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Increased cerebrospinal fluid progranulin correlates with interleukin-6 in the acute phase of neuromyelitis optica spectrum disorder

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ABSTRACT

We examined progranulin (PGRN) levels in cerebrospinal fluid (CSF) samples during the acute phase in 15 patients with neuromyelitis optica spectrum disorders (NMOSD) and compared the results with those from 17 patients with multiple sclerosis (MS), 30 patients with other inflammatory neurological diseases (OIND), and 20 non-inflammatory controls (NIC). CSF PGRN levels of NMOSD patients were significantly higher than those of MS patients and NICs. These levels correlated with CSF interleukin-6 levels, CSF cell counts, CSF protein levels, improvements in the Expanded Disability Status Scale score, and affected total spinal cord lesion length in the NMOSD patients.

1. Introduction

Progranulin (PGRN) is a secreted glycosylated protein with important functions in several processes, including immune responses and cancer growth (Cenik et al., 2012; Eriksen and Mackenzie, 2008; Jian et al., 2013a; Toh et al., 2011). PGRN binds directly to tumor necrosis factor receptors (TNFRs) and disturbs TNFα-TNFR interactions (Liu and Bosch, 2012; Jian et al., 2013b; Tang et al., 2011). PGRN-deficient mice are susceptible to collagen-induced arthritis, and administration of PGRN reverses inflammatory arthritis (Tang et al., 2011). A significant increase in serum PGRN levels has been observed in patients with rheumatoid arthritis and osteoarthritis compared with age-matched healthy controls (Yamamoto et al., 2014). In the central nervous system (CNS) PGRN has neurotrophic effects that enhance neuronal survival and axonal outgrowth (Eriksen and Mackenzie, 2008; Toh et al., 2011). Mutations in the PGRN gene were recently found to be associated with certain forms of frontotemporal lobar degeneration (FTLD) (Baker et al., 2006; Petkau and Leavitt, 2014). It has been reported that PGRN is primarily expressed in neurons and microglia in the CNS (Eriksen and Mackenzie, 2008; Yin et al., 2010). Several studies have shown that, in response to injury or insult, activated microglia upregulate PGRN expression (Moisse et al., 2009; Naphade et al., 2010). Recent reports have demonstrated that PGRN is strongly expressed in macrophages/microglia in active lesions, and by activated microglia in normal-appearing white matter (Vercellino et al., 2011) in the brains of multiple sclerosis (MS) patients. Vercellino et al. also found that PGRN levels in the cerebrospinal fluid (CSF) of MS patients were correspondingly increased in conditions of enhanced macrophage/microglia activation, such as during relapses and in progressive MS. In contrast, another report showed that PGRN levels were not altered in the CSF of patients with MS compared with controls (De Riz et al., 2010).

Neuromyelitis optica spectrum disorders (NMOSD) are inflammatory disorders affecting the CNS. These disorders were previously thought to be closely related to MS but more recently have been demonstrated to represent a distinct clinical and pathophysiologic entity (Sand, 2016). The discovery of a disease-specific serum NMO-IgG antibody

Abbreviations: AQP4, aquaporin-4; CNS, central nervous system; CSF, cerebrospinal fluid; EDSS, Expanded Disability Status Scale; ELISA, enzyme-linked immunosorbent assay; FTLD, frontotemporal lobar degeneration; IL, interleukin; mPSL, methylprednisolone; MRI, magnetic resonance imaging; MS, multiple sclerosis; NIC, non-inflammatory controls; NMOSD, neuromyelitis optica spectrum disorders; OIND, other inflammatory neurological diseases; PBS, phosphate buffered saline; PGRN, progranulin; TNFRs, tumor necrosis factor receptors.

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that selectively binds aquaporin-4 (AQP4) has led to increased understanding of a diverse spectrum of disorders (Lennon et al., 2005). At present, there are no reports examining PGRN levels in NMOSD. In this study, we examined PGRN levels in the CSF of NMOSD patients and compared them with those of patients with MS, other inflammatory neurological diseases (OIND), and non-inflammatory controls (NIC).

2. Patients and methods

2.1. Patients

Fifteen patients with NMOSD were recruited from the Department of Neurology and Geriatrics, Gifu University Graduate School of Medicine from 2005 to 2015. Recruitment was limited to patients who were seropositive for anti-AQP4 antibodies. The diagnosis of NMOSD was made according to the criteria defined by Wingerchuk et al. (2015). This study also enrolled a total of 17 MS patients, 30 OIND patients, and 20 NICs. The diagnosis of MS was confirmed using the 2010 revisions to the McDonald criteria (Polman et al., 2011). The 30 OIND patients had viral meningitis and/or meningoencephalitis including nine patients with herpes simplex encephalitis. The 20 NIC patients consisted of 16 patients with psychosomatic disorders and four patients with migraine. We show the demographic features of the patients in Table 1. This study was approved by the Institutional Review Board of Gifu University Graduate School of Medicine, Gifu City, Japan (28–146).

2.2. Clinical assessment of NMOSD and MS patients

We evaluated the Expanded Disability Status Scale (EDSS) scores of NMOSD patients at the time of CSF sampling during the acute phase and the time of follow-up office visits during the remission phase (10–12 months after CSF sampling). We assessed improvements in EDSS scores (ΔEDSS) defined as follows: EDSS score at the time of CSF sampling − EDSS score at the time of follow-up office visit. There were no relapses during these two timepoints. Data on the affected sites, affected total spinal cord lesion length (length of vertebral body segments), number of relapses prior to sampling, and CSF cell counts and protein levels were acquired from our internal database. All NMOSD patients were treated with high-dose (1 g/day) intravenous methylprednisolone (mPSL) for three consecutive days during the acute phase, followed by oral prednisolone treatment. Oral prednisolone treatment was started at a dose of 1 mg/kg/day and subsequently gradually tapered to maintain the patients in remission at a dose of ≤15 mg/day. In addition to mPSL therapy, three NMOSD patients were treated with immunoadsorption plasmapheresis, two NMOSD patients were treated with plasma exchange, and two NMOSD patients were treated with intravenous immunoglobulin during the acute phase. In addition to low dose prednisolone, one NMOSD patient was treated with azathioprine at a dose of 0.1 g/day during the remission phase. The clinical characteristics of the NMOSD patients are shown in Table 2.

We evaluated the EDSS scores of all 17 MS patients at the time of CSF sampling during the acute phase. Five of the 17 MS patients were assessed at their first episode and the others were assessed during relapse episodes. We also evaluated the EDSS scores at the time of follow-up office visits during the remission phase (10–14 months after CSF sampling) and assessed the ΔEDSS of 14 of the 17 MS patients. All MS patients except one were treated with high-dose intravenous mPSL at a dose of 1 g/day for three consecutive days, followed by oral prednisolone treatment. Oral prednisolone treatment was started at a dose of 1 mg/kg/day and subsequently gradually tapered and discontinued after several months, with only one MS patient maintained on prednisolone (15 mg/day) after that. Regarding disease-modifying therapies, five patients were treated with interferon β and one was treated with fingolimod (0.5 mg/day) during the follow-up period. The clinical characteristics of the MS patients are shown in Table 3.

2.3. Evaluation of magnetic resonance imaging (MRI) findings

We evaluated the affected sites of all NMOSD and MS patients during the acute phase using brain and spine MRI. In NMOSD patients, we also evaluated the affected total spinal cord lesion length on T2-weighted images, including sagittal sections.

2.4. Cerebrospinal fluid sampling

CSF samples were obtained for routine diagnostic evaluations from all 82 patients by atraumatic lumbar puncture. Lumbar puncture was performed using a 21-gauge spinal needle. The samples were collected into tubes, then transferred into polypropylene vials and stored at −30 °C until analysis. All CSF samples from the patients with NMOSD, MS, and OIND were collected during the acute phase. All CSF samples from NMOSD and MS patients were collected before high-dose intravenous mPSL therapy. Four NMOSD and three MS patients had been treated with oral prednisolone before lumbar puncture. One NMOSD and three MS patients had been treated with interferon β before lumbar puncture. One NMOSD patient had been treated with azathioprine (100 mg/day), one MS patient had been treated with mizoribine (50 mg/day), and one MS patient had been treated with fingolimod (0.5 mg/day) before lumbar puncture. On the day before lumbar puncture, one NMOSD patient received mPSL at a dose of 250 mg intravenously. We examined the PGRN levels of CSF samples from all 82 patients. We also examined the IL-6 levels in CSF samples from all MS and NMOSD patients except for one NMOSD patient because the lack of a CSF sample for this patient precluded analysis. IL-6 levels were measured using a commercially available Human IL-6 chemiluminescent enzyme immunoassay kit (Fujirebio, Tokyo, Japan).

2.5. Measurement of progranulin

We determined CSF PGRN levels by sandwich enzyme-linked immunosorbent assay (ELISA) as previously established by Tkaczuk et al. (Serrero et al., 2012). The assay is specific for full-length PGRN but does not recognize processed granulin. The system was designed using a pair of antibodies: anti-PGRN monoclonal and anti-PGRN rabbit antibodies (A&G Pharmaceutical, Inc, Maryland, USA). A total of 100 μl of CSF in 1:2 dilutions (1% skim milk) was applied to each well, which

Table 1
Demographic features and CSF PGRN and IL-6 in all patients.

Characteristic	NMOSD	MS	OIND	NIC
Number of patients	15	17	30	20
Age (years)[a]	47.9 ± 14.6	44.2 ± 12.7	45.6 ± 21.8	35.2 ± 15.7
Sex				
Female	14	13	9	8
Male	1	4	21	12
EDSS score at sampling[b]	6 (3.75 to 8.5)	5 (3.5 to 6.5)	NE	NE
Disease duration at sampling (years)[b]	0.3 (0.14 to 5)	5 (3 to 7)	NE	NE
CSF level[a]				
PGRN (ng/mL)	4.33 ± 5.43	1.15 ± 0.42	2.90 ± 1.46	0.83 ± 0.24
IL-6 (pg/mL)	706 ± 1570	4.65 ± 9.15	NE	NE

[a] Data reported as number or mean ± SD.
[b] Data reported as median (interquartile range). CSF: cerebrospinal fluid, EDSS: Expanded Disability Status Scale, IL-6: interleukin 6, MS: multiple sclerosis, NE: not examined, NMOSD: neuromyelitis optica spectrum disorder, OIND: other inflammatory neurological diseases, NIC: non-inflammatory controls, PGRN: progranulin.