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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PROINFLAMMATORY CYTOKINES AND T CELLS REACTIVE AGAINST MYELIN PROTEINS PLAY AN IMPORTANT ROLE IN THE PATHOGENESIS OF MULTIPLE SCLEROSIS (MS) AND ITS EXPERIMENTAL MODEL, EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS (EAE).1,2

Osteopontin (OPN) is a phosphoprotein containing an arginine-glycine-aspartate integrin binding motif, with important roles in inflammation and immunity to infection.3 Osteopontin enhances interferon γ and interleukin 12 (IL-12), proinflammatory cytokines implicated in MS, and diminishes IL-10, a cytokine protective in MS and other inflammatory diseases. Osteopontin increases myelin-reactive T cell survival.4

Osteopontin is highly upregulated in the brains of persons with MS,5,6 and in the spinal cords of mice with EAE.3 In OPN-deficient mice, EAE shows a greater number of remissions, less progression, and higher IL-10 levels.7 Moreover, OPN induces relapses and progression of EAE.4

Osteopontin levels are higher in the serum of MS patients during relapse than during remission; however, no differences in OPN levels were found between patients with relapsing-remitting MS and control group participants.7,8 Plasma OPN levels are higher in patients with secondary progressive MS than in healthy controls.9

We measured OPN levels in the cerebrospinal fluid (CSF) of MS patients and compared them with the levels in patients with other inflammatory and non-inflammatory neurological diseases.

METHODS

PATIENTS

Twenty-seven patients (17 women, 10 men; mean [SD] age, 42.1 [10.1] years; range, 23-62 years) with MS were investigated. Twenty-two had relapsing-remitting MS, 4 primary progressive MS, and 1 secondary progressive MS. We also investigated 11 patients (7 men, 4 women; mean [SD] age, 50.5 [15.8] years; range, 22-79 years) with other inflammatory neurological diseases (OIND), including 3 with chronic inflammatory demyelinating polyradiculoneuropathy, 3 with neurosarcoidosis, 2 with acute disseminating encephalomyelitis, and 1 each with ankylosing spondylitis with C1 transverse myelitis, multifocal motor neuroloneuropathy, and chronic inflammatory demyelinating polyradiculoneuropathy.
Osteopontin levels in the cerebrospinal fluid of the 3 patient groups. OPN indicates osteopontin; MS, multiple sclerosis; NIND, noninflammatory neurological diseases; and OIND, other inflammatory neurological diseases.

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, and with the finding of high OPN levels in MS patients’ brains.

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. Interestingly, OPN activates pro–MMP-9 and its conver-
sion to an active enzyme. Therefore, the similar patterns in the CSF of patients with MS may be explained by OPN activating the destructive effects of MMP-9. This hypothesis remains plausible despite the fact that, in our samples, levels of MMP-9 were below the ELISA detection limit. In a number of CSF samples from patients of Leppert et al.,12 MMP-9 was undetectable by ELISA but detectable by zymography; however, we had insufficient CSF to conduct zymographies.

Osteopontin is related to T helper 1–response types. 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 radiologically active disease.14 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,7 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.15 A positive OPN effect in remyelination was suggested by its ability to stimulate oligodendrocytes.15 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,3 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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