The neural cell adhesion molecule (NCAM) present in the cerebrospinal fluid of multiple sclerosis patients is unsialylated

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We wish to dedicate this paper to prof. Rita Levi Montalcini, pioneer in neuro-regeneration studies, for her hundredth birthday

Abstract. – The neural cell adhesion molecule (NCAM) is a glycoprotein localised in the plasma membrane of neural and glial cells, which plays a role in myelination and remyelination. It increases in the cerebrospinal fluid (CSF) of acute multiple sclerosis (MS) patients treated with corticosteroids who are improving after an attack, but it has not been shown if it appears in its sialylated (PSA) or unsialylated form. We studied the NCAM and the PSA-NCAM in serum and CSF samples of 16 acute and non-acute MS patients and of 10 non-neurological controls. The NCAM and the PSA-NCAM were dosed by two different ELISA previously set-up.

The NCAM in the serum and in the CSF of the control group presented mean levels similar to those shown in previous papers: 1620 ± 216 and 970 ± 210 ng/ml. In the MS patient group the means were 1700 ± 546 in the sera and 926 ± 285 in the CSFs. All the sera were PSA-NCAM-positive: the mean PSA-NCAM concentration in the control group was 3150 ± 950 ng/ml, while in the MS patient group it was 3570 ± 905 ng/ml. The correlation between serum levels of NCAM and PSA-NCAM was highly significant (p<0.001). Student’s “t” test did not show any significant difference between serum levels of the two groups, both for the NCAM and for the PSA-NCAM.

CSF samples did not show any positive results for the PSA-NCAM, in either controls or in MS patients.

These results demonstrate that the high levels of NCAM we previously found in the CSF of improving MS patients treated with steroids did not contain a quota of PSA-NCAM, but only the unsialylated soluble form of the molecule.

Key Words:
Multiple Sclerosis, MS, CSF, NCAM, PSA-NCAM, Demyelination, Remyelination, Astrocyte.

Introduction

The neural cell adhesion molecule (NCAM) is a glycoprotein localised in the plasma membrane of neural and glial cells. It belongs to the immunoglobulin gene superfamily and presents different isoforms resulting from alternative mRNA splicing as the products of a single gene. These isoforms, of 180, 140 and 120 kD, are structurally distinct and have different important roles in the central nervous system (CNS): interneuronal and glia-neuronal homophilic adhesion phenomena, cell-cell recognition, development of the nervous system, synaptic plasticity, memory and learning, post-injury regeneration.1

The NCAM increases in the cerebrospinal fluid (CSF) of acute multiple sclerosis (MS) patients treated with corticosteroids who are improving after an attack2,3. It has been shown that this is a consequence of phenomena specifically occurring within the CNS, not linked to high levels of NCAM in sera nor to a leakage through a disrupted blood-brain barrier (BBB)3,4. The inhibition ELISA we previously used4 was unable to detect whether the NCAM found in the CSF was the unsialylated form of the molecule or one of the polysialylated isoforms (PSA-NCAM) or their fragments. In order to further elucidate this problem, the CSF samples of acute MS patients were tested with a method capable of detecting the sialic acid portion of the PSA-NCAM. Further, to localize the possible presence of the PSA antigen within the plaque, we prepared histological sections of acute plaques from the brain of a patient dead for MS, immunostaining the slices with an anti-PSA antibody.
Patients and Methods

Serum and CSF samples of 7 acute and 9 non-acute MS patients and of 10 non-neurological controls were studied. Specimens of acute MS patients were collected before and after steroid treatment, while specimens from non-neurological controls (patients who received a lumbar puncture for surgical reasons) and from non-acute MS patients were collected only once. NCAM was tested according to the method previously set up and described\(^2\): an inhibition ELISA which uses solubilized human brain membranes adsorbed to polystyrene microtest plates. The anti-NCAM antibodies were produced by the Protein Laboratory of the University of Copenhagen, Denmark, and were raised against the whole NCAM molecule. PSA-NCAM was tested by an ELISA assay previously described by Figarella-Branger et al\(^5\). In this method the immunocapture of PSA-bearing molecules is first effected by means of a monoclonal antibody (anti-MenB)\(^6\), directed against sialic acid polymers, which is adsorbed into plastic wells. Linked PSA-NCAM is then revealed by means of a secondary antibody, directed against an aminooacidic sequence of NCAM, labelled with peroxydase\(^5\). The anti-MenB antibody was purchased from AbCys SA (Paris, France), and produced according to Rougon et al\(^6\). It is a mouse monoclonal IgM raised against the capsula of Meningococcus-B, which contains the polymer PSA\(^6\). The immunostaining for detecting the PSA portion of the NCAM on MS plaques was done using the same anti-MenB antibody, specific for the PSA portion\(^6\), used for the ELISA assay. Formalin-fixed and paraffin-embedded sections of white matter with acute plaques of demyelination were tested with this antibody. The final staining of the sections was performed by avidin-biotin complex (ABC) procedure and with diaminobenzidine.

Results

The NCAM in the serum and in the CSF of the control group presented mean levels similar to those shown in previous papers: 1620 ± 216 and 970 ± 210 ng/ml\(^3\). In the MS patients group the means were 1700 ± 546 in the sera and 926 ± 285 in the CSFs. All the sera were PSA-NCAM-positive: the mean PSA-NCAM concentration in the control group was 3150 ± 950 ng/ml, while in the MS patients group it was 3570 ± 905 ng/ml. The correlation between serum levels of NCAM and PSA-NCAM was highly significant (\(p<0.001\)) (Figure 1). Student’s “t” test did not show any significant difference between serum levels of the two groups, both for the NCAM and for the PSA-NCAM.

CSF samples did not show any positive results for the PSA-NCAM, in either controls or in MS patients. At the microscopic examination, the apparently normal white matter resulted negative for the PSA-NCAM antigen, while the astrocytes within the plaque were PSA-NCAM-positive (Figure 2). The macrophages were PSA-NCAM-negative. Few smaller PSA-NCAM-positive reactive astrocytes were detected in the not yet demyelinated white matter surrounding the plaque.

![Figure 1](image_url). Correlation: A, PSA-NCAM vs. NCAM in serum of normal controls. B, PSA-NCAM vs. NCAM in serum of MS patients.
Conclusions

Re-expression of the PSA-NCAM on reactive astrocytes in the brain of animals experimentally injured by kainic acid was reported by other investigators\(^7\). This could indicate that the presence of NCAM in the cytosol of reactive astrocytes might be a general feature of astrocyte response to various injuries to the CNS, not necessarily linked to demyelination or to reparative mechanisms which follow demyelination. On the other hand, this possibility cannot be completely excluded, and a possible release in the CSF of a sialylated form of NCAM could be expected. The data of the present study show that PSA-NCAM is not present in the CSF of MS patients, independently of whether in the acute or non-acute phase, if treated or non-treated. This implies that the high levels of NCAM we previously found in the CSF of improving MS patients treated with steroids\(^1,2,8\) did not contain a quota of PSA-NCAM, but only the unsialylated soluble form of the molecule. We could speculate about the origin of such unsialylated molecule: a possible catabolic process through the macrophages has been excluded, being these cells clearly PSA-NCAM-negative in our immunohistologic study. The release by the reactive astrocytes present in the acute plaques remains an open possibility, but in this case a mechanism of desialylation of the molecule within the astrocytes, preceding the release, has to be hypothesized.

References

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Acknowledgements

This paper received partial financial support by Associazione Patologie Autoimmuni Internazionale (APAI), Italy.