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Antibodies to neural and non-neural autoantigens in Japanese patients with CNS demyelinating disorders

Mitsuru Watanabe [a,1], Takayuki Kondo [a,b,*], Kenji Murakata [a], Takashi Kageyama [c], Yoko Shibata [a], Toshiyuki Takahashi [d], Kyoichi Nomura [e], Sadayuki Matsumoto [a]

[a] Department of Neurology, Kitano Hospital Medical Research Institute, 2-4-20 Ohgimachi, Kita-ku, Osaka 530–8480, Japan
[b] Department of Community Network and Collaborative Medicine, Kyoto University Hospital, 54 Kawaharacho Shogoin, Sakyo-ku, Kyoto 606–8507, Japan
[c] Department of Neurology, Tenri Hospital, 200 Mishimacho, Tenri, Nara 632–8552, Japan
[d] Department of Neurology, Tohoku University School of Medicine, 1–1 Seiryomachi, Aoba-ku, Sendai, Miyagi 980–8574, Japan
[e] Department of Neurology, Saitama Medical Center, 1981 Kamoda, Kawagoe, Saitama 350–8550, Japan

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ABSTRACT

Anti-aquaporin 4 (AQP4) antibodies (Abs) are essential in neuromyelitis optica spectrum disorders (NMOSD), but the relationship between CNS demyelinating disorders (CNSDD) and other neural Abs remains unclear. Here we screened anti-neural Abs in the sera of 70 Japanese CNSDD patients. While two had only demyelinating events among three anti-*N*-methyl-D-aspartate receptor (NMDAR) Ab-positive subjects, the other subject who also had anti-AQP4 Abs experienced episodes of anti-NMDAR encephalitis and of NMOSD. Major lesions in the three anti-contactin-associated protein 2 Ab-positive subjects were infratentorial, including one co-carrying anti-AQP4 Abs. Thus, autoantibodies can be clinically silent, but multiple autoantibodies may participate in the pathogenesis.

1. Introduction

Central nervous system demyelinating disorders (CNSDD)—e.g. multiple sclerosis (MS), neuromyelitis optica (NMO), and NMO spectrum disorders (NMOSD)—are assumed to be autoimmune disorders. However, the etiology of CNSDD and the relationship between CNSDD and anti-neural antibodies (Abs) have not been fully elucidated. MS is the most common CNSDD and autoreactive T-cell participation in the pathogenesis of MS has been widely and deeply analyzed (McFarland and Martin, 2007). However, histopathologically prominent deposition of immunoglobulins (Ig) in some MS demyelinating lesions (Lucchinetti et al., 2000), consistent intrathecal synthesis of oligoclonal IgGs (Frohman et al., 2006) and therapeutic effects of apheresis and depletion of B cells by monoclonal Ab such as rituximab (Keegan et al., 2005; Hauser et al., 2008) all indicate that B cells and Abs contribute to the pathogenesis of MS (Uccelli et al., 2005; Meinl et al., 2006). Despite this, no specific target antigen has been defined in MS. Recent technological advances have permitted the identification of anti-neural Abs such as contactin-2 and KIR4.1 in a subpopulation of patients with MS (Boronat et al., 2012; Srivastava et al., 2012). However, the specificity and roles of these Abs in MS remains controversial (Watanabe et al., 2013).

Diagnosis of MS depends in part on the exclusion of other established diseases, allowing heterogeneous pathomechanisms.

Abbreviations: Ab, antibody; AChR, acetylcholine receptor; AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; ANA, anti-nuclear antibody; AQP4, aquaporin 4; CASPR2, contactin-associated protein 2; CIS, clinically isolated syndrome; CNS, central nervous system; CNSDD, central nervous system demyelinating disorder; CREST, calcinosis, Raynaud phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia; CSF, cerebrospinal fluid; DRM, delayed irradiation-induced myelopathy; $GABA_BR$, γ-aminobutyric acid B receptor; GAD, glutamic acid decarboxylase; GlycineR, glycine receptor; IFN, interferon; Ig, immunoglobulin; IIFA, indirect immunofluorescence assay; LAC, lupus anticoagulant; LESCL, longitudinally extensive spinal cord lesion; LGI1, leucine-rich glioma-inactivated protein 1; MAG, myelin associated glycoprotein; MG, myasthenia gravis; MPO-ANCA, myeloperoxidase-anti-neutrophil cytoplasmic antibody; MS, multiple sclerosis; NMDAR, *N*-methyl-D-aspartate receptor; NMO, neuromyelitis optica; NMOSD, neuromyelitis optica spectrum disorder; OCB, oligoclonal band; TAG, transiently expressed axonal glycoprotein; VGKC, voltage-gated potassium channel.

* Corresponding author at: Department of Community Network and Collaborative Medicine, Kyoto University Hospital, 54 Kawaharacho Shogoin, Sakyo-ku, Kyoto 606–8507, Japan. Tel.: +81 75 751 3110; fax: +81 75 751 3115.
E-mail addresses: watanabe-kyu@umin.ac.jp (M. Watanabe), takakon78@hotmail.com (T. Kondo), murakata.kenji@c2.kepco.co.jp (K. Murakata), gohach65@yahoo.co.jp (T. Kageyama), yokosbt7645@yahoo.co.jp (Y. Shibata), t-toshiyuki@mta.biglobe.ne.jp (T. Takahashi), drno@saitama-med.ac.jp (K. Nomura), smatsu@kitano-hp.or.jp (S. Matsumoto).
[1] Present address: Department of Neurology, Neurological Institute, Kyushu University Graduate School of Medicine, 3-1-1 Maidashi, Higashi-ku, Fukuoka, 812–8582, Japan.

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Indeed, subjects diagnosed as having MS can have heterogeneous patterns of pathology (Lucchinetti et al., 2000; Polman et al., 2005, 2011). Some patients with a diagnosis of MS do not have a favorable response to MS disease-modifying drugs. Most importantly, NMO/NMOSD was considered as a subtype of MS before the discovery of NMO-IgG as a specific biomarker that binds to aquaporin (AQP) 4 (Lennon et al., 2004, 2005). Therefore, we thought it reasonable to pursue the possibility that the observed heterogeneity within MS is associated with the presence of anti-neural Abs.

After the discovery of NMO-IgG, discrimination between MS and NMO/NMOSD was clarified (Lennon et al., 2004, 2005; Wingerchuk et al., 2006, 2007). However, unanswered questions remain. First, anti-AQP4 Abs are not necessarily detected in the sera of patients who follow similar clinical courses to subjects with anti-AQP4 Abs (Lennon et al., 2004, 2005; Wingerchuk et al., 2006, 2007). Second, whether AQP4 alone is an exclusive target to produce heterogeneous lesions remains unknown, although AQP4 is clearly a major target antigen in NMO/NMOSD in seropositive patients. Considering that the presence of a spectrum of autoantibodies against different autoantigens is well known in rheumatologic disorders (Routsias et al., 2004), it is fair to assume that such a spectrum of autoantibodies against different neural antigens may also appear in NMO/NMOSD.

In this study we screened for a variety of anti-neural Abs including anti-AQP4 Abs in the sera of Japanese patients with CNSDD, and examined what clinical features were present when particular anti-neural Abs were found.

2. Materials and methods

2.1. Subjects

We enrolled 70 hospital patients with CNSDD including those with MS, NMO/NMOSD, and clinically isolated syndrome (CIS). In all persons with MS, the disease was diagnosed according to the 2005 McDonald criteria (Polman et al., 2005). The diagnoses of NMO/NMOSD were based on the criteria advocated by Wingerchuck in 2006 and 2007 (Wingerchuk et al., 2006, 2007). A diagnosis of CIS was assigned in cases that did not fit any criteria for MS or NMO/NMOSD with a single neurological event that resulted from a CNS lesion.

We reviewed the medical records for all these patients including brain and spinal cord MRI performed using a 1.5 or 3 T MRI system (Philips, Best, The Netherlands).

The study was approved by the Medical Ethics Committee of Kitano Hospital.

2.2. Detection of antibodies

We analyzed serum for Abs against the following neural antigens: AQP4, glutamate receptors (types *N*-methyl-D-aspartate receptor (NMDAR) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR)), contactin-associated protein 2 (CASPR2), leucine-rich glioma-inactivated protein 1 (LGI1), glutamic acid decarboxylase (GAD), myelin associated glycoprotein (MAG), γ-aminobutyric acid B receptor ($GABA_BR$), glycine receptor (GlycineR), CV2, amphiphysin, Hu, Ri, Yo, Tr, Ma2, and myelin (Table 1). These anti-neural Abs were detected using indirect immunofluorescence assays (IIFA) (Euroimmun, Lübeck, Germany) (Wandinger et al., 2011). Note that anti-myelin Ab was detected using IIFA by incubating serum samples on peripheral and central nervous system tissues. Thus, the detailed specificity of anti-myelin Ab remains unknown. Anti-AQP4 Ab was also analyzed at Tenri Hospital and/or Tohoku University using a cell-based assay (Takahashi et al., 2007). A sample was considered positive for anti-AQP4 Ab if either analysis was positive.

The anti-nuclear Abs (ANA), and anti-SSA and anti-SSB Abs were determined by a commercial clinical laboratory service in all cases (SRL, Tokyo, Japan). However, the decision of whether to measure other systemic Abs was made by the physicians in charge of the patients. A high positive cut-off value for ANA (1:160 serum dilution) was used to exclude 95% of normal individuals (Tan et al., 1997). Anti-acetylcholine receptor (AChR) Abs were only checked for subjects who had weakness not caused by CNS lesions.

Table 1
List of antigens of anti-neuronal antibodies screened.

Aquaporin 4 (AQP4)
N-methyl-D-aspartate receptor (NMDAR)
α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR)
Contactin-associated protein 2 (CASPR2)
Leucine-rich glioma-inactivated protein 1 (LGI1)
Glutamic acid decarboxylase (GAD)
Myelin-associated glycoprotein (MAG)
Gamma-aminobutyric acid B receptor ($GABA_BR$)
Glycine receptor (GlycineR)
CV2
Amphiphysin
Hu
Ri
Yo
Tr
Ma2
Myelin

2.3. Data analysis

Statistical analyses were conducted to compare differences between the MS and NMO/NMOSD groups. χ^2 or Fisher tests were used for the analysis of frequency data, and the *t* test was used for continuous variables. $P < 0.05$ was considered significant. Statistical calculations were performed using JMP 9.0.2 software (SAS Institute, NC, USA).

3. Results

3.1. Patient demographics

Seventy patients with CNSDD were enrolled in this study. Twenty-nine had been diagnosed with MS, 28 with NMO/NMOSD, and 13 with CIS. Demographic information for these patients is presented in Table 2. The age of onset and age at survey were younger in patients with MS than in patients with NMO/NMOSD ($P < 0.001$, <0.001, respectively).

Five (17.2%) patients diagnosed with MS lacked cerebral lesions. Their diagnosis was made by demonstrated dissemination in time and space and absence of longitudinally extensive spinal cord lesion (LESCL) and anti-AQP4 Ab. Ovoid lesions were detected more often in patients with MS than in those with NMO/NMOSD (65.5% vs. 23.1%, $P = 0.003$).

Five (17.2%) patients with MS also had other autoimmune diseases, most commonly autoimmune thyroid disorder (3/29). Sjögren syndrome and myasthenia gravis (MG) were each identified in a single patient with MS. In patients with NMO/NMOSD, 4 patients (14.3%) had other autoimmune diseases. Calcinosis, Raynaud phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia (CREST) syndrome, mixed connective tissue disease and MG were each identified in a single patient, and type 1 diabetes mellitus and Sjögren syndrome coexisted in a patient with NMO/NMOSD. Of patients with CIS, two (15.4%) had an autoimmune thyroid disorder, and any other autoimmune diseases were not identified.

3.2. Antibody profiles

Table 2 shows the incidence of Abs measured in patients with MS, NMO/NMOSD, and CIS. ANA were detected more frequently in individuals with NMO/NMOSD than in those with MS, but the difference was not significant (20.0% vs. 3.9%, respectively; $P = 0.099$). For patients

Table 2
Comparison of MS, NMO/NMOSD, and CIS.

	MS ($n = 29$)	NMO/NMOSD ($n = 28$)	CIS ($n = 13$)
Sex: No. of women/total (%)	18/29 (62.1)	23/28 (82.1)	6/13 (46.1)
Age of onset, mean (SD) years[a]	33.7 (9.1)	46.1 (14.7)	39.5 (15.1)
Age of survey, mean (SD) years[a]	41.1 (9.5)	53.0 (14.5)	40.2 (14.8)
Disease duration, mean (SD) years	8.1 (6.6)	7.2 (7.9)	1.2 (1.2)
No. (%) with coexisting autoimmune diseases	5/29 (17.2)	4/28 (14.3)	2/13 (15.4)
Example of coexisting autoimmune disease (No.)	ATD (3), SjS (1), MG (1)	SjS (1), CREST syndrome (1), MCTD (1), MG (1), type 1 DM (1)	ATD (2)
No. (%) with following lesions detected by MRI[b, c]			
Cerebral lesions	24/29 (82.8)	26/27 (96.3)	9/12 (75.0)
Big cerebral lesions (>3 cm)	4/29 (13.8)	2/26 (7.7)	1/11 (9.1)
Periventricular lesions	24/29 (82.8)	19/27 (70.4)	7/11 (63.6)
Ovoid lesions[a]	19/29 (65.5)	6/26 (23.1)	5/11 (45.5)
Corpus callosum lesions	18/28 (64.3)	10/23 (43.5)	4/10 (40.0)
Cerebellar lesions	5/29 (17.2)	1/27 (3.7)	1/11 (9.1)
Brainstem lesions	16/29 (55.2)	14/27 (51.9)	7/12 (58.3)
Spinal cord lesions	18/29 (62.1)	22/28 (78.6)	3/13 (23.1)
LESCL[a]	0/27 (0.0)	15/26 (57.7)	0/8 (0.0)
No. (%) with VEP abnormality	17/29 (58.6)	14/27 (51.9)	5/13 (38.5)
No. (%) with following systemic antibodies[b]			
Anti-nuclear antibodies	1/26 (3.8)	5/25 (20.0)	0/13 (0.0)
(In IFNβ-free condition)[a]	0/20 (0.0)	5/19 (26.3)	0/12 (0.0)
Anti-SSA	4/26 (15.4)	6/25 (24.0)	0/12 (0.0)
Anti-SSB	1/22 (4.5)	2/21 (9.5)	0/10 (0.0)
No. (%) with following anti-neural antibodies[b]			
Anti-AQP4[a]	0/29 (0.0)	24/28 (85.7)	0/13 (0.0)
Anti-NMDAR	1/29 (3.4)	2/28 (7.1)	0/13 (0.0)
Anti-CASPR2	0/29 (0.0)	2/28 (7.1)	1/13 (7.7)
Anti-Ma2	1/29 (3.4)	0/28 (0.0)	0/13 (0.0)
Anti-Tr	1/29 (3.4)	0/27 (0.0)	0/13 (0.0)
Anti-myelin	2/29 (6.9)	2/27 (7.4)	2/13 (15.4)

Abbreviations: ATD, autoimmune thyroid disease; ANA, anti-nuclear antibodies; AQP4, aquaporin-4; CASPR, contactin-associated protein; CIS, clinically isolated syndrome; CREST, calcinosis, Raynaud phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia; DM, diabetes mellitus; IFN, interferon; LESCL, longitudinally extensive spinal cord lesion; MCTD, mixed connective tissue disease; MG, myasthenia gravis; MS, multiple sclerosis; NMDAR, *N*-methyl-D-aspartate receptor; NMO, neuromyelitis optica; NMOSD, neuromyelitis optica spectrum disorder; SjS, Sjögren syndrome; VEP, visual evoked potential.

[a] $P < 0.05$ (MS vs. NMO/NMOSD).

[b] Not all patients were examined.

[c] Lesions were detected by T2-weighted MRI.

who had not been exposed to interferon (IFN) β therapy at the time samples were obtained, ANA were detected with significantly greater frequency in the NMO/NMOSD group than in the MS group (26.3% vs. 0.0%, respectively; $P = 0.020$). Although the percentage of individuals positive for anti-SSA Abs and anti-SSB Abs was slightly higher in the NMO/NMOSD group than in the MS group, the differences were not significant (MS vs. NMO/NMOSD, $P = 0.50$, 0.61, respectively).

Anti-NMDAR Abs were detected in two patients with NMO/NMOSD and one with MS. While two of these three individuals had no known episodes of anti-NMDAR encephalitis (Dalmau et al., 2008), the other patient experienced an episode of psychotic delusion (presented below). Anti-CASPR2 Abs were detected in two patients with NMO/NMOSD and one with CIS. Only one of these three patients with anti-CASPR2 Abs was positive for anti-AQP4 Abs. We detected Abs against Ma2 in one patient with MS and Abs against Tr in another MS patient. Anti-myelin Abs were detected in sera from two patients from each of the three groups. Other anti-neural Abs were not detected in any patient. Cases of interest are described below.

3.3. Cases of interest (Table 3)

3.3.1. Patients with anti-NMDAR Abs

Case 1 was in a 34-year-old woman. She was admitted to our hospital because of complete tetraplegia. She had experienced an episode of intractable hiccups for 3 weeks at the age of 22, and left optic neuritis at the age of 27. One month before admission, she was unresponsive and presented with delirium and hallucination preceded by fatigue, headache, and generalized seizure with a high temperature. A T2-weighted brain MRI image revealed lesions of slightly increased intensity in the bilateral putamina (Fig. 1A). The lesions disappeared 1 month later. Her psychiatric symptoms resolved spontaneously in 1 month, and shortly thereafter the patient developed complete tetraplegia,

Table 3
Clinical data of selected cases.

Case	Diagnosis	Anti-neural Abs for:			Anti-systemic Abs for:				CSF			
		AQP4	NMDAR	CASPR2	ANA	SSA	SSB	Others	Cell count (/mm^3)	Protein (mg/dL)	IgG index	OCB
1	NMO	+	+	−	−	+	−	MPO-ANCA, LAC	16	56.4	0.92	−
2	NMOSD	+	+	−	−	−	−	Centromere	3	41.7	1.07	−
3	MS	−	+	−	−	−	−	−	2	69.8	0.86	−
4	NMO	+	−	+	+	−	−	−	NE	NE	NE	NE
5	NMOSD	−	−	+	−	−	−	−	0	29	0.62	−
6	CIS	−	−	+	−	−	−	−	NE	NE	NE	NE

Abbreviations: ANA, anti-nuclear antibodies; AQP4, aquaporin 4; CASPR, contactin-associated protein; CIS, clinically isolated syndrome; LAC, lupus anticoagulant; MPO-ANCA, myeloperoxidase-anti-neutrophil cytoplasmic antibodies; MS, multiple sclerosis; NE, not examined; NMDAR, *N*-methyl-D-aspartate receptor; NMO, neuromyelitis optica; NMOSD, neuromyelitis optica spectrum disorder; OCB, oligoclonal band; +, positive; −, negative.

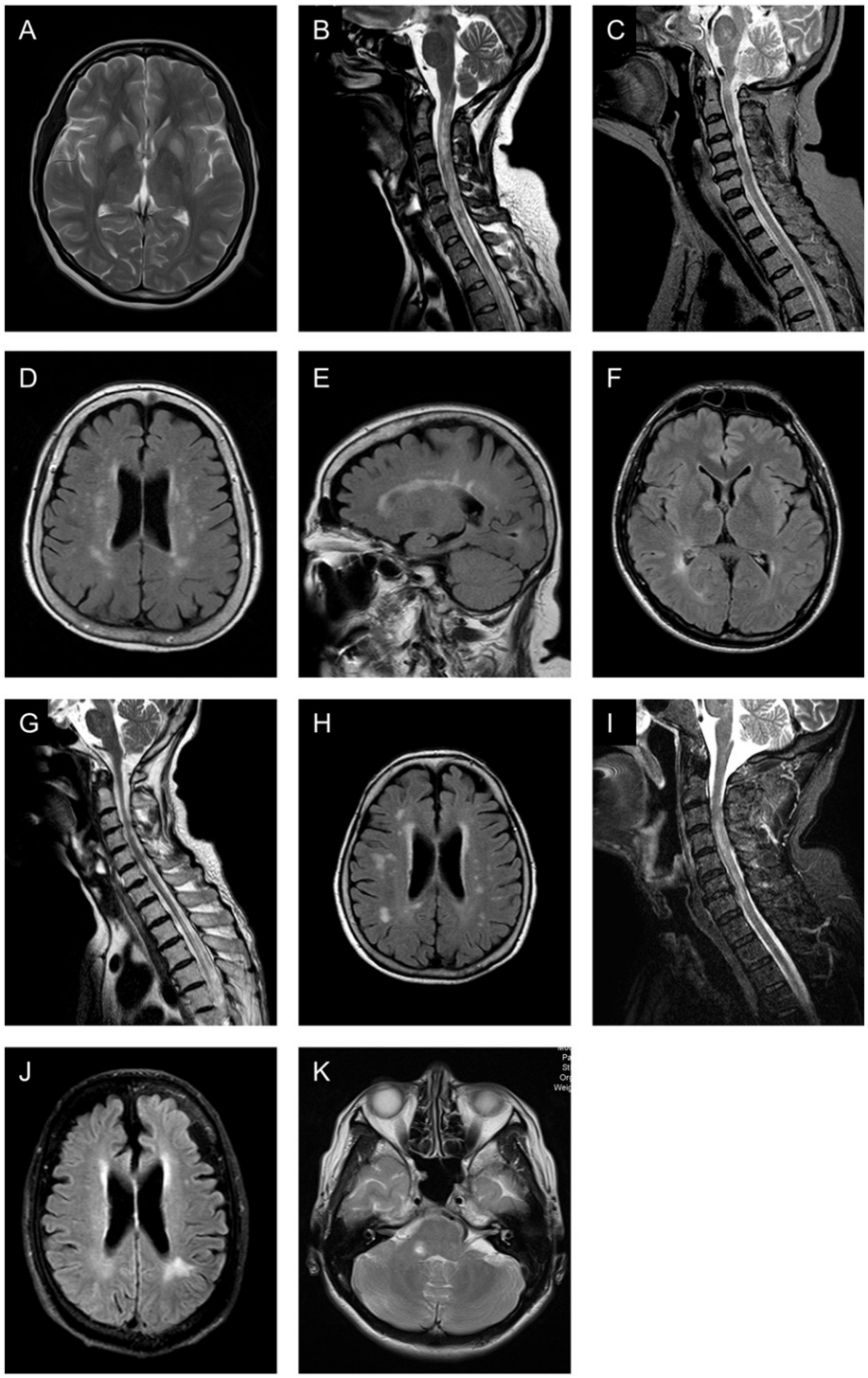

Fig. 1. CNS lesions of some patients with CNS demyelinating disorder. (A and B) MRI images from a 34-year-old woman who was AQP4-seropositive NMO with anti-NMDAR Abs. (A) T2-weighted brain MRI showing bilateral putamina lesions. (B) T2-weighted cervical cord MRI showing multiple long lesions. (C–E) MRI images from a 69-year-old woman who was AQP4-seropositive NMO with anti-NMDAR Ab. (C) T2-weighted cervical cord MRI showing multiple lesions. (D and E) Brain MRI (fluid-attenuated inversion recovery, FLAIR; axial and sagittal) showing multiple periventricular and corpus callosum lesions characteristic of MS. (F) Brain MRI image (FLAIR) from a 39-year-old MS patient with anti-NMDAR Ab showing a few periventricular lesions. (G and H) MRI from a 65-year-old woman who was AQP4-seropositive NMO with anti-CASPR2 Ab. (G) T2-weighted spinal cord MRI showing a long lesion at the thoracic level with cord atrophy. (H) Brain MRI (FLAIR) showing nonspecific disseminated lesions. (I and J) MRI images from a 65-year-old man who was NMOSD with anti-CASPR2 Ab. (I) T2-weighted cervical cord MRI showing LESCL in C3–C7. (J) Brain MRI (FLAIR) showing a nonspecific periventricular lesion characteristic of leukoaraiosis. (K) T2-weighted brain MRI image from a 36-year-old woman who was CIS, showing right-sided pontine and middle cerebellar peduncle lesions.

accompanied by decreases of sensation in all extremities and the body trunk, followed in a few days by bladder and bowel disturbance. Spinal MRI revealed multiple lesions from the upper cervical spine to the conus medullaris (Fig. 1B). The lesions appeared to be discontinuous and exhibited a moth-eaten appearance. Ab analysis revealed the presence of anti-AQP4, anti-SSA, and anti-NMDAR Ab; myeloperoxidase-anti-neutrophil

cytoplasmic Ab (MPO-ANCA); and lupus anti-coagulant (LAC). Steroid therapy and plasma exchange ameliorated her symptoms, and she was able to walk with a cane in 3 months.

Case 2 was in a 69-year-old woman. She experienced multiple episodes of myelitis since the age of 64. The spinal lesions, as shown by MRI, spanned fewer than three vertebrae (Fig. 1C). Brain MRI revealed many lesions in the periventricular area and corpus callosum. These lesions were similar to typical MS lesions (Fig. 1D and E). However, this patient was diagnosed with NMOSD because she was positive for anti-AQP4 Ab. She was also positive for ANA and anti-centromere Abs. A cerebrospinal fluid (CSF) study showed an elevated IgG index, but absence of oligoclonal bands (OCB).

Case 3 was in a 39-year-old man who had two episodes of hemisensory disturbance on his left side and dizziness caused by brainstem and cerebral lesions. Fig. 1F shows brain MRI after the second episode. He was diagnosed with MS, but typical brain lesions such as periventricular or juxtacortical lesions were not found. His CSF showed an elevated IgG index, but absence of OCB. IFNβ therapy was initiated after the second relapse. He was stable afterwards.

3.3.2. Patients with anti-CASPR2 Abs

Case 4 was in a 65-year-old woman who had a 23-year history of frequent relapse–remission episodes with bilateral optic neuritis and myelitis, with an LESCL at the thoracic level (Fig. 1G). She was positive for anti-AQP4 Ab. Brain MRI showed nonspecific disseminated lesions (Fig. 1H). She remains bedridden, but is stable with oral steroid therapy.

Case 5 was in a 63-year-old man who had received radiation therapy at age 58 years to treat hypopharyngeal cancer and had developed tetraplegia with bladder and bowel disturbance over 2 days. Anti-AQP4 Abs were not detected in his serum, but spinal MRI revealed an LESCL in the cervical spinal cord (Fig. 1I). Brain MRI also showed leukoaraiosis, not indicative of MS or NMO/NMOSD (Fig. 1J). His symptoms were ameliorated immediately after steroid-pulse therapy.

Case 6 was in a 36-year-old woman who had a history of facial numbness on her right side. MRI showed a right-sided pontine lesion, but no cerebral lesions (Fig. 1K). The woman did not consent to a detailed examination, and observation was continued without medication.

4. Discussion

It is thought that both MS and NMO/NMOSD can occur in the context of rheumatologic autoimmune diseases or nonorgan-specific Abs, or both (Wingerchuk et al., 2007; Solomon et al., 2013). In this study, 5 (17.2%) patients with MS, 4 (14.3%) patients with NMO/NMOSD and 2 (15.4%) patients with CIS had other autoimmune diseases (Table 2). We found that MS patients with accompanying rheumatologic autoimmune diseases exhibit some atypical features when subjects with autoimmune thyroiditis were excluded. One patient with MS complicated by MG had few brain lesions, and IFNβ therapy appeared to increase MS attacks. Another patient with MS complicated by Sjögren syndrome had concentric lesions, and relapses were not observed under oral steroid therapy. OCB was not seen in their CSF.

ANA was found much more frequently in the NMO/NMOSD group than in the MS group, and the difference was significant when patients exposed to IFNβ were excluded. There were no differences between groups in the rate of detection of anti-myelin Abs, suggesting that anti-myelin Abs are not helpful in differentiating MS from NMO/NMOSD.

The diagnostic procedure for CNSDD routinely includes an analysis for the presence of nonorgan specific Abs and the coexistence of rheumatologic autoimmune diseases. However, the possibility that immunity to multiple CNS antigens coexists in a single patient usually attracts little attention in a clinical setting. This study shows that at least three individuals with CNSDD in our analysis carried autoantibodies to more than one neural antigen. In the case 1 patient, seizure and psychosis was consistent with anti-NMDAR encephalitis (Dalmau et al., 2008), while hiccups, optic neuritis, and myelitis are regarded as symptoms of NMO (Wingerchuk et al., 2007). MPO-ANCA and LAC were also detected. Demonstrated by the literature on myelitis with these two Abs (Nakashima et al., 1998; Birnbaum et al., 2009), MPO-ANCA and LAC can also contribute to the CNS manifestation of this patient. Therefore, case 1 could be regarded as representative of cases in which more than one anti-neural autoantibody can participate in the pathogenesis of a patient with CNSDD. Similarly, it was previously reported that a patient who had both anti-AQP4 and glutamate receptor ε2 subtype Abs exhibited LESCL and transient amnesia (Honda and Yuasa, 2008). In cases 2 and 3, the patient histories lacked episodes known to be related to anti-NMDAR encephalitis (Dalmau et al., 2008). Taken together with the fact that 30–50% of patients with systemic lupus erythematosus carry anti-NMDAR Abs (Watson et al., 2012), anti-NMDAR Abs may be clinically silent in these patients, although the possibility that anti-NMDAR Abs play some role in the demyelinating process is not completely excluded. There are some reports of CNSDD patients with anti-NMDAR Abs (Ishikawa et al., 2007; Kruer et al., 2010; Motoyama et al., 2010). Although the authors noted that anti-NMDAR Abs may contribute to the pathogenesis of these patients with CNSDD, it is also likely that unidentified Abs, other than anti-NMDAR Abs, play important roles in the pathogenesis. Taken together, there are possibilities in the setting of autoimmune disorders that autoantibodies are not necessarily pathogenic, that unidentified pathogenic Abs may exist, and that multiple CNS autoantibodies may work together in a single patient.

One patient carried anti-AQP4 Abs among a total of three with anti-CASPR2 Abs, while, of the Abs screened, the other two only carried anti-CASPR2 Abs; in other words, no other responsible Abs other than anti-CASPR2 Abs were identified. Moreover, the distribution of the lesions was common to these three patients, and was limited to the brainstem or spinal cord. Because roles for anti-CASPR2 Abs in CNSDD have not been established, there remains a possibility that case 5 may be a delayed irradiation-induced myelopathy (DRM). However, there is agreement that no treatment has been conclusively shown to be of value in DRM and that steroid therapy shows only a temporary benefit (Rampling and Symonds, 1998), although steroid therapy was reported to be effective for limited cases of irradiation-induced myelopathy (Uchida et al., 2009). The patient was diagnosed as having NMO/NMOSD because the myelopathy developed acutely 5 years after irradiation, and had a good response to steroid therapy and the presence of anti-neural Abs such as anti-CASPR2 Abs that indicated autoimmune phenomena had occurred in this patient.

To date, anti-CASPR2 Abs have been reported to be associated with limbic encephalitis, Morvan's syndrome, acquired neuromyotonia, and nonparaneoplastic cerebellar ataxia (Irani et al., 2010; Lancaster et al., 2011; Wandinger et al., 2011; Becker et al., 2012). However, three cases of CNSDD associated with anti-CASPR2 Abs in this study have raised the possibility that these Abs can also play a role in CNS demyelination. CASPR2 is localized on myelinated axons in the juxtaparanodal region in the CNS and peripheral nervous system and forms a tripartite complex with voltage-gated potassium channels (VGKCs) and transiently expressed axonal glycoprotein (TAG)-1/contactin-2 (Poliak et al., 1999, 2003; Boronat et al., 2012; Zoupi et al., 2013). These molecular interactions mediate the axo–glial contact and these components including neurofascin isoforms and TAG-1/contactin-2 were also identified as auto-antigens in a subset of MS patients (Mathey et al., 2007; Derfuss et al., 2009; Boronat et al., 2012; Kawamura et al., 2013). Moreover, juxtaparanodal proteins including CASPR2 are diffusely distributed on denuded axons in MS lesions and in a CNS demyelination animal model (Coman et al., 2006; Zoupi et al., 2013). Immunity to potassium channels or their related proteins may be an important target in CNSDD. KIR4.1, a recently identified MS-associated autoantigen (Srivastava et al., 2012), is another example of immunity to potassium channels in CNSDD. With these points in mind, we speculate that CASPR2 can be a candidate antigen in CNSDD through a mechanism

involving anti-CASPR2 Abs and can affect the myelin–axon interaction. However, further research in vivo and in vitro is needed to test whether anti-CASPR2 Ab can be a pathogenic factor in CNSDD.

There are some limitations to this research. One limitation is that the screening for the various Abs in the sera of patients with CNSDD was conducted at arbitrary timings; many of the sera were obtained at the initial medical examination, but some were obtained after the administration of treatment. A second limitation is that we only screened sera for the Abs, but not CSF, which may be more directly associated with CNS pathophysiology. Finally, the role each detected Ab plays in disease pathogenesis remains undetermined. A detected Ab (i) may be directly pathogenic, (ii) may simply reflect B cell dysfunction and generation of multiple Abs because Ab-mediated diseases are more likely to coexist, (iii) may simply be produced as the result of CNS tissue damage, and (iv) may not be truly associated with observed diseases.

We screened a broad profile of anti-neural Abs in Japanese patients with CNSDD and demonstrated that immunity against multiple autoantigens can participate in the pathogenesis of CNSDD. In a clinical setting, we recommend considering the presence of unidentified or undetected Abs even if some Abs have already been detected. In conclusion, there is a possibility that hidden autoimmunity may have a more direct association with CNSDD than the detected autoimmunity does.

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