Increased Osteopontin Levels in the Cerebrospinal Fluid of Patients With Multiple Sclerosis

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Objective: To determine cerebrospinal fluid levels of osteopontin (OPN), a proinflammatory cytokine that was found to be overexpressed in multiple sclerosis lesions and increased in plasma during relapses and in secondary progressive multiple sclerosis.

Design: Case series. Osteopontin, interleukin 12p40 (IL-12p40), IL-10, and matrix metalloproteinase 9 were measured by enzyme-linked immunosorbent assay by an investigator unaware of the patients' diagnoses.

Patients: Consecutive patients with multiple sclerosis (n=27), or other inflammatory (n=11) or non-inflammatory (n=23) neurological diseases, undergoing lumbar puncture, were investigated.

Results: Osteopontin was significantly elevated in the cerebrospinal fluid of patients with multiple sclerosis (mean [SD], 415 [186] ng/mL) and other inflammatory diseases (563 [411] ng/mL) compared with those with noninflammatory neurological diseases (286 [150] ng/mL). Cerebrospinal fluid OPN levels were slightly higher than plasma OPN levels. Cerebrospinal fluid OPN levels positively correlated with the ability to detect cerebrospinal fluid IL-12p40.

Conclusion: Osteopontin in the cerebrospinal fluid may be, in part, of central nervous system origin, and may play an important role in central nervous system inflammation.

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Cerebrospinal fluid was collected at the time of elective lumbar puncture. Multiple sclerosis patients were not in relapse and systemic lupus erythematosus. Twenty-four patients had noninflammatory neurological diseases (NIND) (18 women, 6 men; mean [SD] age, 42.1 [18.7] years; range, 17-74 years), comprising 11 patients with idiopathic intracranial hypertension, 4 with cerebrovascular disease, 2 with tension headaches, 2 with motor neuron disease, and 1 each with progressive ophthalmoplegia, complex partial seizures, nonepileptic seizures, dystonia, and normal-pressure hydrocephalus. The Nottingham Research Ethics Committee approved the study. All patients gave informed consent. In MS patients, disability was scored using Kurtzke’s expanded disability status scale.10

MEASUREMENT OF OPN, IL-12p40, IL-10, AND MATRIX METALLOPROTEASE 9

Cerebrospinal fluid and plasma were collected simultaneously, aliquoted, and stored immediately at -80°C until use. Osteopontin was measured in the plasma and CSF by sandwich enzyme-linked immunosorbent assay (ELISA) (Assay Designs, Ann Arbor, Michigan; sensitivity, 3.5 ng/mL). We measured CSF matrix metalloproteinase 9 (MMP-9) (R+D Systems, Abingdon, England; sensitivity, 0.156 pg/mL), IL-10 (R+D Systems, Abingdon, England; sensitivity, 0.5 pg/mL), and IL-12p40 (Diaclone, Boldon, England; sensitivity, 20 pg/mL), all by ELISA, following the manufacturer’s instructions. Cerebrospinal fluid was collected at the time of elective lumbar puncture. Multiple sclerosis patients were not in relapse or receiving disease-modifying treatment at the time of sample collection.

STATISTICAL ANALYSIS

Cytokine levels were compared between groups using the Mann-Whitney U test. Correlations were explored using the Pearson coefficient.

RESULTS

The mean (SD) levels of OPN in plasma were 380 (236) ng/mL in the MS group, 386 (271) ng/mL in the OIND group, and 377 (121) ng/mL in the NIND group. Differences were not statistically significant. In the MS patients, the mean (SD) level of disability, using Kurtzke’s expanded disability status scale, was 2.8 (1.6) (range, 1-6.5).

In the relapsing-remitting MS group, the mean (SD) interval between the last clinical relapse and sample collection was 5.8 (7) months.

Multiple sclerosis patients had a mean (SD) CSF OPN level of 415 (186) ng/mL, similar to OIND patients (563 [411] ng/mL) (difference not statistically significant; \( P = .5 \)). Both were higher than those of the NIND patients (286 [150] ng/mL) \( (P = .02 \) for patients with MS; \( P = .02 \) for patients with OIND) (Figure). Patients with MS and patients with OIND together had higher OPN levels than the NIND control group (457 [271] ng/mL vs 289 [146] ng/mL; \( P = .008 \)).

Levels of CSF IL-12p40 were detectable in 15 patients, of whom 8 had MS, 4 had OIND (1 with sarcoidosis, 1 with multifocal motor neuropathy, 2 with polyradiculoneuropathy), and 3 had NIND (motor neuron disease, tension headache, or benign intracranial hypertension). Levels varied between 0.1 and 985 pg/mL (mean [SD], 71.8 [244] pg/mL), with the outlier of 985 pg/mL in a patient with neurosarcoidosis. Without this outlier, the mean (SD) IL-12p40 level was 10.9 (13) pg/mL. The median IL-12p40 level was 6.4 pg/mL. Levels of CSF OPN were higher in patients with detectable IL-12p40 than in those with undetectable IL-12p40 (mean [SD], 416 [184] pg/mL vs 235 [169] pg/mL; \( P = .02 \)).

Cerebrospinal fluid IL-10 was detectable in 15 patients (mean [SD], 3.6 [2.9] pg/mL; range, 0.02-7.9 pg/mL) of whom 8 had MS (3 with simultaneously detectable IL-12p40; all with high IL-12p40 and low IL-10). Osteopontin levels did not correlate with either IL-10 levels or IL-10 detection. Levels of MMP-9 were below the ELISA detection limit in all samples.

There was no correlation between OPN levels in CSF and plasma. Levels of CSF OPN appeared consistently higher than plasma levels, although differences were not statistically significant (\( P = .1 \)). There was no correlation between the CSF OPN levels and MS type, though with 1 secondary progressive MS patient, analysis for secondary progressive MS was not possible. There was no correlation between OPN levels and Kurtzke’s expanded disability status scale or the interval since the last relapse.

COMMENT

We found significantly elevated levels of OPN in the CSF of patients with MS and OIND compared with those with NIND. Levels of OPN were slightly higher in MS CSF than in plasma, suggesting a component of CSF OPN was contributed to by central nervous system (CNS)–derived OPN. This is consistent with the ability of CNS cells to express OPN,11 and with the finding of high OPN levels in MS patients’ brains.3

The fact that OPN levels were increased in the CSF of relapsing-remitting MS patients who were in clinical remission suggests continuous upregulation of OPN in the CNS of patients with MS, regardless of clinical disease activity. The same pattern of upregulation was found for MMP-9 in the CSF of patients with MS, suggesting the destructive proteolytic process is continuous.12 Interestingly, OPN activates pro–MMP-913 and its conver-
Osteopontin is related to T helper 1–type responses. We therefore explored the correlation between OPN and IL-12p40 levels. Only 15 patients (8 with MS) had detectable IL-12p40 levels, probably reflecting the fact that our patients were in clinical remission, and consistent with previous findings associating CSF IL-12p40 detection with radioactively active disease. Similarly, only 8 MS patients had detectable levels of CSF IL-10, making correlation analysis difficult.

Osteopontin was increased in the CSF of OIND patients, indicating that OPN upregulation is not MS-specific but is inflammation-specific. The higher levels seen in CSF compared with plasma also suggest a contribution from CNS OPN. In these studies, we did not have the opportunity to compare CSF OPN levels between clinical relapse and remission. However, although previous studies showed no significant difference in plasma OPN levels between MS and healthy participants, we detected differences in CSF OPN levels between stable MS patients and participants in the control group. The majority of our patients had a mild to moderate disability; this, together with CSF OPN being increased regardless of the clinical disease activity, further suggests that OPN contributes continuously, and possibly from early stages, to MS pathology.

Osteopontin has been implicated in remyelination after experimental demyelination. Osteopontin was significantly upregulated in both the demyelination and remyelinating phases, suggesting a dual role. A positive OPN effect in remyelination was suggested by its ability to stimulate oligodendrocytes. Whether this dual OPN role is also played in MS, where demyelination and remyelinating lesions coexist, remains to be established. Our data, including the correlation of CSF OPN levels with the detection of proinflammatory IL-12p40, but not of antiinflammatory IL-10, and recent observations in EAE, however, strongly suggest that in CNS inflammatory demyelination, OPN net effect is detrimental.

In conclusion, we found elevated OPN levels in the CSF of patients with MS and OIND which tend to exceed plasma levels, suggesting that the sources of OPN in these conditions include a contribution from CNS resident or infiltrating cells.

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