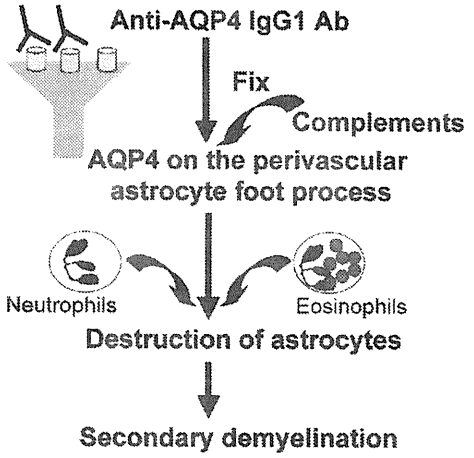

Fig. 1. Destruction of an astrocyte foot process by complement-activating anti-aquaporin-4 (AQP4) antibody. Once the anti-AQP4 IgG1 antibody crosses the blood–brain barrier, it binds to AQP4 on the astrocyte foot process, and fixes and activates complements. Activated complements mobilize neutrophils and eosinophils, which then produce severe tissue damage, and cause secondary demyelination. Disruption of the astrocyte foot process prolongs resolution from vasogenic edema caused by inflammation.

12. Proposed mechanism of NMO based on anti-AQP4 autoimmunity

Based on the high specificity of anti-AQP4 antibody and the selective loss of AQP4 in NMO lesions, it is postulated that the complement-activating anti-AQP4 antibody plays a pivotal role in the development of NMO lesions [76]. Once anti-AQP4 antibody gets across the BBB, it binds to AQP4 molecules on the astrocyte foot processes and activates complements (Fig. 1). Activated complements mobilize neutrophils and eosinophils that then facilitate tissue destruction. The observation that the anti-AQP4 antibodies so far examined are all IgG1 subclass [7] that can efficiently fix complements is compatible with such a hypothesis. The *in vitro* and *in vivo* pathogenic effects of anti-AQP4 antibody further support this notion. Here, demyelination is secondary to destruction of astrocytes, which is supposed to be fundamentally distinct from the primary demyelinating mechanism executed by myelin antigen-specific T cells and anti-myelin autoantibodies.

13. Concerns about the proposed mechanism of NMO based on anti-AQP4 antibody

There are several concerns surrounding the above-mentioned hypothesis based on anti-AQP4 antibody. First, in the presence of high titers of anti-AQP4 antibodies, some patients remained in remission [7], and there are cases who carry anti-AQP4 antibody without showing NMO presentation [56]. Because AQP4 is present in the astrocyte foot processes behind the BBB, additional factors that disrupt the BBB and render the antibody able to enter the CNS across the BBB may be necessary to induce relapse. In addition, the fact that anti-AQP4 antibody titers [6,7,34,35] appear to have no correlation with clinical parameters in most studies to date further support the prerequisite for some additional factor to induce relapse. Indeed, in animal models, there is a requirement for myelin antigen-specific T cells for anti-AQP4 antibody to operate *in vivo* in the CNS [74,75]. We found that in OSMS and NMO patients' peripheral blood T cells reactive to myelin antigens, such as MBP, proteolipid protein, and myelin-oligodendrocyte glycoprotein, showed intra- and inter-molecular epitope spreading [76], suggesting that T cells are already stimulated with myelin antigens *in vivo* in these patients. Second, AQP4 is present in retina, distal collecting tubules, gastric mucosa, muscle and lung, and NMO-IgG binds to these structures [4,77]; however, no impairments in these organs have been observed to date. In particular, although Müller cells, which are equivalent to astrocytes, abundantly express AQP4 in the foot process adjacent to the blood vessels in retina, no severe inflammation has ever been reported in anti-AQP4 antibody-positive NMO patients, suggesting that the presence of complement-fixing anti-AQP4 antibody is not enough to produce tissue damage. Moreover, AQP4 expression is ubiquitous throughout the CNS, although its expression level varies, being high in the gray matter of the spinal cord [66]. Cerebral gray matter and cerebellum abundantly express AQP4; however, these sites are seldom involved in NMO. Such a ubiquitous presence of AQP4 cannot explain the selectiveness of lesion distribution, namely in the optic nerves and spinal cord. Third, the deposited immunoglobulins in postmortem NMO lesions are mainly IgM [64] while the anti-AQP4 antibodies described are all IgG. We observed that some NMO lesions show perivascular deposition of complements and IgG in acute lesions, while no AQP4 loss is found (submitted for publication) [68]. Thus, perivascular complement and IgG deposition does not strictly correlate with AQP4 loss. Finally, AQP4 loss has been observed in MS plaques by researchers at several independent institutions, while preservation of AQP4 in NMO lesions is also seen by several groups [68,69]. These observations suggest that AQP4 loss is not completely linked to NMO lesion formation, and that there are two subtypes of NMO/OSMS with LESCLs, at least in Asians (Fig. 2).

14. Alternative mechanisms of OSMS in Asians based on CSF and peripheral blood cytokine/chemokine profiles

In peripheral blood, OSMS shows a pronounced T-helper-1 (Th1) and T-cytotoxic-1 (Tc1) shift, where IFNγ-producing T cells predominate over IL-4-producing T cells throughout the relapse and remission phases [78,79]. We previously reported that IL-17 is upregulated in the CSF of OSMS patients and that levels of both IL-17 and the downstream cytokine IL-8 in CSF show a significant positive correlation

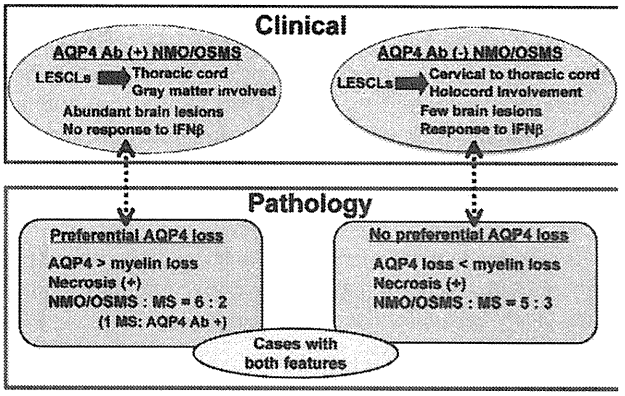

Fig. 2. Two subtypes of NMO/OSMS with LESCLs in Asians. There are NMO-IgG-seropositive and -seronegative cases with NMO/OSMS, which show some difference in clinical features. Pathologically, there are also two subtypes of NMO: one presenting with preferential AQP4 loss and one that does not. Even in MS plaques, AQP4 loss is occasionally seen. Some cases show both features in different lesions. Ab: antibody; AQP4: aquaporin-4; LESCLs: longitudinally extensive spinal cord lesions; MS: multiple sclerosis; NMO: neuromyelitis optica; OSMS: opticospinal multiple sclerosis.

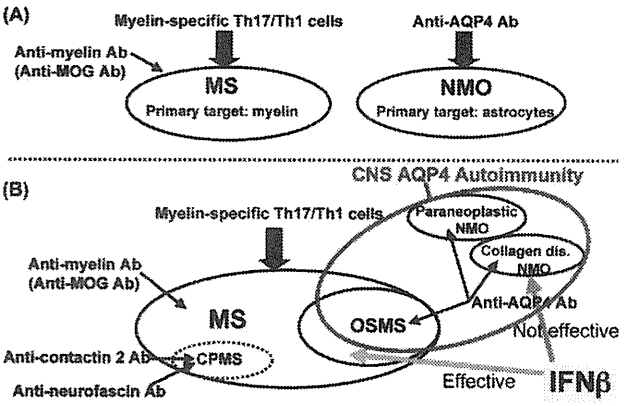

Fig. 3. Two hypothetical mechanisms of MS and NMO. In (A), myelin is a primary target of T cells and antibodies in MS, whereas in NMO, astrocytes are a primary target of anti-AQP4 antibody. In (B), myelin-specific T cells initiate CNS inflammation and antibodies recognizing various components of CNS antigens modify the clinicopathological features. Ab: antibody; AQP4: aquaporin-4; CPMS: chronic progressive multiple sclerosis; MOG: myelin-oligodendrocyte glycoprotein; MS: multiple sclerosis; NMO: neuromyelitis optica; OSMS: opticospinal multiple sclerosis.

with spinal cord lesion length [65]. Recently, by simultaneously measuring the levels of 27 cytokines and chemokines in CSF from patients with various causes of myelitis, we found that IL-17, IFNγ, and G-CSF were specifically elevated in OSMS patients, irrespective of the presence or absence of anti-AQP4 antibody [80]. IL-17 (IL-17A) is exclusively produced by Th17 cells, which are CD4+ T cells recently shown to be a distinct lineage from Th1 and Th2 cells [81]. Increasing evidence suggests that Th17 cells, but not Th1 cells, are responsible for organ-specific autoimmune diseases, such as EAE [81,82]. IL-8 is a chemokine for neutrophils. In OSMS patients, CSF neutrophilia and infiltration of neutrophils to severe lesions are characteristic [65]. Hence, elevated IL-8 may be partly responsible for such neutrophil activation and mobilization in OSMS. Indeed, the level of myeloperoxidase, an activated neutrophil product, is increased in sera from OSMS patients, especially in those with LESCLs at relapse [42]. Th17 cells carrying granzyme B have recently been shown to efficiently disrupt BBB tight junctions and loosen the BBB [83]. Therefore, autoimmune Th17 cells may initiate BBB disruption and inflammation in OSMS (Fig. 3), causing vasogenic edema in the CNS, regardless of the anti-AQP4 antibody status. After myelin-specific T cells initiate CNS inflammation, antibodies recognizing various components of CNS antigens might modify the clinicopathological features of MS. In such a scenario, NMO without overt autoimmune diseases or paraneoplastic conditions may represent one extreme end of an MS spectrum.

Regarding other factors with possible effects on vascular permeability, we previously reported that the levels of vascular endothelial growth factor (VEGF) in sera were significantly elevated in OSMS patients, showing a significant positive correlation with spinal cord lesion length [43]. IL-17 has also been shown to induce VEGF production in target tissues [84]. Moreover, a mutation in the *platelet activating factor* (*PAF*) *acetylhydrolase* (*PAF-AH*) gene, which inactivates its enzymatic activity (required to metabolize PAF into an inactive form), is significantly more frequent in OSMS patients than healthy controls [85]. Indeed, PAF-AH activity in peripheral blood is decreased in OSMS patients [85]. This likely prolongs PAF activity and increases vascular permeability as well as vascular growth. These vascular-acting factors may also enhance tissue edema in OSMS.

Given that AQP4 knockout mice showed prolongation of vasogenic edema [86], but a decrease in the level of cytotoxic edema [87], anti-AQP4 antibody produced either by a heightened humoral autoimmune background or secondarily by tissue breakdown may prolong resolution of tissue edema, thereby contributing to further tissue destruction in NMO and OSMS patients. NMO-IgG/anti-AQP4 antibody-positive patients show a significantly higher frequency of severe optic nerve damage (permanent complete blindness) than anti-AQP4 antibody-negative CMS patients [5,6]. Optic nerves are especially vulnerable to the detrimental effects of tissue edema in the optic canal where space is tight and increased tissue pressure easily causes circulatory insufficiency. In the spinal cord, the thoracic cord is prone to developing LESCLs in anti-AQP4 antibody-positive patients, although AQP4 is widely expressed from the cervical to sacral cord. Because the thoracic cord corresponds to the watershed of vascular supply in the spinal cord, even in the spinal cord, vulnerability to ischemia may be one of the factors contributing to the development of LESCLs. Prolongation of vasogenic edema at sites where the surrounding space is tight or the vascular supply is poor may cause poor recovery from tissue damage in patients with anti-AQP4 antibody.

15. Concluding remarks

The discovery of anti-AQP4 antibody has surely opened a new exciting research area in the field of human demyelinating diseases. There are two major hypotheses concerning the role of anti-AQP4 antibody: first, anti-AQP4 antibody directly causes NMO through astrocyte destruction, in a process that is distinct from that underlying MS; and second, anti-AQP4 antibody is a secondary modifying factor in OSMS and NMO. It remains to be elucidated whether MS and NMO are distinct diseases.

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Reappraisal of brain MRI features in patients with multiple sclerosis and neuromyelitis optica according to anti-aquaporin-4 antibody status

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ABSTRACT

Brain lesions are not uncommon in neuromyelitis optica (NMO) patients with anti-aquaporin-4 (AQP4) antibody; however, the appearance of these lesions is said to be different from that of those in Western patients with multiple sclerosis (MS). To clarify the similarities and dissimilarities of brain lesions in anti-AQP4 antibody-positive and -negative MS and NMO patients, we examined the presence of anti-AQP4 antibody in the sera of 148 consecutive patients fulfilling Poser's criteria for clinically definite MS, of whom 38 also met the revised NMO criteria, using an immunofluorescence method, and analyzed brain lesions by magnetic resonance imaging (MRI). Brain lesions fulfilling the Barkhof criteria were significantly more common in 121 patients without anti-AQP4 antibody than in 27 patients with anti-AQP4 antibody (57.0% vs. 33.3%, $P=0.033$), while the frequency of those that met the Paty criteria was not different between the two groups (74.4% vs. 73.5%). Ovoid lesions were detected more commonly in patients without anti-AQP4 antibody than in those with the antibody (72.3% vs. 48.2%, $P=0.022$). The anti-AQP4 antibody-positive patients had significantly more atypical brain lesions, such as extensive brain lesions, than the anti-AQP4 antibody-negative ones (18.5% vs. 1.7%, $P=0.0023$). Thus, although MS-like brain lesions are more common in anti-AQP4 antibody-negative patients than anti-AQP4 antibody-positive patients, approximately 30 to 50% of patients with anti-AQP4 antibody harbour brain MRI lesions indistinguishable from those present in typical MS patients, such as periventricular ovoid lesions, suggesting the existence of considerable overlap in brain MRI features between anti-AQP4 antibody-positive and -negative Asian patients. In the present study, NMO patients with brain lesions showed a significantly higher annualized relapse rate ($P^{corr}=0.017$) and higher frequency of anti-AQP4 antibody ($P^{corr}<0.0001$) than typical NMO patients without brain lesions, suggesting that development of brain lesions in NMO may reflect high disease activity and thus be a warning sign.

1. Introduction

Neuromyelitis optica (NMO) is characterised by severe and selective involvement of the optic nerves and spinal cord, which frequently shows longitudinally extensive spinal cord lesions (LESCLs) extending over three or more vertebral segments. NMO was originally defined as a disease involving only the optic nerves and spinal cord with a monophasic course [1]. However, the concept of NMO has changed along with the collection and analysis of samples from many NMO patients, and a relapsing form of NMO is widely accepted in Western countries [2,3]. Additionally, a highly specific IgG against NMO, designated NMO-IgG, has been described [4], and its relevant antigen was reported to be aquaporin-4 (AQP4) [5]. The presence of NMO-IgG/anti-AQP4 antibody has also influenced the concept of NMO. Some patients with NMO-IgG also show atypical brain lesions, such as large confluent lesions (>3 cm) and diencephalic lesions, during their clinical course [6]. In fact, 60% of patients fulfilling the 1999 criteria for NMO show brain lesions on MRI [6]. Thus, based on such evidence, the 2006 revised criteria for NMO include the presence of NMO-IgG and do not preclude patients with brain lesions [3].

The distribution of atypical brain lesions on MRI reflects the distribution of high expression of AQP4, and histopathological analyses of NMO show perivascular IgM and IgG deposition with complement activation and loss of immunoreactivity to AQP4 [7–9]. These findings suggest a role for humoral immunity in the pathogenesis of NMO and a direct etiological role for NMO-IgG.

However, either NMO-IgG or anti-AQP4 antibody is detected in around 10% of MS patients who fulfil the established clinical criteria

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for MS [4,10,11]. We have also reported that anti-AQP4 antibody-positive patients occasionally have MS-like brain lesions, such as periventricular ovoid lesions [11]. Thus, it remains to be elucidated whether brain MRI lesions are distinguishable between MS and NMO patients, and between those with and without anti-AQP4 antibody, or if considerable overlaps exist between these conditions. Because the opticospinal form of MS (OSMS) in Asians is now claimed to be the same as NMO in Westerners, it is especially problematic to differentiate NMO from MS in Asian patients, who frequently show severe and selective involvement of the optic nerve and spinal cord, irrespective of the presence or absence of anti-AQP4 antibody [11]. Therefore, it is critical to compare brain MRI lesions in a large unbiased series of Asian MS and NMO patients. In the present study, we aimed to compare the frequencies of typical MS-like brain lesions and atypical ones between Japanese MS and NMO patients, and between those with and without anti-AQP4 antibody.

2. Methods

2.1. Patients

For the present study, we enrolled 148 consecutive patients with clinically definite relapsing–remitting or secondary-progressive MS based on the Poser criteria [12], seen at the MS clinic of the Department of Neurology, Kyushu University Hospital during 1987–2007, and whose sera were available for anti-APQ4 antibody assays. From a retrospective review of the medical records of all patients, we recorded demographic and imaging data. There were 137 relapsing–remitting and 11 secondary-progressive MS patients. All patients were residents of Kyushu Island, the southernmost part of mainland Japan. None were seropositive for human T cell leukaemia virus type I. No patients with primary progressive MS were included in the present study. Patients with monophasic NMO without subsequent relapse were also excluded to avoid including patients with acute disseminated encephalomyelitis. Among the 148 patients, 27 (18.2%, 24 female and 3 male) were positive for anti-AQP4 antibody, and 38 also met the revised Wingerchuk's criteria for NMO [3]. We then classified these NMO patients into "typical NMO" and "NMO with brain lesions" based on whether they had brain lesions meeting the Paty criteria [13]. We adopted the Paty criteria [13] because in the Wingerchuk's revised criteria for NMO [3] the Paty criteria were recommended for determining the presence of MS-like brain lesions. Therefore, "typical NMO" patients were those who showed only optic neuritis and myelitis without brain lesions fulfilling the Paty criteria [13]. "NMO patients with brain lesions" were those who had only optic neuritis and myelitis and also had brain lesions fulfilling the Paty criteria [13]. "MS patients" were those who met the Poser [12] and the revised McDonald criteria [14] and did not meet the definition of either "typical NMO" or "NMO with brain lesions".

2.2. Magnetic resonance imaging

All MRI studies were performed using 1.5-T Magnetom Vision and Symphony units (Siemens Medical Systems, Erlangen, Germany) as described previously [15]. The typical imaging parameters for the brain were as follows: axial T2-weighted turbo spin-echo imaging using TR/TE = 2800/90 ms, flip angle = 180°; axial turbo-fluid-attenuated inversion recovery (FLAIR) imaging using TI/TR/TE = 2200/9000/110 ms, flip angle = 180; and sagittal and axial pre-contrast and axial and coronal post-contrast T1-weighted spin-echo imaging using TR/TE range = 400–460/12–17 ms, and flip angle range = 80–90°. One excitation, with a matrix of 256 × 256, slice thickness of 5 mm, and slice gap of 2.5 mm, was used for all brain studies. Gadopentetate dimeglumine at 0.1 mmol/kg body weight was administered intravenously for contrast-enhanced studies.

MRI scans were taken at the time of clinical relapse (within 30 days of the onset of acute exacerbation) or in the remission phase. Brain MRI scans from 87 patients at relapse and 136 patients in remission were examined. Brain MRI lesions were evaluated according to the Barkhof criteria [16] and Paty criteria [13] for MS. Atypical brain lesions, such as extensive brain lesions (>3 cm), bilateral diencephalic (thalamic/hypothalamic) lesions, cavity formation and extension from the cervical cord into the brainstem were defined based on previous reports [6,11]. At the time of brain MRI, treatment was being received by the patients being imaged in 108 out of 223 scans (58 on IFNβ-1b, 42 within one month of steroid pulse therapy and eight on both).

2.3. Anti-AQP4 antibody assay

Green fluorescence protein (GFP)-AQP4 fusion protein–transfected human embryonic kidney cells (HEK-293) were produced as previously described [11]. AQP4-expressing cells were initially incubated with human serum samples diluted 1:4 with DMEM for 1 h at 37.0 °C without cell fixation, washed in phosphate-buffered saline, and then visualized with an Alexa 594-conjugated goat anti-human IgG antibody (Invitrogen). The fluorescence of non-fixed cells was observed using a confocal laser-scanning microscope (FLUOVIEW FV300; Olympus Optical Co., Tokyo, Japan). The anti-AQP4 antibody assay was carried out at least twice for each sample, and those that gave a positive result twice were deemed to be positive.

2.4. Statistical analysis

Statistical analyses of numerical variables were performed using the Mann–Whitney *U* test. Differences in frequencies between the two subgroups were tested for significance using Fisher's exact probability test. When multiple comparisons were performed, uncorrected *P* values (P^{uncorr}) were corrected by multiplying them by the number of comparisons (Bonferroni–Dunn's correction) to calculate corrected *P* values (P^{corr}).

Table 1
Comparison of demographic features between patients with NMO and those with MS.

	NMO patients ($n=38$)	MS patients ($n=110$)
No. of females/males	32/6 (5.3:1)	75/35 (2.1:1)
Age at onset (years)[a]	35.4 ± 14.2	31.3 ± 12.4
Disease duration (years)[a]	11.8 ± 8.7	11.9 ± 9.9
Annualized relapse rate[a]	0.99 ± 0.58*	0.68 ± 0.58*
EDSS score[a]	5.2 ± 2.6*	3.4 ± 2.6*
Anti-AQP4 antibody	24/38 (63.2%)*	3/110 (2.7%)*
Frequency of symptoms:		
Optic neuritis	38/38 (100.0%)*	62/110 (56.7%)*
Bilateral optic neuritis	6/38 (15.8%)	13/110 (11.9%)
Severe optic neuritis (≥FS 5)	28/38 (73.7%)*	38/110 (34.5%)*
Myelitis	38/38 (100.0%)	91/110 (82.7%)
Acute transverse myelitis	23/38 (60.5%)*	22/110 (20.0%)
Secondary progression	0/38 (0.0%)	11/110 (10.0%)
CSF:		
Marked pleocytosis (≥50/μl)	4/35 (11.4%)	6/101 (5.9%)
Neutrophilia (≥5/μl)	4/35 (11.4%)	4/94 (4.3%)
OB	5/34 (14.7%)*	37/90 (41.1%)*
IgG index (≥0.658)[b]	14/33 (42.4%)	42/83 (50.6%)
LESCLs during the entire course	32/38 (84.2%)*	28/110 (25.5%)*

AQP4 = aquaporin-4; CNS = central nervous system; CSF = cerebrospinal fluid; EDSS = Kurtzke's Expanded Disability Status Scale [17]; FS = Kurtzke's Visual Functional Scale [17]; LESCLs = longitudinally extensive spinal cord lesions; MS = multiple sclerosis; NMO = neuromyelitis optica; OB = oligoclonal IgG bands.

[a] Means ± SD.

[b] The upper normal range of IgG index was derived from our previous study [24].

* $P<0.05$.

Table 2
Comparison of demographic features among patients with typical NMO, patients with NMO with brain lesions, and patients with MS.

	Typical NMO patients ($n=21$)	NMO with brain lesions ($n=17$)	MS patients ($n=110$)
No. of females/males	18/3 (5.7:1)	14/3 (5:1)	75/35 (2.1:1)
Age at onset (years)[a]	33.4 ± 14.5	37.8 ± 14.0	31.3 ± 12.4
Disease duration (years)[a]	11.6 ± 8.8	12.1 ± 8.7	11.9 ± 9.9
Annualized relapse rate[a]	0.77 ± 0.43*	1.26 ± 0.62*,**	0.68 ± 0.58**
EDSS score[a]	5.6 ± 2.3*	4.7 ± 2.9	3.4 ± 2.6*
Anti-AQP4 antibody	7/21 (33.3%)*,***	17/17 (100.0%)*,**	3/110 (2.7%)**,***
Frequency of symptoms:			
Optic neuritis	21/21 (100.0%)*	17/17 (100.0%)**	62/110 (56.6%)*,**
Bilateral optic neuritis	4/21 (19.0%)	2/17 (11.8%)	13/110 (11.9%)
Severe optic neuritis (≥FS 5)	14/21 (66.7%)*	14/17 (82.3%)**	38/110 (34.5%)*,**
Myelitis	21/21 (100.0%)	17/17 (100.0%)	91/110 (82.7%)
Acute transverse myelitis	15/21 (71.4%)*	8/17 (47.1%)	22/110 (20.0%)*
Secondary progression	0/21 (0.0%)	0/17 (0.0%)	11/110 (10.0%)
CSF:			
Marked pleocytosis (≥50/μl)	1/18 (5.6%)	3/17 (17.6%)	6/101 (5.9%)
Neutrophilia (≥5/μl)	1/18 (5.6%)	3/17 (17.6%)	4/94 (4.3%)
OB	3/18 (16.7%)	2/16 (12.5%)	37/90 (41.1%)
IgG index (≥0.658)[b]	8/17 (47.1%)	6/16 (37.5%)	42/83 (50.6%)
LESCLs during the entire course	17/21 (81.0%)*	15/17 (88.2%)**	28/110 (25.5%)*,**

AQP4 = aquaporin-4; CSF = cerebrospinal fluid; EDSS = Kurtzke's Expanded Disability Status Scale [17]; FS = Kurtzke's Visual Functional Scale [17]; LESCLs = longitudinally extensive spinal cord lesions; MS = multiple sclerosis; NMO = neuromyelitis optica; OB = oligoclonal IgG bands.
*, **, *** Corrected $P<0.05$.
[a] Means ± SD.
[b] The upper normal range of IgG index was derived from our previous study [24].

3. Results

3.1. Demographic features

The demographic features of the 148 patients are summarized in Table 1. The disease duration was similar between NMO and MS patients. Although relapse rate, Kurtzke's Expanded Disability Status Scale (EDSS) scores [17], and frequencies of severe optic neuritis, ATM, and LESCLs during the entire course were significantly greater in the 38 patients who satisfied the revised NMO criteria [3] than in the remaining 110 MS patients, the frequency of oligoclonal bands (OB) was significantly higher in MS patients than that in NMO patients. Among MS patients, 65.2% had OBs and/or an elevated IgG index (OB/high IgG index). Although none of the four MS patients with CSF neutrophilia had anti-AQP4 antibodies, all of them had LESCLs.

When clinical features were compared among patients with typical NMO, NMO with brain lesions and MS, EDSS score and frequencies of severe optic neuritis, ATM and LESCLs during the entire course were significantly greater in typical NMO patients than in MS patients ($P^{corr}=0.0024$, $P^{corr}=0.023$, $P^{corr}<0.0001$, and $P^{corr}<0.0001$, respectively) (Table 2). Annualized relapse rates were significantly higher in NMO patients with brain lesions than in MS patients ($P^{corr}<0.001$) and typical NMO patients ($P^{corr}=0.017$). Frequencies of severe optic neuritis and LESCLs were also significantly higher in NMO patients with brain lesions than in MS patients ($P^{corr}<0.001$ and $P^{corr}<0.001$, respectively). Anti-AQP4 antibody positivity rate was highest in NMO patients with brain lesions and the rate was significantly higher in NMO patients with brain lesions (100%) than typical NMO patients (33.3%, $P^{corr}<0.0001$) and MS patients (2.7%, $P^{corr}<0.0001$). It was also significantly higher in

Table 3
Comparison of demographic features between anti-AQP4 antibody-positive and -negative patients with NMO and MS.

	Anti-AQP4 antibody-positive patients ($n=27$)	Anti-AQP4 antibody negative patients ($n=121$)
No. of female/male patients	24/3 (8.0:1)*	83/38 (2.2:1)*
Age at onset (years)[a]	36.3 ± 13.8	31.4 ± 12.7
Disease duration (years)[a]	13.7 ± 9.2	11.5 ± 9.6
Annualized relapse rate[a]	1.0 ± 0.62*	0.71 ± 0.57*
EDSS score[a]	4.7 ± 2.6	3.7 ± 2.7
Frequency of symptoms:		
Optic neuritis	27/27 (100.0%)*	72/121 (59.5%)*
Bilateral optic neuritis	4/27 (14.8%)	15/121 (12.4%)
Severe optic neuritis (FS≥5)	21/27 (77.8%)*	45/121 (37.2%)*
Myelitis	26/27 (96.3%)	103/121 (85.1%)
Acute transverse myelitis	12/27 (44.4%)	33/121 (27.3%)
CSF:		
Marked pleocytosis (≥50/μl)	3/25 (12.0%)	7/111 (6.3%)
Neutrophilia (≥5/μl)	3/25 (12.0%)	5/104 (4.8%)
OB	5/24 (20.8%)	37/100 (37.0%)
IgG index (≥0.658)[b]	9/23 (39.1%)	47/93 (50.5%)
LESCLs during the entire course	20/27 (74.1%)*	40/121 (33.1%)*

AQP4 = aquaporin-4; CSF = cerebrospinal fluid; EDSS = Kurtzke's Expanded Disability Status Scale [17]; FS = Kurtzke's Visual Functional Scale [17]; LESCLs = longitudinally extensive spinal cord lesions; MS = multiple sclerosis; NMO = neuromyelitis optica; OB = oligoclonal IgG bands.
[a] Means ± SD.
[b] The upper normal range of IgG index was derived from our previous study [24].
* $P<0.05$.

Table 4
Comparison of brain MRI findings between NMO and MS.

	NMO patients ($n=38$)	MS patients ($n=110$)
Barkhof brain lesions[a]	7/38 (18.4%)*	71/110 (64.6%)*
≥9 T2 brain lesions	8/38 (21.1%)*	76/110 (69.1%)*
≥1 Gd-enhanced lesion	3/36 (8.3%)*	35/108 (32.4%)*
≥1 juxtacortical lesion	15/38 (39.5%)*	81/110 (73.6%)*
≥1 periventricular lesion	11/38 (29.0%)*	81/110 (73.6%)*
≥1 infratentorial lesion	13/38 (34.2%)*	72/110 (65.5%)*
Paty brain lesions[b]	17/38 (44.7%)*	92/110 (83.6%)*
Ovoid lesions	12/38 (31.6%)*	87/108 (80.6%)*
Atypical brain lesions	10/38 (26.3%)	23/110 (20.9%)
Extensive brain lesions	5/38 (13.2%)*	2/110 (1.8%)*
Bil. diencephalic lesions	0/38 (0.0%)	6/110 (5.5%)
Cavity formation	3/38 (7.9%)	16/110 (14.6%)
Extension from the cervical cord into brainstem	3/38 (7.9%)*	0/110 (0.0%)*

Bil. = bilateral; Gd = gadolinium; MS = multiple sclerosis; NMO = neuromyelitis optica.
[a] Brain lesions fulfilling the Barkhof criteria [16].
[b] Brain lesions fulfilling the Paty criteria [13].
* $P<0.05$.

typical NMO patients than MS patients ($P^{corr}<0.001$). The frequencies of OBs and an elevated IgG index were higher in MS patients than in typical NMO patients and NMO patients with brain lesions, but the differences did not reach statistical significance (Table 2).

The demographic features of patients with and without anti-AQP4 antibody are shown in Table 3. Female to male ratio, annualized relapse rate and frequencies of optic neuritis, severe optic neuritis, and LESCLs during the entire course, were significantly higher in anti-AQP4 antibody-positive patients than in anti-AQP4 antibody-negative patients ($P=0.034$, $P=0.0070$, $P<0.0001$, $P=0.00018$, and $P=0.00015$, respectively).

3.2. Brain MRI findings

The frequencies of brain lesions fulfilling the Barkhof [16] or Paty [13] criteria were significantly higher in MS patients than in NMO patients (64.6% vs. 18.4%, $P<0.0001$, and 83.6% vs. 44.7%, $P<0.0001$, respectively). The frequency of ovoid lesions was similarly higher in MS patients than that in NMO patients (80.6% vs. 31.6%, $P<0.001$). Although the frequency of total atypical brain lesions was not different between the two groups, extensive brain lesions (13.2% vs. 1.8%, $P=0.012$) and lesions extending from the cervical cord into the brainstem (7.9% vs. 0.0%, $P=0.016$) were significantly more common in NMO patients than in MS patients (Table 4).

When brain MRI features were compared among typical NMO patients, NMO patients with brain lesions, and MS patients, atypical brain lesions were most frequently found in NMO patients with brain lesions (Table 5). The frequencies of atypical brain lesions and extensive brain lesions were significantly higher in the NMO patients with brain lesions (52.9% and 29.4%, respectively) than in MS patients (20.9%, $P^{corr}=0.038$, and 1.8%, $P^{corr}=0.0013$, respectively) and typical NMO patients (4.8%, $P^{corr}=0.0056$, and 0%, $P^{corr}=0.037$ respectively). By contrast, ovoid lesions were significantly more commonly found in MS patients (80.6%) and NMO patients with brain lesions (64.7%) than in typical NMO patients (4.8%, $P^{corr}<0.001$ and $P^{corr}<0.001$, respectively).

In comparisons between those with and without anti-AQP4 antibody, brain lesions fulfilling the Barkhof criteria were significantly less common in anti-AQP4 antibody-positive patients (33.3%) than in anti-AQP4 antibody-negative patients (57.0%) ($P=0.033$), while fulfilment of the Paty criteria during the entire clinical course was observed nearly as frequently in anti-AQP4 antibody-positive patients as in anti-AQP4 antibody-negative ones (74.1% vs. 73.5%) (Table 6). Among the items of Barkhof's criteria, the frequency of patients having ≥nine T2 hyperintense lesions was significantly higher among anti-AQP4 antibody-negative patients than among anti-AQP4 antibody-positive patients ($P=0.031$) while the frequencies of patients with ≥1 gadolinium-enhanced lesion, those with ≥1 juxtacortical lesion, those with ≥1 periventricular lesion and those with ≥1

Table 5
Comparison of brain MRI findings among patients with typical NMO, NMO with brain lesions and MS.

	Typical NMO patients ($n=21$)	NMO with brain lesions ($n=17$)	MS patients ($n=110$)
Barkhof brain lesions[a]	0/21 (0.0%)*,**	7/17 (41.2%)**	71/110 (64.6%)*
≥9 T2 brain lesions	0/21 (0.0%)*,**	8/17 (47.1%)**	76/110 (69.1%)*
≥1 Gd-enhanced lesion	1/19 (5.3%)*	2/17 (11.8%)	35/108 (32.4%)*
≥1 juxtacortical lesion	6/21 (28.6%)*	9/17 (52.9%)	81/110 (73.6%)*
≥1 periventricular lesion	0/21 (0.0%)*,**	11/17 (64.7%)**	81/110 (73.6%)*
≥1 infratentorial lesion	3/21 (14.3%)*,**	10/17 (58.8%)**	72/110 (65.5%)*
Paty brain lesions[b]	0/21 (0.0%)*,**	17/17 (100.0%)**	92/110 (83.6%)*
Ovoid lesions	1/21 (4.8%)*,**	11/17 (64.7%)**	87/108 (80.6%)*
Atypical brain lesions	1/21 (4.8%)*	9/17 (52.9%)*,**	23/110 (20.9%)**
Extensive brain lesions	0/21 (0.0%)*	5/17 (29.4%)*,**	2/110 (1.8%)**
Cavity formation	0/21 (0.0%)	3/17 (17.7%)	16/110 (14.6%)
Bil. diencephalic lesions	0/21 (0.0%)	0/17 (0.0%)	6/110 (5.5%)
Extension from the cervical cord into brainstem	1/21 (4.8%)	2/17 (11.8%)	0/110 (0.0%)

Bil. = bilateral; Gd = gadolinium; MS = multiple sclerosis; NMO = neuromyelitis optica.
*, ** Corrected $P<0.05$.
[a] Brain lesions fulfilling the Barkhof criteria [16].
[b] Brain lesions fulfilling the Paty criteria [13].

Table 6
Comparison of brain MRI findings between anti-AQP4 antibody-positive and -negative patients with NMO and MS.

	Anti-AQP4 antibody-positive patients ($n=27$)	Anti-AQP4 antibody-negative patients ($n=121$)
Barkhof brain lesions[a]	9/27 (33.3%)*	69/121 (57.0%)*
≥9 T2 brain lesions	10/27 (37.0%)*	74/121 (61.2%)*
≥1 Gd-enhanced lesion	4/27 (14.8%)	34/117 (29.1%)
≥1 juxtacortical lesion	13/27 (48.2%)	83/121 (68.6%)
≥1 periventricular lesion	13/27 (48.2%)	78/121 (64.5%)
≥1 infratentorial lesion	12/27 (44.4%)	73/121 (60.3%)
Paty brain lesions[b]	20/27 (74.1%)	89/121 (73.5%)
Ovoid lesions	13/27 (48.2%)*	86/119 (72.3%)*
Atypical brain lesions	10/27 (37.0%)	23/121 (19.0%)
Extensive brain lesions	5/27 (18.5%)*	2/121 (1.7%)*
Bil. diencephalic lesions	0/27 (0.0%)	6/121 (5.0%)
Cavity formation	4/27 (14.8%)	15/121 (12.4%)
Extension from the cervical cord into brainstem	2/27 (7.4%)	1/121 (0.8%)

Bil. = bilateral; Gd = gadolinium; MS = multiple sclerosis; NMO = neuromyelitis optica.
[a] Brain lesions fulfilling the Barkhof criteria [16].
[b] Brain lesions fulfilling the Paty criteria [13].
* $P<0.05$.

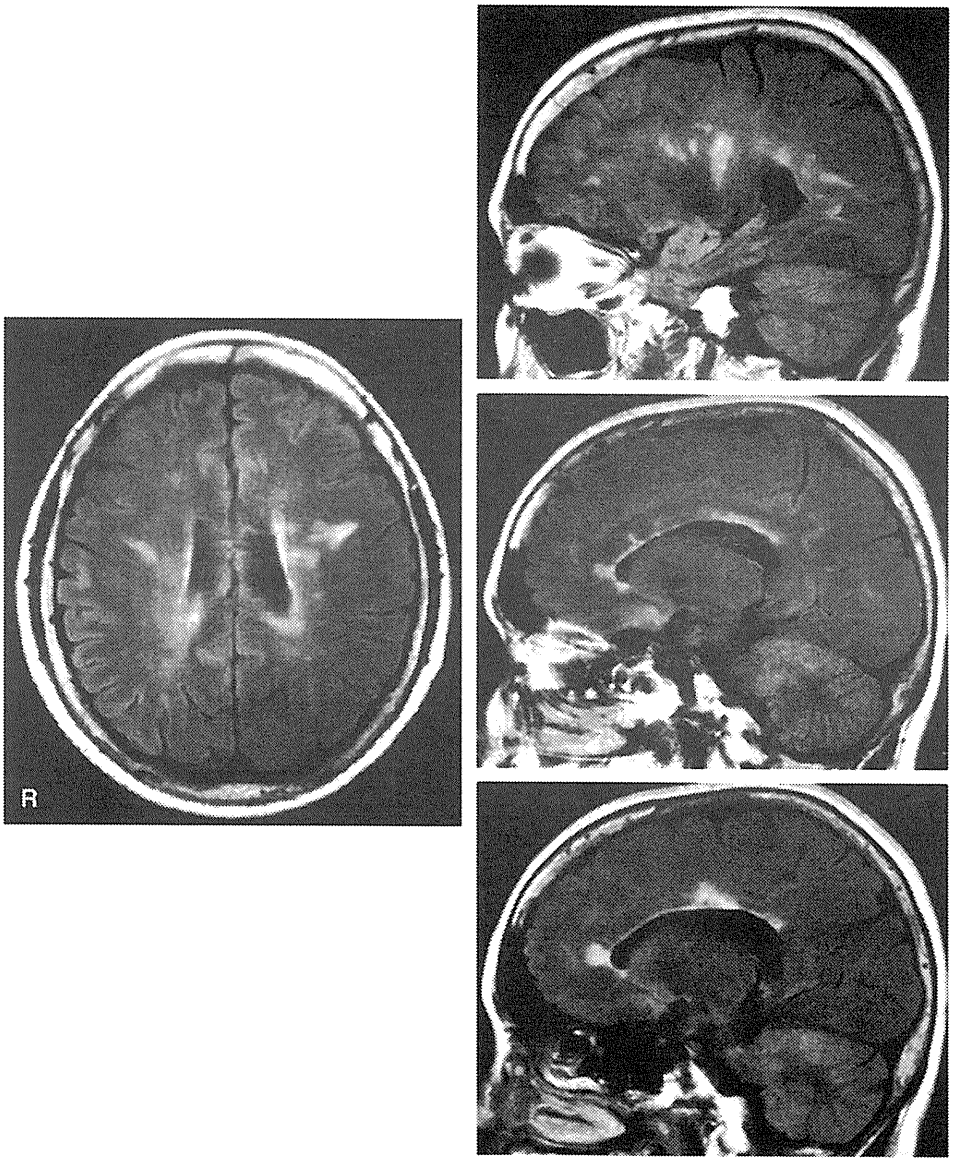

Fig. 1. Axial and sagittal fluid-attenuated inversion recovery (FLAIR) images in an MS patient with a high titre of anti-AQP4 antibody (1:4096). She had 15 years of illness and her EDSS score was 1 at the time of the MRI scan. Note the presence of ovoid periventricular lesions typical of MS.

Table 7
Comparison of demographic features between MS patients with and without CSF OB/high IgG index.

	MS with OB/high IgG index ($n=54$)	MS without OB/high IgG index ($n=30$)
No. of female/male patients	38/16 (2.4:1)	18/12 (1.5:1)
Age at onset (years)[a]	28.7 ± 10.4*	35.9 ± 15.5*
Disease duration (years)[a]	12.0 ± 10.1	10.9 ± 8.6
Relapse rate[a]	0.67 ± 0.42	0.71 ± 0.56
EDSS score[a]	3.8 ± 2.6	3.2 ± 2.5
Frequency of symptoms:		
Optic neuritis	31/54 (57.4%)	16/30 (53.3%)
Bilateral optic neuritis	6/54 (11.1%)	4/30 (13.3%)
Severe optic neuritis (FS≥5)	18/54 (33.3%)	11/30 (36.7%)
Myelitis	44/54 (81.5%)	26/30 (86.7%)
Acute transverse myelitis	8/54 (14.8%)	8/30 (26.7%)
Secondary progression	8/54 (14.8%)	1/30 (3.3%)
CSF:		
Marked pleocytosis (≥50/μl)	2/52 (3.9%)	2/30 (6.7%)
Neutrophilia (≥5/μl)	3/48 (6.3%)	1/30 (3.3%)
LESCLs during the entire course	15/54 (27.8%)	9/30 (30.0%)

The upper normal range of the IgG index was derived from our previous study [24]. CSF = cerebrospinal fluid; EDSS = Kurtzke's Expanded Disability Status Scale [17]; FS = Kurtzke's Visual Functional Scale [17]; LESCLs = longitudinally extensive spinal cord lesions; MS = multiple sclerosis; OB = oligoclonal IgG bands.
[a] Means ± SD.
* $P<0.05$.

infratentorial lesion did not differ significantly between the two groups. About a half of patients with anti-AQP4 antibody had ovoid periventricular lesions (Fig. 1), but the frequency of ovoid lesions was less common in anti-AQP4 antibody-positive patients than in anti-AQP4 antibody-negative patients (48.2% vs. 72.3%, $P=0.022$). By contrast, atypical brain lesions were present more frequently in patients with anti-AQP4 antibody (37.0%) than in those without the antibody (19.0%) ($P=0.070$). Among these, extensive brain lesions were observed more commonly in anti-AQP4 antibody-positive patients (18.5%) than in antibody-negative patients (1.7%) ($P=0.0023$). However, the frequencies of other atypical lesions did not differ significantly between anti-AQP4 antibody-positive and -negative patients.

Finally, we compared clinical features between MS patients with and without CSF OB/high IgG index, and found that age at onset was significantly younger in MS patients with CSF OB/high IgG index than in MS patients without it ($P=0.036$) (Tables 7 and 8). Moreover, the frequencies of brain lesions fulfilling the Barkhof and Paty criteria and ovoid lesions were significantly higher in those with CSF OB/high IgG index than in those without it ($P=0.00040$, $P=0.014$, and $P=0.030$, respectively).

4. Discussion

By extensive analyses of bpboard1rain MRIs of Japanese patients with MS and NMO, we found that MS-like brain lesions were more common in anti-AQP4 antibody-negative patients than in those with the antibody, while extensive brain lesions were more frequently observed in the latter than in the former; however, about 30 to 50% of either NMO or anti-AQP4 antibody-positive patients had brain MRI lesions that were indistinguishable from those associated with MS. Surprisingly, anti-AQP4 antibody-positive patients had periventricular ovoid lesions more frequently than atypical brain lesions. Even in patients who met the revised NMO criteria [3], MS-like brain lesions, including periventricular ovoid lesions, were more frequently observed than atypical brain lesions in the present series. The presence of typical MS-like brain lesions, such as periventricular ovoid lesions, suggests that considerable overlap exists in MRI appearance between patients with NMO who have anti-AQP4 antibody and classical MS patients without anti-AQP4 antibody. The fact that we [11] and others [4,10] observed that around 10% of classical MS patients harbour NMO-IgG/anti-AQP4 antibody further supports such an overlap between the two conditions. In fact, among

Table 8
Comparison of brain MRI findings between MS patients with and without CSF OB/high IgG index.

	MS with OB/high IgG index ($n=54$)	MS without OB/high IgG index ($n=30$)
Barkhof brain lesions[a]	45/54 (83.3%)*	13/30 (43.3%)*
≥9 T2 brain lesions	46/54 (85.2%)*	16/30 (53.3%)*
≥1 Gd-enhanced lesion	19/53 (35.9%)	8/30 (26.7%)
≥1 juxtacortical lesion	46/54 (85.2%)*	19/30 (63.3%)*
≥1 periventricular lesion	46/54 (85.2%)*	19/30 (63.3%)*
≥1 infratentorial lesion	40/54 (74.1%)	17/30 (56.7%)
Paty brain lesions[b]	51/54 (94.4%)*	22/30 (73.3%)*
Ovoid lesions	48/53 (90.6%)*	21/30 (70.0%)*
Atypical brain lesions	15/54 (27.8%)	3/30 (10.0%)
Extensive brain lesions	0/54 (0.0%)	0/30 (0.0%)
Bil. diencephalic lesions	5/54 (9.3%)	0/30 (0.0%)
Cavity formation	11/54 (20.4%)	3/30 (10.0%)
Extension from the cervical cord into brainstem	0/54 (0.0%)	0/30 (0.0%)

Bil. = bilateral; Gd = gadolinium; MS = multiple sclerosis.
[a] Brain lesions fulfilling the Barkhof criteria [16].
[b] Brain lesions fulfilling the Paty criteria [13].
* $P<0.05$.

patients with anti-AQP4 antibody, ovoid lesions were more commonly encountered than atypical brain lesions. It is therefore suggested that a common mechanism may in part be operative in these two conditions, especially in producing periventricular ovoid lesions, irrespective of the presence or absence of anti-AQP4 antibody. Ovoid periventricular lesions are said to be caused by T cells invading along the postcapillary high endothelial venules, which perpendicularly radiate from the lateral ventricular walls [18]. Thus, T cells might also play an important role in producing the brain lesions in the patients with anti-AQP4 antibody, but the target antigens could be different from those in patients without the antibody. We also previously reported cases showing seroconversion during the course of MS [11]. All of these findings support the notion that there are cases in whom anti-AQP4 antibody can be produced during the course of idiopathic demyelinating diseases, such as MS, and secondarily modify the clinical features, like anti-neurofascin antibody [19].

Among atypical brain lesions, only the extensive brain lesions seemed to be significantly more frequent in anti-AQP4 antibody-positive patients than in anti-AQP4 antibody-negative patients in our series. We previously reported that extensive brain lesions showed a vasogenic oedema pattern on diffusion-weighted MRI [11,20]. In AQP4 knock-out mice, cytotoxic oedema is ameliorated [21] while vasogenic oedema becomes worse [22]. Destruction of AQP4 on astrocyte foot processes by complement activation by anti-AQP4 antibody might well retard the resolution of vasogenic oedema, which tends to cause extensive oedematous brain lesions associated with inflammation in patients with anti-AQP4 antibody.

In the present study, NMO patients with brain lesions showed a significantly higher annualized relapse rate than typical NMO patients, suggesting a high disease activity in the former. Indeed, frequencies of severe optic neuritis, LESCLs and cavity formation were all higher in the former than in the latter, although this difference was not statistically significant. In addition, anti-AQP4 antibody positivity rate was highest in NMO patients with brain lesions among the three groups examined. Therefore, development of brain lesions in NMO patients may reflect high disease activity and the presence of anti-AQP4 antibody, and thus be regarded as a warning sign for a grave clinical course.

The positivity rate of CSF OB/high IgG index in our MS patients was lower than those reported for Caucasians with MS [23]. However, the positivity rate was similar to those previously reported in Asian patients with MS [24–26]. The disparities between Western and Asian MS patients may be related to differences in genetic backgrounds. Interestingly, MS patients with CSF OB/high IgG index showed not only a significantly younger age at onset but also higher frequency of brain lesions fulfilling the Barkhof criteria [16] than those without it. These findings suggest that MS with CSF OB/high IgG index has similar features to classical Western-type MS, even in Asians.

In summary, up to a half of anti-AQP4 antibody-positive patients could develop classical MS-like brain lesions, which is even more frequent than the development of so-called atypical brain lesions. Because the presence of anti-AQP4 antibody can modify treatment response, as shown previously [11], anti-AQP4 antibody should be tested for even in patients with classical MS-like features, especially in Asians.

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Clinical/Scientific Notes

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B-CELL ACTIVATING FACTOR OF THE TNF FAMILY IS UPREGULATED IN NEUROMYELITIS OPTICA

Neuromyelitis optica (NMO) is characterized by optic neuritis and longitudinally extensive transverse myelitis, and serum NMO-IgG antibody against aquaporin-4 (AQP4) has been implicated in the pathogenesis.[1] It has been reported that NMO patients are often complicated by either serum non-organ-specific autoantibodies and autoimmune diseases such as systemic lupus erythematosus (SLE) or Sjögren syndrome (SS).[1] Efficacy of interferon (IFN)-β for the prevention of NMO relapses is not supported in contrast to that for multiple sclerosis (MS).[2] These findings suggest that humoral immunity plays a pivotal role in the pathogenesis of NMO.

B-cell activating factor of the tumor necrosis factor family (BAFF) is a key molecule involved in the differentiation and survival of B cells, and this molecule also promotes immunoglobulin production.[3] Previous studies have shown that serum level of BAFF is increased and correlates with disease activity and titers of pathogenic autoantibodies in SLE and SS.[3] BAFF is implicated in the establishment and maintenance of autoantibody-associated autoimmune diseases.

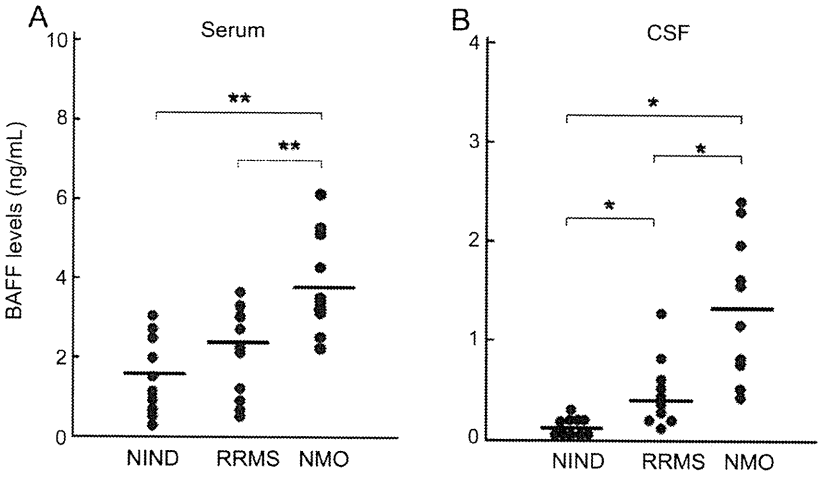
Since it has been speculated that more intense humoral immune responses are involved in the pathogenesis of NMO than MS, we investigated whether BAFF in serum and CSF is increased in NMO.

Methods. Serum and CSF were collected from patients with relapsing-remitting MS (RRMS) and patients with NMO during the acute relapse phase. Patients with NMO included 7 women and 3 men with an age range of 24 to 55 years (median 43), Expanded Disability Status Scale range of 4.0–8.5 (median 6.0), and disease duration range of 2.0–10.5 years (median 5.2) at the sampling of CSF and serum. Patients with RRMS included 5 women and 5 men with an age range of 19–55 years (median 37), EDSS range of 2.0–5.0 (median 3.0), and disease duration range of 2.2–6.3 years (median 4.2) at the sampling of CSF and serum. None of the patients with NMO or RRMS had any history of treatment with any immunosuppressants or IFN-β at the time of sampling. All patients with NMO demonstrated longitudinally extending transverse myelitis (LETM) and serum anti-AQP4 antibody which was assayed using AQP4-transfected HEK293 cells previously described.[4] Two patients with NMO and 2 patients with RRMS were SS-A antibody positive without clinical SS. As a control, we examined 5 women and 5 men with an age range of 28–56 years (median 43) who had various noninflammatory neurologic diseases, including 2 patients with amyotrophic lateral sclerosis, 3 patients with migraine, 3 patients with multiple system atrophy, and 2 patients with Alzheimer disease. Diagnosis of NMO and RRMS was based on the revised criteria for NMO and McDonald criteria for RRMS.[5,6] CSF and serum samples were stored at −80°C until assay. The internal review board of our institution approved the study. BAFF was measured by ELISA kit (Bender MedSystems, Vienna, Austria) according to the manufacturer's protocol. Mann-Whitney test was performed for statistical analysis and $p < 0.05$ was considered significant.

Results. The serum BAFF level was significantly elevated in patients with NMO (mean ± SD: 3.85 ± 1.18 ng/mL) than patients with RRMS (2.28 ± 1.0 ng/mL) and patients with noninflammatory neurologic diseases (NIND) (1.60 ± 0.97 ng/mL) (figure). The CSF BAFF level also was significantly higher in patients with NMO (1.34 ± 0.68 ng/mL) than patients with RRMS (0.49 ± 0.32 ng/mL) and patients with NIND (0.10 ± 0.09 ng/mL). Patients with RRMS show a higher level of CSF BAFF than patients with NIND. There was no significant difference in the serum BAFF level between patients with RRMS and patients with NIND.

Discussion. The finding that patients with NMO showed increased serum levels of BAFF, while patients with RRMS and patients with NIND did not, may support the pivotal roll of B cells and humoral immunity in the pathogenesis of NMO, because BAFF is a key molecule for the differentiation and survival of B cells and immunoglobulin production.[3] Upregulation of BAFF is thought to promote and maintain NMO–immunoglobulin G production in

Figure B-cell activating factor of the tumor necrosis factor family (BAFF) levels in serum (A) and CSF (B)



BAFF levels detected by ELISA in CSF and serum of neuromyelitis optica (NMO) patients (n = 10), relapsing-remitting MS (RRMS) patients (n = 10), and noninflammatory neurologic diseases (NIND) patients (n = 10). The line denotes the mean value. $^{*}p < 0.05$, $^{**}p < 0.01$.

the systemic immune system of patients with NMO. Moreover, the finding may be in line with previous reports of the excellent prevention of NMO relapses by rituximab, which depletes the CD20-positive B cell lineage, and the augmentation of NMO relapses by IFN-β, which induces BAFF and augments systemic autoimmune diseases such as SLE and SS.[2,3,7]

Increased levels of BAFF in CSF were found in both patients with RRMS and patients with NMO. Previous study has shown that BAFF expression is increased in MS lesions and activated astrocytes are a strong source of BAFF.[3] Upregulated BAFF in the CSF may reflect the local production in lesions of the CNS in both RRMS and NMO.

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AUTHOR CONTRIBUTIONS

Statistical analysis was conducted by Dr. Kazumasa Okada.

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Autoimmunity in neuromyelitis optica and opticospinal multiple sclerosis: Astrocytopathy as a common denominator in demyelinating disorders

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ABSTRACT

Neuromyelitis optica (NMO) selectively affects the optic nerves and spinal cord. In Asians, multiple sclerosis (MS) is rare; however, when it appears, the selective and severe involvement of the optic nerves and spinal cord is characteristic. This form, termed opticospinal multiple sclerosis (OSMS), has similar features to the relapsing form of NMO in Westerners. The discovery that NMO-IgG, an NMO-specific IgG, targets aquaporin-4 (AQP4), suggested that NMO is a distinct disease entity with a fundamentally different etiology from MS. Because NMO-IgG is present in 30–60% of OSMS patients, OSMS in Asians is suggested to be the same entity as NMO. Pathologically, perivascular immune complex (IgM, IgG and C9neo) deposition and extensive loss of AQP4 in active lesions are reported hallmarks of NMO. However, we found that some autopsied NMO cases showed selective AQP4 loss while others showed preservation of AQP4, despite extensive tissue destruction. Vasculocentric deposition of complement and immunoglobulin was detected only in NMO patients, with less than 30% of actively demyelinating lesions showing AQP4 loss. Such heterogeneity of AQP4 expression and immunoglobulin deposition suggests a heterogeneous disease process in NMO. We recently reported that AQP4 was extensively lost in glial fibrillary acidic protein-positive hypertrophic astrocytes, both in demyelinated and myelinated layers of actively demyelinating lesions in Baló's disease, a variant of MS. We also found that in some acute MS lesions, AQP4 was lost extensively far beyond the areas of myelin loss. Active demyelinating lesions involved perivascular lymphocyte cuffings, consisting mainly of T cells in Baló's disease and MS, while the same was true for approximately half of the active lesions in NMO. This review proposes that anti-AQP4 antibody-dependent AQP4 loss occurs in some NMO patients while antibody-independent AQP4 astrocytopathy can occur in heterogeneous demyelinating conditions, including Baló's disease, NMO and MS. The latter may be mediated by T cells and other cell-mediated mechanisms, and should be tested in future experimental studies.

1. Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS), which is thought to occur following autoimmune attack of CNS myelin. On the other hand, neuromyelitis optica (NMO) is an inflammatory disease of the CNS selectively affecting the optic nerves and spinal cord. In this condition, longitudinally extensive spinal cord lesions (LESCLs) extending three or more vertebral segments are regarded to be characteristic hallmarks following magnetic resonance imaging (MRI) [1]. Pathologically, both axons and myelin are involved, resulting in necrotic cavitation.

In Asians, MS is rare; however, when it appears the selective but severe involvement of the optic nerves and spinal cord is characteristic [2]. This form, termed opticospinal MS (OSMS), has similar features to the relapsing form of NMO in Westerners [1]. The nosological position of NMO has long been a matter of debate; however, the recent discovery of a specific IgG against NMO, designated NMO-IgG, suggests that NMO is a distinct disease entity with distinct etiology from MS [3,4]. Because NMO-IgG has been reported to be present in approximately 50–60% of OSMS patients [3,5], OSMS in Asians is claimed to be the same entity as NMO. However, the observation that NMO-IgG is not found in all cases of NMO or OSMS [5–7], and that 5–10% of classical MS patients also carry the antibody [3,5,7] cast doubt on the homogeneity of NMO and the simple dichotomy of categorizing human demyelinating disease into MS and NMO. In Asians, the mechanism underlying the formation of LESCLs appears to be heterogeneous, and the disease condition in those with NMO-IgG does not completely overlap with OSMS in Asians [6,7].

On the other hand, Baló's concentric sclerosis is relatively frequently reported among Asians, especially in Filipinos, Southern Han Chinese and Taiwanese [8]. This disease is said to be a rare variant of MS with huge brain lesions showing concentric rings of alternating demyelination and preserved myelin layers. Although the mechanism

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of Baló's disease still remains to be elucidated, we revealed extensive aquaporin-4 (AQP4) loss in demyelinated and myelinated layers of Baló's lesions without perivascular immunoglobulin and complement deposition [9]. Recently, Graber et al. [10] reported the occurrence of concentric rings of Baló in the brainstem in an NMO patient with NMO-IgG. These findings collectively suggest a link exists among NMO, Baló's disease and MS. Therefore, we recently reappraised AQP4 expression patterns in NMO and MS and found selective loss of AQP4 without vasculocentric deposition of complement and immunoglobulins, which is also detected in a fraction of MS and NMO patients [11], suggesting a common underlying mechanism among the three conditions. In this review, from an immunohistopathological, and humoral and cellular autoimmune point of view, possible underlying mechanisms of human demyelinating diseases, encompassing MS, NMO and Baló's disease, are discussed.

2. Recent immunohistopathological studies on NMO and MS

The pathological hallmark in MS is sharply demarcated demyelinating plaques with axons relatively preserved. By contrast, in NMO, intense demyelination, a great loss of axons, perivascular lymphocytic infiltration, microglial proliferation and vascular proliferation are seen in optic nerve and spinal cord lesions; these can occasionally lead to cystic cavities in severely involved areas [12–15]. Astrocytosis is scarce in some necrotic lesions but considerable in others. On the other hand, the neuropathological features of MS in Asians are as follows: (1) preferential occurrence of lesions in the optic nerves and spinal cord; (2) necrotizing lesions with occasional cavity formation not only in the spinal cord and optic nerves but also in the cerebrum; (3) poor gliosis; and (4) poor perivascular cuffing in the necrotic form [16–19]. Perivascular cuffing and gliosis varied regionally. Spinal cord lesions were usually most severe in the lower cervical to the mid thoracic cord. Polymorphonuclear leukocyte infiltration was occasionally seen in severe lesions in Asian MS patients, but eosinophil infiltration, as described in Western NMO patients [20], was not reported in early [16–19] or more recent literature [21].

More recently, Lucchinetti et al. [20] described perivascular immune complex deposition (IgM, IgG and C9neo) in a rim or rosette pattern. Misu et al. [22] reported that extensive loss of AQP4 was accompanied by decreased glial fibrillary acidic protein (GFAP) staining in active perivascular lesions where myelin basic protein (MBP) staining was relatively preserved in postmortem Japanese NMO cases. MBP loss with an AQP4 preservation pattern was not observed in any of the 22 active inflammatory lesions. Based on the presence of immunoglobulin and complement deposition in active perivascular lesions, Misu et al. [22] postulated that astrocytic impairment associated with the loss of AQP4 by humoral immunity is the primary event in NMO, suggesting a primary role for the anti-AQP4 antibody in NMO pathology. Roemer et al. [23] made similar observations regarding novel NMO lesions in the spinal cord, optic nerves and medullary tegmentum extending to the area postrema where the blood–brain barrier (BBB) is absent.

3. Questions relating to AQP4 immunohistopathology in NMO

Contradictory to the pivotal reports by Misu et al. [22] and Roemer et al. [23], Kobayashi et al. [24] reported an autopsied case of NMO showing preservation of AQP4 in severe lesions in the spinal cord and medulla, and in the demyelinated lesions in the optic nerve. In MS plaques, according to Misu et al. [22], AQP4 was never lost but was somewhat upregulated, reflecting astrogliosis, while in Roemer's report [23], some chronic MS plaques showed selective AQP4 loss. Therefore, there are considerable inconsistencies concerning AQP4 expression among pathology reports on NMO and MS. With respect to AQP4 pathology in NMO, I raise the following questions (Table 1): (1) Is AQP4 loss specific to NMO? (2) Is AQP4 uniformly lost in NMO lesions? (3) Is AQP4 loss always accompanied with vasculocentric deposition of immunoglobulin and complement? (4) Are there any T cell components in NMO? (5) How does such a tiny perivascular deposition of immunoglobulin and complement produce such a large lesion in the spinal cord and occasionally in the brain [25]?

Table 1
Questions and our answers relating to AQP4 immunohistopathology in NMO.

No.	Questions	Answers
1	Is AQP4 loss specific to NMO?	No
2	Is AQP4 uniformly lost in NMO lesions?	No
3	Is AQP4 loss always accompanied with vasculocentric deposition of immunoglobulin and complement?	No
4	Are there any T cell components in NMO?	Yes
5	How does such a tiny perivascular deposition of immunoglobulin and complement produce such a large lesion in the spinal cord and occasionally in the brain?	Astrocytopathy may play a role.

4. Reappraisal of AQP4 immunohistopathology in NMO, Baló's disease and MS

To address the above-mentioned questions, we first assessed AQP4 expression in the concentric demyelinating lesions of Baló's disease [9]. We evaluated AQP4 expression relative to another astrocytic marker (GFAP), the extent of demyelination, and lesion staging and perivascular deposition of complement and immunoglobulin in four Filipino cases with Baló's disease. All cases with Baló's disease demonstrated extensive AQP4 loss in demyelinated and myelinated layers of all actively demyelinating lesions, with perivascular lymphocytic cuffings of T cells but no deposition of immunoglobulins or complement around vessels. None of the patients with MRI-confirmed Baló's disease were seropositive for anti-AQP4 antibody [26]. AQP4 loss is thus supposed to be induced independently from the anti-AQP4 antibody. We therefore proposed autoantibody-independent AQP4 astrocytopathy in Baló's disease [26].

Second, we reappraised AQP4 expression patterns in NMO and MS [11]. We evaluated AQP4 expression relative to GFAP, the extent of demyelination, lesion staging (CD68 staining for macrophages), and perivascular deposition of complement and immunoglobulin in 11 patients with NMO and NMO spectrum disorders (NMOSD), five with MS, and 30 with other neurological diseases. Six NMO/NMOSD and two MS cases showed preferential AQP4 loss beyond the demyelinated areas, irrespective of lesion staging. Importantly, even in the same NMO patients, AQP4 loss was observed in some active lesions while AQP4 was preserved in other active lesions where numerous myelin-laden macrophages had infiltrated. This was true even for an NMO patient who was confirmed to be seropositive for the anti-AQP4 antibody. The other five NMO and three MS cases showed AQP4 preservation even in actively demyelinating lesions, despite grave tissue destruction. Vasculocentric deposition of complement and immunoglobulin was detected only in NMO/NMOSD patients, with less than 30% of actively demyelinating lesions showing AQP4 loss. These findings collectively suggest that AQP4 loss without perivascular complement and immunoglobulin deposition can occur in heterogeneous demyelinating conditions, including NMO, Baló's disease and MS (Fig. 1).

From the above findings, AQP4 down-modulation does not seem to be specific for NMO, and the mechanisms underlying AQP4 down-modulation could be heterogeneous (Table 1). Thus, an answer to question 1 is NO. AQP4 loss does not occur uniformly throughout NMO lesions, even in actively demyelinating lesions, suggesting that the mechanism of lesion formation is heterogeneous, even in the same patient. Consequently, the answer to question 2 is also NO. AQP4 loss was less frequently observed in optic nerve lesions as compared with lesions in other CNS sites (Fig. 2) [11]. Because AQP4 loss was not always accompanied with vasculocentric deposition of immunoglobulin and complement, the answer to question 3 is again

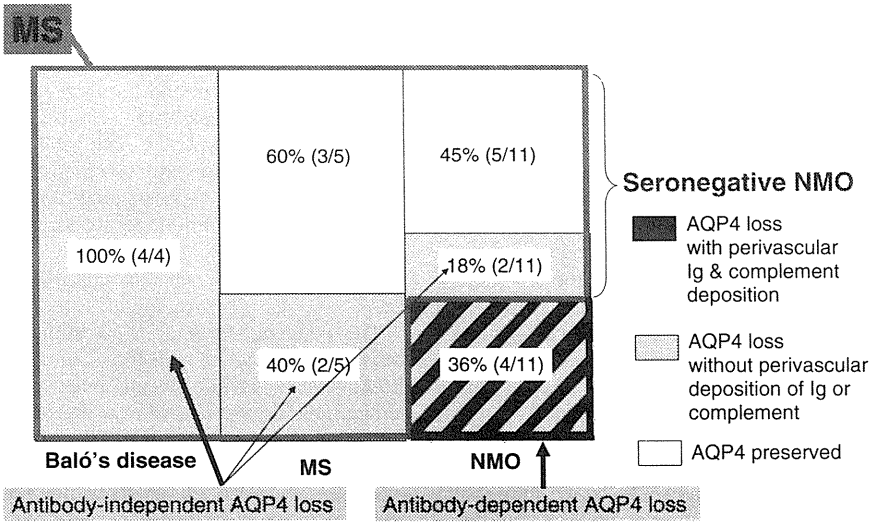

Fig. 1. AQP4 expression pattern in MS, NMO and Baló's disease. Even NMO cases presenting AQP4 loss with perivascular immunoglobulin and complement deposition in active lesions demonstrated AQP4 loss without perivascular immunoglobulin and complement deposition in other lesions. NMO cases having AQP4 loss without perivascular immunoglobulin and complement deposition may correspond to anti-AQP4 antibody-seronegative NMO or could be OSMS with LESCLs. Number of cases positive/number of cases examined in parenthesis. AQP4 = aquaporin-4; LESCLs = longitudinally extensive spinal cord lesions; MS = multiple sclerosis; NMO = neuromyelitis optica; OSMS = opticospinal multiple sclerosis.

NO. We did find T cell infiltration in half of the NMO lesions showing more or less AQP4 down-modulation. Therefore, the answer to question 4 is YES.

5. Humoral immunity in NMO, MS and Baló's disease

The high specificity of NMO-IgG for NMO suggests that NMO is a distinct disease entity from MS [3,4]. Because NMO-IgG recognizes AQP4 on astrocyte endfeet, astrocytic destruction by anti-AQP4 antibodies, which fix and activate complement, is thought to be the primary pathogenic process in NMO [4].

5.1. Specificity of anti-AQP4 antibody

NMO-IgG has not been described in other inflammatory diseases in Westerners; however, 9% of MS cases in Lennon's original series did have the antibodies [3]. To date, 5–15% of tested MS cases were found to be positive for NMO-IgG or anti-AQP4 antibody [3,5–7,27] while 10% of NMO-IgG-positive patients had brain lesions that were indistinguishable from MS lesions [28]. This indicates the existence of considerable overlap between NMO and MS, which should not be ignored.

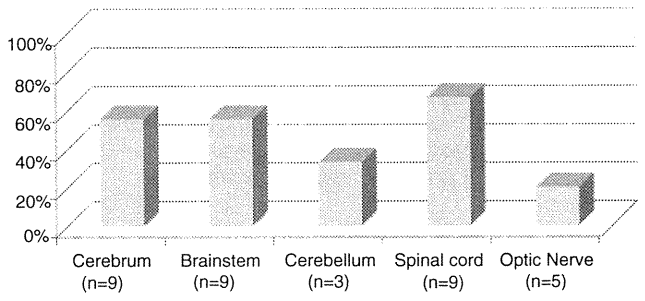

Fig. 2. Frequency of AQP4 loss according to CNS sites of active and chronic active lesions in nine autopsied NMO cases. AQP4 = aquaporin-4; CNS = central nervous system; NMO = neuromyelitis optica.

5.2. Sensitivity of the anti-AQP4 antibody

One of the confounding problems is that NMO-IgG is not detected in all NMO patients (Table 2). In Caucasians, 73% were positive in Lennon's original report [3] and similar figures have also been reported elsewhere: 61.1% in Jarius et al. [29] and 56.72% in Paul et al. [27]. Recently, Fazio et al. [30] conducted immunofluorescence, flow cytometry and radioimmunoprecipitation assays in Italian patients with NMO and found up to 47% were positive. In Africans and their descendants, much lower positivity rates were reported: 33.3% in Caribbean patients with NMO [31] and 5% in African-American patients with OSMS [32]. In Japan, Nakashima et al. [5] reported the detection of NMO-IgG in 63% of OSMS patients and 15% of CMS patients. Recently, the same group also reported that 20 of 22 NMO patients had the anti-AQP4 antibody, while none of the 53 MS patients tested positive (90% vs. 0%) [33]. Tanaka et al. [34], in their selected series of MS patients, independently reported that the anti-AQP4 antibody positivity rate was 61.5% in OSMS patients with LESCLs and 0% in CMS patients without LESCLs. We reported that the positivity rate for the anti-AQP4 antibody was 27.1% (13/48) in OSMS patients, 5.6% (3/54) in CMS patients, 0% (0/52) in patients with other neurological diseases and 0% (0/35) in healthy controls [6]. Among OSMS patients, the antibody positivity rate was highest (55.6%) in OSMS patients with both LESCLs and MS-like brain lesions fulfilling the Barkhof criteria for MS [35], although NMO-IgG was originally described in patients with exclusive optic nerve and spinal cord lesions.

Table 2
Positivity rates for NMO-IgG/anti-AQP4 antibody among races.

Race	Disease	NMO-IgG/anti-AQP4 antibody (%)
Caucasians [3,27–30]	NMO	30–73[a]
Northern Japanese [5]	OSMS	63[a]
Southern Japanese [6]	OSMS	27[a]
Caribbean [31]	NMO	33
African-American [32]	OSMS	5

[a] Measured by Mayo Clinic.

There are obvious discrepancies in the detection rates among the above-mentioned series. The reasons for these may relate to differences in the subjects used: selected versus consecutive patients; NMO versus OSMS patients with LESCLs; Northern versus Southern Japanese patients that have been shown to have somewhat distinctive features in clinical phenotype by a recent nationwide survey [36,37]. They could also relate to the methods used: AQP4-transfected versus GFP-AQP4 fusion protein-transfected; fixed transfected cell specimens versus unfixed ones; 1:4 dilution versus 1:400 dilution. However, even in the studies done by the Mayo clinic, there are considerable differences in positivity rate, indicating that the difference is partly attributable to differences among subjects (Table 2) [3,5,6,27–32]. It remains to be elucidated whether the 30–70% of NMO patients who fulfilled the NMO diagnostic criteria and did not carry the antibodies are truly seronegative NMO patients or false negatives due to the low sensitivity of the assay employed.

In the case of Baló's disease, the anti-AQP4 antibody in sera was negative in six MRI-confirmed Baló's cases by standard cell-based immunofluorescence and flow cytometric assays [26], suggesting that AQP4 loss occurs through anti-AQP4 antibody-unrelated mechanisms in this condition.

5.3. Titers and IgG subclass

Takahashi et al. [33] claimed that anti-AQP4 antibody titers showed a very strong positive correlation with spinal cord lesion length ($R=0.9108$), while others have not confirmed this result [6,7,38,39]. Although the NMO-IgG/anti-AQP4 antibody usually appears in the early course of the disease [40], seroconversion of NMO-IgG/anti-AQP4 antibody during the course of illness is observed in some patients [6,7]. This may indicate the possibility that the antibody is produced after tissue destruction on some occasions, as seen in MS patients in whom various autoantibodies emerge during the clinical course; some of them target even neural antigens, such as neurofascin, and are shown to be functional in vivo [41]. Considering that OSMS patients with a low titer of anti-AQP4 antibody showed similar clinical and immunological features to those of OSMS patients without the antibody, it is possible that a low titer anti-AQP4 antibody is secondary to severe tissue destruction [7]. However, once it appeared, even though secondarily, it could exert pathogenic effects in vivo.

To clarify the clinical relevance of anti-AQP4 antibody titers and the IgG subclass, we developed a bridging enzyme-linked immunosorbent assay (ELISA) and a flow cytometric assay [42]. In the latter, each IgG subclass was quantitated using the mean fluorescence intensity ratio of AQP4-transfected and -untransfected human embryonic kidney cells. By ELISA, levels of the anti-AQP4 antibody were observed to positively correlate with the number of relapses presenting with optic neuritis while they showed a steady rise over time without concomitant relapse in seven of twelve longitudinally studied patients over an average of 8 years. Anti-AQP4 antibody levels determined by standard immunofluorescence assay, ELISA and flow cytometry had no correlation with disease severity, such as Expanded Disability Status Scale (EDSS) scores.

By flow cytometry, IgG1, 2, 3 and 4 anti-AQP4 antibodies were found in 97.8, 39.1, 13.0 and 8.7%, respectively. In patients not receiving corticosteroids, the levels of IgG1 anti-AQP4 antibody correlated positively with disease duration, while those of IgG2 antibodies correlated negatively with maximum spinal cord lesion length, being lower in patients with longitudinally extensive spinal cord lesions compared with those without, but higher in patients with anti-SS-A/B antibodies than in those without. Moreover, IgG2 anti-AQP4 antibody carriers showed a younger age of onset and a lower Progression Index than those with other subclasses. We hypothesize that a longer disease duration and a higher number of relapses increase total anti-AQP4 antibody levels, especially IgG1, possibly through affinity maturation. A similar observation was made by other authors, in which total anti-AQP4 antibody titers increased as relapse numbers increased and disease duration became longer [43,44]. There also exists a unique subgroup of IgG2 anti-AQP4 antibody carriers with a younger age of onset and humoral autoimmune background who demonstrate a relatively benign course, in spite of having high levels of total anti-AQP4 antibody. Such IgG2 anti-AQP4 antibody carriers may correspond to cases with so-called "benign NMO" in the literature [45,46] and may not require a long-term administration of low dose corticosteroids and immunosuppressants.

5.4. Autoimmune background for anti-AQP4 antibody production

The relapsing form of NMO with the anti-AQP4 antibody is frequently associated with other autoantibodies and autoimmune diseases, such as Sjögren syndrome, systemic lupus erythematosus, autoimmune thyroiditis and myasthenia gravis, in Westerners [1,47]. Even in Asian patients with anti-AQP4 antibodies, other autoantibodies, such as SSA and SSB, as well as other autoimmune diseases, such as Sjögren syndrome, are frequently present [6,7,48–50]. Therefore, an autoimmune-prone background, especially heightened humoral autoimmunity, seems to be an important factor in the production of the anti-AQP4 antibody. We found that, among anti-AQP4 antibody-positive individuals, Th1 cell percentage showed a significant negative correlation with anti-AQP4 antibody titer, and that those with SSA/SSB antibody had significantly higher titers of the anti-AQP4 antibody [7]. Therefore, a high titer anti-AQP4 antibody seems to be produced in those with a heightened humoral autoimmune background, a Th2-prone condition; however, an anti-AQP4 antibody titer itself does not necessarily correlate with severity of the disease, rather curiously tends to show an inverse correlation with EDSS scores [6]. This result is probably due to the existence of NMO patients with mild disability but with a high anti-AQP4 antibody titer.

5.5. Pathogenicity of NMO-IgG/anti-AQP4 antibody

Sera and IgG from NMO patients with NMO-IgG/anti-AQP4 antibody induce astrocyte damage and death in primary cultures only in the presence of complement [51–53], while in the absence of complement they do not affect AQP4 water channel function in astrocytes [54]. IgG containing the anti-AQP4 antibody from NMO-IgG-seropositive NMO patients reproduces astrocyte loss in vivo only when MBP-specific T cells are transferred to cause experimental autoimmune encephalomyelitis (EAE) [55–57]. However, when the AQP4 antibody was injected into young rats with a leaky BBB, or after transfer of non-encephalitogenic T cells, it did not induce any disease or neuropathological alterations in the CNS [57]. Direct injection of NMO-IgG together with human complement into the brain of mice induced AQP4 loss with extensive inflammatory infiltrates, having some similarity to human NMO lesions [58]. Inflammation might be exaggerated by the fact that mice complement inhibitor existing in the CNS has no prohibiting effects on human complement and it still implies some factor might trigger breakdown of the BBB at first. Recently, Kinoshita et al. [59] reported that following pre-treatment with complete Freund's adjuvant, injection of anti-AQP4 antibody-containing IgG can induce inflammation and astrocyte damage in the CNS. Thus, if non-specific inflammation renders the BBB leaky, anti-AQP4 antibody may enter into the CNS and cause astrocyte destruction. However, animals never demonstrated any clinical signs and pathological changes never became extensive as seen in human NMO cases.

6. Cellular immunity in NMO and MS

6.1. AQP4-specific T cell response

By immunizing overlapping pentadecameric peptides of AQP4, Kalluri et al. [60] found that the N-terminal region of AQP4 is highly

immunogenic in mice, and that the intracellular epitope AQP4 (22–36) was a major immunogenic determinant. AQP4 (22–36) and AQP4 (289–303)-specific T cells were present in the natural T cell repertoire of C57BL/6 mice and T cell lines could be raised. However, active immunization with these AQP4 peptides did not produce any signs of disease, despite induction of antigen-specific T cells [60]. These observations imply that AQP4 is not encephalitogenic. Human T cell epitopes on AQP4 remain to be elucidated.

6.2. Myelin protein-specific T cell response

We reported the establishment of major myelin protein-derived T cell lines (TCLs) from anti-AQP4 antibody-seropositive NMO patients as well as anti-AQP4 antibody-seronegative MS patients [61,62]. TCLs from most of these patients reacted with multiple epitopes on the plural myelin proteins, such as MBP, proteolipid protein and myelin oligodendrocyte glycoprotein, which was not seen in TCLs from healthy donors that were established in the same way. Therefore, this indicates that inter- or intra-molecular epitope spreading against myelin proteins occurs in MS and NMO patients, irrespective of anti-AQP4 antibody status. These findings suggest that T cells are stimulated in vivo against major myelin proteins even in anti-AQP4 antibody-positive patients with NMO/NMOs, like anti-AQP4 antibody-negative MS patients. Therefore, after myelin-specific T cells initiate CNS inflammation, antibodies recognizing various components of CNS antigens might modify the clinicopathological features of demyelinating diseases (Fig. 3).

6.3. Th17 and Th1 cytokines

In peripheral blood, OSMS shows a pronounced T-helper-1 (Th1) and T-cytotoxic-1 (Tc1) shift, where interferon-γ (IFNγ)-producing T cells predominate over IL-4-producing T cells throughout the relapse and remission phases [63,64]. We previously reported that IL-17 is up-regulated in the cerebrospinal fluid (CSF) of OSMS patients and that levels of both IL-17 and the downstream cytokine IL-8 in CSF show a significant positive correlation with spinal cord lesion length [21]. Recently, by simultaneously measuring the levels of 27 cytokines and chemokines in CSF from patients with various causes of myelitis, we found that IL-17, IFNγ and granulocyte-colony stimulating factor were specifically elevated in OSMS patients, irrespective of the presence or absence of the anti-AQP4 antibody [65]. IL-17 (IL-17A) is exclusively produced by Th17 cells, which are CD4+ T cells recently shown to be a distinct lineage from Th1 and Th2 cells [66]. Increasing evidence suggests that Th17 cells, but not Th1 cells, are responsible for organ-specific autoimmune diseases, such as EAE [66,67]. IL-8 is a chemokine for neutrophils. In OSMS patients, CSF neutrophilia and infiltration of neutrophils in severe lesions are characteristic [21]. Hence, elevated IL-8 may be partly responsible for such neutrophil activation and mobilization in OSMS. Indeed, the level of myeloperoxidase, an activated neutrophil product, is increased in sera from OSMS patients, especially in those with LESCLs at relapse [68]. Th17 cells carrying granzyme B have recently been shown to efficiently disrupt BBB tight junctions and loosen the BBB [69]. Therefore, autoimmune Th17 cells may initiate BBB disruption and inflammation in OSMS, causing vasogenic edema in the CNS, regardless of the anti-AQP4 antibody status.

IL-17 has also been shown to induce vascular endothelial growth factor (VEGF) production in target tissues [70]. Regarding other factors with possible effects on vascular permeability, we previously reported that the levels of VEGF in sera were significantly elevated in OSMS patients, showing a significant positive correlation with spinal cord lesion length [71]. Given that AQP4 knockout mice showed prolonged vasogenic edema [72], but a decrease in the level of cytotoxic edema [73], the anti-AQP4 antibody produced either by a heightened humoral autoimmune background or secondarily by tissue breakdown may prolong resolution of tissue edema, thereby contributing to further tissue destruction in NMO and OSMS patients. Prolonged vasogenic edema at sites where the surrounding space is tight (the bony optic canal portion of the optic nerves) or where vascular supply (thoracic spinal cord) is poor may cause poor recovery from tissue damage in patients with the anti-AQP4 antibody.

6.4. Infections

Relapsing NMO is associated with other autoimmune disorders whereas monophasic NMO is associated with preceding infection [1]. Recently, it was reported that 88% of parainfectious NMOs are monophasic [74]. Hypercomplementemia and elevation of C-reactive protein are seen in anti-AQP4 antibody-positive patients with NMO spectrum disorders at relapse; however, such a systemic inflammatory reaction is rare in classical MS [75]. Considering its relapsing

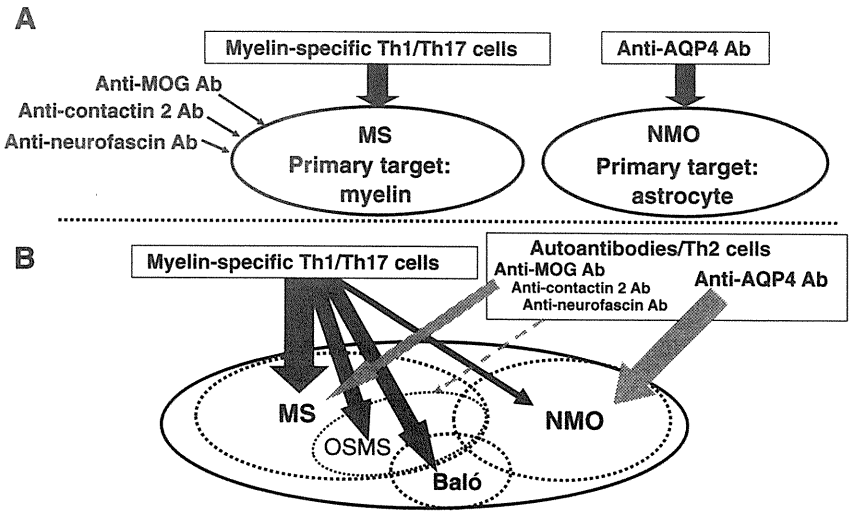

Fig. 3. Two hypothetical mechanisms of MS and NMO. In panel (A), myelin is a primary target of T cells and autoantibodies in MS, whereas in NMO, astrocytes are a primary target of the anti-AQP4 antibody. In panel (B), myelin-specific T cells initiate CNS inflammation and autoantibodies recognizing various components of CNS antigens modify clinicopathological features. In (A), NMO takes a uniform disease process presenting AQP4 loss in all active lesions whereas in (B) heterogeneous disease processes take place even in the same patient, showing heterogeneous pathological features. Ab = antibody; AQP4 = aquaporin-4; CNS = central nervous system; MOG = myelin-oligodendrocyte glycoprotein; MS = multiple sclerosis; NMO = neuromyelitis optica; OSMS = opticospinal multiple sclerosis; Baló = Baló's disease.

nature, specific acute infection is less likely to play a role in causing relapsing NMO with the anti-AQP4 antibody. However, we found that *Helicobacter pylori* infection is more prevalent in anti-AQP4 antibody-positive NMO than anti-AQP4 antibody-negative CMS patients and healthy controls [76]. In such patients, the antibody response to *Helicobacter pylori* neutrophil activating protein (NAP) was found to be markedly exaggerated and anti-NAP antibody titers showed a positive correlation with disease severity (EDSS scores) [77]. Therefore, it is possible that persistent *Helicobacter pylori* infection potentiates Th17/Th1 responses, thereby contributing to the autoimmune response of Th17/Th1 cells to CNS antigens. Alternatively, chronic persistent infection may in part contribute to the development of NMO through molecular mimicry between bacterial AQP and human AQP4, and the products of infectious agents, such as lipopolysaccharides, may render the BBB leaky.

7. Autoimmune mechanisms of NMO, MS and Baló's disease

7.1. Proposed mechanism

Based on the high specificity of the anti-AQP4 antibody and the selective loss of AQP4 in NMO lesions, it is postulated that the complement activating anti-AQP4 antibody plays a pivotal role in the development of NMO lesions [78]. Once the anti-AQP4 antibody gets across the BBB, it binds to AQP4 molecules on the astrocyte foot processes and activates complement. Activated complement mobilizes

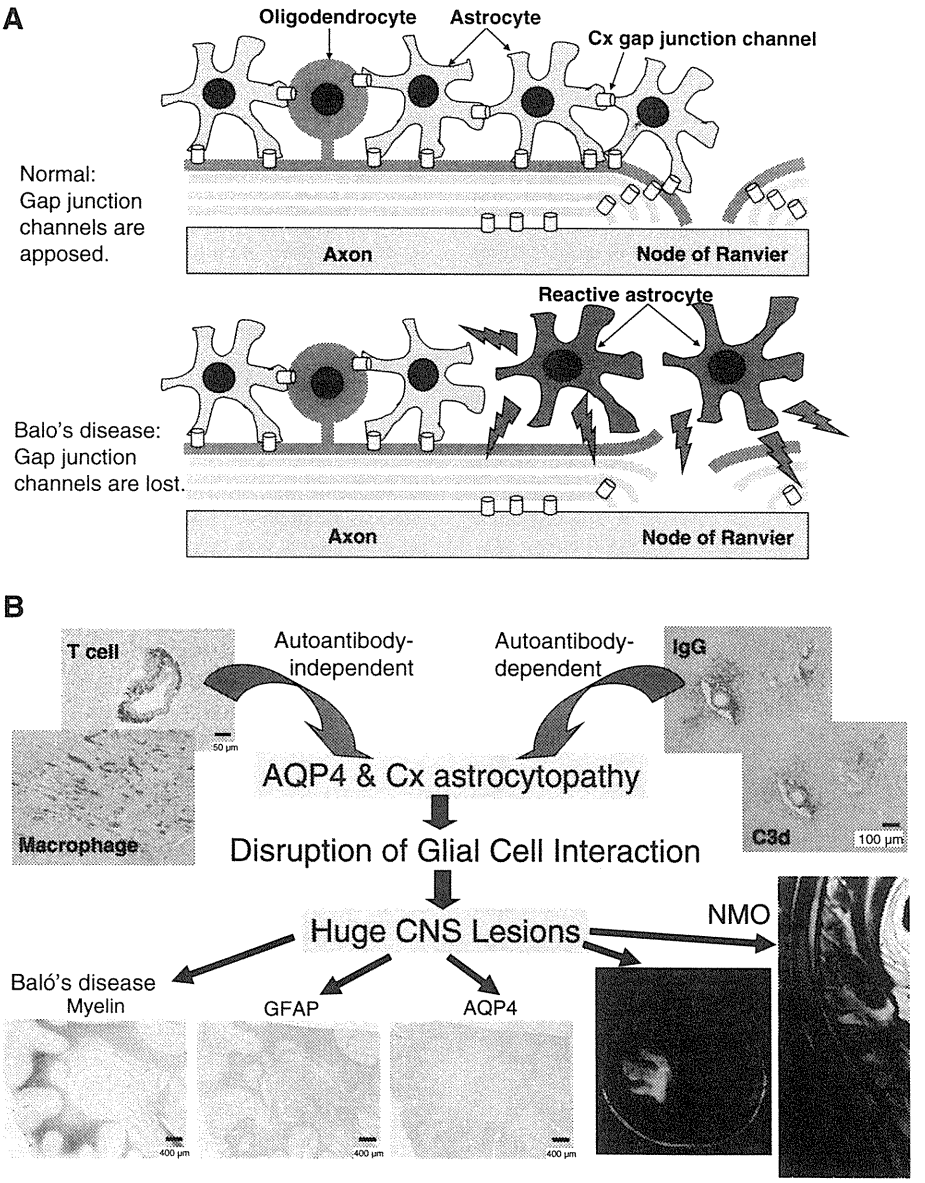

Fig. 4. (A) Hypothetical mechanism of connexin astrocytopathy causing secondary demyelination. Loss of astrocytic connexins, such as connexin 43, may disrupt interactions between astrocyte–astrocyte and astrocyte–oligodendrocyte. Once disruption of connexin gap junction channels occurs in the perivascular areas by either autoantibody-mediated or T cell-mediated mechanisms, disturbance of glial intercellular communications may extensively propagate along with the fiber tracts. (B) Hypothetical mechanism of huge CNS lesions in demyelinating diseases. Connexin loss may cause a widespread disruption of intercellular communications among glial cells and axons while AQP4 loss exacerbates vasogenic edema associated with inflammation. AQP4 and connexin astrocytopathy culminating in huge CNS lesions occurs in Baló's disease, and could also develop in a fraction of MS and NMO patients (light green-colored areas in Fig. 1). AQP4 = aquaporin-4; CNS = central nervous system; Cx = connexin; NMO = neuromyelitis optica.