er in the patients with NMO than in the RRMS patients (table 2). These results suggest that treatment with IFN-β_{1b} is not effective in reducing the relapse number and the disability progression in NMO patients.

This study also showed that the decrease in relapse number after IFN-β_{1b} treatment was not significantly different between the anti-AQP-4-Ab-positive and anti-AQP-4-Ab-negative patients with NMO. We have reported that seropositive patients with a long spinal cord lesion (LCL) had more relapses than seronegative patients with LCL [12]. This study included patients with LCL reported previously, although our previous study included patients who had not been treated with IFN-β_{1b}. Many patients in this study had been treated with IFN-β_{1b} a few years earlier, and the presence of the anti-AQP-4-Ab was not related to disease activity during the periods 2 years before and after the start of treatment.

A Japanese randomized controlled trial has shown that IFN-β_{1b} treatment is effective in decreasing attack frequency even in patients with optic-spinal MS (OSMS) characterized by lesions restricted to the optic nerves and spinal cord [13]. Recent observations including those from our study suggest that OSMS includes a benign phenotype without LCL showing some features commonly seen in classic MS [12, 14, 15], and that these patients may show beneficial effects with IFN-β_{1b} treatment. In a Japanese IFN-β_{1b} MS study [13], classic MS might include NMO with symptoms related to brain lesions. We have found that 70% of the patients with NMO showed brain symptoms [Komori, unpubl. data]. Therefore, the effect of IFN-β_{1b} treatment in patients with Japanese classic MS [13] was underestimated and that in Japanese patients with OSMS [13] was overestimated. To evaluate the therapeutic effects of IFN-β_{1b} in NMO patients, it should be considered that OSMS and NMO are different [12].

Acknowledgment

This work was supported in part by Health and Labor Sciences Research Grants for research on intractable diseases from the Ministry of Health, Labor and Welfare of Japan.

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Acta Ophthalmologica

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Case Series

Anti-aquaporin-4 antibody-positive optic neuritis

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ABSTRACT.

Purpose: **It has recently been reported that the anti-aquaporin-4 antibody (AQP4-Ab) can be a specific marker of neuromyelitis optica. We present three cases of optic neuritis (ON) where the patients tested positive for AQP4-Ab, but showed no neurological signs.**

Methods: **Sera were obtained from 32 Japanese patients with ON and no other neurological abnormalities (mean age 46 ± 20 years). AQP4-Ab was detected by indirect immunofluorescence staining using human-AQP4-transfected HEK 293 cells.**

Results: **AQP4-Ab was positive in three female patients (aged 9, 64 and 82 years). Their illness was characterized by bilateral severe optic nerve involvement, insufficient visual recovery, and autoimmune abnormalities (such as positive antinuclear antibody). Two of these patients experienced recurrent episodes of ON. In at least two episodes, the intracranial portion of the optic nerve showed significant inflammation on magnetic resonance imaging.**

Conclusions: **These cases indicate that some ON patients have an immunological pathogenesis similar to that seen in neuromyelitis optica. In addition, examination for AQP4-Ab positivity in the initial phase of ON is important in predicting the prognosis, including the possibility of the development of transverse myelitis.**

Key words: anti-aquaporin-4 antibody – autoimmune optic neuropathy – neuromyelitis optica – optic neuritis

Acta Ophthalmol. 2009: 87: 562–566

doi: 10.1111/j.1755-3768.2008.01259.x

Introduction

Optic neuritis (ON) is a common ophthalmological disease, but its pathophysiological mechanism is poorly understood. It is thought to be an inflammatory demyelinating disease caused by autoimmune mechanisms, and to be closely related to multiple sclerosis (MS). Multiple sclerosis has been typically classified into two subgroups in Asia – classic MS (C-MS) and optic-spinal MS – but most cases of the latter is now thought to actually be neuromyelitis optica (NMO) (Wingerchuk et al. 1999; Kira 2003; Lennon et al. 2004; Nakashima et al. 2006; Tanaka et al. 2007). Lennon et al. (2004, 2005) recently reported that the serum immunoglobulin G antibody that recognizes the aquaporin-4 water channel (AQP4) is a specific marker of NMO. In this report, we describe AQP4 positivity in three patients with ON and no neurological disturbance.

Materials and Methods

We examined sera from 32 Japanese patients with ON from June to December 2006. The diagnosis of ON was made by the authors (MT and TS, at Niigata University Hospital and Dokkyo Medical University Koshigaya Hospital, respectively), based on the subacute onset of visual loss, sluggish pupillary light reflex, central scotoma, and gadolinium (Gd)-DTPA (diethylene-triamine-penta-acetic-acid) contrast enhancement of the optic nerve under magnetic resonance imaging (MRI). Patients of all ages were included; those with a history of ON during the last 10 years (for whom detailed neuro-ophthalmological records could be reviewed) were also included. None of these patients showed signs of brain or spinal cord lesions. The patient population included 19 females and 13 males

(mean age 47.5 ± 20.0 years). Nine patients were affected in the right eye and seven in the left, and 16 had bilateral ON. The medians of worst and final best-corrected visual acuity (BCVA) were 20/400 and 20/25, respectively. Five patients experienced recurrent episodes of ON. Sera were obtained in accordance with ethical standards in line with the tenets of the Declaration of Helsinki. The study was approved by the ethical committee of Niigata University and written informed consent was acquired from all participants. Antibody testing was performed in a completely blind manner, with no clinical information provided to the examiner.

For the AQP4-Ab detection, we constructed AQP4 antigen-presenting cells as follows (Tanaka et al. 2007): total RNA was extracted from an adult human cerebellum from a donor bank and cDNA-encoding human aquaporin-4 (AQP4 M23 isoform; GenBank accession number U63623) (Lu et al. 1996) was cloned by the reverse transcription-polymerase chain reaction (RT-PCR) technique. Full-length cDNA was inserted into the *Xba*I site of a pEF-BOS expression vector and transfected to HEK 293 cells. The HEK 293 cells were then fixed in 4% paraformaldehyde in 0.1 m phosphate-buffered saline (PBS), pH 7.4. Non-specific binding was blocked with 10% goat serum/PBS, the cells were incubated with the patient serum for 60 mins at room temperature and then incubated with fluorescein isothiocyanate (FITC)-conjugated rabbit anti-human IgG (BD Biosciences, San Jose, CA, USA). A *SlowFade* Gold anti-fade reagent (Molecular Probes, Carlsbad, CA, USA) was then applied to the slide and positivity verified under the fluorescence microscope.

Results

Positivity for AQP4-Ab was observed in three of 32 patients (Fig. 1). All positive patients were female and had episodes of severe bilateral optic nerve involvement, but no complaints of retrobulbar pain with eye movement. No neurological signs were detected by neurologist's examination.

Case 1

A previously healthy 9-year-old girl was seen at the hospital in August 2003 suffering from viral meningitis. A few weeks after her meningitis was resolved, she noticed a bilateral visual disturbance. Her BCVA was 20/400 OD and 20/800 OS. Her light reflex was sluggish OU, and her visual fields were severely impaired with central scotoma. Her anterior segment appeared normal, but funduscopy revealed bilateral disc swelling. Bilateral swelling of the optic nerves, including the intracranial portion, was noted on her MRI. Cerebrospinal fluid (CSF) findings were normal (including myelin basic protein); an oligoclonal band was not observed. The subject was treated with three cycles of i.v. methyl prednisolone pulse therapy (0.5 g/day for 3 days at 1-week intervals), followed by oral corticosteroid. Her BCVA slowly recovered, reaching 20/15 OU. In September 2004, her BCVA dropped to 20/40 OD and hand motion (HM) OS. Her left eye showed a relative afferent pupillary defect (RAPD). Her visual field was severely impaired, and funduscopy revealed left disc swelling. Her MRI showed Gd-enhancement in the left optic nerve, predominantly in the intracranial portion (Fig. 2A). Serological examination revealed high levels of antinuclear antibody (ANA) at an index of 89.6 (normal: < 20), an anti-SS-A antibody (SS-A) index of 130.0 (normal: < 30), and a rheumatoid factor (RF) of 61.31 IU/ml (normal: < 10 IU/ml). She was treated again with i.v. and then oral corticosteroids. Best corrected VA OS slowly recovered to 20/15. In March 2005, BCVA OD had dropped to light perception (LP), but disc swelling was not observed. The patient was treated again with i.v. and oral corticosteroids. Her BCVA OD slowly recovered, but only to 20/200. In September 2005, BCVA OS had dropped to 20/2000. Disc swelling was not observed. She was treated again with i.v. and oral corticosteroids. Her BCVA OS slowly recovered, but only to 20/200. AQP4-Ab positivity was detected on 6 June 2006. Levels for ANA, SS-A and RF remained similar to those seen in the second episode.

Case 2

An 82-year-old woman with mild hypertension reported bilateral visual loss in December 2005. Her BCVA was reduced to HM OU in a few days. Her light reflex was sluggish OU. Her anterior segment appeared normal and the funduscopy revealed normal discs. Her MRI revealed bilateral Gd-enhancement of the optic nerve, predominantly in the intracranial portion (Fig. 2B). She had an elevated RF of 30.4 IU/ml. She was treated with three cycles of i.v. methyl prednisolone pulse therapy (0.5 g/day for 3 days at 1-week intervals), followed by oral corticosteroid. Over several months, her BCVA slowly improved to around 20/25 OS, but reached only 20/200 OD with central scotoma. AQP4-Ab positivity was detected on 18 July 2006.

Case 3

A previously healthy 64-year-old woman reported visual loss in April 2002. Her BCVA was no LP OD and 20/30 OS with right RAPD. Her anterior segment appeared normal, but funduscopy revealed slight swelling in the right optic disc, and her MRI revealed right optic nerve swelling. The patient was not willing to start steroid pulse treatment under hospitalization, and only oral corticosteroid was administered. Visual recovery was insufficient: her BCVA in July 2002 was 20/2000 OD and 20/20 OS with right RAPD. In July 2004, her VA OS had fallen to 20/200 with central scotoma, but disc swelling was not observed. Oral steroids were immediately restarted. Over the next month, the subject's BCVA OS improved to 20/25. AQP4-Ab positivity was detected on 11 September 2006. A serological examination at that time revealed a positive ANA (1 : 80 speckled; normal < 1 : 40) and an elevated SS-A (47.8 IU/ml; normal: < 10 IU/ml).

These patients have been carefully followed, both ophthalmologically and neurologically, using neuro-imaging. Total follow-up periods so far are 4 years and 4 months, 2 years, and 5 years and 7 months for Cases 1, 2 and 3, respectively. To date, the subjects have not developed any neurological abnormalities suggesting myelitis.

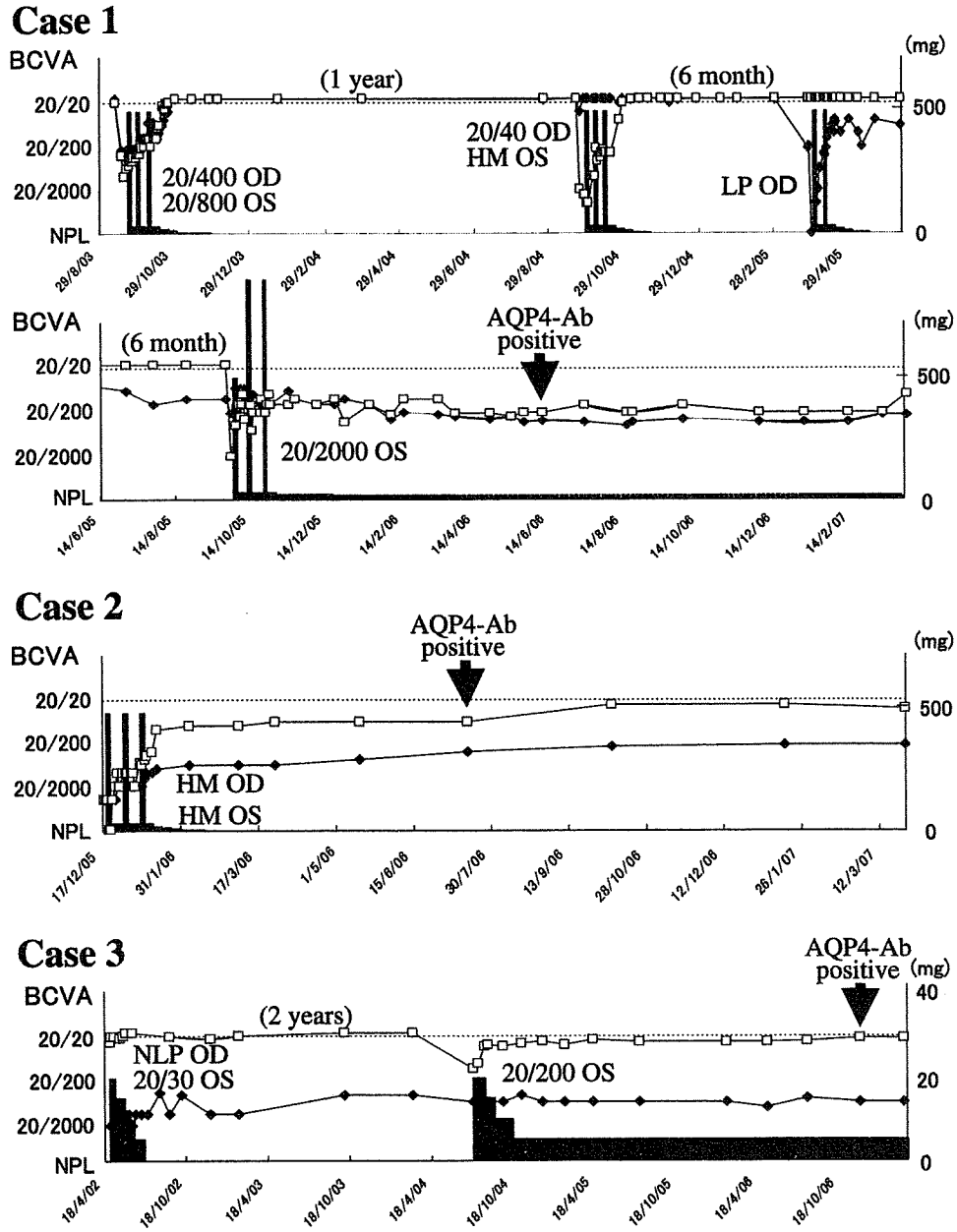

Fig. 1. Clinical course of visual acuity in aquaporin-4 antibody (AQP4-Ab) positive cases, showing dates of evaluation, best corrected visual acuity (BCVA; left ordinate) and dose of corticosteroid (in mg; right ordinate). ♦ and □ = right and left BCVA, respectively. Interval periods between attacks are indicated in parentheses. The worst BCVA in each attack is noted. NLP = no light perception; LP = light perception; HM = hand motion; OS = left eye; OD = right eye. Arrows with 'AQP4-Ab positive' indicate the dates sera were obtained.

Discussion

Our three cases of AQP4-Ab positive ON appear to have distinct clinical characteristics: all were female; all had bilateral eye involvement, and all had severe visual impairment in the acute phase and delayed, insufficient visual recovery. Two of the three patients had recurrent episodes of visual loss. These clinical characteristics could be correlated to some of the distinctive clinical features of neuromyelitis optica (Wingerchuk et al. 1999; Kira 2003; Osoegawa et al. 2005), including a high female : male ratio, high relapse frequency, and severe disability. In a comprehensive study by Lennon et al. (2004), recurrent ON was classified as a 'high-risk syndrome for NMO', and AQP4-Ab was detected in two of eight cases. Post-infection episodes like those seen in our Case 1 are also seen in NMO. However, our patients were outside the typical age range for ON (usually 15–50 years), and they lacked retrobulbar pain with eye movement. These aspects of our cases seem atypical for idiopathic ON.

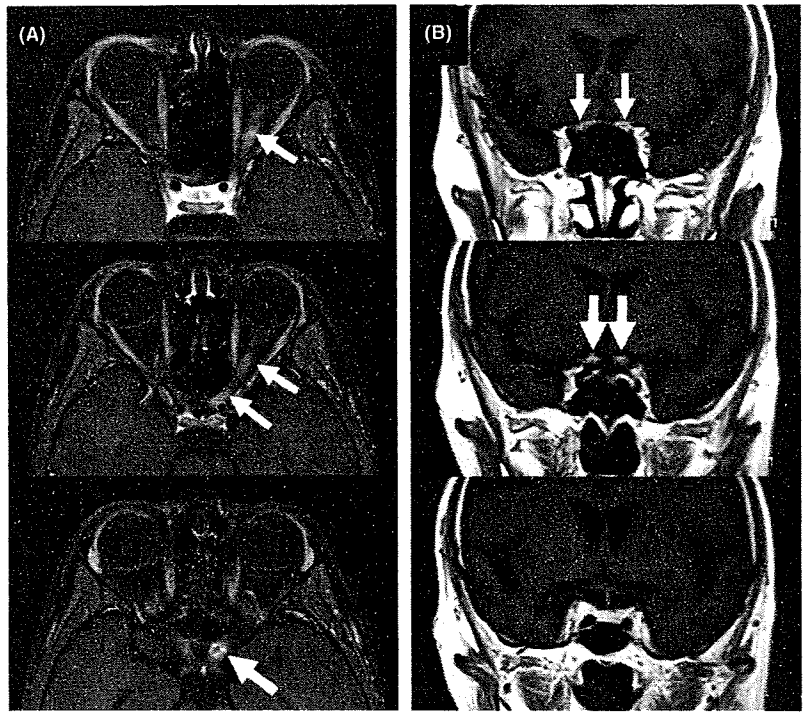

Fig. 2. Magnetic resonance images. (A) Case 1. Axial Gd-enhanced T1-weighted images (with fat suppression) obtained at the onset of the second optic neuritis episode, showing marked enhancement of the left optic nerve (arrows), especially in the intracranial portion close to the optic chiasm (bottom). (B) Case 2. Coronal Gd-enhanced T1-weighted images obtained at the onset of optic neuritis, showing marked enhancement in the intracranial portion of the bilateral optic nerves (arrows).

An autoimmune disorder involving a positive ANA or SS-A is suggested by our three cases, although no systemic diseases were observed. For recurrent episodes and positive autoantibodies, one possible diagnosis is 'autoimmune optic neuropathy' (AON) (Dutton et al. 1982), which is thought to be an entity distinct from demyelinating ON. For AON, in addition to direct inflammatory involvement of the optic nerve, an ischaemic sequel of small vessel vasculitis has been postulated (Goodwin 2006). However, AON is sometimes associated with transverse myelopathy (Goodwin 2006), and NMO is frequently associated with systemic autoimmune disorders (such as connective tissue disease), or with the presence of multiple serum autoantibodies (Wingerchuk 2006). Thus, there could be some clinical overlap between AON and NMO.

Whether the AQP4-Ab is directly related to ON in our cases remains to be elucidated. AQP4 is the predominant water channel expressed in astrocytes and ependymal cells throughout the brain and spinal cord, particularly at sites of fluid transport on the pial and ependymal surfaces in contact with CSF (Nielsen et al. 1997; Rash et al. 1998). As indicated in Fig. 2, the intracranial portion of the optic nerve adjacent to such structures was affected on at least two occasions. This inflammation of the pre-chiasmal portion of the optic nerve could explain the high frequency of bilateral involvement and lack of the retrobulbar pain with eye movement in the early stages of this condition. Preferential expression of AQP4 in the optic chiasm of rats (Venero et al. 1999) and involvement of the optic chiasm revealed by MRI in NMO (Pittock et al. 2006) support this idea.

This study indicates that a subpopulation of patients with ON may have an immunological pathogenesis similar to that seen with NMO. A recent large study (Pirko et al. 2004) revealed that 12.5% of cases of recurrent ON evolved to NMO. It is important to test for the presence of AQP4-Ab in patients with ON, because AQP4-Ab positive patients may have greater potential for developing severe transverse myelitis in the future. In addition, whether or not AQP4-Ab can be detected in mild, monophasic unilateral ON is a topic of interest. A longterm study with a large patient population would help to establish a better understanding of AQP4-Ab positive ON patients.

Acknowledgements

KT was supported by a grant-in-aid for scientific research (no. 17590868; Japan Society for the Promotion of Science) and a Neuroimmunological Disease Research Committee grant (Japanese Ministry of Health, Labour and Welfare). We thank Mitsuko Kaneko and Fumie Higuchi for technical assistance and Dr Takehiro Kobayashi for paediatric care of Case 1.

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Received on May 28th, 2007.
Accepted on February 18th, 2008.

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Sleep Medicine 10 (2009) 253–255

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Case Report

A patient with anti-aquaporin 4 antibody who presented with recurrent hypersomnia, reduced orexin (hypocretin) level, and symmetrical hypothalamic lesions

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Received 6 June 2007; received in revised form 28 August 2007; accepted 23 November 2007
Available online 28 January 2008

Abstract

Recent studies have demonstrated that hypothalamic lesions associated with brain tumor, head trauma, and encephalopathy can cause symptomatic hypersomnia with a reduced orexin (hypocretin) level in the cerebrospinal fluid (CSF). Aquaporin 4 (AQP4), a member of the AQP superfamily, is strongly expressed in the hypothalamus in which orexin (hypocretin)-containing neurons are primarily concentrated. We report the case of a patient with a serum anti-AQP4 antibody who presented with recurrent hypersomnia, symmetrical hypothalamic lesions with long spinal cord lesions on MRI, and a reduced CSF orexin (hypocretin) level, all of which were improved simultaneously by steroid therapy. Further studies should be performed to determine the roles of anti-AQP4 antibody positivity in patients with hypersomnia associated with orexin (hypocretin) deficiency and hypothalamic lesions.

Keywords: Hypersomnia; Hypothalamus; Aquaporin 4; Orexin (hypocretin)

1. Introduction

Aquaporin 4 (AQP4), a member of the AQP superfamily, is strongly expressed in the hypothalamus [1] in which orexin (hypocretin)-containing neurons are primarily concentrated [2]. Recently, the NMO-IgG/anti-AQP4 antibody, which can be detected in the serum of patients with neuromyelitis optica (NMO) and an opticospinal form of multiple sclerosis (OSMS), has been shown to selectively bind to AQP4. [3,4] Herein, we provide the first case report of a patient with the anti-AQP4 antibody who presented with recurrent hypersomnia as the main symptom and a reduced orexin (hypocretin) level in the cerebrospinal fluid (CSF).

2. Case

A 42-year-old Japanese woman developed acute-onset hypersomnia within several days with no apparent causes or triggers. She slept for more than 16 h per day and exhibited excessive daytime sleepiness (Epworth sleepiness score, ESS = 19/24). She experienced no

Abbreviations: AQP, aquaporin; NMO, neuromyelitis optica; ESS, Epworth sleepiness score; CSF, cerebrospinal fluid.

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1389-9457/$ - see front matter © 2009 Published by Elsevier B.V.
doi:10.1016/j.sleep.2007.11.022

symptoms suggestive of cataplexy, sleep paralysis or hypnagogic hallucinations. Her hypersomnia and excessive daytime sleepiness persisted for one month and disappeared spontaneously. At the age of 43, she suffered from acute-onset hypersomnia again with a three-months history of sensory disturbance of her limbs and was admitted to our hospital for the evaluation of her symptoms.

Neurological examinations revealed hypersomnia (about 14 h of sleep per day), excessive daytime sleepiness (ESS = 13/24), and transverse myelopathy at the cervical level. No optic nerve involvement could be detected by ophthalmologic examination or by analyses using visual evoked potential. CSF analysis revealed mild pleocytosis (24/mm^3) and an elevated IgG index of 0.77. The level of CSF orexin (hypocretin), a hypothalamic neuropeptide that regulates arousal and sleep, mildly decreased (191.2 pg/ml; normal range 290 ± 65 pg/ml) [5]. A brain MRI revealed bilateral symmetrical hypothalamic and periaqueductal lesions (Fig. 1A–B) and a spinal MRI revealed an extensively longitudinal spinal cord lesion (Fig. 1C–D).

She was administered intravenous methylprednisolone (1000 mg/day) for three successive days, followed by oral prednisolone at a dose of 60 mg, which was then tapered. Her hypersomnia and excessive daytime sleepiness gradually improved. Two months after the steroid administration, she recovered completely from her sleep disturbance, and slightly from her sensory disturbance. After the treatment, her CSF orexin (hypocretin) level increased to 291.7 pg/ml, and her CSF-IgG index and CSF cell count normalized. For measurement of orexin (hypocretin), all CSF samples were assayed in the same batch to minimize interassay variability. Symmetrical hypothalamic lesions on the MRI became undetectable and the size of the spinal cord lesions was reduced. Her serum taken on admission was tested for the anti-AQP4 antibody [4] and NMO-IgG [6] by the indirect immunofluorescence methods, and turned out to be positive.

3. Discussion

We encountered a patient who presented with recurrent hypersomnia, symmetrical hypothalamic lesions

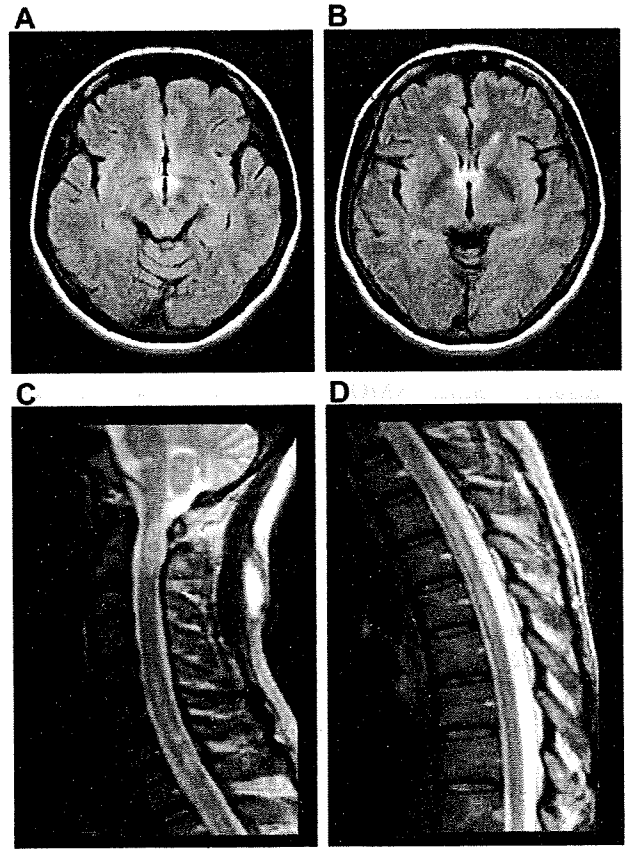

Fig. 1. Brain axial fluid attenuation inversion recovery magnetic resonance images show hyperintensity areas involving bilateral symmetrical hypothalami (A) and periaqueductal lesions (B). Spinal sagittal T2-weighted magnetic resonance images show hyperintensity areas of the cervicothoracic cord from the C6 level to the Th7 level and of the cervical cord at the C2 level (C) with mild swelling (C and D).

and long spinal cord lesions on her MRI, and a reduced CSF orexin (hypocretin) level, all of which were improved simultaneously by steroid therapy. Our final diagnosis of this patient is high-risk syndrome of NMO defined as myelitis with $\geqslant 3$ vertebral segment spinal cord lesions [7]. She did not satisfy the revised NMO diagnostic criteria [8] owing to the lack of evidence of optic neuritis.

Recent studies have demonstrated that hypothalamic lesions associated with brain tumor, head trauma, and encephalopathy can cause symptomatic hypersomnia with a reduced CSF orexin (hypocretin) level [2]. We considered that her hypersomnia might have been caused by hypothalamic lesions, because orexin (hypocretin) neurons, which regulate arousal and sleep, are concentrated in the lateral hypothalamus [2]. In this patient, only mild orexin (hypocretin) deficiency existed while the subject expressed hypersomnolence. We speculate that the hypothalamic lesions might cause a dysfunction in the central histaminergic neurons localized in the tuberomammillary nucleus of the posterior hypothalamus, which also have an important role in wakefulness, in addition to the orexin (hypocretin) neurons [9]; nonetheless, further study is required to confirm this speculation.

AQP4 is expressed throughout the central nervous system, especially in periaqueductal and periventicular regions involving the hypothalamus of the brain [1,10] and is found in non-neuronal structures, such as astrocytes and ependymocytes, but is absent from neurons [1]. Hypothalamic lesions are observed in 3 of 89 NMO patients and 31 high-risk syndrome of NMO patients seropositive for NMO-IgG; however, no patients presenting hypersomnia or other sleep disturbances were described [10]. Poppe et al. have reported the cases of two NMO patients presenting excessive somnolence with symmetrical hypothalamic lesions [11], although they measured neither NMO-IgG nor anti-AQP4 antibody nor CSF orexin (hypocretin) level. This is the first report of a patient presenting symptomatic hypersomnia with symmetrical hypothalamic lesions associated with anti-AQP4 antibody positivity and a reduced CSF orexin (hypocretin) level. Taken together, these findings suggest that the anti-AQP4 antibody may have a role in her hypothalamic lesions, although it still remains undetermined how the anti-AQP4 antibody can cause hypothalamic lesions.

From our experience, we consider that patients with the anti-AQP4 antibody might develop hypersomnia, which can be improved by steroid therapy. Further studies should be performed to determine the roles of anti-AQP4 antibody positivity in patients with hypersomnia associated with orexin (hypocretin) deficiency and hypothalamic lesions.

Funding/support

This study was supported in part by the Ministry of Education, Culture, Sports, Science, and Technology, Japan and a Neuroimmunological Disease Research Committee grant from the Ministry of Health, Labor, and Welfare, Japan.

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Case Report

Neuromyelitis Optica Preceded by Brain Demyelinating Episode

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ABSTRACT

Neuromyelitis optica (NMO) is considered a distinct disease from multiple sclerosis (MS) because of its pathogenesis. It is well accepted that NMO selectively affects the spinal cord and optic nerve and is not associated with brain lesions at the onset of the disease, unlike MS. We present a unique case where the patient's initial lesion was in the brain, and optic neuritis and myelitis were revealed 6 years after the brain lesion. In addition, the patient's serum antiaquaporin 4 (AQP4) antibody was positive. We consider the brain lesion to precede abnormal lesion of NMO, and the AQP4 measurement is important for diagnostics, even if it occurs with brain lesions.

Keywords: Neuromyelitis optica, aquaporin 4, brain lesion, MRI.

Acceptance: Received June 5, 2008, and in revised form June 5, 2008. Accepted for publication June 19, 2008.

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J Neuroimaging 2008;xx:1-3.
DOI: 10.1111/j.1552-6569.2008.00288.x

Introduction

Neuromyelitis optica (NMO) is an inflammatory demyelinating disease of the central nervous system that most commonly disturbs the spinal cord and optic nerves, selectively. A highly specific serum antibody (NMO-IgG) has recently been found in NMO patients,[1] and NMO-IgG reacts specifically with the water channel protein aquaporin 4 (AQP4), which is concentrated in astrocytic foot processes at the blood-brain barrier.[2] In addition, a high rate of anti-AQP4 antibody was detected in Asian patients with multiple sclerosis (MS). This was characterized by the selective involvement of the optic nerve and long spinal cord lesions extending over three vertebral segments.[3]

Unlike MS, NMO is not associated in most patients with brain lesions at disease onset. However, brain lesions do occur over time in the majority of NMO patients. Until now, there has been no report of an NMO case where brain lesions presented before the onset of optic neuritis and myelitis. We present here a unique NMO case where brain lesions were revealed 6 years before the onset of optic neuritis and myelitis.

Case report

A 21-year-old woman was admitted to our hospital because of her transient mild neurological symptoms, such as gait disturbance and dizziness, which continued for several days. Her medical history revealed that she had experienced aseptic meningitis on three occasions as a teenager. She did not reveal any symptoms that would suggest spinal cord or optic nerve disturbance. Magnetic resonance imaging (MRI) of her brain revealed several T2 hyperintense lesions in her corpus callosum (genu, splenium), left internal capsule, and left corona radiata (Fig 1). Her cerebrospinal fluid (CSF) revealed normal findings. She was retrospectively classified with acute disseminated encephalomyelitis (ADEM) or the initial episode of MS. For the next 6 years without medication, she did not present any neurological symptoms.

At age 27, she was admitted to our hospital again because of neurological symptoms gradually developed between about a week. She had bilateral poor eyesight, visual field disturbance, severe paraplegia with difficult walking, bilateral extensor plantar responses, severe disturbance of all sensory modalities of her trunk and bilateral legs below the T4 level, and urinary retention. She had no erythema or arthritis. The laboratory tests revealed high levels of serum antinuclear antibody, anti-Ro antibody, and antithyroid antibodies. The Schirmer test was positive (right 2 mm, left 2 mm); however, her minor salivary gland biopsy showed no inflammatory infiltration. Her CSF on admission revealed marked pleocytosis (482/ul, 60% polymorphonuclear leukocytes), high protein (220 mg/dl), and IgG (45.7 mg/dl) concentrations, however, negative for oligoclonal IgG bands. Her serum collected in the acute phase was examined by the method previously described [3] and was found to be positive for anti-AQP4 antibody. An MRI of her brain presented similar findings to 6 years earlier (Fig 2) and that of the cervical and thoracic spinal cord revealed a T2 hyperintense lesion within the central area of the cord extending from C6 to T8 without contrast enhancing (Fig 3). We diagnosed her as NMO. She did not meet the criteria of any collagen disease

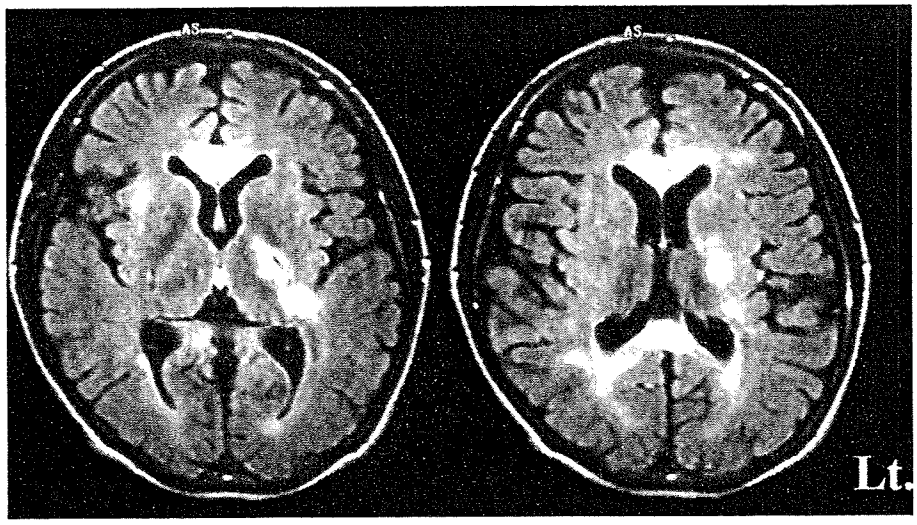

Fig 1. Brain MRI (fluid attenuated inversion recovery images) at age 21 revealed several T2 hyperintense lesions in her corpus callosum (genu, splenium), left internal capsule, and left corona radiata.

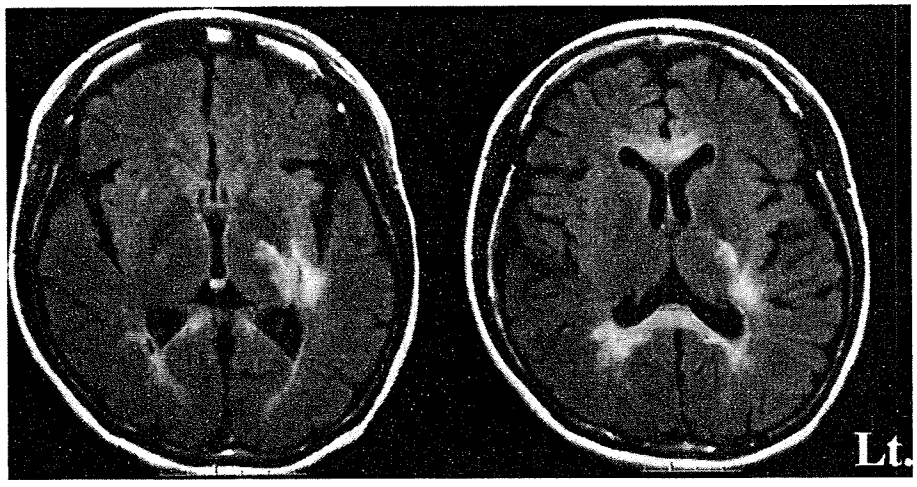

Fig 2. Brain MRI (fluid attenuated inversion recovery images) at age 27 revealed presented similar findings to 6 years before.

such as Sjogren syndrome or systematic lupus erythematosus. A 3-day course of 1 mg/day intravenous methylprednisolone followed by oral prednisolone was administered immediately after admission. On the 14th day of admission she was able to walk unassisted, and her eyesight and visual field disturbance significantly improved. Her spinal cord MRI showed a reduction in the T2 hyperintense area, and CSF findings were normalized.

She was treated with low-dose oral prednisolone after discharge and no relapse was noted over the subsequent 18 months.

Discussion

Wingerchuk et al. proposed diagnostic criteria for defining NMO that require optic neuritis, myelitis, and at least two of three supporting criteria as follows: MRI evidence of a contiguous spinal cord lesion three or more segments in length, onset brain MRI non-diagnostic for MS, or NMO-IgG.[4] Especially concerning the brain MRI, patients with NMO present normal or few non-specific minor subcortical white matter changes at the onset of the disease, and they usually do not fulfill Barkhof radiological criteria for MS.[5] Pittock et al. showed brain MRI lesions in 36 patients among 60 reviewed NMO patients. Most lesions were non-specific, but 6 patients had MS-like lesions without symptoms, and 5 patients had diencephalic, brainstem, or cerebral lesions atypical for MS. It should be noted that half of the patients in this study with a normal initial brain MRI developed abnormalities on a subsequent MRI.[6] Nakashima et al. also reported that brain lesions were seen in 71% of the patients with NMO-IgG, but most were medullary lesions as an extension of cervical myelitis or non-specific cerebral white matter lesions.[7] Brain lesions are no longer an exclusionary criterion in the most recently proposed diagnostic criteria for NMO.[4] In these reported cases, brain lesions did not precede optic nerve

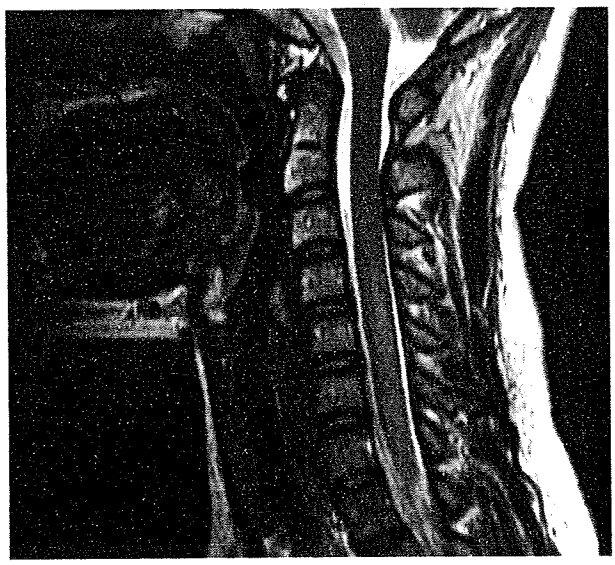

Fig 3. Cervical and thoracic spinal cord MRI (T2-weighted images) at age 27 revealed T2 hyperintense lesion within the central area of cord extending from C6 to T8.

or spinal cord lesions. This makes our case unique because the patient showed brain lesions before optic nerve and spinal cord lesions. It indicates brain lesions can precede the abnormality of later diagnosed NMO.

There is strong evidence that immunomodulatory therapy such as interferon beta is effective for MS patients to prevent recurrence. However, Warabi et al. reported interferon beta-1b treatment was not successful for MS with genetic and clinical characteristics mimicking NMO.[8] In addition, Matsuoka et al. reported that anti-AQP4 antibody-positive MS patients fulfilling definite NMO criteria showed less frequent responses to interferon beta-1b than anti-AQP4 antibody-negative opticospinal MS patients with longitudinally extensive spinal cord lesions.[9] In fact, immunosuppressive treatment is currently the most commonly used drug for the acute and long-term treatment of NMO patients.[10-12] These findings are consistent with findings that NMO is an autoantibody-mediated disease. Accordingly, intravenous corticosteroids and oral prednisolone therapy were very effective for our patient, and she has maintained without relapse using low-dose oral prednisolone. NMO prognosis is generally poorer than MS prognosis because most attacks are moderate or severe and usually follow a relapsing course. Therefore, it is important to discriminate between NMO and MS at the early stage of the disease to select an appropriate treatment, especially in the case present chronologically or spatially atypical brain lesions.

In conclusion, we consider the measurement of anti-AQP4 antibody provides not only the additional diagnostic certainty but also the best therapy and prognostic conclusion in cases with clinically atypical presentation.

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Pathologic and immunologic profiles of a limited form of neuromyelitis optica with myelitis

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ABSTRACT

Background: Neuromyelitis optica (NMO) is a demyelinating syndrome characterized by myelitis and optic neuritis. Detection of anti-NMO immunoglobulin G antibody that binds to aquaporin-4 (AQP4) water channels allows the diagnosis of a limited form of NMO in the early stage with myelitis, but not optic neuritis. However, the detailed clinicopathologic features and long-term course of this limited form remain elusive.

Methods: We investigated 8 patients with the limited form of NMO with myelitis in comparison with 9 patients with the definite form.

Result: All patients with limited and definite form showed uniform relapsing-remitting courses, with no secondary progressive courses. Pathologic findings of biopsy specimens from the limited form were identical to those of autopsy from the definite form, demonstrating extremely active demyelination of plaques, extensive loss of AQP4 immunoreactivity in plaques, and diffuse infiltration by macrophages containing myelin basic proteins with thickened hyalinized blood vessels. Moreover, the definite form at the nadir of relapses displayed significantly higher amounts of the inflammatory cytokines interleukin (IL)-1β and IL-6 in CSF than the limited form and multiple sclerosis.

Conclusion: This consistency of pathologic findings and uniformity of courses indicates that aquaporin 4–specific autoantibodies as the initiator of the neuromyelitis optica (NMO) lesion consistently play an important common role in the pathogenicity through the entire course, consisting of both limited and definite forms, and NMO continuously displays homogeneity of pathogenic effector immune mechanisms through terminal stages, whereas multiple sclerosis should be recognized as the heterogeneous 2-stage disease that could switch from inflammatory to degenerative phase. This report is a significant description comparing the pathologic and immunologic data of limited NMO with those of definite NMO. *Neurology*® 2009;73:1628–1637

GLOSSARY

AQP4 = aquaporin 4; **definite NMO** = definite form of neuromyelitis optica; **EDSS** = Extended Disability Status Scale; **GFAP** = glial fibrillary acidic protein; **HIMP** = high-dose IV methylprednisolone; **IFN** = interferon; **Ig** = immunoglobulin; **IL** = interleukin; **IP** = interferon-inducible protein; **LEM** = longitudinally extensive myelitis; **limited NMO (MY)** = limited form of neuromyelitis optica with myelitis; **limited NMO (ON)** = limited form of neuromyelitis optica with optic neuritis; **MBP** = myelin basic protein; **MIG** = monokine induced by interferon γ; **MCP** = monocyte chemoattractant protein; **MS** = multiple sclerosis; **NMO** = neuromyelitis optica; **NMO-IgG** = neuromyelitis optica immunoglobulin G; **OSMS** = optic-spinal multiple sclerosis; **RANTES** = regulated on activation, normal T-cell expressed, and secreted; **TNF** = tumor necrosis factor.

Neuromyelitis optica (NMO) is an inflammatory and demyelinating syndrome of the CNS characterized by severe attacks of optic neuritis and myelitis.[1,2] Recently, a disease-specific serum autoantibody, NMO immunoglobulin G (NMO-IgG), that binds aquaporin 4 (AQP4), the main channel regulating water homeostasis in the CNS, was discovered in sera from patients with NMO by indirect immunohistochemical and fluorescence methods using mouse brain tissue.[3] Subsequently, our group[4] and others[5-7] have independently established highly

Supplemental data at www.neurology.org

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Supported by a Grant-in-Aid for Scientific Research (C) from the Ministry of Education, Culture, Sports, Science and Technology of Japan (20590991, I.K.), a Grant for the Promotion of Niigata University Research Projects (I.K.), and Research Grants for research on intractable diseases (Neuroimmunological Disease Research Committee) from Ministry of Health, Labor and Welfare of Japan (M.N.).

Disclosure: The authors report no disclosures.

sensitive assays for anti-human AQP4 antibody using human AQP4-transfected human embryonic kidney 293 cells as substrates of the indirect immunofluorescence assay. The highly specific NMO-IgG and anti-AQP4 antibody for NMO should allow early detection of longitudinally extensive myelitis (LEM) without optic involvement, or optic neuritis without spinal cord involvement, with stringent diagnostic accuracy.

A few patients with LEM and seropositivity of anti-AQP4 antibody without optic involvement, corresponding to a limited form of NMO with myelitis (limited NMO [MY]), have been described previously.[8-11] However, details of the clinicopathologic features and long-term course remain elusive. We therefore investigated 8 patients with limited NMO (MY) in comparison with 9 patients showing the definite form of NMO (definite NMO) using clinical, immunologic, radiologic, and pathologic data from biopsy and autopsy specimens. We clarified the homogeneity of characteristic features of NMO through long-term entire courses consisting of both the limited and definite form.

METHODS **Patients and diagnostic criteria.** We retrospectively reviewed the medical records of 306 consecutive patients (219 women, 87 men) between 1980 and 2008 at the multiple sclerosis (MS) clinic in the Department of Neurology at Niigata University Hospital. We stringently defined definite NMO cases as fulfilling all items of the 2006 NMO criteria[12] and limited NMO as either 1) optic neuritis with seropositivity for anti-AQP4 antibody, but without brain, brainstem, or spinal cord lesions (limited NMO [ON]); or 2) myelitis with seropositivity of anti-AQP4 antibody, but without optic nerve involvement (limited NMO [MY]). As a disease control, MS (n = 13) was defined as clinically definite MS according to the criteria of Poser et al.[13] and International Panel criteria for MS,[14,15] excluding definite NMO and limited NMO, between 2006 and 2008 at the MS clinic. The present study was approved by the institutional review board of the Niigata University School of Medicine, Niigata, Japan. Written informed consent was obtained from all patients or guardians of patients participating in the study.

MRI examinations. MRI was performed with 5-mm-thick slices using a 1.5-T scanner (GE Medical Systems, Milwaukee, WI). MRI scans were performed at the time of clinical relapse.

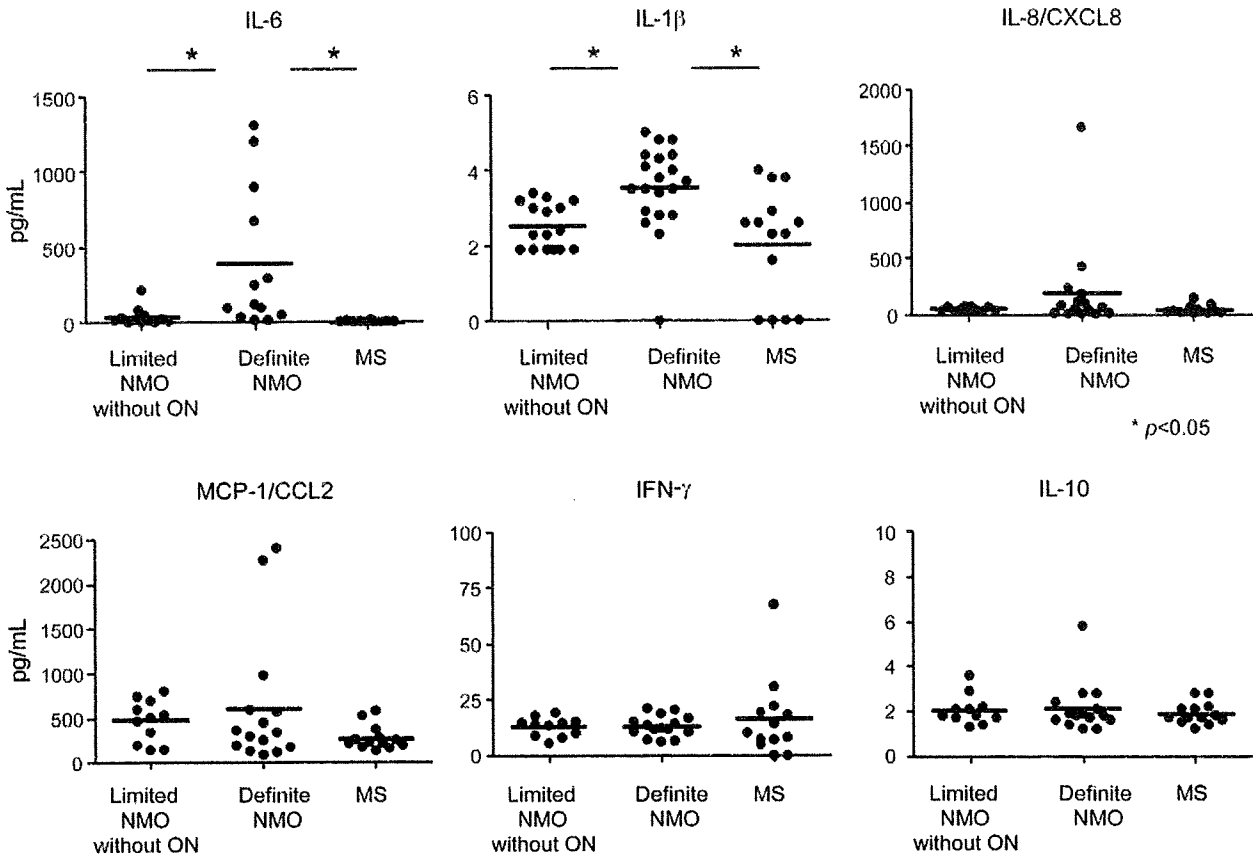
Anti-AQP4 antibody assay in sera and profiles of cytokines and chemokines in CSF. We examined anti-AQP4 antibody using the method described in our previous report[4] and titrated specimens in sera. CSF supernatants were analyzed simultaneously for 14 different cytokines and chemokines, namely, interleukin (IL)-1β; IL-2; IL-4; IL-5; IL-6; IL-8/CXCL8; IL-10; IL-12p70; interferon (IFN)-γ; tumor necrosis factor (TNF)-α; regulated on activation, normal T-cell expressed, and secreted (RANTES)/CCL5; monokine induced by IFN-γ (MIG)/CXCL9; monocyte chemoattractant protein (MCP)-1/CCL2; and IFN-inducible protein (IP)-10/CXCL10, using BD™ cytometric bead arrays (BD Pharmingen, San Diego, CA) according to the instructions from the manufacturer.

Neuropathologic techniques and immunohistochemistry. The study was performed on brain, optic nerve, and spinal cord materials from 1 patient (case 15) with definite NMO at autopsy and 1 patient (case 2) with limited NMO (MY) at diagnostic spinal cord biopsy. Materials were processed for 4-μm-thick, paraffin-embedded slides. Sections were stained with hematoxylin & eosin and Klüver-Barrera (KB). Immunohistochemistry was performed without modification using avidin-biotinylated enzyme complex (Vectastain; Vector Laboratories, Burlingame, CA). Primary antibodies were specific for myelin basic protein (MBP; DAKO, Glostrup, Denmark), neurofilament protein (NF/SMI-31; Sternberger Monoclonals, Baltimore, MD), glial fibrillary acidic protein (GFAP; DAKO) for astrocytes, and AQP4 (Chemicon, Temecula, CA). Diaminobenzidine was used as the chromogen. Selected sections were counterstained with a filtered solution of hematoxylin (blue).

Statistical analyses. We compared clinical features of the diagnostic categories and subgroups, such as patients with limited NMO and definite NMO. Statistical analyses between the 2 subgroups of limited NMO and definite NMO were performed using the Mann-Whitney U test or Fisher exact probability test, as appropriate. Statistical analyses among 3 subgroups of 1) limited NMO, 2) early phase with myelitis exclusively, and 3) progressive phase with both myelitis and optic neuritis (with 2 and 3 extracted from definite NMO) were performed using analysis of variance, the Kruskal-Wallis H test, or the χ^2 test. When significant results were obtained, multiple comparisons between each subgroup were performed using the Bonferroni, Bonferroni-Dunn, or Tukey multiple comparison test. Changes in Extended Disability Status Scale (EDSS) score at from relapse to remission were analyzed by the Wilcoxon signed-ranks test. All statistical analyses were considered significant for values of $p < 0.05$.

RESULTS **Demographics, age at onset, disease duration, and index events.** All patients were Japanese in our series. A female predominance was seen for both limited NMO (MY) (male/female = 0/8) and definite NMO (male/female = 1/8) (table e-1 on the *Neurology*® Web site at www.neurology.org). The median age at disease onset in the limited NMO (MY) cohort (44.5 years) was older than that in definite NMO cohort (24.0 years). In our series, the duration of the disease for limited NMO (MY) (mean ± SD, 4.8 ± 4.5 years) was significantly shorter than that for definite NMO (16.7 ± 10.5 years), and the number of exacerbations of limited NMO (MY) (2.1 ± 1.3) was significantly fewer than that of definite NMO (9.1 ± 5.5). Limited NMO (MY) showed either a recurrent (n = 4) or a monophasic (n = 4) course, whereas all cases of definite NMO had recurrent courses (n = 9) (figure e-1). Meanwhile, annual relapse rates did not differ between limited NMO (MY) (0.5 ± 0.3/y) and definite NMO (0.6 ± 0.3/y). Even if we retrospectively

Figure 1 **Cytokine and chemokine levels in CSF from patients with limited NMO (MY), definite NMO, and MS at the nadir of attacks, assessed by the multiplexed fluorescent bead-based immunoassay**



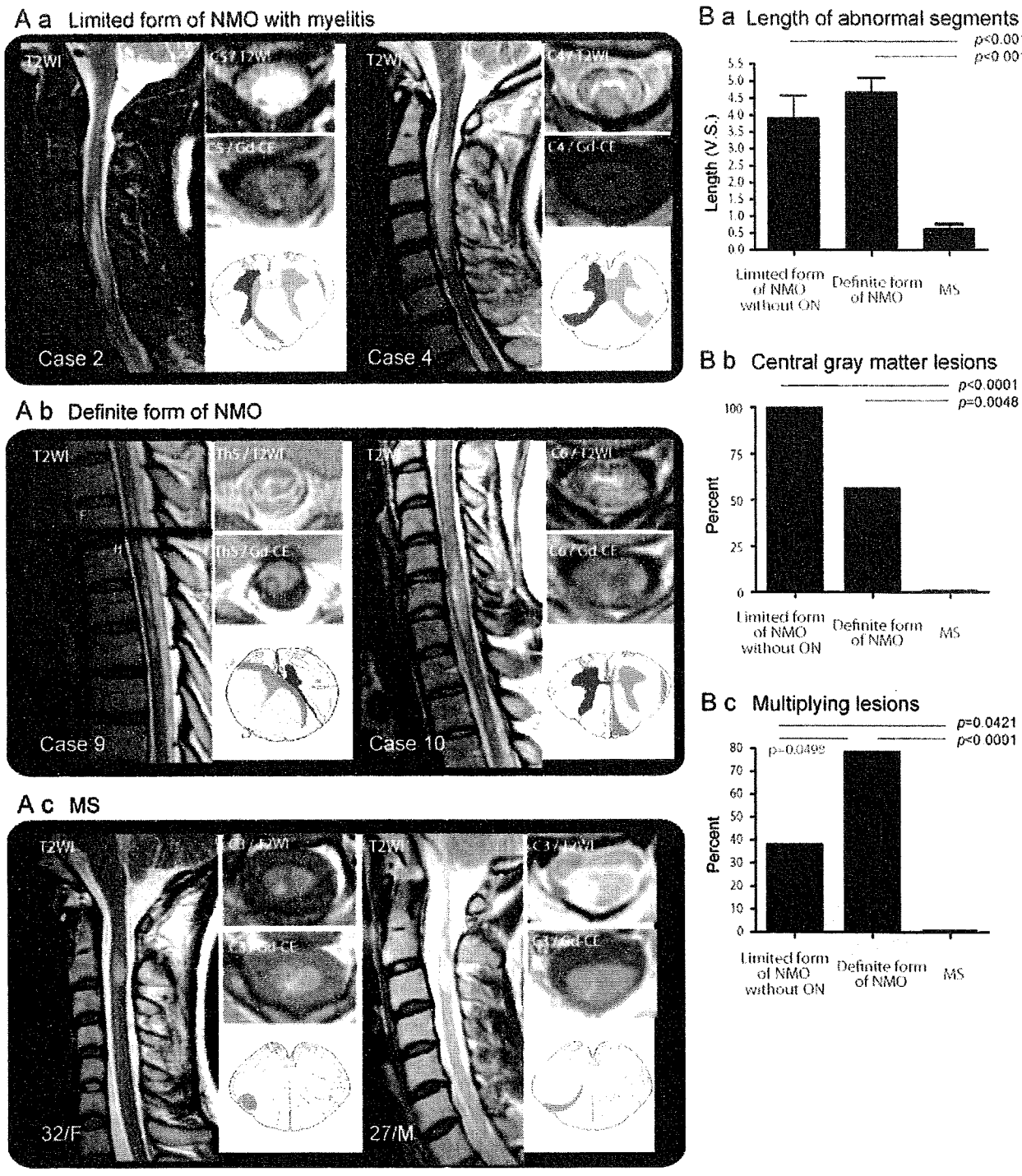
We determined that definite neuromyelitis optica (NMO) at the nadir of relapses showed significantly higher levels of interleukin (IL)-1β and IL-6 inflammatory cytokines in CSF than the limited form of NMO with myelitis (limited NMO [MY]) and multiple sclerosis (MS). The definite form of NMO (definite NMO) also tended to display higher levels of IL-8/CXCL8 in CSF than limited NMO (MY) and MS, but not significantly so. Other cytokine and chemokine levels, including IL-2; IL-4; IL-5; IL-10; IL-12p70; interferon (IFN)-γ; tumor necrosis factor (TNF)-α; regulated on activation, normal T-cell expressed, and secreted (RANTES)/CCL5; monokine induced by IFN-γ (MIG)/CXCL9; and IFN-inducible protein (IP)-10/CXCL10 did not differ between groups (data not shown). Bars indicate the mean for each group. ON = optic neuritis; MCP = monocyte chemoattractant protein.

categorized the definite NMO group as early phase with only myelitis or complete phase with both myelitis and optic neuritis, no differences in annual relapse rates were recognized depending on disease phase, and no subjects showed a secondary progressive clinical course in limited NMO (MY) or definite NMO. Relapses and disease progression phenotypes of limited NMO (MY) and definite NMO were similar.

Serologic and CSF findings. All cases of limited NMO (MY) (100.0%) and most cases of definite NMO (88.9%) showed seropositivity for anti-AQP4 antibodies in sera (table e-1). No patients with limited NMO (MY) or definite NMO had marked pleocytosis including neutrophilia in CSF. Immunoglobulin (Ig) G index and albumin leakage in CSF[16] were slightly increased in both limited NMO (MY) and definite NMO, compared with disease control subjects. Moreover, we determined that definite NMO at the nadir of relapses displayed significantly higher amounts of the inflammatory cytokines IL-1β and IL-6 in CSF than limited NMO (MY) and MS (figure 1).

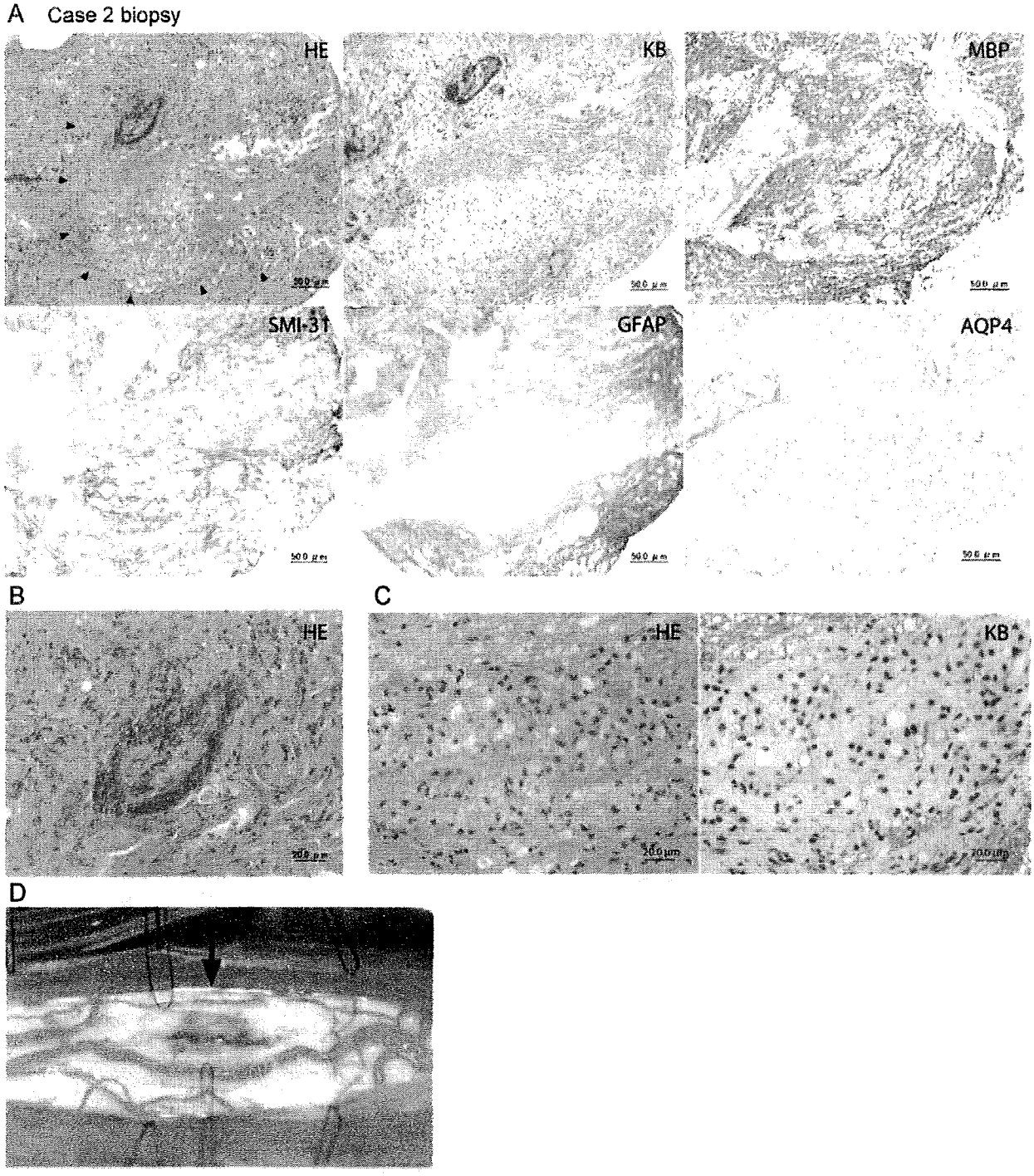
MRI findings. No patients with MS, 75.0% of patients with limited NMO (MY), and 77.8% of patients with definite NMO showed LEM (≥3 vertebral segments) on MRI (table e-2). In regard to characteristic figures on axial images, a central gray matter–predominant lesion was revealed as an H-shape on T2-weighted axial imaging, based on the anatomic structure of central gray matter in the spinal cord (figure 2, A and B, and figure e-2). All limited NMO (MY) cases (100.0%) and half of definite NMO cases (55.6%) had this H-shaped sign at the nadir of attacks, whereas no cases of classic MS showed the sign. Moreover, we also recognized a subpial peripheral white matter lesion in both limited and definite NMO on axial images (figures 2A and

Figure 2 **MRI findings for the limited form of NMO with myelitis, definite NMO, and MS**



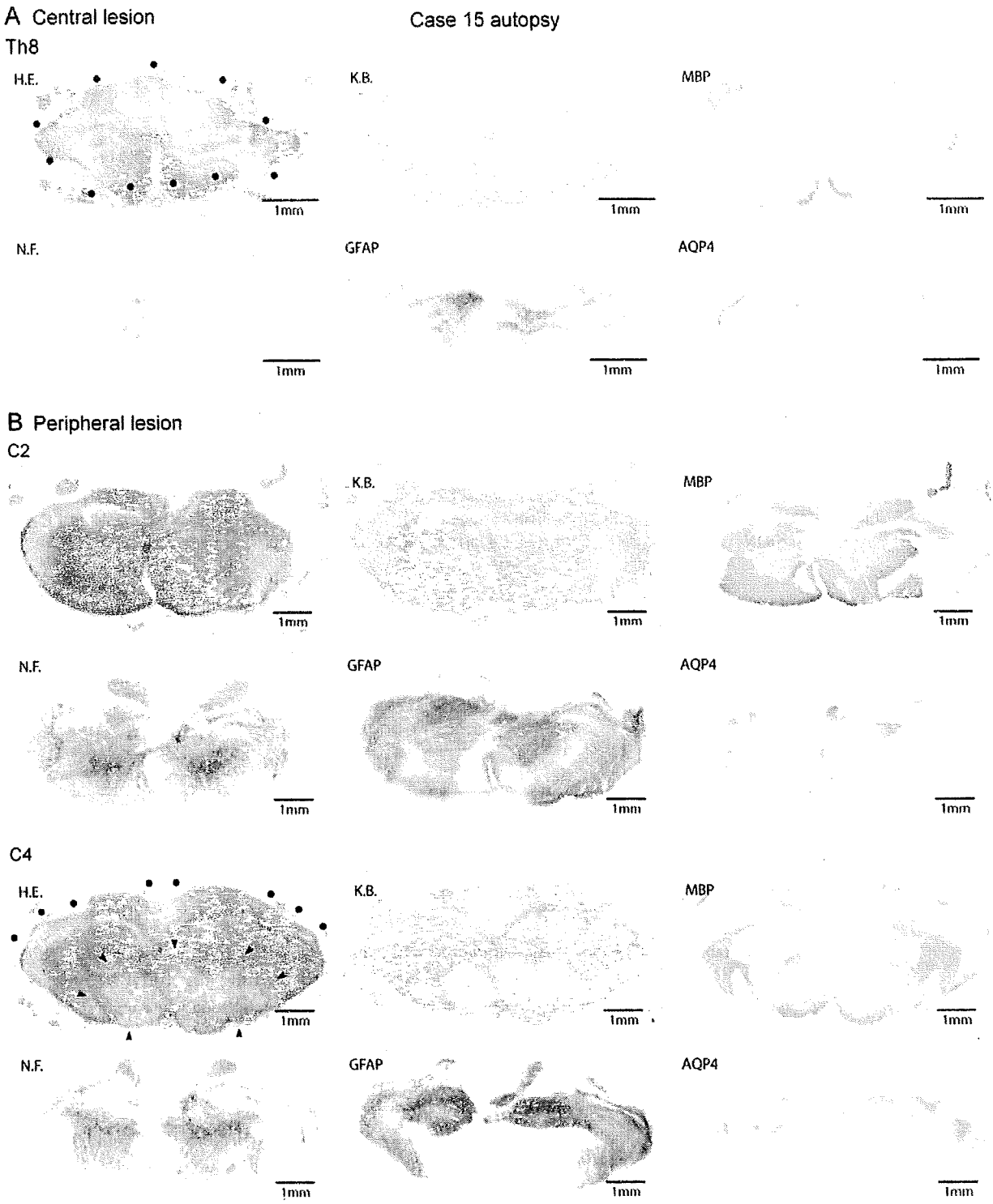
(A) MRI findings for the limited form of neuromyelitis optica (NMO) with myelitis (cases 2 and 4; A, a), definite neuromyelitis optica (cases 9 and 10; A, b), and multiple sclerosis (MS; a 32-year-old woman and a 27-year-old man; A, c). Regions in lower right corners: red indicates abnormal gadolinium-enhancement; yellow indicates abnormal hyperintensity on T2-weighted imaging (T2WI) with subpial peripheral white matter lesions; and blue indicates central gray matter-predominant legions, showing an H-shape on T2-weighted axial imaging, based on the anatomic structure of central gray matter of the spinal cord. These MRI images were representative for each group. (B) Summary of MRI findings for the limited form of NMO with myelitis (limited NMO [MY]; n = 8), definite NMO (n = 9), and MS (n = 13). Patients with limited or definite NMO had significantly longer length of abnormal segments (B, a) than patients with MS. In regard to characteristic figures on axial images, a central gray matter-predominant lesion (B, b) was revealed as an H-shape on T2-weighted axial imaging, based on the anatomic structure of central gray matter in the spinal cord (A, a–c). All cases of limited NMO (MY) (100.0%) and half of definite NMO cases (55.6%) had this H-shaped sign at the nadir of attacks, whereas no cases of classic MS showed the sign. Moreover, we confirmed that definite NMO (77.8%) had significantly more multiplying lesions (B, c), referring to accumulation of more than 2 lesions at the same spinal cord level, compared with patients with limited NMO (MY) (37.5%) or MS (0%).

Figure 3 **Macroscopic and pathologic findings from a patient with limited form of neuromyelitis optica with myelitis**



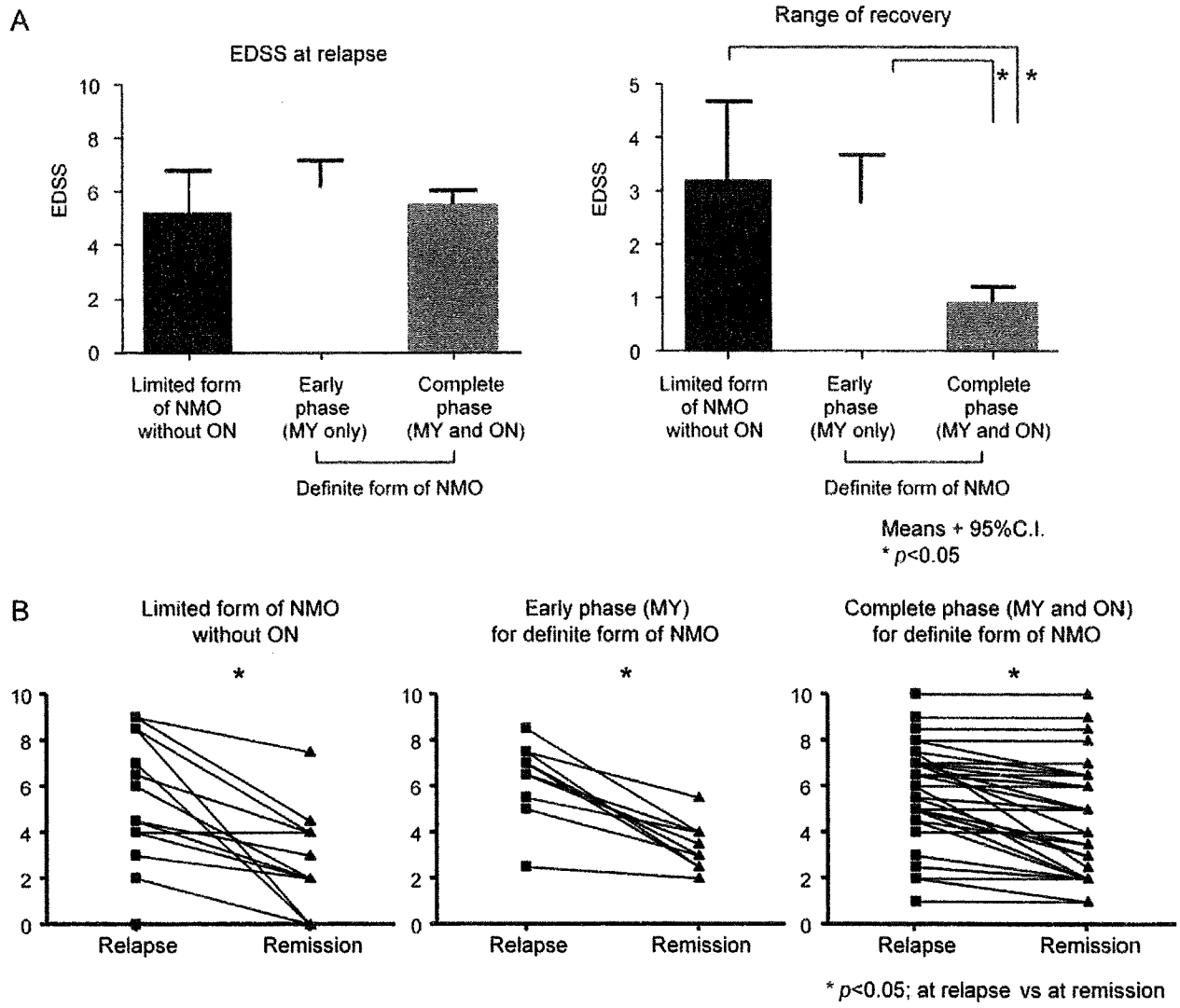
A woman (case 2; A) with Brown-Séquard syndrome at onset, who was 65 years old at onset, presented with progressive neurologic deficits involving both gray and white matter of the spinal cord at the C4 level over 17 days. Because such a progressive course over several weeks was unusual for neuromyelitis optica (NMO),[17] the possibility of spinal cord tumor with malignant features needed to be excluded, and she underwent surgery for biopsy of spinal cord lesions at the posterior funiculus. Macroscopically, the posterior funiculus of the spinal cord was edematous with brownish coloration at the open biopsy (D, arrow). Spinal cord biopsy specimens from a patient with limited NMO with myelitis demonstrated early active demyelinating lesions (arrowheads), with demyelination of a plaque (Klüver-Barrera [KB] staining) and relatively preserved axons (SMI-31 staining), extensive loss of aquaporin-4 (AQP4) expression in both plaques and periplaque white matter, limited loss of expression of glial fibrillary acidic protein (GFAP) within plaques, and diffuse infiltration by numerous macrophages (C) containing immunoreactive products for myelin basic protein (MBP) with thickened, hyalinized blood vessels (B). These findings from biopsy specimens of limited NMO with myelitis mirror the definite form of NMO (figure 4). Subsequently, anti-AQP4 antibody showed positive results in serum. Limited NMO (MY) was diagnosed. The patient was able to walk with a cane after repeated high-dose IV methylprednisolone therapy and tacrolimus (3 mg/d) without relapses. HE = hematoxylin & eosin.

Figure 4 **Pathologic findings from a patient with definite form of neuromyelitis optica**



Demyelinating lesions exist not only in central gray matter lesions (A) but also in peripheral white matter lesions (B) in definite neuromyelitis optica (NMO), although most previous reports have emphasized only central spinal cord lesions in NMO. This finding mirrored MRI findings in both limited and definite forms of NMO (figure 2, A and B, and table e-2). Furthermore, we recognized selective loss of both aquaporin-4 (AQP4) and glial fibrillary acidic protein (GFAP) immunoreactivity in early active demyelinating NMO lesions with preserved myelin basic protein (MBP) staining (arrows in B). However, loss of GFAP immunoreactivity was always considerably smaller than loss of AQP4 in our series (arrows). Inactive NMO lesions (dots) indicate selective loss of AQP4 immunoreactivity, although GFAP staining was inconsistent. These data suggest loss of AQP4 immunoreactivity as a prominent feature through all NMO stages. HE = hematoxylin & eosin; KB = Klüver-Barrera; NF = neurofilament protein.

Figure 5 **Responsiveness to immunotherapy for patients with limited and definite forms of neuromyelitis optica**



(A) Limited form of neuromyelitis optica with myelitis (limited NMO [MY]). (B) Definite form of neuromyelitis optica (definite NMO). EDSS = Expanded Disability Status Scale; ON = optic neuritis; CI = confidence interval.

e-2 and table e-2). This peripheral pattern on MRI reflected the pathologic findings (figure 4), indicating the presence of demyelinating lesions in not only central gray matter lesions, but also subpial white matter lesions in spinal cords of patients with NMO, although most reports have emphasized only central lesions.[17,18] Moreover, we confirmed that definite NMO (77.8%) had significantly more multiplying lesions, referring to accumulation of more than 2 lesions at the same spinal cord level, compared with patients with limited NMO (MY) (37.5%) or MS (0%) (figure 2B and table e-2).

Pathologic findings. Spinal cord biopsy specimens of limited NMO (MY) (case 2) with early active demyelinating lesions, classified as previously described,[19] demonstrated demyelination of a plaque (KB staining) with relatively preserved axons (NF/SMI-31 staining), extensive loss of AQP4 expression in both plaque and periplaque white matter, limited loss of expression of GFAP within a plaque, and diffuse infiltration by numerous macrophages containing immunoreactive products for MBP with thickened, hyalinized blood vessels (figure 3). Moreover, this sample showed deposits of IgG and IgM colocalizing with products of complement activation in a vasculocentric pattern (data not shown). All these pathologic findings in a biopsy sample derived from a patient with limited NMO (MY) (figure 3) were consistent with autopsy materials derived from patients with definite NMO (case 15) (figure 4). Although the duration from initial symptoms of a patient with definite NMO (case 15) was 28 years at the autopsy, he

still kept both a high amount of inflammatory cytokines in CSF (figure 1) and a lot of active inflammatory demyelinating lesions in the spinal cord (figure 4), optic nerves, and brain. These findings indicated that NMO has continuous pathogenic effector immune reactions until the terminal stage.

Impairment through the entire course of limited NMO. As previously reported,[2,17,20] 80% to 90% of patients with definite NMO had relapsing episodes of optic neuritis and myelitis, and most attacks of definite NMO worsened over several days and then slowly and incompletely improved in the weeks or months after reaching maximum clinical deficit. However, treatment response, disease course, and long-term impairment in limited NMO (MY) with stringent diagnostic criteria or definite NMO in the earlier phase remain elusive. First, we compared variations in EDSS scores before and after high-dose IV methylprednisolone (HIMP; 1 g/d for 3 days) at each acute attack between limited NMO (MY) and definite NMO. EDSS scores at the nadir of relapse did not differ between limited NMO (MY) (mean ± SD, 5.2 ± 2.8) and definite NMO (5.3 ± 2.2) (figure 5 and table e-1). However, EDSS scores at remission after repeated HIMP differed significantly between limited NMO (MY) (2.5 ± 2.2) and definite NMO (4.2 ± 2.4). Degrees of EDSS score change before and after repeated HIMP in patients with limited NMO (MY) (3.2 ± 2.4) were higher than in definite NMO (1.2 ± 1.4). Moreover, we retrospectively divided the phase of definite NMO into early phase with only myelitis and complete phase with both myelitis and optic neuritis. EDSS score at relapse of early-phase definite NMO with only myelitis (6.2 ± 1.6) was similar to that in complete phase with both myelitis and optic neuritis (5.5 ± 2.1). However, degrees of EDSS score change before and after repeated HIMP in patients with definite NMO with only myelitis at the early phase (2.8 ± 1.4) were higher than with definite NMO at complete phase (0.9 ± 1.1). These data suggest that limited NMO (MY) or early-phase definite NMO with only myelitis shows better prognosis with treatment using IV steroid therapy than definite NMO.

DISCUSSION Our findings of clinical, pathologic, radiologic, and immunologic features indicated that NMO at the early stage shows a common pathogenesis to NMO at the complete stage, contrasting with large population-based classic MS natural history cohorts,[21-23] as described below.

First, with regard to clinical features, the extreme predominance of females, annual relapsing rates, and a uniform relapsing-remitting course through the entire disease course, but not a secondary progressive course, and concomitant serology of several autoimmune conditions showed the common features of both limited and definite NMO. In particular, extreme uniform disease courses for both limited and definite NMO, as previously reported for definite NMO,[20] differed considerably from the courses seen with large population-based MS natural history cohorts, because more than two-thirds of patients with relapsing-remitting MS (inflammatory phase with autoimmune reactions) eventually experience secondary progression as defined by gradual, unremitting clinical deterioration of neurologic function (degenerative phase with degeneration of both myelin sheath and underlying axon), with or without superimposed relapses.[21-23]

Second, all pathologic findings for both limited and definite NMO showed common features in terms of demyelinating lesions with myelin degradation products within numerous macrophages, pronounced perivascular deposition of immunoglobulins and complement,[24] pattern-specific loss of AQP4 immunoreactivity,[25,26] and relatively preserved MBP-stained myelinated fibers despite the loss of AQP4 and GFAP staining, as previously reported.[26] These findings thus strongly emphasized that limited NMO (MY) shows the same pathologic findings as definite NMO, independent of disease duration, on the basis of a common pathogenesis for complement-activating AQP4-specific autoantibodies as the initiator of the NMO lesion.[25]

Third, in regard to immunologic conditions, limited and definite NMO showed no differences in CSF abnormalities of pleocytosis, IgG index, albumin leakage, oligoclonal IgG bands, MBP, and serum abnormalities of seropositivity (anti-AQP4 antibodies) and other autoimmune status, including antinuclear antibodies, consistent with previous reports.[27,28] These data suggest that definite NMO and limited NMO (MY) throughout the entire course are associated with broad activation of humoral immunity including B-cell and plasma cell activations without the consumption of any complement in sera at the peak of disease activity.

Finally, in terms of radiologic findings, existence of LEM and the H sign on axial images, suggesting preferential involvement of spinal central gray matter, were consistent with previous pathologic and radiologic studies,[17,18] and subpial peripheral white matter lesions represent a common feature between limited NMO and definite NMO. Subpial peripheral white matter lesions in spinal cords on MRI (figure 2, A and B, table e-2, and figure e-2) and pathologic findings (figure 4) were marked for definite and limited NMO, although this finding has never been stressed in previous reports.[24] Such sub-