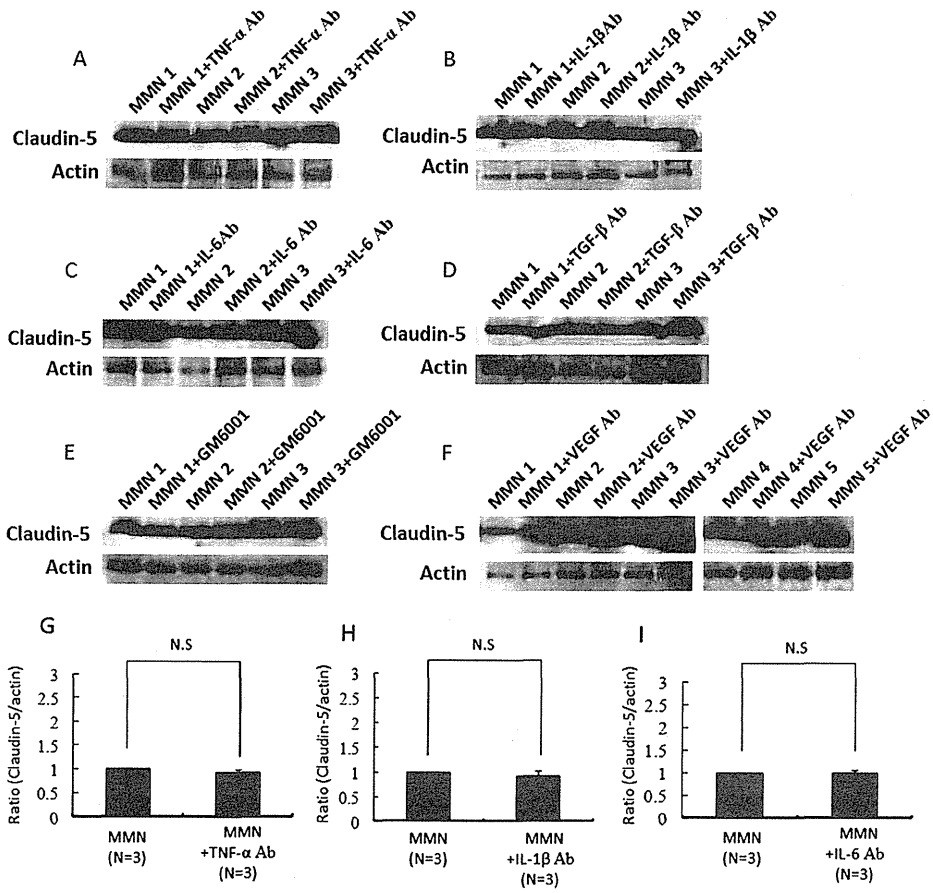

Figure 3 (A–F) The effects of anti-TNF-α, IL-1β, IL-6, TGF-β or vascular endothelial growth factor (VEGF) neutralising antibodies or a matrix metalloproteinase inhibitor on the amount of tight junction proteins in human peripheral nerve microvascular endothelial cells (PnMECs) after exposure to the sera from patients with multifocal motor neuropathy (MMN) was determined by a Western blot analysis. (G–L) Each bar graph reflects the combined densitometry data from each independent experiment (mean±SEM, n=3, *: p<0.01). (F and L) Preincubation with an anti-VEGF neutralising antibody increased the amount of claudin-5 protein in PnMECs (mean±SEM, n=5, *: p<0.01). The transendothelial electrical resistance value of PnMECs significantly increased (M) or the NaF permeability of PnMECs significantly decreased (N) after incubation with the sera from patients with MMN that were pretreated with an anti-VEGF neutralising antibody (mean±SEM, n=5). (O) The serum VEGF concentration was analysed in patients with MMN or amyotrophic lateral sclerosis, or from healthy control subjects. The bars indicate the mean of each group. No significant differences were observed between the three groups. (P–T) The expression of VEGF by PnMECs after exposure to the sera from patients with MMN. The amount of VEGF protein in the PnMECs was significantly increased after exposure to the sera from patients with MMN (P), although it did not change after exposure to the sera from healthy controls (Q). (R and S) Each bar graph reflects the combined densitometry data from each independent experiment (mean±SEM, MMN n=11, healthy control n=10, p<0.01). (T) The presence of anti-GM1 IgM antibodies did not influence the changes in the amounts of VEGF proteins in the PnMECs. MMN: conditioned medium with 10% MMN sera diluted with DMEM containing 10% fetal bovine serum (FBS); MMN+TNF-α Ab: conditioned medium with 10% MMN sera pretreated with an anti-TNF-α neutralising antibody; MMN +IL-1β Ab: conditioned medium with 10% MMN sera pretreated with an anti-IL-1β neutralising antibody; MMN+IL-6 Ab: conditioned medium with 10% MMN sera pretreated with an anti-IL-6 neutralising antibody; MMN+TGF-β Ab: conditioned medium with 10% MMN sera pretreated with an anti-TGF-β neutralising antibody; MMN+GM6001: conditioned medium with 10% MMN sera pretreated with a GM6001; MMN+VEGF Ab: conditioned medium with 10% MMN sera pretreated with an anti-VEGF neutralising antibody. Control: non-conditioned DMEM containing 20% FBS; MMN: conditioned medium with 10% serum from a patient with MMN diluted with non-conditioned DMEM containing 10% FBS; Normal: conditioned medium with 10% serum from a healthy control diluted with non-conditioned medium of DMEM containing 10% FBS; GM1-IgM positive MMN: conditioned medium with 10% serum samples of MMN patients with anti-GM1 IgM antibodies; GM1-IgM negative MMN: conditioned medium with 10% serum samples of MMN patients without anti-GM1 IgM antibodies.

impaired remyelination caused by the disruption of the BNB was the mechanism responsible in this case. Oh *et al*[17] reported perivascular lymphocytic infiltration in the endoneurial or perineurial microvessels of the BNB in the motor nerves from an autopsy case of a patient with MMN. Thus, a leaky BNB that allows the intrusion of circulating pathogenic antibodies and inflammatory cytokines may play a crucial role in the development of MMN. Some studies have indeed demonstrated anti-GM1 antibody-mediated focal demyelination and blockade of voltage-dependent Na^+ channels at the node of Ranvier in vivo and in vitro[24–26] and reported that the sera obtained from patients with MMN can block nerve conduction in distal motor nerves in mice.[27] However, the molecular mechanism of BNB breakdown in MMN has not been adequately explained as yet. In the present study, we used conditionally immortalised human BNB-derived endothelial cells to analyse the effects of the sera from patients with MMN on the impairment of the BNB function.[14] We have also previously reported that VEGF disrupts the BNB and that sera obtained from patients with Bickerstaff's brainstem encephalitis and Miller Fisher syndrome did not influence the barrier

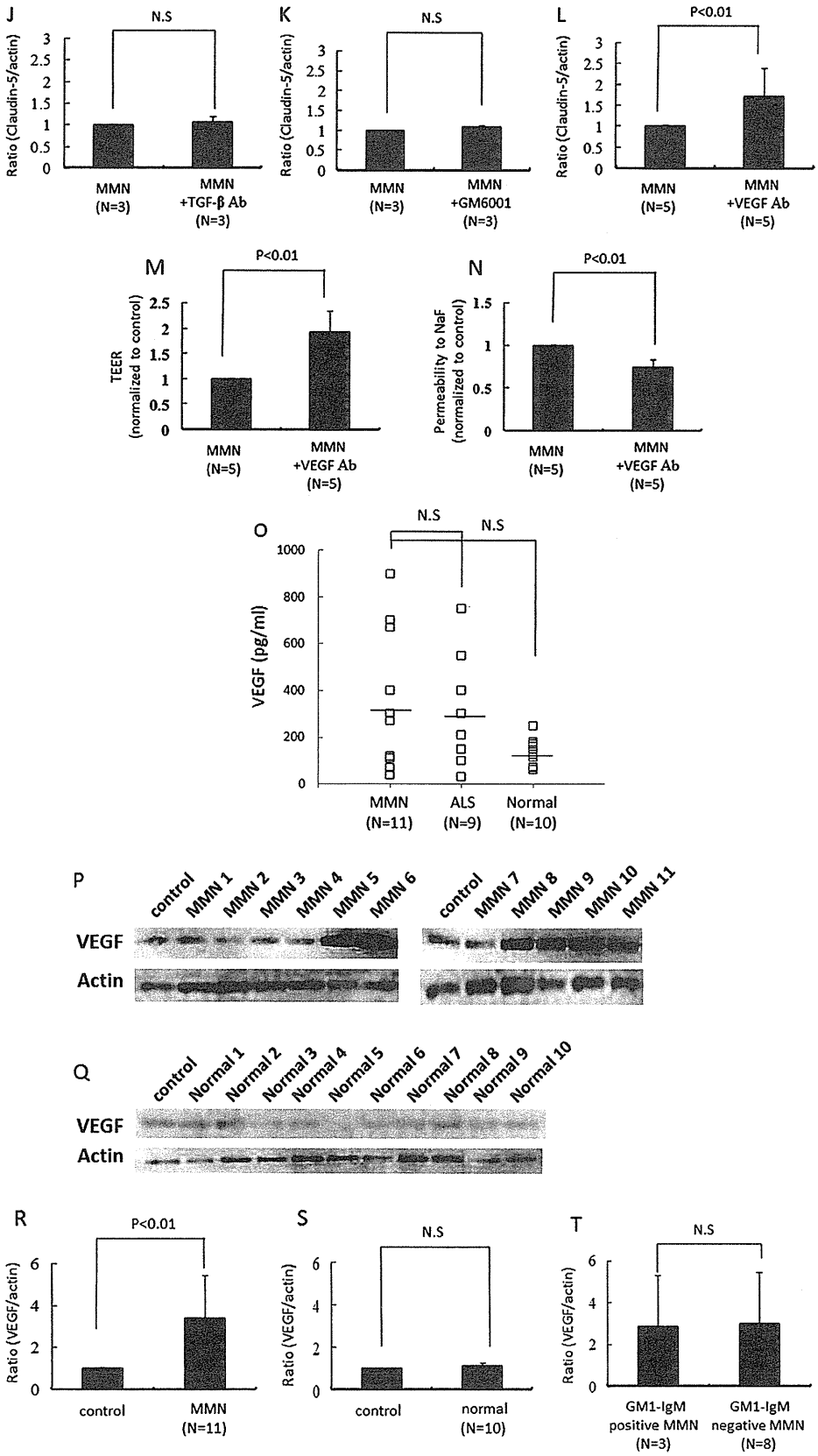

Figure 3 Continued.

function in the same in vitro BNB model.[21 28] Our present study is the first to demonstrate that the sera from patients with MMN can disrupt the BNB. The expression of claudin-5 and the TEER values were decreased, and the NaF permeability of PnMECs was increased after exposure to the MMN sera. Together, these results indicate that humoral factors in the MMN sera

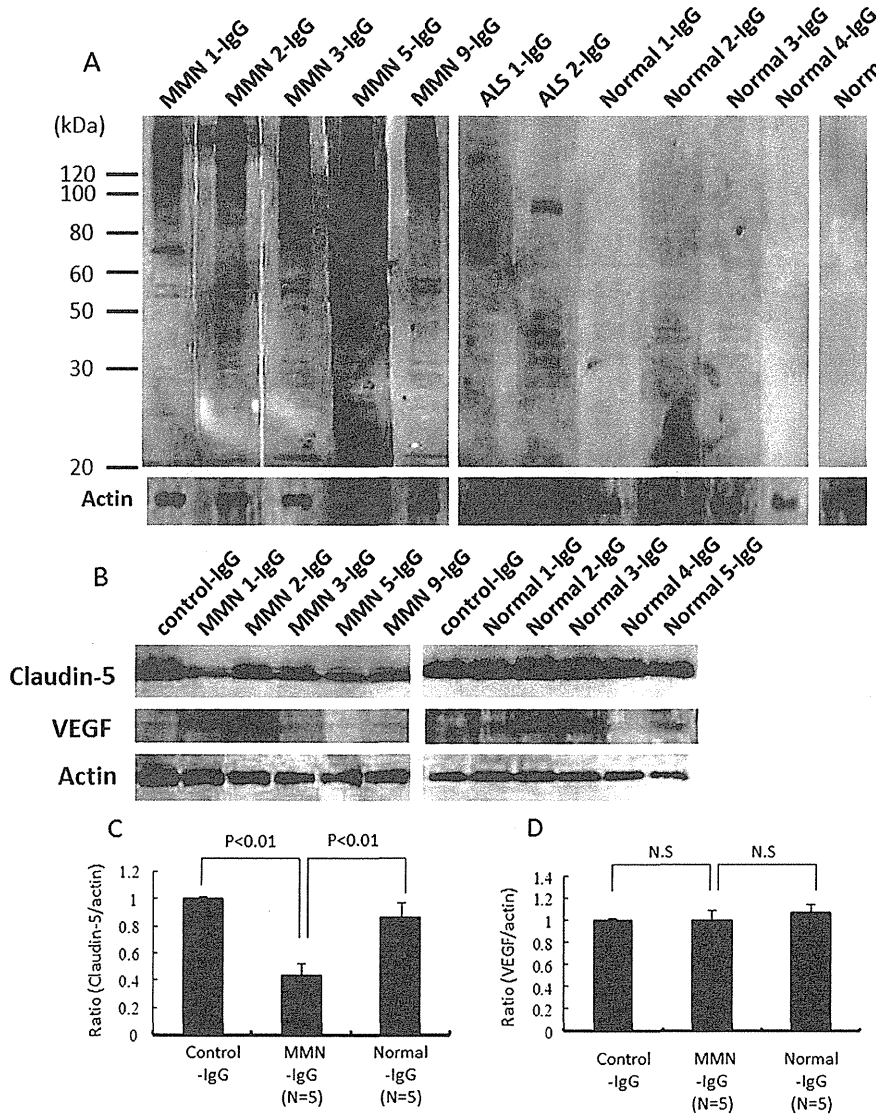

Figure 4 (A) Representative results obtained by immunoblotting of human peripheral nerve microvascular endothelial cell (PnMEC) lysates. The blots were exposed to the purified serum IgG from five patients with multifocal motor neuropathy (MMN), two patients with amyotrophic lateral sclerosis (ALS) and five healthy controls after loading 20 mg of protein lysates from PnMECs. The purified IgG fractions of the sera from patients with MMN predominantly reacted with one or more antigens of approximately 30, 45, 50, 54, 56 and 70 kDa in the PnMEC lysates. The purified serum IgG samples from the two patients with ALS also reacted with approximately 30, 40, 45, 50 and 90 kDa antigens of PnMECs. The two bands corresponding to the 40 and 45 kDa antigens of PnMECs were detected from the purified IgG fractions from the five healthy controls. The expression of actin was used as an internal standard. (B and C) The effects of the purified serum IgG from patients with MMN without anti-GM1 IgM antibodies on the expression of tight junction proteins and vascular endothelial growth factor (VEGF) in human peripheral nerve microvascular endothelial cells (PnMECs) were determined by a Western blot analysis. (B) The amount of claudin-5 in PnMECs was significantly decreased after exposure to the purified IgG fractions of patient's sera, whereas it was not affected by the purified IgG fractions from healthy controls, as determined by a Western blot analysis. The amount of VEGF proteins did not change following exposure to the purified serum IgG fractions obtained from the patients with MMN and healthy controls. (C and D) Each bar graph reflects the combined densitometry data from independent experiments (mean±SEM, n=5, *: p<0.01). The transendothelial electrical resistance value of PnMECs was significantly decreased (E) and the NaF permeability of PnMECs was significantly increased (F) after exposure to the purified IgG fraction from patients with MMN, although it was not changed by incubation with the purified IgG fractions from healthy controls. (G) The amount of VCAM-1 and NF-κB p65 in PnMECs was significantly increased after exposure to the purified IgG fraction from patients with MMN, whereas it was not changed by the purified IgG fractions from healthy controls, as determined by a Western blot analysis. (H and I) Each bar graph reflects the combined densitometry data from independent experiments (mean±SEM, n=5, p<0.01). Control-IgG: conditioned medium containing purified IgG fractions obtained from fetal bovine serum; MMN-IgG: conditioned medium with containing purified IgG fractions obtained from the sera of patients with MMN; ALS-IgG: conditioned medium with containing purified IgG fractions obtained from the sera of patients with ALS; Normal-IgG: conditioned medium with containing purified IgG fractions obtained from the sera of healthy individuals.

disrupt the BNB. We therefore first tried to identify the most important substance involved in disrupting the BNB in patients with MMN.

The presence of circulating cytokines, including TNF-α, IL-1β and VEGF, appears to be linked to the pathogenesis of the BNB breakdown in patients with MMN. Recent data suggest that

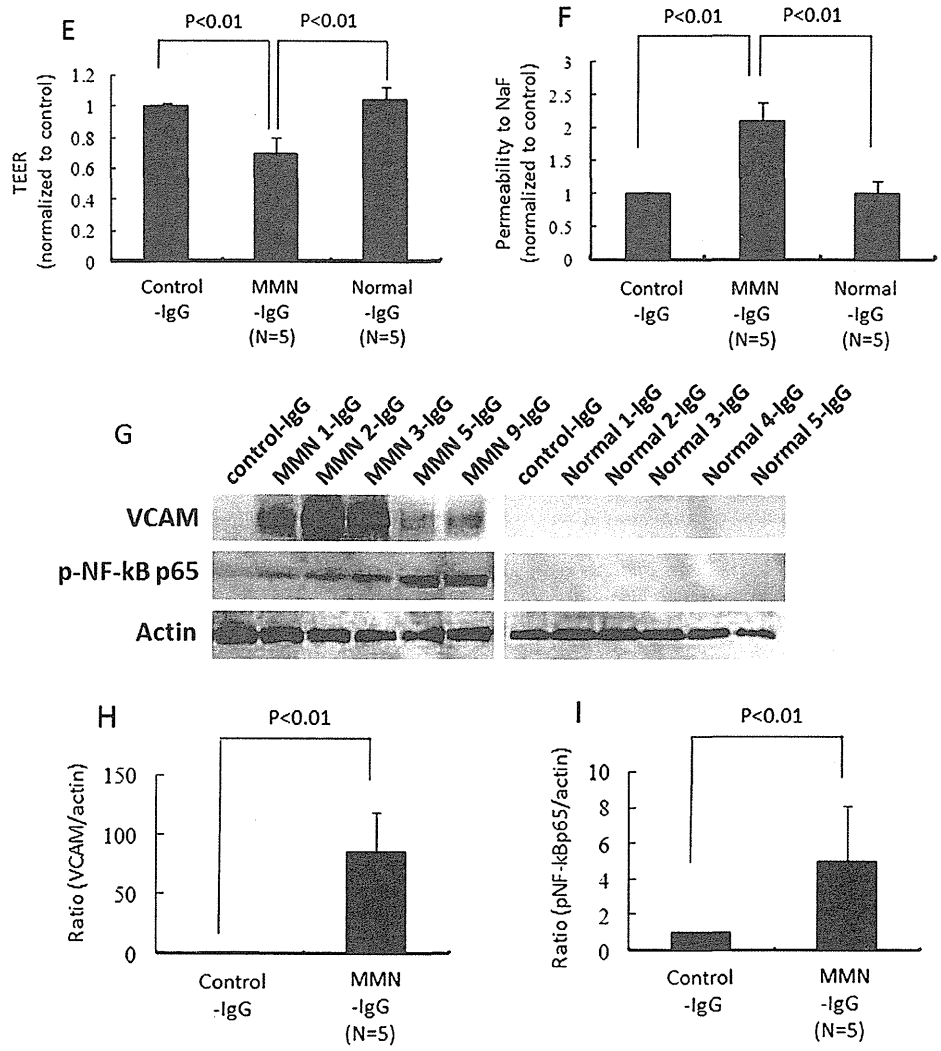

Figure 4 Continued.

these cytokines can disrupt the BNB; in particular, VEGF was able to induce BNB impairment.[21] Our present study demonstrated that the BNB function was restored after adding a neutralising anti-VEGF antibody to the MMN sera, indicating that VEGF was the key molecule responsible for the disruption of the BNB in the patients with MMN in our study. Although the serum concentration of VEGF was not increased in the patients with MMN compared to that from healthy controls, the secretion of VEGF by PnMECs was increased after exposure to the MMN sera. This finding suggests that the effect of VEGF occurred via an autocrine mechanism; thus, minimal secretion may lead to a significant effect. Our present studies demonstrated that the neutralising anti-VEGF antibody may also have therapeutic potential for restoring the BNB integrity in MMN. We were unable to identify which humoral factors in the MMN sera caused the increased VEGF secretion observed in the present study; however, we demonstrated that the amount of VEGF proteins did not change following exposure to IgG obtained from the MMN sera in our study, thus indicating that unknown humoral factors other than IgG in the MMN sera are key mediators of increased VEGF secretion (figure 5).

We next hypothesised that antibodies binding to PnMECs might be involved in the BNB disruption in patients with MMN, because antibody-mediated immunological therapies including high-dose IVIg are effective against MMN. We thus determined whether purified IgG from the MMN sera without complement would have a direct influence on the BNB properties. Our results demonstrated that the purified IgG from the MMN sera decreased the amount of claudin-5 and the TEER value, and increased the permeability of the BNB, thus indicating that unknown antibodies, possibly IgG, against PnMECs from the MMN sera cause the disruption of the BNB (figure 5). This finding supports our hypothesis concerning the etiopathogenesis of MMN: MMN has an antibody-mediated immunological basis.

The proportion of patients with MMN with anti-GM1 antibodies in our present study (~27%), was lower than that reported in several previous studies, in which these antibodies were detected in 22–85% of patients with MMN.[2] The wide variation observed in the incidence of these antibodies is likely derived from the different ELISA assays used in the different studies.[2–6] We believe that this fact did not influence the possible consequences of the interpretation of our present study because the absence of these antibodies does not exclude a diagnosis of MMN. The frequent presence of anti-GM1 IgM antibodies in the sera of patients with MMN and their decrease during improvement induced by cyclophosphamide[29–31] have

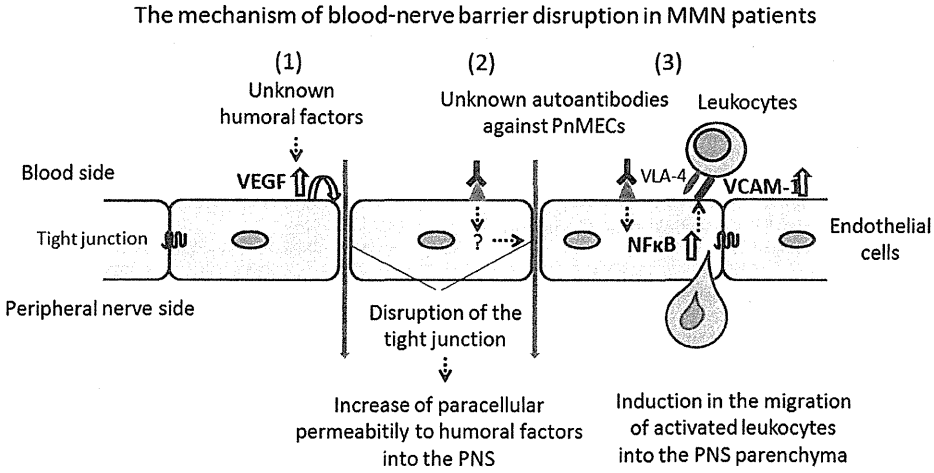

Figure 5 Schematic depiction of the assumed molecular mechanisms underlying the blood-nerve barrier (BNB) disruption observed in patients with multifocal motor neuropathy (MMN). The disruption of the BNB caused by humoral factors present in the sera of patients with MMN involves two differently regulated steps, including the disruption of tight junction proteins via the autocrine secretion of vascular endothelial growth factor from PnMECs induced by unknown humoral factors (1) and exposure to unknown autoantibodies against PnMECs (2), and the upregulation of VCAM-1 via NF-κB signalling in PnMECs induced by exposure to unknown autoantibodies against PnMECs in MMN sera (3).

favoured the hypothesis that GM1, which is present on the endothelial cells forming the BNB, may be the target of this immune response. GM1 is indeed present on the endothelial cells forming the BNB,[32] and anti-GM1 monoclonal antibodies can open the bovine BNB without the help of complement in vitro.[33] However, we found that the presence of anti-GM1 IgM antibodies in the patients' sera did not influence the BNB function in the PnMECs. Although the anti-GM1 IgM antibody is still a candidate cause of the disruption of the BNB, we consider that untested factors or unknown antibodies against PnMECs in the sera of patients with MMN other than the anti-GM1 IgM antibodies may be the key players that upset the BNB.

The interaction of VCAM-1 and very late activating antigen-4 (VLA-4) has a unique role in the pathogenesis of multiple sclerosis because it is involved in rolling and the arrest of leucocytes, which is a prerequisite for the activation of all further steps of transendothelial leucocyte migration.[34 35] We demonstrated that the sera from patients with MMN increased the amount of VCAM-1 protein, and this effect was reversed by exposure to an NF-κB inhibitor. The present study also has shown that the sera from patients with ALS had increased amounts of VCAM-1 protein. This finding can be explained by the hypothesis concerning a complicated pathogenesis of ALS, wherein immunological factors, including cytokines, chemokines and MMPs, and the disruption of the blood-brain barrier (BBB) and the blood-spinal cord barrier may play key roles in the development of the disease.[36 37] We also have indicated that the purified IgG of the sera from patients with MMN increased the amount of VCAM-1 proteins. This indicated that the unknown antibodies against PnMECs in the sera from patients with MMN may increase the VCAM-1 protein expression by upregulating the NF-κB signalling, thus causing the migration of activated leucocytes to the PNS parenchyma (figure 5), supporting the previous observation that perivascular lymphocytic infiltration in endoneurial microvessels of the BNB was present in autopsy cases of MMN.[17 18] Natalizumab is a humanised monoclonal antibody against the VLA-4 and inhibits the binding of leucocytes to the VCAM-1 expressed on activated brain vessels.[38 39] Our results provide the theoretical basis for applying natalizumab clinically in patients with MMN. In case of CIDP, natalizumab cannot be recommended at present, because Wolf *et al*[40] reported the case of a patient with CIDP in whom natalizumab treatment was not beneficial. Novel therapy directed specifically towards the reduction of VCAM-1 in the BNB could also be a possible therapeutic strategy for the treatment of MMN.

In conclusion, our study demonstrated that the disruption of the BNB caused by the humoral factors present in the sera of patients with MMN involves two differently regulated steps; the disruption of BNB function via the autocrine secretion of VEGF from PnMECs induced by unknown humoral factors or exposure to unknown autoantibodies against PnMECs, and the up-regulation of VCAM-1 in PnMECs induced by exposure to unknown autoantibodies against PnMECs in MMN sera. These data may provide novel explanations concerning the etiopathogenesis and the triggers of the BNB breakdown in MMN. A further analysis of the molecular mechanisms underlying the BNB breakdown observed in MMN, including the identification of the unknown molecules responsible for the disruption of BNB, and clarification of the effects of natalizumab and neutralising anti-VEGF antibodies against the disruption of the BNB, using an ex vivo or in vivo experimental model would assist in the development of therapies for this disabling disease.

Contributors FS performed the experiments, analysed and interpreted the data and wrote the manuscript. YS, TM and AT performed experiments and analysed the data. MO, NM and AM recruited patients. MK and RK evaluated the data and edited the manuscript. TK conducted and supervised the study, evaluated the data and wrote the manuscript.

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Competing interests All authors declare no potential conflicts of interest.

Patient consent Obtained.

Ethics approval The study was approved by the ethics committee of Yamaguchi University.

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RESEARCH PAPER

A nationwide survey of combined central and peripheral demyelination in Japan

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ABSTRACT

Objectives To clarify the clinical features of combined central and peripheral demyelination (CCPD) via a nationwide survey.

Methods The following characteristics were used to define CCPD: T2 high-signal intensity lesions in the brain, optic nerves or spinal cord on MRI, or abnormalities on visual-evoked potentials; conduction delay, conduction block, temporal dispersion or F-wave abnormalities suggesting demyelinating neuropathy based on nerve conduction studies; exclusion of secondary demyelination. We conducted a nationwide survey in 2012, sending questionnaires to 1332 adult and paediatric neurology institutions in Japan.

Results We collated 40 CCPD cases, including 29 women. Age at onset was 31.7±14.1 years (mean±SD). Sensory disturbance (94.9%), motor weakness (92.5%) and gait disturbance (79.5%) were common. Although cerebrospinal fluid protein levels were increased in 82.5%, oligoclonal IgG bands and elevated IgG indices were detected in 7.4% and 18.5% of cases, respectively. Fifteen of 21 patients (71.4%) had abnormal visual-evoked potentials. Antineurofascin 155 antibodies were positive in 5/11 (45.5%). Corticosteroids, intravenous immunoglobulins and plasmapheresis resulted in an 83.3%, 66.7% and 87.5% improvement, respectively, whereas interferon-β was effective in only 10% of cases. CCPD cases with simultaneous onset of central nervous system (CNS) and peripheral nervous system (PNS) involvement exhibited greater disability, but less recurrence and more frequent extensive cerebral and spinal cord MRI lesions compared to those with temporarily separated onset, whereas optic nerve involvement was more common in the latter.

Conclusions CCPD shows different characteristics from classical demyelinating diseases, and distinctive features exist between cases with simultaneous and temporarily separated onset of CNS and PNS involvement.

INTRODUCTION

Inflammatory demyelinating diseases are immune-mediated inflammatory disorders of the nervous system, which are divided into two categories: those affecting the central nervous system (CNS), such as acute disseminated encephalomyelitis and multiple sclerosis (MS) and those affecting the peripheral nervous system (PNS), including Guillain-Barré syndrome and chronic inflammatory demyelinating polyradiculoneuropathy (CIDP).

Demyelinating diseases usually affect either the CNS or PNS, possibly because the relevant autoimmune cells recognise only CNS or PNS antigens. However, it has occasionally been reported that patients with demyelination in the CNS or PNS also exhibit demyelination in the other nervous system. It was reported that 13 of 150 patients with MS had symptoms related to peripheral neuropathy and 4 had demyelinating polyneuropathy.[1] In addition, 5 of 100 patients with CIDP had symptomatic CNS involvement.[2] Demyelinating conditions affecting both the CNS and PNS are described using various diagnostic names, such as combined central and peripheral demyelination (CCPD), CIDP with CNS involvement and CIDP with multifocal CNS demyelination.[3] Although case reports or a small series of studies of such cases have been repeatedly found in the literature,[4–17] whether such conditions represent a distinct disease entity remains to be determined. Since large-scale epidemiological studies on this condition have never before been performed, we conducted a nationwide survey in Japan to uncover the demographic features of CCPD.

METHODS

Procedures

In this survey, CCPD was defined as fulfilling the following criteria:

1. CNS involvement criterion: T2 high-signal intensity lesions in the brain, optic nerves or spinal cord on MRI, or abnormalities on visual-evoked potentials (VEPs).
2. PNS involvement criterion: conduction delay, conduction block, temporal dispersion or F-wave abnormalities, suggesting peripheral demyelinating neuropathy according to nerve conduction studies (NCS). In the present study, it was mandatory that among median, ulnar and tibial nerves, at least two nerves had the aforementioned abnormal findings suggestive of demyelination.
3. Exclusion criterion: secondary demyelinating diseases or changes, such as infectious diseases (eg, human T lymphocyte trophic virus type 1-associated myelopathy, syphilis, neuroborreliosis, HIV infection or progressive multifocal leucoencephalopathy), pre-existing inflammatory diseases (eg, sarcoidosis, Behçet's disease, Sjögren's syndrome, vasculitis or other collagen diseases), mitochondrial disease, metabolic/toxic

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diseases (eg, vitamin deficiency, amyloidosis, chronic alcoholism, diabetes mellitus or subacute myelo-opticoneuropathy due to clioquinol intoxication, cervical spondylotic myelopathy, syringomyelia, spinocerebellar degeneration, multiple myeloma, other tumours, inherited diseases (eg, leucodystrophies), cerebrovascular disease and non-specific lesions on T2-weighted MRI (eg, leucoaraiosis). In our previous study on CCPD,[18] all seven CCPD cases fulfilled the EFNS/PNS criteria for CIDP and six cases met the McDonald criteria (2011) for MS.[19 20] Therefore, we did not exclude patients who eventually met either MS or CIDP criteria for the present survey.

Patients with CCPD who visited adult or paediatric neurologists between 2007 and 2011, and met the aforementioned diagnostic criteria were surveyed in 2012. The survey was conducted in two steps. First, a primary questionnaire sheet was sent to 1332 institutions in Japan, which included educational facilities accredited by the Japanese Society of Neurology, neurology departments with two or more board-certified neurologists, neurology departments in hospitals with more than 500 beds, paediatric departments in hospitals with any board-certified paediatric neurologist and departments of paediatrics in medical schools. A response was received from 671 institutions (50.3%), of which 41 institutions reported 57 cases. In the second step, a survey using a detailed questionnaire sheet about each patient was sent to the institutions that reported the CCPD cases. This questionnaire requested the age at onset, sex, history of preceding diseases, habitation area, mode of onset, clinical signs and symptoms, Hughes functional scale scores (grade 0: normal; grade 1: minimal symptoms and signs, able to run; grade 2: able to walk 5 m independently; grade 3: able to walk 5 m with the use of aids; grade 4: chairuser or bedbound; grade 5: requires assisted ventilation; grade 6: dead)[21] at the peak and in remission, laboratory findings, MRI findings of the brain and spinal cord, VEP and NCS findings, differential diagnosis, clinical course, treatment and outcomes. In this second survey, 54 of 57 cases (94.7%) were collated from 38 institutions (92.7%).

Among 54 cases collated, 14 cases were excluded for the following reasons: four cases did not meet CNS involvement criteria; four cases did not meet PNS involvement criteri/a; two cases lacked basic clinical data; two cases were experienced outside the term of this survey; and two cases were strongly suspected of having other diseases (cerebral vascular disease in one and leucodystrophy in another). In the present survey, CNS and PNS symptoms developed less than 2 months apart were regarded as simultaneous or sequential onset of both CNS and PNS involvement. The mode of onset was defined as acute (reaching a maximum intensity within 1 week), subacute (reaching a maximum intensity after 1 week to 1 month) or chronic (reaching a maximum intensity after 1 month).

Statistical analysis

Continuous variables were summarised by descriptive statistics, and categorical variables were summarised using counts of patients and percentages. For comparisons between two groups, qualitative variables were analysed using Fisher's exact test. Continuous variables that followed a parametric distribution were analysed with Student's t tests, whereas non-parametric variables were analysed with the Mann-Whitney U test.

Table 1 Demographic features of 40 patients with CCPD

Basic demographics	N=40
Sex ratio (male/female)	11:29
Age at onset (years, mean±SD)	31.7±14.1
Age at examination (years, mean±SD)	36.5±14.6
Follow-up period (months, mean±SD)*	93.0±91.8
Disease duration (months, mean±SD)*	137.9±124.8
Mode of onset	n/N (%)
Acute	6/31 (19.4)
Subacute	14/31 (45.2)
Chronic	11/31 (35.5)
Clinical course	n/N (%)
Monophasic	10/38 (26.3)
Relapse–remitting	20/38 (52.6)
Chronic progressive	8/38 (21.1)
Initial symptoms	n/N (%)
Related to CNS involvement	15/38 (39.5)
Related to PNS involvement	15/38 (39.5)
Simultaneous or sequential	8/38 (21.0)
Fulfilment of MS or CIDP criteria	n/N (%)
McDonald criteria for MS	27/40 (67.5)
EFNS/PNS definite criteria for CIDP	35/40 (87.5)
Symptoms and signs during the entire course	n/N (%)
Seizure†	3/40 (7.5)
Mental disturbance†	5/40 (12.5)
Visual disturbance†	19/40 (47.5)
Right	1/19 (5.3)
Left	8/19 (42.1)
Bilateral	10/19 (52.6)
Cranial nerve involvement (other than the optic nerves)	17/39 (43.6)
Motor weakness‡	37/40 (92.5)
Hemiplegia†	10/36 (27.8)
Paraplegia†	6/36 (16.7)
Weakness of 4 extremities§	24/36 (66.7)
Muscle atrophy§	11/40 (27.5)
Respiratory disturbance	3/40 (7.5)
Gait disturbance	31/39 (79.5)
Cerebellar ataxia†	10/38 (26.3)
Sensory disturbance	37/39 (94.9)
Half-body involvement†	5/37 (13.5)
Sensory level†	14/37 (37.8)
Glove and stocking type§	22/37 (59.4)
Other types	3/37 (8.1)
Deep tendon reflexes	
Hyporeflexia§	26/40 (65.0)
Normal	1/40 (2.5)
Hyper-reflexia†	9/40 (22.5)
Both hyporeflexia and hyper-reflexia	4/40 (10.0)
Pathological reflexes†	18/40 (45.0)
Sphincter disturbance†	18/38 (47.4)

*Two patients' data were missing.
†Symptoms derived from CNS involvement.
‡Detail of motor weakness in one patient was unknown.
§Symptoms derived from PNS involvement.
CCPD, combined central and peripheral demyelination; CIDP, chronic inflammatory demyelinating polyradiculoneuropathy; CNS, central nervous system; n, number of involved cases; N, number of cases collated; MS, multiple sclerosis; PNS, peripheral nervous system.

RESULTS

Baseline characteristics

The demographic features of 40 patients with CCPD are summarised in table 1. The mean age at onset was 31.7±14.1 years (range: 8–59 years), with disease duration of 137.9±124.8 months. The male to female ratio was 1:2.6 (11/29). The mode of onset was acute in 19.4%, subacute in 45.2% and chronic in 35.5%. Clinical courses were monophasic in 10 (26.3%), relapsing remitting in 20 (52.6%) and chronic progressive in 8 (21.1%)

cases. Four patients had antecedent infections, of which three had respiratory infections and one had an alimentary infection. Only one patient developed CCPD after a vaccination (details of the vaccination are unknown). In the present survey, 67.5% (27/40) of the patients with CCPD met the McDonald criteria[20] for MS, while 87.5% (35/40) fulfilled the EFNS/PNS definite criteria for CIDP.[19]

Neurological symptoms and signs

The initial symptoms related to CNS involvement, such as visual disturbance, hemiplegia and hemibody sensory disturbance, were observed in 15 cases (39.5%), those related to PNS involvement, such as weakness and sensory disturbance of four extremities, in 15 cases (39.5%), and those related to both CNS and PNS involvement (simultaneous or sequential occurrence) in 8 cases (21%). The most common symptom/sign during the entire course was sensory disturbance (94.9%), the second most common symptom/sign was motor weakness (92.5%) and the third was gait disturbance (79.5%). Visual disturbance was observed in nearly half of the patients, with approximately 50% exhibiting bilateral involvement. Overall, cranial nerves were affected in 30/40 (75%) cases and optic nerves were the most commonly affected (19/30, 63.3%; see online supplementary table). Hyporeflexia and hyper-reflexia were seen in 65% and 22.5%, respectively, while four patients had both, depending on what was examined. Pathological reflexes were found in 45% and sphincter disturbance was present in 47.4%. About one-fourth of the patients showed muscle atrophy and cerebellar ataxia. Mental disturbance, seizure and respiratory disturbance were only occasionally observed.

Laboratory findings of peripheral blood and cerebrospinal fluid

Increased C reactive protein levels were found in only 10.5% of the cases and none of the patients had abnormal glycated haemoglobin levels (table 2). Few patients had common autoantibodies. Antiaquaporin 4 (AQP4) antibodies were not detected in any of the patients, whereas antineurofascin155 antibodies were found in 5/11 (45.5%). Epstein-Barr virus, herpes simplex virus, varicella zoster virus and mycoplasma were negative in all examined cases. Cerebrospinal fluid (CSF) protein levels were increased in 82.5% of the cases, while pleocytosis was present in 27.5%, indicating albuminocytological dissociation in 57.5%. The CSF oligoclonal IgG band positivity rate was only 7.4% and an elevated IgG index was found in 18.5% of the cases.

Table 2 Laboratory findings in 40 patients with CCPD

	n/N (%)
Blood	
High HbA1c level	0/37 (0)
CRP level >1.0 mg/dL	4/38 (10.5)
Hyperthyroidism	1/37 (2.7)
Hypothyroidism	3/37 (8.1)
Rheumatoid factor	1/31 (3.2)
ANA ≥1:160	1/31 (3.2)
Anti-SS-A Ab	0/35 (0)
Anti-SS-B Ab	0/35 (0)
MPO-ANCA	1/27 (3.7)
PR3-ANCA	0/25 (0)
Anti-AQP4 Ab	0/29 (0)
Antiganglioside Ab	2/24 (8.3)
Antineurofascin155 Ab	5/11 (45.5)
Monoclonal gammopathy	1/28 (3.6)
CSF	
Amounts of protein >40 mg/dL	33/40 (82.5)
Cell counts >5/μL	11/40 (27.5)
Albuminocytological dissociation	23/40 (57.5)
OB	2/27 (7.4)
Increased IgG index level	5/27 (18.5)

Ab, antibodies; ANA, antinuclear antibody; AQP4, aquaporin 4; CCPD, combined central and peripheral demyelination; CRP, C reactive protein; CSF, cerebrospinal fluid; HbA1c, glycated haemoglobin; MPO-ANCA, myeloperoxidase-antineutrophil cytoplasmic antibody; N, number of cases collated; N, number of involved cases; OB, oligoclonal IgG bands; PR3-ANCA, proteinase-3-antineutrophil cytoplasmic antibody.

Neuroimaging, VEP and NCS findings

Following MRI examination, cerebral, cerebellar, brainstem and optic nerve lesions were detected in 75%, 15%, 32.5% and 17.5%, respectively (table 3). Among cases with cerebral lesions, 36.7% had nine or more lesions. Large lesions (>3 cm in diameter) were observed in 25% and gadolinium (Gd)-enhanced lesions were found in only 17.5%. Spinal cord lesions were found in 30/40 (75%) and the lesions in 11 cases were Gd-enhanced. Longitudinally extensive spinal cord lesions (LESCLs), extending three or more vertebral segments, were present in 3/40 (7.5%). VEPs were abnormal in 15/21 patients (71.4%) and bilaterally observed in 53.3% of these. Based on neurological, MRI and VEP findings, the involvement of multiple affected CNS sites (either two or three sites among the brain, optic nerves and spinal cord) was found in 70% of

Table 3 MRI and VEP findings in 40 patients with CCPD

	n/N (%)
MRI	
Cerebral lesions	30/40 (75.0)
≤3	6/30 (20.0)
4–8	13/30 (43.3)
≥9	11/30 (36.7)
Gd-enhancement	7/40 (17.5)
Lesions larger than 3 cm	10/40 (25.0)
Cerebellar lesions	6/40 (15.0)
Gd-enhancement	2/40 (5.0)
Brainstem lesions	13/40 (32.5)
Gd-enhancement	3/40 (7.5)
Optic nerve lesions	7/40 (17.5)
Gd-enhancement	1/40 (2.5)
Spinal cord lesions	30/40 (75.0)
LESCLs	3/40 (7.5)
Gd-enhancement	11/40 (27.5)
VEPs	
Abnormal findings	15/21 (71.4)
Right	2/15 (13.3)
Left	5/15 (33.3)
Bilateral	8/15 (53.3)
Affected CNS sites	
Brain only	4/40 (10.0)
Optic nerves only	1/40(2.5)
Spinal cord only	7/40 (17.5)
Brain+optic nerves	5/40 (12.5)
Brain+spinal cord	13/40 (32.5)
Optic nerves+spinal cord	2/40 (5.0)
Brain+optic nerves+spinal cord	8/40 (20.0)

CCPD, combined central and peripheral demyelination; CNS, central nervous system; Gd, gadolinium; LESCLs, longitudinally extensive spinal cord lesions; N, number of cases collated; N, number of involved cases; VEPs, visual-evoked potentials.

Table 4 Abnormal findings of NCS in 40 patients with CCPD

	Total†	Median‡	Ulnar‡	Tibial‡	Sural‡
Motor nerve					
Decreased MCV	31/40 (77.5)	55/69 (79.7)	47/66 (71.2)	46/63 (73.0)	
Prolonged distal latency	21/40 (52.5)	31/67 (46.3)	28/62 (45.2)	22/59 (37.3)	
Decreased or absent CMAP	22/40 (55.0)	19/70 (27.1)	26/69 (37.7)	44/70 (62.9)	
Conduction block	11/40 (27.5)	20/64 (31.3)	22/61 (36.1)	20/59 (33.9)	
Temporal dispersion	16/40 (40.0)	23/67 (34.3)	27/64 (42.2)	23/58 (39.7)	
Prolonged F-wave latency	28/40 (70.0)	38/54 (70.4)	29/45 (64.4)	34/41 (82.9)	
Decreased F-wave occurrence	19/40 (47.5)	28/58 (48.3)	24/50 (48.0)	21/50 (42.0)	
Sensory nerve					
Decreased SCV	17/40 (42.5)	20/53 (37.7)	30/49 (61.2)		17/38 (44.7)
Decreased or absent SNAP	35/40 (87.5)	41/66 (62.1)	50/68 (73.5)		43/60 (71.7)

†Patients with indicated abnormalities in any one of the three nerves were regarded as abnormal (numbers of abnormal patients/total numbers of patients examined).
‡Numbers of abnormal nerves/total numbers of nerves examined.
CCPD, combined central and peripheral demyelination; CMAP, compound muscle action potential; MCV, motor nerve conduction velocity; NCS, nerve conduction study; SCV, sensory nerve conduction velocity; SNAP, sensory nerve action potential.

patients with CCPD, while isolated involvement of the brain, optic nerve lesions or spinal cord was present in 10%, 2.5% and 17.5%, respectively. Devic type (optic-spinal) involvement was observed in only 5%. In motor NCS, decreased motor nerve conduction velocity and prolonged F-wave latency were the most common findings, and were observed in 77.5% and 70% of patients with CCPD, respectively (table 4). Abnormal compound muscle action potential amplitude, prolonged distal latency and decreased F-wave occurrence were detected in approximately half of the patients. Conduction block and temporal dispersion were detected in 27.5% and 40%, respectively. In sensory NCS, decreased or absent sensory nerve action potential was recognised in as much as 87.5%, while decreased sensory nerve conduction velocity was present in 42.5%.

Treatment and prognosis

Patients with CCPD were most commonly treated with either intravenous or oral corticosteroids, followed by intravenous immunoglobulins, resulting in 83.3%, 75% and 66.7% improvement, respectively (table 5). Plasmapheresis was performed in only eight patients, of whom seven (87.5%) improved. By contrast, interferon-β (IFN-β) was effective in only one patient and the disease was actually exacerbated in three patients. At the illness peak, 16/40 (40%) patients with CCPD had severe disability, with a Hughes functional scale score of 4 or more, and three required artificial ventilation (figure 1). However, after treatment, 26 of 40 (65%) patients had no or only mild disabilities (≤1 Hughes functional scale score).

Table 5 Treatment response in 40 patients with CCPD

Treatment	Efficacy n/N (%)
Corticosteroid pulse therapy*	30/36 (83.3)
Oral corticosteroids	21/28 (75.0)
IVIg	18/27 (66.7)
Plasmapheresis	7/8 (87.5)
IFN-β	1/10 (10.0)

*500 mg/day for three consecutive days were administered to two patients, while 1000 mg/day for three consecutive days were administered to the remaining patients.
CCPD, combined central and peripheral demyelination; IFN-β, interferon β; IVIg, intravenous immunoglobulin; N, number of cases collated; n, number of efficacious cases.

Comparison of clinical features between patients with CCPD with simultaneous or temporarily separated onset of CNS and PNS involvement

We classified the collated patients into two subgroups according to the pattern of onset: simultaneous or sequential involvement of both CNS and PNS at onset (simultaneous onset group), or temporarily separated onset of CNS and PNS involvement (temporarily separated onset group). Follow-up period and disease duration were significantly shorter in the simultaneous onset group than in the temporarily separated onset group (44.6 ±45.0 months vs 112.0±97.7 months, p=0.0316 and 56.9 ±58.2 vs 169.3±128.5 months, p=0.0055, respectively; table 6). In the temporarily separated onset group, patients who had already been diagnosed as MS when PNS demyelination developed were seen in 9/15 (60%), while those who had already been diagnosed as CIDP when CNS demyelination developed were seen in 7/15 (46.7%) cases. The Hughes functional scale scores at the peak of illness were significantly greater in the simultaneous onset group than the temporarily separated onset

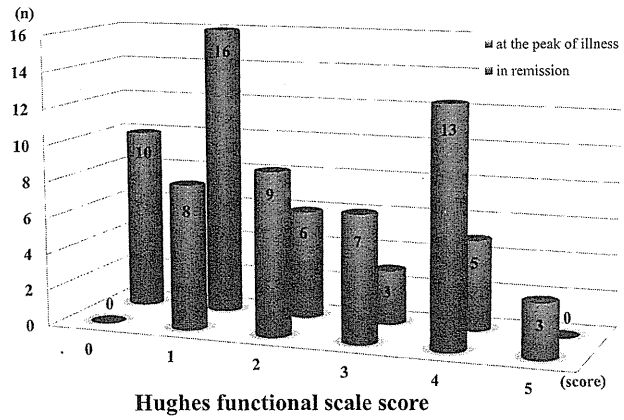

Figure 1 Hughes functional scale scores at the peak of illness and in remission. Forty patients with combined central and peripheral demyelination were evaluated by the Hughes functional scale score at the peak of illness and in remission. No one died because of the disease. The post-treatment scores became significantly less than the pretreatment scores (2.85±1.29 to 1.43±1.30, p<0.0001). All three patients with grade 5 at the peak of illness belonged to the simultaneous onset group.

Table 6 Comparison of clinical features between patients with CCPD with simultaneous or temporarily separated onset of CNS and PNS involvement*

	Temporarily separated onset group	Simultaneous onset group	p Value†
Demographics	N=30	N=8	
Sex ratio (male/female)	7:23 (1:3.3)	2:6 (1:3)	NS
Age at onset (years, mean±SD)	29.4±13.2	35.0±14.9	NS
Age at examination (years, mean±SD)	35.5±14.8	36.0±14.1	NS
Follow-up period (months, mean±SD)‡	112.0±97.7	44.6±45.0	0.0316
Disease duration (months, mean±SD)‡	169.3±128.5	56.9±58.2	0.0055
Mode of onset	n/N (%)	n/N (%)	
Acute	4/22 (18.2)	2/8 (25.0)	NS
Subacute	9/22 (40.9)	4/8 (50.0)	NS
Chronic	9/22 (40.9)	2/8 (25.0)	NS
Clinical course	n/N (%)	n/N (%)	
Monophasic	3/29 (10.3)	6/8 (75.0)	0.0008
Relapsing–remitting	19/29 (65.5)	1/8 (12.5)	0.0140
Chronic progressive	7/29 (24.1)	1/8 (12.5)	NS
Fulfilment of MS or CIDP criteria	n/N (%)	n/N (%)	
McDonald criteria for MS	22/30 (73.3)	4/8 (50.0)	NS
EFNS/PNS definite criteria for CIDP	26/30 (86.7)	7/8 (87.5)	NS
The number of patients who had already been diagnosed as MS when PNS demyelination developed	9/15 (60.0)		
The number of patients who had already been diagnosed as CIDP when CNS demyelination developed	7/15 (46.7)		
Hughes functional scale score	N=30	N=8	
At the peak of illness	2.73±1.14	3.75±1.39	0.0457
In remission	1.43±1.28	1.50±1.60	NS
Score changes after treatment	1.30±0.99	2.25±1.16	0.0427
Symptoms and signs	n/N (%)	n/N (%)	
Visual disturbance	19/30 (63.3)	0/8 (0.0)	0.0015
Cranial nerve involvement (other than optic nerves)	12/29 (41.4)	5/8 (62.5)	NS
Motor weakness	29/30 (96.7)	7/8 (87.5)	NS
Muscle atrophy	9/30 (30.0)	2/8 (25.0)	NS
Respiratory disturbance	0/30 (0.0)	3/8 (37.5)	0.0066
Gait disturbance	22/29 (75.9)	7/8 (87.5)	NS
Cerebellar ataxia	8/30 (26.7)	2/6 (33.3)	NS
Sensory disturbance	30/30 (100.0)	5/7 (71.4)	0.0315
Pathological reflexes	13/30 (43.3)	5/8 (62.5)	NS
Sphincter disturbance	14/29 (48.3)	3/7 (42.9)	NS
Blood	n/N (%)	n/N (%)	
Antineurofascin 155 Ab	3/8 (37.5)	2/3 (66.7)	NS
CSF	N=30	N=8	
Amounts of protein	85.3±64.9	126.5±88.3	NS
Cell counts	4.61±6.06	26.0±52.3	NS
	n/N (%)	n/N (%)	
Amounts of protein >40 mg/dL	24/30 (80.0)	7/8 (87.5)	NS
Cell counts >5/μL	7/30 (23.3)	3/8 (37.5)	NS
OB	2/21 (9.5)	0/5 (0.0)	NS
Increased IgG index level	4/20 (20.0)	1/6 (16.7)	NS
MRI	n/N (%)	n/N (%)	
Brain lesions	23/30 (76.7)	8/8 (100.0)	NS
Cerebral lesions	21/30 (70.0)	8/8 (100.0)	NS
Lesions more than 3 cm	5/30 (16.7)	5/8 (62.5)	0.0186
Cerebellar lesions	6/30 (20.0)	0/8 (0.0)	NS
Brainstem lesions	10/30 (33.3)	2/8 (25.0)	NS
Optic nerve lesions	6/30 (20.0)	1/8 (12.5)	NS
Spinal cord lesions	24/30 (80.0)	4/8 (50.0)	NS
LESCLs	0/30 (0.0)	3/8 (37.5)	0.0066
VEPs	n/N (%)	n/N (%)	
Abnormal VEP findings	14/17 (82.4)	1/4 (25.0)	0.0526§
Affected CNS sites	n/N (%)	n/N (%)	
Brain only	1/30 (3.3)	3/8 (37.5)	0.0237
Optic nerves only	1/30 (3.3)	0/8 (0.0)	NS
Spinal cord only	6/30 (20.0)	0/8 (0.0)	NS
Brain+optic nerves	4/30 (13.3)	1/8 (12.5)	NS
Brain+spinal cord	9/30 (30.0)	3/8 (37.5)	NS
Optic nerves+spinal cord	2/30 (6.7)	0/8 (0.0)	NS
Brain+optic nerves+spinal cord	7/30 (23.3)	1/8 (12.5)	NS
Treatment efficacy	n/N (%)	n/N (%)	
Corticosteroid pulse therapy	25/27 (92.6)	6/8 (75.0)	NS
Oral corticosteroids	17/20 (85.0)	4/6 (66.7)	NS
IVIg	13/20 (65.0)	4/5 (80.0)	NS
Plasmapheresis	5/6 (83.3)	2/2 (100.0)	NS

*Two patients were excluded because their patterns of onset were undetermined.
†A p value<0.05 is regarded as significant. Qualitative variables were analysed by Fisher exact test. Continuous variables that follow a parametric distribution were analysed by Student's t test, while non-parametric variables were analysed by Mann-Whitney U test.
‡Two patients' data in the temporarily separated onset group were missing.
§Indicates a trend (ie, p<0.1).
Ab, antibodies; CCPD, combined central and peripheral demyelination; CIDP, chronic inflammatory demyelinating polyradiculoneuropathy; CNS, central nervous system; CSF, cerebrospinal fluid; IFN-β, interferon β; IVIg, intravenous immunoglobulin; LESCLs, longitudinally extensive spinal cord lesions; MS, multiple sclerosis; N, number of cases collated; n, number of involved cases; NS, not significant; OB, oligoclonal IgG bands; PNS, peripheral nervous system; VEPs, visual-evoked potentials.

group (2.73±1.14 vs 3.75±1.39, p=0.0457). The monophasic course was more frequently observed in the simultaneous onset group than the temporarily separated onset group (75% vs 10.3%, p=0.0008), whereas the relapsing–remitting course was more common in the temporarily separated onset group than the simultaneous onset group (65.5% vs 12.5%, p=0.0140). Visual disturbance and sensory disturbance were more commonly present in the temporarily separated onset group than the simultaneous onset group (63.3% vs 0%, p=0.0015 and 100% vs 71.4%, p=0.0315, respectively), while respiratory disturbance occurred more often in the simultaneous onset group than in the temporarily separated onset group (37.5% vs 0%, p=0.0066). On MRI, cerebral lesions >3 cm and LESCLs were more frequently found in the simultaneous onset group than in the temporarily separated onset group (62.5% vs 16.7%, p=0 0.0186, and 37.5% vs 0%, p=0.0066, respectively). For the CNS affected sites, there were significantly more patients with PNS involvement and isolated brain involvement in the simultaneous onset group than in the temporarily separated onset

group (37.5% vs 3.3%, p=0.0237). By contrast, no patients in the simultaneous onset group had PNS involvement and isolated spinal cord involvement, while six patients in the temporarily separated group showed PNS and isolated spinal cord involvement. Abnormal VEPs tended to be more frequently detected in the temporarily separated onset group than in the simultaneous onset group (82.4% vs 25%, p=0.0526). The Hughes functional scale scores were significantly lower following immunotherapies compared with pretreatment scores in the temporarily separated onset group and the simultaneous onset group (2.73 ±1.14 to 1.43±1.28, p=0.0002, and 3.75±1.39 to 1.50±1.60, p=0.0203, respectively). However, the improvement in these scores was more remarkable in the simultaneous onset group than in the temporarily separated onset group (2.25±1.16 vs 1.30±0.99, p=0.0427; figure 2). Even when we excluded the patient with a history of vaccination, we obtained essentially the same results, although the difference in the Hughes grade scores at the peak, and the score changes after treatment between the temporarily separated onset group and the simultaneous onset group, were no longer statistically significant because of the smaller sample size (data not shown).

DISCUSSION

CCPD is an extremely rare and devastating disease. We identified 40 patients throughout Japan during the study period. The numbers of registered MS and patients with CIDP in Japan in 2011 were 16 140 and 2986, respectively.[22] Even taking into consideration the response rates (50.3% in the first survey and 94.7% in the second), patients with CCPD were a very minor population (84 at most) among those with idiopathic demyelinating disorders (likely less than 0.52% of MS and 2.8% of patients with CIDP in Japan). The present nationwide survey is valuable for determining the characteristic features of CCPD. However, the study had some limitations. Many neurologists answered the questionnaires before the CCPD diagnostic criteria were established. In addition, because there are no specific biomarkers for either MS or CIDP, we could not differentiate these conditions from CCPD; instead, the number of patients who eventually met either the established MS or CIDP criteria was indicated. Nevertheless, the present study analysing the largest number of patients with CCPD defined by the same criteria is significant.

According to results from this study, CCPD was found in a preponderance of females and young adults. However, the age of onset ranged from 8 to 59 years, suggesting CCPD occurrence in a wide age range, except for elderly people. Thus, the ages of onset for CCPD overlap with those for MS and CIDP. Subacute or chronic onset was observed more often than acute onset, while a relapsing remitting or chronic progressive course was more common than a monophasic course. This suggested that a persisting inflammation affecting both the CNS and PNS was the main form of the disease. Indeed, most patients with CCPD reported in the literature to date show a relapsing remitting or chronic progressive course.[4–6 8–11 13–16] Initial symptoms that related to either CNS or PNS involvement were equally observed. CCPD had very high frequencies of motor weakness (>90%), as well as sensory disturbance with various distributions. Cranial nerve involvement that included optic nerves was also commonly seen in CCPD (75%).

The presence of widespread peripheral demyelination, as revealed by NCS and high frequency of albuminocytological dissociation, is compatible with CIDP. The abundant discrete CNS lesions which include the optic nerves and spinal cord on MRI are consistent with MS. However, several features distinct from

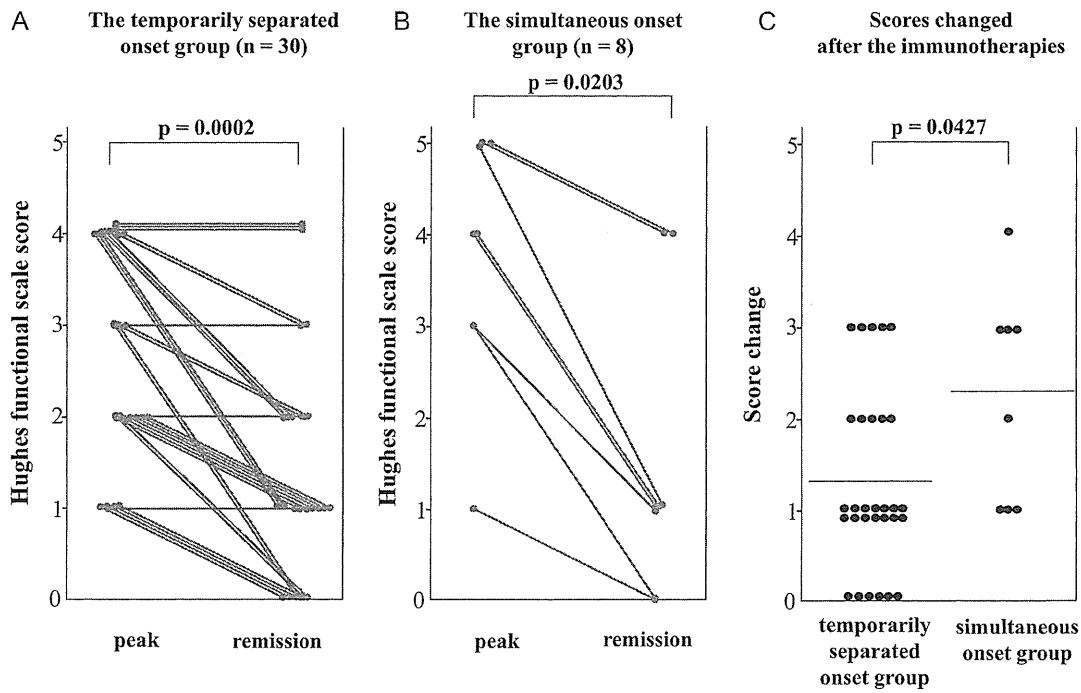

Figure 2 Comparison of treatment response in patients with combined central and peripheral demyelination with temporarily separated onset and those with simultaneous onset of central nervous system; and peripheral nervous system involvement. CCPD Hughes functional scale scores were significantly lower after immunotherapies compared with pretreatment scores in the temporarily separated onset group and simultaneous onset group (2.73±1.14 to 1.43±1.28, p=0.0002 and 3.75±1.39 to 1.50±1.60, p=0.0203, respectively). By contrast, score changes were more prominent in the simultaneous onset group than in the temporarily separated onset group (2.25±1.16 vs 1.30±0.99, p=0.0427). n=30 in the temporarily separated onset group and n=8 in the simultaneous onset group.

MS were observed in the present study, including a low frequency of CSF IgG abnormalities and poor response to IFN-β. Zéphir *et al*[16] also reported an absence of CSF oligoclonal IgG bands in five cases with CCPD. Collectively, these findings suggest that at least some mechanisms are distinct from MS function in CCPD.

Most patients with CCPD responded well to immunotherapies, regardless of acute or chronic onset, suggesting a contributory immune/inflammatory mechanism. Although we found only one paper reporting on the efficacy of the combined use of intravenous immunoglobulin and plasma exchange in a case of CCPD,[12] the present study disclosed for the first time a high efficacy for plasma exchange in CCPD, which may suggest humoral immunity involvement. A female preponderance in CCPD is also consistent with the nature of autoimmune diseases, although common systemic autoantibodies and antiganglioside antibodies were infrequent, as in previous reports.[8–12 16 23] The unresponsiveness or even disease exacerbation following IFN-β therapy found in our study was consistent with previous reports of CIDP development after IFN-β introduction in patients with MS.[24 25] Such a phenomenon may also support an autoantibody-mediated mechanism because type I IFNs potently stimulate the production of all subclasses of IgG antibodies.[26] These findings suggest the involvement of specific autoantibodies reactive to antigens that are commonly present in CNS and PNS tissues in CCPD. Additional large-scale studies will be needed to clarify the relevant antigens in this condition.

There were several distinctive features between cases of simultaneous and temporarily separated onset of CCPD. A relapsing remitting course was observed more often in the latter than in the former, whereas a monophasic course was observed more often in the simultaneous onset. This difference may be because of the classification criteria as well as the shorter observation period of the simultaneous onset group. However, Adamovic *et al*[17] reported that among 13 paediatric patients with acute simultaneous inflammatory demyelination of both the CNS and PNS, recurrence was seen only in 2 (15.4%) cases. Accordingly, as in our series, the simultaneous onset cases were followed-up nearly 4 years on average. Therefore, simultaneous onset CCPD may be less likely to recur. In addition, the difference in clinical and laboratory manifestations cannot be explained solely by the difference in observation times. For example, visual disturbance and VEP abnormalities were observed more frequently in the temporarily separated onset group than in the simultaneous onset group, in which none of the cases showed apparent visual impairment. By contrast, frequencies of other cranial nerve involvements did not differ between the simultaneous onset and temporarily separated onset groups. Thus, frequent optic nerve involvement appears to be one characteristic feature of CCPD with temporarily separated onset. This suggestion is consistent with previous case reports examining relapsing CIDP with optic nerve lesions,[27–29] as well as the relatively high frequency of VEP abnormalities in relapsing or progressive patients with CIDP (8/17, 47%).[23] Therefore, this may be a useful laboratory test for suspected CCPD cases, especially those with relapsing CIDP as a presenting feature. By contrast, the simultaneous onset group had a significantly higher frequency of patients with PNS involvement and isolated brain involvement than the temporarily separated onset group, and no patients in the simultaneous onset group had PNS involvement and isolated spinal cord involvement, whereas 20% of the temporarily separated group patients did. Collectively, such differences in the CNS sites of involvement further indicate that distinct mechanisms are operating in these two subgroups.

It is interesting to note that LESCLs were exclusively found in the simultaneous onset group and extensive cerebral lesions were also more common in the simultaneous onset group than in the temporarily separated onset group. Since no AQP4 antibodies were detected in any CCPD cases examined, LESCLs are likely produced by a mechanism distinct from that in neuromyelitis optica (NMO). Indeed, Devic type (optic–spinal) involvement was seen only in the temporarily separated group but not in the simultaneous onset group, further suggesting that LESCLs in the simultaneous onset group are produced by mechanisms distinct from those in NMO. Although the mechanisms for such extensive lesions remain unknown, it is important to raise CCPD as a differential diagnosis for LESCLs and extensive brain lesions.

In the present series, compared with the temporarily separate onset cases, the simultaneous onset cases exhibited more severe disabilities at the peak of illness, such as higher frequencies of respiratory disturbance and greater Hughes functional scale scores, which were likely a reflection of the high frequency of extensive brain and spinal cord MRI lesions. These findings were consistent with those of Adamovic *et al*,[17] who showed that among 13 paediatric patients with acute simultaneous inflammatory demyelination of the CNS and PNS, 6 were bedbound or wheelchair users and one remained on mechanical ventilation at discharge. In our series, however, the simultaneous onset group showed improvements similar to or better than the temporarily separated onset group after immunotherapy, suggesting a high efficacy of immunotherapy for simultaneous onset CCPD, despite severe manifestations. Further studies and characterisation of simultaneous onset and temporarily separated onset CCPD cases may support the existence of two CCPD subtypes and help to shed light on the distinct mechanisms between the two subtypes.

In conclusion, CCPD exhibits distinctive features from those of the classical demyelinating diseases and, therefore, may be a distinct disease, but it is not a simple coexistence of MS and CIDP. Simultaneous onset CCPD is characterised by severe disability with a relatively high frequency of respiratory disturbance, as well as extensive brain and spinal cord lesions observed in MRI scans. By contrast, temporarily separated onset CCPD features a high frequency of optic nerve involvement. Although CCPD is extremely rare, awareness of this condition is important because responses to disease-modifying drugs, such as IFN-β, for patients with CCPD are different from those in patients with MS, and appropriate immunotherapies may well produce satisfactory outcomes with minimal disabilities.

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Contributors HO, DM, TY and JK conceived the study, supervised the analyses and wrote the paper. RY, NK, TM, MH and HM participated in procedure development and collated the data.

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Commentary

Acquired and genetic channelopathies: *In vivo* assessment of axonal excitability☆

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ABSTRACT

Neuronal or axonal ion channel function can be impaired or altered in a number of disorders, such as acquired (autoantibody-mediated, toxic, and metabolic) and genetic channelopathies, and even neurodegenerative (motor neuron disease) or inflammatory diseases (multiple sclerosis, immune-mediated neuropathies). When specific channels are affected, axonal/neuronal excitability primarily alters according to original function of the corresponding channels. Separately, in the 1990s, axonal excitability testing was developed to assess ion channel function, membrane potential, and passive membrane properties non-invasively in human subjects. Using this technique, numerous papers on altered axonal excitability in a variety of disorders have been published since 2000. In a recent issue of *Experimental Neurology*, Park et al. demonstrated changes in peripheral axonal excitability in limbic encephalitis and acquired neuromyotonia with anti-voltage gated potassium channel antibodies. Unexpectedly, the results were not consistent with those caused by simple potassium channel blockade, suggesting that multiple other factors contribute to altered axonal excitability. In contrast it was reported that patients with episodic ataxia type 1 (genetic channelopathy with mutation of Kv1.1 channel gene) show prominent excitability changes exactly compatible with fast potassium channel blockade. This commentary aims to highlight findings of this study in a broader context, and provides possible explanations for the discrepancy of patterns of axonal excitability changes in acquired and genetic potassium channelopathies.

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Introduction

Ion channelopathies are caused by dysfunction of channels due to hereditary or acquired disorders. Channelopathies affect almost all areas of neurological practice, including epilepsy, movement disorders, migraine, peripheral neuropathy, pain syndrome, and myopathy (Kullmann and Waxman, 2010). Over the past 2 decades, the concept of ion channelopathy has been significantly expanded. In addition to genetic channelopathies, neuronal or axonal ion channel function can be altered in a number of conditions, such as acquired (autoantibody-mediated, toxic, and metabolic) and even neurodegenerative (motor neuron disease) or inflammatory diseases (multiple sclerosis, immune-mediated neuropathies) (Krishnan et al., 2009; Kuwabara and Misawa, 2004, 2008).

Furthermore, ionic conductances are largely affected by membrane potential and trans-axonal ionic concentration. For example, in chronic dialysis patients, axons are depolarized by hyperkalemia, resulting in increased axonal sodium and potassium conductances (Kiernan et al., 2002). Conversely under hypokalemia axonal membrane is hyperpolarized, and the ionic conductances are reduced (Kuwabara et al., 2002b). Another example is diabetic neuropathy; under hyperglycemia, the activation of the polyol pathway leads to reduced Na^+/K^+ pump activity, and the resulting intra-axonal sodium accumulation decreases sodium currents due to decreased trans-axonal sodium gradient. In this regard, uremic or diabetic neuropathy is a type of channelopathy (Kitano et al., 2004; Misawa et al., 2006a,b). Therefore ionic conductances and axonal excitability are dependent on the environmental conditions, as well as the channel function itself.

Separately, an exciting development has been the identification of neurological disorders that are associated with specific antibodies to ion channels. The most common CNS syndrome associated with voltage-gated potassium channel (VGKC) antibodies is a form of limbic encephalitis (Irani et al., 2010). Another example of anti-VGKC antibody-associated syndrome is acquired neuromyotonia, also termed as Isaacs syndrome and cramp-fasciculation syndrome, that is characterized by muscle cramp, myokymia, and fasciculations due to spontaneous repetitive firing of motor axons. The motor axonal hyperexcitability is caused by suppression of fast potassium channels by anti-VGKC antibodies (Hart et al., 2002).

☆ Commentary on: Park SB et al. Axonal dysfunction with voltage gated potassium channel complex antibodies. Experimental Neurology 261 (2014) 337-342.

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In this issue of *Experimental Neurology*, Park et al. reported changes in peripheral axonal excitability in patients with limbic encephalitis or acquired neuromyotonia, whose sera had high-titer of anti-VGKC antibodies (Park et al., 2014). Axonal excitability testing was performed at the wrist of the median nerve motor axons. Patients with limbic encephalitis demonstrated prominent abnormalities in peripheral axonal excitability during the acute phase, but the pattern of excitability property changes was not consistent with blockade of VGKC, and was possibly explained by reduced sodium currents because most of the patients had hyponatremia due to a syndrome of inappropriate antidiuretic hormone secretion.

They also showed that patients with acquired neuromyotonia demonstrated no significant changes at the site of stimulation. The total findings suggest that serum anti-VGKC antibodies did not affect excitability properties at the site of stimulation (tested site), largely because the antibodies cannot assess the tested motor axons by the blood–nerve barrier (see below). The findings indicate that not only the effects of anti-VGKC antibodies, but also a complex interaction of multiple factors should be taken into consideration in the clinical situation, and therefore this study is interesting and of clinical significance.

Nerve excitability testing

Testing the excitability of axons can provide insights into the ionic mechanisms underlying the pathophysiology of axonal dysfunction in humans. The technique of threshold tracking was developed in the 1990s, to non-invasively measure a number of axonal excitability indices, which depend on membrane potential and on the sodium and potassium conductances. By delivering a conditioning stimulus, which alters membrane potential or activates specific ion channels, the current required to produce a target potential (threshold) will change. The techniques have been extensively applied to the study of the biophysical properties of human peripheral nerves *in vivo* and have provided important insights into axonal ion channel function in health and disease. This commentary focused on assessment of potassium channel function (for the details on methodology and interpretations of nerve excitability testing, please refer to previous excellent review articles [Bostock et al., 1998; Krishnan et al., 2009]).

There are many types of potassium channels on axons (Reid et al., 1999), but it is convenient, for clinical purposes, to restrict discussion to two groups that are dependent on the membrane potential, those with fast kinetics (fast potassium channels) and those with slow kinetics (slow potassium channels). Fast potassium channels are located in the juxta-paranodal region, where they contribute to the resistance of the internodal membrane and limit the depolarizing afterpotential responsible for supernormality. Slow potassium channels have a density at the node 25 times that at the internode, but their kinetics is too slow to allow them to affect the action potential directly. They help to determine the resting membrane potential, contribute to accommodation to depolarizing stimuli, and are responsible for the late subnormality. In excitability testing, the S1d phase of threshold electrotonus and supernormality are limited by fast potassium conductance, and the S2 phase and subnormality are caused by slow potassium conductance. Therefore, patterns of combined findings of the threshold electrotonus and recovery cycle can provide information about fast and slow potassium conductances (Table 1; Fig. 1).

Briefly, in the threshold electrotonus studies, the membrane potential was altered by the use of DC polarizing currents that were 40% of the unconditioned threshold. Depolarizing and hyperpolarizing currents were used, each lasting 100 ms, and their effects on the threshold current for the test motor responses were examined. The recovery cycle of axonal excitability after a single supramaximal stimulus was measured by delivering the test stimulus at different intervals after the conditioning stimulus. The intervals between the conditioning and test stimulation were changed systematically from 2 to 200 ms. When fast potassium channels are blocked, both the S1d phase and supernormality should increase (Bostock et al., 1998).

Table 1
Axonal excitability indices and potassium channel conductance.

Parameter	Ion channel
Threshold electrotonus	
S1d	(Limited by) fast potassium channel
S2	Slow potassium channel
S3	Inward rectifying channel
Recovery cycle	
Refractoriness	Recovery from inactivation of sodium channel
Supernormality	(Limited by) fast potassium channel
Late subnormality	Slow potassium channel

S1d = peak of the slow phase in the depolarizing direction, see Fig. 1.

Genetic potassium channelopathy (episodic ataxia type 1) and excitability

Detailed nerve excitability findings in episodic ataxia type 1, a representative genetic potassium channelopathy, have been reported (Tomlinson et al., 2010); episodic ataxia type 1 is a neuronal channelopathy caused by mutations in the KCNA1 gene encoding the fast potassium channel subunit Kv1.1. The disorder presents with brief episodes of cerebellar dysfunction and persistent neuromyotonia, and is associated with an increased incidence of epilepsy. The S1d phase in threshold electrotunus, and supernormality in the recovery cycle were prominently greater than those in normal controls. The findings exactly show loss of function of fast potassium channels. Using these two parameters, the patients with episodic ataxia type 1 and controls could be clearly separated into two non-overlapping groups. The authors concluded that nerve excitability testing is useful in diagnosis, since it can differentiate patients with episodic ataxia type 1 from normal controls with high sensitivity and specificity.

In a study by Park et al. (2014), the authors presumably expected the same findings, but results of actual recordings with the same techniques in patients with anti-VGKC antibodies were very different from those in patients with episodic ataxia type 1. The S1d phase of threshold electrotonus, and supernormality in the recovery cycle were smaller than those in normal controls, the opposite patterns to those of episodic ataxia type 1 (see Fig. 1 in their paper). The findings indicate that changes in axonal excitability in patients with anti-VGKC antibodies are not caused by potassium channel blocking, and other factors should have contributed to the altered excitability. The unexpected results are considered to be due to multiple factors, and the inaccessibility of the site (at the wrist portion of the median nerve) to the antibodies because of the blood–nerve barrier is one of the factors.

The blood–nerve barrier and autoantibodies

Because of the blood–nerve barrier, large molecule substances such as immunoglobulin (antibodies) cannot access the nerve trunk. The internal microenvironment in the peripheral nerves is highly regulated. In humans, this regulation is facilitated by specialized tight junction-forming endoneurial microvascular endothelial cells. The endoneurial endothelial cells come in direct contact with circulating blood and, thus, can be considered the blood–nerve barrier.

However, the blood–nerve barrier is anatomically deficient in the distal nerve terminals, nerve roots, and dorsal root ganglia (Olsson, 1990). In immune-mediated neuropathies, such as Guillain–Barré syndrome and chronic inflammatory demyelinating neuropathy, it is established that the distal nerve terminals and nerve roots, where the blood–nerve barrier is deficient, are preferentially affected (Brown

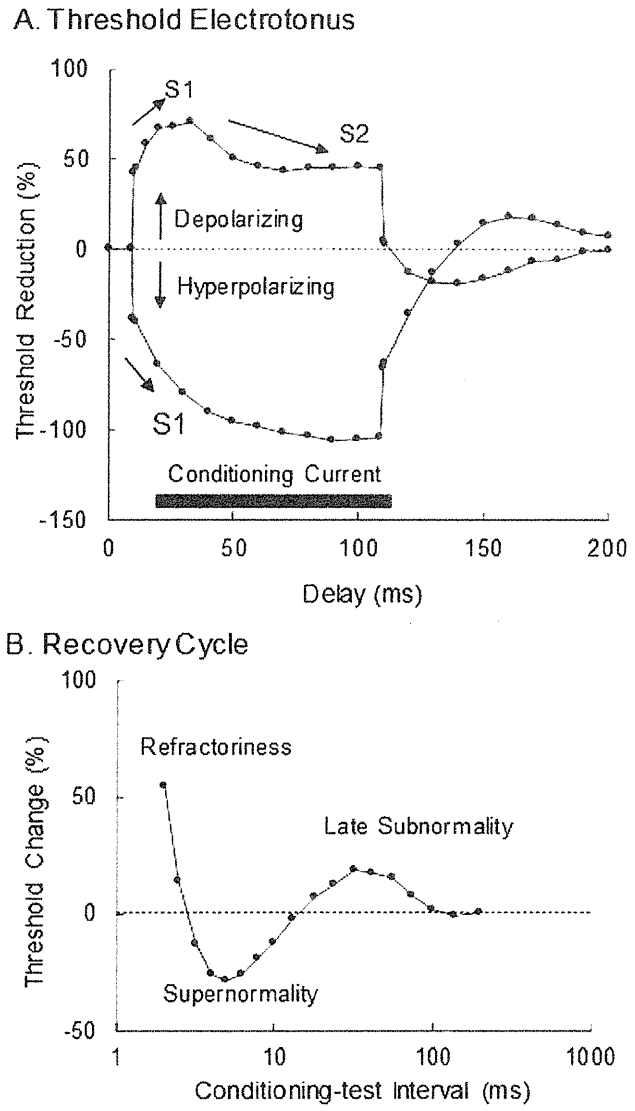

Fig. 1. Threshold electrotonus and recovery cycle measured in the median nerve at the wrist of a normal subject. A. Threshold electrotonus; changes in the threshold stimulus current for the target potential produced by subthreshold depolarizing or hyperpolarizing currents lasting 100 ms, with the intensity of the polarizing currents being 40% of the threshold. B. The recovery cycle of axonal excitability following a single supramaximal conditioning stimulus. At a short-conditioning test interval, the axons fall within the relatively refractory period, and the threshold current increases. At 3–4 ms, axons enter the supernormal period, and less current is required to activate them. Then axons enter the late subnormal period. The size of S1d and supernormality is limited by fast potassium currents, and therefore the size increases when potassium channels are impaired.

and Snow, 1991; Kuwabara et al., 2002a; Kuwabara and Yuki, 2013), and the blood–nerve barrier partly determines the distribution of demyelinative lesions. Motor nerve conduction studies frequently show prolonged distal latencies and temporal dispersion of distally evoked compound muscle action potential, suggesting demyelination in the distal nerve segments in Guillain-Barré syndrome and chronic inflammatory demyelinating neuropathy.

In anti-VGKC antibody-related disorders, the circulating antibodies can access the motor nerve terminals and roots, and fast potassium channels would be affected in these regions. Actually patients with acquired neuromyotonia in that study had motor nerve hyperexcitability evidenced by muscle cramp and fasciculations, and spontaneous motor unit activity on EMG examinations. Nerve excitability testing used in the study assesses the excitability at the stimulus site (the wrist portion of the median nerve), where blood–nerve barrier functions, and therefore anti-VGKC antibodies did not affect the tested site (nerve trunk).

Factors affecting axonal excitability

As described in the Introduction, many factors can affect axonal ionic conductances and excitability. Ion channel dysfunction itself reasonably impairs the ionic currents, but many other factors, such as membrane potential, trans-axonal ionic gradient, exposure of channels by demyelination, and increased channels in axonal regeneration, can alter ionic conductances and thereby axonal excitability.

In a paper by Park et al. (2014), altered excitability properties in limbic encephalitis patients may be caused by hyponatremia due to a syndrome of inappropriate antidiuretic hormones, and the resulting reduced trans-axonal sodium gradient and reduced sodium currents. In patients with acquired neuromyotonia, nerve excitability testing was not significantly affected by the lack of other factors influencing axonal excitability. In both cases, anti-VGKC antibodies did not affect the results. The results of this study are basically negative from the view of potassium channel blockade by autoantibodies, but sufficiently educational, telling us that in clinical practice, the determinants of axonal excitability include many other factors, some of which are secondary or co-incident, and patients show the total effects of multiple factors.

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Moesin is a possible target molecule for cytomegalovirus-related Guillain-Barré syndrome

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ABSTRACT

Objective: Previous histochemical studies in the demyelinating form of Guillain-Barré syndrome (GBS), acute inflammatory demyelinating polyneuropathy (AIDP), have shown complement deposition on the surface of Schwann cells, and therefore unknown epitopes would be present on the outer surface of Schwann cells.

Methods: We used a proteomic-based approach to search for the target molecules of AIDP in the extracted proteins from schwannoma cells. Sera were obtained from 40 patients with GBS, 31 controls with inflammatory disease, and 46 normal controls.

Results: We found that patients with AIDP after cytomegalovirus (CMV) infection have serum autoantibodies against membrane-organizing extension spike protein (moesin), which is expressed in the Schwann cell processes at the nodes of Ranvier and is crucial for myelination. Of the 40 patients with GBS, 6 had recent CMV infection and 5 of them (83%) had high levels of serum immunoglobulin G antibodies against moesin. The anti-moesin antibodies were found in none of the control subjects with disease including 5 with CMV infection but no neuropathy, and only 2 (4%) of the 46 normal control subjects. Immunocytochemistry showed that moesin was stained at the distal tips of schwannoma cells by sera from the patients with CMV-related AIDP but not by sera from controls.

Conclusion: Moesin is a possible immunologic target molecule of pathogenic autoantibodies in patients with CMV-related AIDP.

Classification of evidence: This study provides Class II evidence that levels of serum anti-moesin antibodies accurately distinguishes CMV-related AIDP from non-CMV-related AIDP (sensitivity 83%, specificity 93%). *Neurology*® 2014;83:113-117

GLOSSARY

AIDP = acute inflammatory demyelinating polyneuropathy; **AMAN** = acute motor axonal neuropathy; **CMV** = cytomegalovirus; **GBS** = Guillain-Barré syndrome; **Ig** = immunoglobulin; **MS** = multiple sclerosis; **2-DE** = 2-dimensional electrophoresis.

Guillain-Barré syndrome (GBS) is classified into 2 major categories: acute inflammatory demyelinating polyneuropathy (AIDP, a classical demyelinating form) and acute motor axonal neuropathy (AMAN, an axonal variant) based on electrophysiologic criteria.[1] Whereas it is now established that the epitopes of AMAN are gangliosides GM1 and GD1a expressed on the motor axolemma,[2] the target molecules in AIDP are still unknown.

Autopsy studies of patients with early-stage AIDP revealed complement activation marker C3d and terminal complement complex neoantigen C5b-9 along the outer surface of Schwann cells in association with vesicular degeneration of the outermost myelin lamellae. These observations suggest that AIDP may stem from complement activation by autoantibodies bound to epitopes on the Schwann cell outer surface.[3]

To identify the target antigen in AIDP, we used a proteomic-based approach to search for the surface molecules expressed by schwannoma cell line YST-1 that reacted to serum antibodies of AIDP.

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Supplemental data at Neurology.org

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METHODS **Subjects.** We recruited patients with GBS who visited Chiba University Hospital from 1998 to 2011. Serum samples were collected from 40 patients with GBS within 3 weeks of the onset before therapy who fulfilled the clinical criteria of GBS.[4] According to electrodiagnostic criteria,[1] patients were classified as having AIDP (n = 20) or AMAN (n = 9), or were unclassified (n = 11). Five patients with cytomegalovirus (CMV) infection without neuropathy, 16 with chronic inflammatory demyelinating polyneuropathy, and 10 with multiple sclerosis (MS) served as disease controls. Forty-six healthy subjects were also included.

Serum immunoglobulin (Ig)G and IgM antibodies against CMV antigen were measured using ELISA (Denka Seiken Co., Ltd., Tokyo, Japan). Following the manufacturer's instructions, serum was positive if its optical density was more than 4 SDs above the mean value for the 190 normal control samples. Patients with increased IgM antibodies were diagnosed as having CMV infection, and 6 patients with AIDP had CMV infection. All 6 patients with CMV-related AIDP had prominent sensory loss, and 5 of them showed facial palsy. Two of 6 CMV-related AIDP samples were positive (more than mean + 2 SDs of the 9 normal controls) for IgM GM2 antibodies using the ELISA kit (MyBioSource, San Diego, CA).

Standard protocol approvals, registrations, and patient consents. All subjects gave informed consent, and procedures were approved by the Ethics Committee of Chiba University School of Medicine.

Protein identification after agarose 2-dimensional electrophoresis and immunoblotting. The extracted proteins from YST-1 cells, which were insoluble with 4% 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid and soluble with 7 M urea, 2 M thiourea, 2% (w/v) CHAPS, and 0.1 M DTT, were applied. Agarose 2-dimensional electrophoresis (2-DE) was performed as previously described.[5] Separated proteins were transferred to polyvinylidene difluoride membranes, and immunoblotting with diluted serum samples (1:500) and horseradish peroxidase–conjugated goat anti-human IgG antibodies (1:10,000; Jackson ImmunoResearch Laboratories, West Grove, PA) was performed. Stained bands with Western blotting detection reagent (ECL Plus; GE Healthcare, Waukesha, WI) were detected using an LPR-400EX chemiluminescence imager (Taitec, Tokyo, Japan). The immunoreactive spots were matched to Coomassie Brilliant Blue–stained total protein spots.

Proteins were identified using in-gel tryptic digestion followed by LTQ-XL (Thermo Scientific, San Jose, CA) according to the previously described methods.[6] The Mascot search engine (version 2.2.6; Matrix Science, London, UK) was used to identify proteins from the MS and MS/MS peptide spectra. Peptide mass data were matched by searching the UniProtKB human database (SwissProt 2011x, August 2011, 7,645 entries).

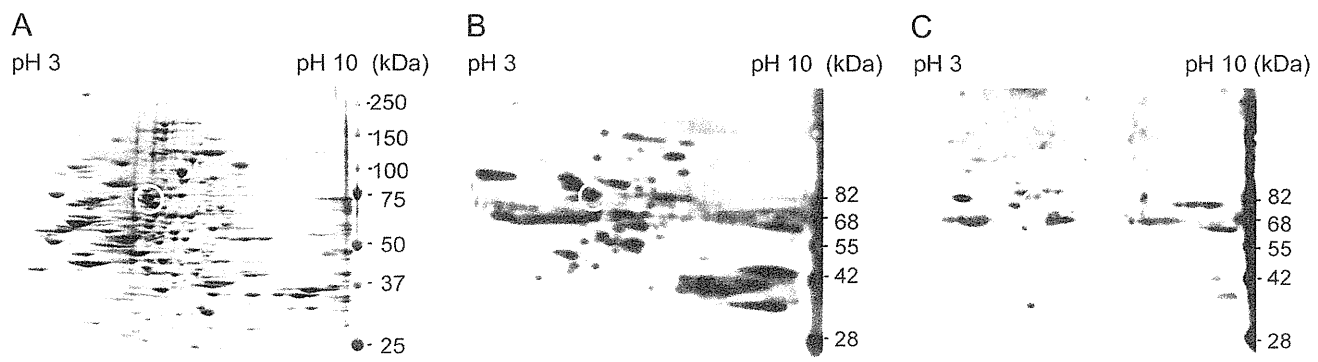
Western blot validation using human recombinant moesin protein. Full-length human recombinant moesin (OriGene, Rockville, MD) was separated (100 ng per lane) on 7.5% polyacrylamide gels and transferred to polyvinylidene difluoride membranes, and which were immunoblotted with diluted serum samples (1:500) and horseradish peroxidase–conjugated goat anti-human IgG antibodies (1:10,000; Jackson ImmunoResearch Laboratories). Anti-moesin IgG antibodies were detected using an LPR-400EX chemiluminescence imager (Taitec) after 5-minute incubation with ECL Plus (GE Healthcare), and densitometric measurements of the Western blot band images were performed using LumiVision Imager imaging analysis software (Taitec) in 5 minutes. The cutoff was mean + 2 SDs of healthy control values.

Immunocytochemistry. YST-1 cells were stained with a monoclonal mouse anti-human moesin antibody (1:250; Abnova, Taipei, Taiwan) or patient serum (1:1,000) in phosphate-buffered saline. The moesin antibody was visualized with an Alexa Fluor 568–conjugated goat anti-mouse IgG (Molecular Probes, Inc., Eugene, OR) and human serum IgG with an Alexa Fluor 488 goat anti-human IgG (Molecular Probes, Inc.).

Level of evidence. We set out to determine whether there is Class II evidence that CMV-related GBS is associated with serum anti-moesin IgG antibodies in a single center study.

RESULTS In AIDP, the most common antecedent infectious agent is CMV, and therefore we used serum obtained from a patient with CMV-related AIDP for the 2-DE immunoblot screen. Eight immunoreactive spots were shown in 2-DE Western blotting with the serum from a patient with CMV-related AIDP, and 6

Figure 1 **Identification of protein spots immunoreactive to serum from a patient with cytomegalovirus-associated Guillain-Barré syndrome using 2-dimensional electrophoresis and Western blotting**



(A) Polyvinylidene difluoride (PVDF) membrane blotted with separated proteins extracted from YST-1 cells were stained with Coomassie Brilliant Blue. (B, C) PVDF membrane reacted with 1:10,000-diluted serum from a patient with cytomegalovirus-associated acute inflammatory demyelinating polyneuropathy: B; and normal control: C. Circles indicate the spot seen in A and B, not in C, that was subsequently identified as moesin by mass spectrometry and database analysis.