A Prominent Elevation of Glial Fibrillary Acidic Protein in the Cerebrospinal Fluid during Relapse in Neuromyelitis Optica

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Neuromyelitis optica (NMO) is a neurologic disease characterized by severe optic neuritis, longitudinally extended, transverse myelitis and serum aquaporin-4 (AQP4) antibody. Our recent neuropathological study revealed the extensive loss of AQP4 and glial fibrillary acidic protein (GFAP), an astrocyte-specific protein, in NMO lesions, but not in MS lesions, suggesting that severe astrocytic damage or dysfunction may be related to the pathogenesis of NMO. Here we report a patient of NMO, in which the cerebrospinal fluid (CSF) levels of GFAP were measured both during relapse of myelitis and after high-dose intravenous methylprednisolone (HIMP). The patient was a 34-year old woman with two previous episodes of optic neuritis. She developed myelitis longitudinally extending from C3 to T12 with contrast enhancement, and was AQP4 antibody-positive. In the acute phase, the GFAP level in the cerebrospinal fluid (CSF) was prominently elevated (18,966.7 ng/ml) as compared with controls (0.6 ± 0.33 ng/ml). However, following HIMP, the clinical and MRI findings improved, and the CSF-GFAP level was near-normal (2.1 ng/ml). The CSF of myelin basic protein was also elevated in relapse (1,016.0 pg/ml), and became lower but still remained high (158.7 pg/ml) after HIMP compared with controls (3.36 ± 3.83 pg/ml). The prominent elevation of the CSF-GFAP level in relapse of NMO, followed by its sharp decline after therapy, suggests severe astrocytic damage with a temporal profile distinct from that of the demyelinating process in NMO. CSF-GFAP may be useful as a biomarker of NMO.

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negative oligoclonal IgG bands, and longitudinally extensive myelitis preferentially involving the central gray matter (Mandler et al. 1993; O’Riordan et al. 1996; Wingerchuk et al. 1999; Misu et al. 2002; Nakamura et al. 2008).

Moreover, a serum autoantibody (NMO-IgG) exclusively detected in NMO and its high-risk syndrome was discovered (Lennon et al. 2004), and later the target antigen of NMO-IgG was identified as aquaporin-4 (AQP4) (Lennon et al. 2005). AQP4 is a predominant water channel protein in the central nervous system, and is richly expressed in astrocytes, especially on the foot processes (Jung et al. 1994). AQP4 antibody is negative in MS, and thus this autoantibody is a useful laboratory test for the differential diagnosis of NMO (Takahashi et al. 2007).

In addition to the diagnostic value of AQP4 antibody, evidence that AQP4 is targeted in NMO was recently published, that is, we and a group from the Mayo Clinic reported the extensive loss of AQP4 in the perivascular lesions of NMO with deposition of immunoglobulins and activated complements (Misu et al. 2006; Misu et al. 2007; Roemer et al. 2007). In our report, we also showed that glial fibrillary acidic protein (GFAP), an astrocyte-specific protein, was lost in the areas lacking AQP4-immunoreactivity, suggesting that astrocytes are primarily affected or damaged in NMO (Misu et al. 2007). The GFAP levels in the cerebrospinal fluid (CSF) have been measured in various neurologic diseases (Petzold et al. 2004), and could have pathological implications in NMO.

Here we report a patient of typical NMO in which the CSF-GFAP levels were measured both in relapse and after high-dose intravenous methylprednisolone (HIMP).

**Clinical Findings**

A previously healthy 34-year-old woman experienced acute visual impairment in her right eye in 2004. MRI demonstrated a short tau inversion recovery (STIR)-hyperintense lesion in the right optic nerve with gadolinium (Gd)-enhancement. She was treated with HIMP (1 g/day × 3 days) twice and recovered with mild visual impairment.

Two years later, left optic neuritis developed, but the visual impairment was relieved after HIMP. Serum AQP4 antibody (in an assay using AQP4-transfected human embryonic kidney cells [Takahashi et al. 2006, 2007]) and oligoclonal IgG bands were positive.

At 37 years of age, she had back pain and numbness in both legs. Then she developed urinary retention and had difficulty in walking. Due to these neurological symptoms, she was admitted to a local hospital. On examination, she was paraparetic, and hypesthesia from T4 down was noted. A CSF (CSF1) examination revealed pleocytosis (87 cells/μl, mononuclear cells[M]: polymorphonuclear cells[P] = 78:9), elevated protein concentration (80 mg/dl), and slightly high IgG index (0.73). Spinal cord MRI demonstrated a T2-hyperintense lesion extending from C3 to T12 localized at the center of the cord with Gd-enhancement and cord swelling (Fig. 1A, C). Then she was treated with HIMP (1 g/day × 3 days) twice. After the treatment, her leg muscle power improved, she became able to stand up but couldn’t walk without support. The urinary disturbance was resolved. In accordance with the clinical improvement, the T2-hyperintense lesion on MRI shrank (Fig. 1B, D).

She was then transferred to our hospital. Neurological examination on admission revealed a sensory disturbance from T5 down and moderate spastic paraparesis. Serum AQP4 antibody titers were lower (1024 ×) than that in relapse, and CSF (CSF2) abnormalities were milder (8 cells/μl, protein level 20 mg/dl). We added plasma exchange to hasten the functional recovery. After two months from the onset of myelitis, she was gradually able to walk with a cane and was discharged.

**CSF-GFAP and CSF-myelin basic protein (MBP) levels (Fig. 2)**

The GFAP and MBP levels in CSF obtained both in relapse (CSF1) and after HIMP (CSF2) in the NMO patient were measured using commercially available ELISA kits (A05188, SPIbio, France). CSF samples of five control patients
with non-inflammatory diseases (headache \([n = 3]\), conversion disorder \([n = 1]\), somatoform disorder \([n = 1]\)) were also assayed for GFAP and MBP. The CSF-GFAP level in relapse of NMO (18,966.7 ng/ml) was remarkably higher than the levels in controls (0.6 ± 0.33 ng/ml), but in remission the level was near-normal (2.1 ng/ml). Meanwhile, the CSF-MBP level in relapse of NMO (1,016.0 pg/ml) was also higher than the controls (3.36 ± 3.83 pg/ml), but in remission (158.7 pg/ml) still remained higher than those in controls.

**DISCUSSION**

The present patient had two attacks of unilateral optic neuritis followed by transverse myelitis which was longitudinally extended from C3 to T12 on MRI. Moreover, she was seropositive for AQP4 antibody. Thus, the patient fulfilled the Wingerchuk criteria of NMO (Wingerchuk 2006).

A striking finding in the NMO patient was that the CSF-GFAP level in relapse was extraordinarily elevated (several ten thousand-fold higher than the levels in controls), and rapidly returned to normal after HIMP. In a previous study of the CSF-GFAP levels in patients with various neurologic diseases, mild elevations of CSF-GFAP (324 ± 155 pg/ml; normal control 142 ± 26 pg/ml) were reported in MS, and were considered due to astrogliosis in chronic MS lesions (Rosengren et al. 1995). Meanwhile, quite high levels of CSF-GFAP have been detected in destructive brain diseases including brain trauma (16,678 ± 15,022 pg/ml) and cerebral infarction (680-174,000 pg/ml) (Rosengren et al. 1994; Petzold et al. 2004). Thus, the remarkable elevation of CSF-GFAP in our patient suggests that astrocytes are severely damaged or affected in the transverse myelitis of NMO, which is probably consistent with the extensive loss of GFAP-immunoreactivities in the NMO lesions shown in neuropathological studies.

Fig. 1. Longitudinally extensive myelitis in the patient with neuromyelitis optica. The spinal cord lesion extended from C3 to T12 on the sagittal view of T2-weighted MRI (arrowheads) (A), and was localized in the central portion of the cord on the axial view (T3/4 level) (arrowhead) (C). After two courses of high-dose intravenous methylprednisolone, the lesion apparently shrank (arrowheads) (B, D).
of autopsied cases of NMO (Misu et al. 2006, 2007; Roemer et al. 2007). Since the loss of GFAP and AQP4-immunoreactivities are particularly profound in the perivascular regions deposited with immunoglobulins and activated complements, and since the potential cytotoxic effect of AQP4 antibody and activated complement against AQP4-expressing cells was recently shown in an in vitro study (Hinson et al. 2007), it is highly likely that AQP4 antibody along with activated complement was directly involved in damaging astrocytes in the acute phase of the longitudinally extensive myelitis in our NMO patient.

The fact that the CSF-GFAP level after HIMP therapy for myelitis returned to a near-normal level may imply that the pathological process of severe astrocytic damage was effectively suppressed by HIMP in the present patient. In accordance with the normalized CSF-GFAP level, clinical improvement and shrinkage of the longitudinally extensive spinal cord lesion were observed. It would be of interest to determine whether changes of the CSF-GFAP levels in relapse of NMO reflect the therapeutic efficacy in larger scale studies. In contrast to the rapid reduction of the CSF-GFAP, CSF-MBP level in the NMO patient remained high after HIMP. This result suggests that myelin destruction accompanies astrocytic damage but lasts longer in the acute exacerbation of NMO. A similar continuing demyelinating process has been reported in MS in which CSF-MBP could be detected 4 to 6 weeks after treatment for relapse (Lamers et al. 1998; Ohta et al. 2000). Therefore, we speculate that the prominent elevation of the CSF-GFAP levels in relapse of NMO followed by its sharp decline after HIMP may reflect the severe astrocytic damage whose temporal profile is distinct from that of a demyelinating process, and that astrocytic damage may be essential in the pathogenesis of NMO.

In summary, the present report is the first to demonstrate a prominent elevation of the CSF-GFAP level in the acute phase of longitudinally extensive myelitis in NMO. The results suggest that CSF-GFAP may be useful as a biomarker of the pathological process, especially astrocytic damage, of NMO. Larger scale studies are needed to verify the significance of the elevated CSF-
GFAP level in NMO.

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