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Short communication

Increase of complement fragment C5a in cerebrospinal fluid during exacerbation of neuromyelitis optica

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ABSTRACT

Complement is thought to play a pivotal role in neuromyelitis optica (NMO) pathogenesis. Anaphylatoxins (C3a, C4a, and C5a), produced in complement activation, have proinflammatory potential, and thereby may play an important role. We measured concentrations of anaphylatoxins in CSF and sera, obtained from patients with NMO (n = 15), multiple sclerosis (MS) (n = 15), and other neurological disease (OND) (n = 12), and evaluated their clinical implications. The CSF–C5a levels were elevated significantly in NMO patients, especially in patients with multiple enhanced lesions on MRI. The CSF–C5a levels correlated with the severity of exacerbation. Our results may provide a rationale for anti-complement therapies of NMO.

1. Introduction

Neuromyelitis optica (NMO) is a neuroinflammatory disease characterized by severe optic neuritis and longitudinally extensive transverse myelitis. An autoantibody against aquaporin-4 (AQP4), a water channel densely expressed on astrocytic endfeet, has been detected exclusively in sera from patients with NMO and its spectrum disorders (Lennon et al., 2004, 2005). Active NMO lesions are characterized by severe astrocytic damage with extensive loss of AQP4 and glial fibrillary acidic protein (GFAP), often accompanied by perivascular deposition of activated complement and immunoglobulin, as well as tissue necrosis, massive edema, and infiltration of inflammatory cells (Lucchinetti et al., 2002; Misu et al., 2007). In vitro studies showed that immunoglobulin derived from AQP4 antibody-positive NMO patients destroyed cultured astrocytes in the presence of complement (Hinson et al., 2007; Kinoshita et al., 2009; Sabater et al., 2009). Furthermore, in vivo studies showed that AQP4 antibody-positive immunoglobulin fractions induced central nervous system (CNS) pathology in rodents similar to that in NMO patients (Bradl et al., 2009; Saadoun et al., 2010). Thus, antibody- and complement-mediated astrocytopathy probably constitutes an important part in the pathogenesis of NMO.

Complement plays a major role in immune surveillance system, and orchestrates innate and adaptive immunity (Manthey et al., 2009; Ricklin et al., 2010). In complement activation, membrane-attack complex (MAC: C5b–C9), a terminal product of the activation process, directly damages cellular membrane; meanwhile, anaphylatoxins (C3a, C4a, and C5a), derived from C3, C4, and C5, do not directly damage cellular membrane, but have such biological effects as enhancing endothelial permeability, potentiating chemotaxis of inflammatory cells, inducing release of reactive oxygen species, cytokines and chemokines from cells, and enhancing phagocytosis (Manthey et al., 2009; Ricklin et al., 2010). Taken together with the neuropathological features of NMO, the activation of complement including anaphylatoxins may contribute to the unique pathogenesis of NMO. In this study, we aimed to evaluate whether anaphylatoxins were increased in cerebrospinal fluid (CSF) and sera during acute exacerbation of NMO and to explore their clinical implications.

2. Materials and methods

We collected CSF and sera from patients with AQP4 antibody-positive NMO in relapse (n = 15: 10 with myelitis, 4 with optic neuritis, and 1 with brain lesions), multiple sclerosis (MS) in relapse (n = 15: 7

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with myelitis, 2 with optic neuritis, and 6 with brain lesions), and other neurological disease (OND) (n = 12: 4 with Parkinson's disease, 4 with spinocerebellar degeneration, 3 with headache, and 1 with conversion disorder). We also collected CSF from two other patients with NMO in remission. Clinical diagnoses were made according to Wingerchuk criteria in 2006 for definite NMO, and McDonald criteria in 2005 for MS. Patients within two weeks after the onset of relapse were enrolled. CSF and serum samples were frozen and stored at −80 °C until measurement. We obtained written informed consent from each patient. This study was approved by the Ethics Committee of Tohoku University School of Medicine.

We measured concentrations of C3a, C4a, and C5a in CSF and sera, using a bead-based immunosorbent assay (Human Anaphylatoxin Kit, BD Biosciences, San Jose, CA, USA) with a flowcytometer (FACSCalibur, BD Biosciences). The assay procedure was according to the manufacturer's instruction. All assays were done by duplicate. When the concentrations of any anaphylatoxin were elevated in CSF/sera from particular patient group, we evaluated the relationship between the anaphylatoxin levels and the clinical characteristics of exacerbation, including site of lesion, longitudinal length of spinal cord lesion, number of enhanced lesions on MRI, the Expanded Disability Status Scale (EDSS) score at the nadir of exacerbation, and the increment of EDSS score during exacerbation (ΔEDSS = the EDSS at the nadir of exacerbation − the EDSS before exacerbation).

Comparisons of values were made by chi-square test or Fisher's exact test on qualitative values, and two-tailed Kruskal–Wallis test or Mann–Whitney test on quantitative values. When the difference was significant by Kruskal–Wallis test, multiple comparisons post hoc analysis were made by Dunn's test. For correlation analysis, we used Spearman's correlation coefficient by rank. Statistical significance was determined by p-value <0.05.

3. Results

Demographic data of patients with NMO, MS, and OND are shown in Table 1. Significant differences among the groups were detected in gender and CSF cell counts. By post hoc analysis, the differences in CSF cell counts were detected between NMO and OND ($p<0.01$) and between MS and OND ($p<0.05$). Significant differences between NMO and MS were apparent in the prevalence of longitudinally extended spinal cord lesion (LESCL: spinal cord lesion >3 vertebral segments) ($p<0.001$), and serum AQP4-antibody positivity ($p<0.0001$). There were no significant differences among the groups in serum-C3a [median (range), ng/ml; NMO: 46.0 (27.4–54.4), MS: 36.1 (15.5–66.4), and OND: 33.5 (24.5–55.9)], serum-C4a [median (range), ng/ml; NMO: 27.8 (25.2–44.4), MS: 24.6 (22.4–33.1), and OND: 31.3 (23.1–40.9)], or serum-C5a [median (range), ng/ml; NMO: 43.5 (31.2–65.5), MS: 48.2 (34.4–61.8), and OND: 47.0 (38.1–67.9)] (Table 2). A significant difference was detected in CSF–C5a [median (range), pg/ml; NMO: 188 (29.5–1000), MS: 168 (41–516), and OND: 61.2 (25.9–190)] ($p<0.05$), and the difference was still significant after correction by CSF protein concentrations. By post-hoc analysis, the difference was significant between NMO and OND ($p<0.05$). No significant differences were apparent among the groups in CSF–C3a [median (range), pg/ml; NMO: 1700 (740–2320), MS: 1550 (760–2160), and OND: 1780 (1580–2500)], or CSF–C4a [median (range), pg/ml; NMO: 1720 (1530–2060), MS: 1670 (1290–2160), and OND: 1690 (1320–1980)]. In two patients with NMO in remission, CSF–C3a [1684 (1523, 1844)], CSF–C4a [1739 (1715, 1762)], and CSF–C5a [125 (116, 133)] were not different from those in OND (Fig. 1). In NMO patients, the CSF–C5a concentrations were increased significantly in patients with multiple enhanced lesions on MRI than those in patients with single or no enhanced lesion ($p<0.001$). The CSF–C5a levels correlated with the number of enhanced lesions ($r=0.524$, $p=0.045$) (Fig. 2). The CSF–C5a levels did not correlate with the peak EDSS scores during the exacerbation ($r=0.502$, $p=0.057$), but correlated with the ΔEDSS ($r=0.592$, $p=0.020$) (Fig. 3).

Table 1
Demographic data of patients with NMO, MS, and OND.

	NMO	MS	OND	Group differences
No. of patients	15	15	12	
Female/male	15/0	11/4	7/5	$p<0.05$
Age, year	48 (17–78)	35 (18–54)	57 (13–77)	N.S.
CSF cell counts,/mm^3	4.0 (0–207)	3.0 (0–10)	0 (0–3)	$p<0.001$
CSF protein, mg/dl	32 (23–142)	25 (21–42)	29 (18–32)	N.S.
Myelitis	10 (67%)	7 (47%)	N.A.	†N.S.
Optic neuritis	4 (27%)	2 (13%)	N.A.	†N.S.
LESCL in myelitis	9 (90%)	0 (0%)	N.A.	†$p<0.001$
EDSS	3.5 (2.0–8.5)	3.0 (1.0–6.5)	N.A.	†N.S.
Serum AQP4-Ab positive	15 (100%)	0 (0%)	N.A.	†$p<0.0001$

Values are presented as median (range).
N.A. = not applicable; N.S. = not significant.
† Difference between NMO and MS.

4. Discussion

The present study showed that the CSF–C5a concentrations were elevated significantly in patients with NMO during acute exacerbation, and the concentrations correlated with the number of enhanced lesions and with the increment of EDSS score during the relapse; these results suggest that the CSF–C5a elevation in NMO correlates with the severity of the attack.

Heretofore, it has been reported that serum CH50 and complement derivatives were elevated in NMO patients (Doi et al., 2008; Tuzun et al., 2011). These reports suggested that systemic activation of complement occurred in NMO, similarly to other systemic diseases such as systemic lupus erythematosus (SLE) and vasculitis (Smith, 1995; Spronk et al., 1995), but did not show directly the activation in CNS. Meanwhile, our results suggested that the complement activation occurs in CNS during exacerbation of NMO. The CSF–C3a, –C4a, and –C5a levels in remission of two cases of NMO were not different from those in OND, although the data in both relapse and remission in the same NMO patients were not available. We also showed that the increase of CSF–C5a in relapse of NMO was not significant as compared with that in MS, because the CSF–C5a levels were elevated in a fraction of MS patients. It may be explained by the fact that the antibody- and complement-mediated pathology is also observed in MS (Lucchinetti et al., 2000).

In general, the complement activation occurs via such four pathways as the classical, the alternative, the mannan-binding lectin, and the extrinsic protease pathways. Through these pathways, cleavage of C5 into C5a, the most potent and stable in anaphylatoxins, and C5b, an initial element forming MAC, by C5 convertase is a final step of the complement activation. Therefore, C5–C5a axis is a key of the complement activation in all pathways. For example, the stimulation of C5a enhances the release of superoxide and granule enzymes in neutrophils, phagocytosis in macrophages, chemotaxis in eosinophils, co-stimulation in T lymphocytes, and vasodilation and adhesion molecule expression in endothelial cells (Manthey et al., 2009). Thus, in CNS as well as other tissues, the increase of C5a and other anaphylatoxins probably accelerates fluid leakage through the blood vessels, extravasation of immunoglobulin and complement, and migration of inflammatory cells. Furthermore,

Table 2
Serum-C3a, C4a, and C5a concentrations in patients with NMO, MS, and OND.

	NMO	MS	OND	Group differences
C3a (ng/ml)	46.0 (27.5–54.4)	36.2 (13.2–66.4)	33.5 (24.3–75.0)	N.S.
C4a (ng/ml)	27.8 (25.2–44.4)	24.6 (22.5–33.2)	31.3 (23.1–40.9)	N.S.
C5a (ng/ml)	43.5 (31.2–65.5)	48.2 (34.5–61.8)	47.0 (34.5–67.9)	N.S.

Values are presented as median (range).
N.S. = not significant.