を画像に取り込み, さらに抗原抗体反応施行後に検出試薬を用いて抗体反応スポットを検出し画像として別に保存する. 最後に抗体反応スポットと抗原抗体反応施行前のメンブレンおよびゲル上の全蛋白染色スポットを画像解析ソフトを用いてマッチングする. 次に一致したスポットにつきin gel消化を行い, 液体クロマトグラフィー (liquid chromatography : LC) とタンデム質量分析 (tandem mass spectrometry : MS/MS) 装置を組み合わせたLC-MS/MSシステムによりペプチドのアミノ酸配列を解析し (図3), さらにそこから得られたデータを, 検索サーバーを介しデータベース検索により抗神経抗体の認識抗原蛋白を同定している (図4).

Protein View
Match to : GDIA_RAT Score : 130
Rab GDP dissociation inhibitor alpha - Rattus norvegicus (Rat)
Nominal mass (Mr) : 51074 ; Calculated pI value : 5.00
NCBI BLAST search of GDIA_RAT against nr
Unformatted sequence string for pasting into other applications
Taxonomy : Rattus norvegicus
Fixed modifications : Carbamidomethyl(C)
Variable modifications : Carbamyl (N-term), Oxidation (M)
Cleavage by Trypsin : cuts C-term side of KR unless next residue is P
Sequence Coverage : 10%
Matched peptides shown in **Bold Red**

1 MDEEYDVIVL GTGLTECILS GIMSVNGKKV LHMDRNPYYG GESSSITPLE
51 ELYKR**FQLLE GPPESMGR**GR DWNVDLIPKF LMANGQLVKM LLYTEVTRYL
101 DFKVVEGSFV YKGGKIYKVP STETEALASN LMGMFEKRRF RKFLVFVANF
151 DENDPKTFEG VDPQTTSMRD VYRKFDLGQD VIDFTGHALA LYRTDDYLDQ
201 PCLETINRIK **LYSESLAR**YG KSPYLYPLYG LGELPQGFAR LSAIYGGTYM
251 LNKPVDDIIM ENGKVVGVKS EGEVARCKQL ICDPSYIPDR VRKAGQVIRI
301 ICILSHPIKN TNDANSCQII IPQNQVNRKS DIYVCMISYA HNVAAQGKYI
351 AIASTTVETA EPEKEVEPAL ELLEPIDQKF VAISDLYEPI DDGSESQVFC
401 SCSYDATTHF ETTCNDIKDI YKR**MAGSAFD FENMKRKQND VFGEADQ**

図4. 検索サーバーを利用した蛋白質の同定
Mascot Search Serverに図3の質量分析データを送ってデータベース検索をした結果αGDIが同定された.

4. NPSLE患者と抗神経抗体

我々は，これまで上記プロテオミクス解析の手法を用いてNPSLE患者の血清中よりいくつかの抗神経抗体を同定し報告した[10]．二次元免疫ブロットにより12例の健常者血清に反応せず7例のNPSLE患者の血清にのみ反応した9つのスポットを選択し，このうちの7つのスポットから6つの抗原蛋白を同定した．これらはstress-70 protein, Rab GDP dissociation inhibitor alpha（αGDI），isocitrate dehydrogenase［NAD］subunit alpha, L-lactate dehydrogenase B chain, F-actin-capping protein subunit alpha-2, Rab GDP dissociation inhibitor beta（GDI-2）であった．これらの抗原蛋白のうちαGDIは，神経シナプスに局在し小胞輸送に必要なG蛋白Rab3aのリサイクリングに関与することが知られている．さらにαGDIをコードするGDI-1遺伝子は，精神発達遅滞を唯一の臨床症状とする非特異的X連鎖精神遅滞の原因遺伝子の1つであり，そのノックアウトマウスでは，短期記憶障害，攻撃性の低下，社会行動の変化を示すことが知られている[11]．これらの報告を踏まえ抗αGDI抗体が神経シナプスにおける伝達障害に関与する可能性を考慮し，同抗体の特異性につき検討した．

5. NPSLEと抗αGDI抗体

ヒトαGDIリコンビナント蛋白を用い，NPSLE患者および対照の血清を一次抗体として一次元免疫ブロットを施行した．検索対象はNPSLE患者18例，精神神経症状を合併しないSLE患者19例，多発性硬化症患者12例，感染性髄膜脳炎患者13例，多発性ニューロパチー患者10例，統合失調症患者5例，躁うつ病患者5例，健常者12例であった．結果，NPSLE患者4例と神経症状を合併しないSLE患者1例の計5例で同抗体が陽性でありその他の患者および健常者では全て陰性であった．抗体陽性であった4例のNPSLE患者は臨床的に，ACRの精神神経症状の分類に基づくpsychosisを合併したため，NPSLE全患者18例をpsychosisの有無で2つに分類し改めて抗