Intrathecal sICAM-1 production in multiple sclerosis
Correlation with triple dose Gd-DTPA MRI enhancement and IgG index

Abstract

In this study the aim was to evaluate the intrathecal sICAM-1 production in multiple sclerosis (MS) patients during relapse and remission. In addition to this, we assessed whether there is a correlation between intrathecal sICAM-1 production and other disease activity markers such as IgG index and gadolinium enhancement in magnetic resonance imaging (MRI). Twenty four relapsing-remitting MS patients were included in the study. Serum and cerebrospinal fluid (CSF) samples were obtained both during relapse and remission. The soluble form of ICAM (sICAM) was measured by the ELISA method in serum and CSF. Cranial MRI with triple dose gadolinium injection was performed for each patient both during relapse and remission. Serum levels of sICAM-1 (245.23 ± 92.88 ng/ml) were higher during relapse than those in remission (219.90 ± 110.94 ng/ml), but the difference was not statistically significant. In relapse periods patients had significantly higher sICAM-1 index values (1.76 ± 0.60) than those found during remission periods (1.01 ± 0.44) (p < 0.05). The IgG index values were higher in relapse periods than in remission (0.88 ± 0.37 vs. 0.67 ± 0.28) (p < 0.005). On T1 weighted images following triple dose Gd injection, at least two or more enhancing lesions were present in 22/24 of the patients (91%) in relapse and 4/24 of the patients (19%) in remission. There was strong correlation both between the sICAM-1 index and Gd enhancement (r = 0.72 p < 0.05) and sICAM-1 index and IgG index in relapse (r = 0.69 p < 0.05). In conclusion, there is association between high sICAM-1 and IgG indices, as well as between high sICAM-1 index and Gd enhancing MRI lesions in relapsing MS patients.

Key words multiple sclerosis · sICAM · IgG index · triple dose gadolinium · MRI

Introduction

Multiple sclerosis (MS) is an autoimmune, inflammatory demyelinating disease localized the central nervous system (CNS). Although the entire pathogenetic mechanisms are still obscure, the main pathological event is the adhesion and transmigration predominantly of lymphocytes and macrophages. The recruitment of these cells across the blood brain barrier depends on complex leukocyte-endothelial cell interactions. Surface proteins expressed on leukocyte membranes interact...
with their respective ligands located on the endothelium. Selectins located on leucocytes bind the carbohydrate ligands and capture lymphocytes from the circulation by causing them to roll along the activated endothelial cells. Under the influence of chemokines, integrins provide an adhesive force on lymphocytes and arrest them before diapedesis. In this process, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) are very important proteins that recognize integrin ligands, leukocyte function associated antigen-1 (LFA-1) and very late antigen-4 (VLA-4) [3]. The expression of membrane-bound ICAM-1 is upregulated by proinflammatory cytokines such as IFN-γ and TNF-α in various conditions, including MS [1, 18]. The circulating soluble form of ICAM-1 (sICAM-1) can normally be measured in the serum. However, raised serum sICAM-1 values can be detected in the acute phase of a relapse [6, 7, 11, 12, 14, 15, 17]. Therefore, the circulating level of sICAM-1 is considered to be an in vivo measure of disease activity [4].

The aim of this study was to investigate the intrathecal sICAM-1 production in MS patients during relapse and remission. The secondary aim was to assess whether a correlation exists between intrathecal sICAM-1 production and other disease activity markers such as IgG index and gadolinium enhancement in magnetic resonance imaging (MRI).

Materials and methods

- Patients

Twenty-four (15 female, 9 male) definite relapsing-remitting MS (RRMS) [according to McDonald’s criteria [10]] patients having had two or more relapses in the last two years were included in the study. All patients were judged to have a relapse on admission according to objective criteria; the appearance of new symptoms or worsening of old ones for at least 24 hours. Also the patients were not users of any immuno-suppressive or immunomodulator drug. All of the subjects were informed about the study protocol and gave their consent. This study was approved by the local ethics committee.

- Clinical evaluation and collection of the material

MS patients were admitted within 2–14 (mean 4.23 ± 10.65) days from the beginning of the symptoms. The neurological examination was scored according to the Expanded Disability Status Scale (EDSS) [9]. After collecting CSF (5 ml) and serum samples (5 ml) by lumbar and venipuncture on the day of admission, the treatment protocol for the presenting relapse was started with 1 g/day i.v. methylprednisolone for five days and the oral steroid dose was tapered by 10 mg reduction every 5 days. Three months later when the patients were in remission not only neurological evaluation, but also lumbar and venipunctures were repeated.

- Biochemical analysis

Assay of sICAM-1

Serum and matched CSF samples were separated and stored at −70°C. Repeated freeze-thaw cycles were avoided. Levels of sICAM-1 were measured by sandwich enzyme-linked immunosorbent assay (ELISA), according to the instructions of the manufacturer (Biosource International, Inc. California, U. S. A.). All of the samples were assayed blind. Cerebrospinal fluid samples were used undiluted and sera were diluted with 1:100 diluents buffer provided with the product. Absorbencies were read with an Organon Teknika reader 2305 at 450 nm (reference filter 620 nm).

The sICAM-1 index was calculated according to the formulation below, which is similar to the IgG index formulation. sICAM-1 index = sICAM-1 CSF: sICAM-1 Serum/Albumin CSF:Albumin Serum.

Immunoglobulin G index

Albumin and IgG were measured by automated immunoprecipitation nephelometry. The IgG index was calculated according to the standard formula and the values greater than or equal to 0.7 were considered to reflect abnormal intrathecal IgG synthesis which is a marker of disease activity in MS.

- Magnetic resonance imaging (MRI)

Brain MRI was performed on MS patients on the day of admission during a relapse period and 3 months later during remission. A Siemens Magnetom 42SP with 1 Tesla magnetic field was used to perform T2 weighted axial and sagittal sections with fluid attenuated inversion recovery technique and T1 weighted axial and sagittal sections after i.v. gadolinium (Gd) injection. The slice thickness was 5 mm with a 2.5 mm gap between slices, field of view was 230 mm and 224x256. In order to increase the sensitivity of MRI imaging the demyelinated plaques, triple dose (0.3 mmol/kg) of Gd was administered. The hyperintense lesions in T2 weighted images and contrast enhancing (active) lesions in T1 images following a triple dose Gd injection were counted for each patient during relapse and remission phases.

- Statistical analysis

The non-parametric Wilcoxon signed ranks test was applied in order to analyse the difference between the values obtained in relapse and remission phase. The Pearson correlation test was used to determine the relation between the sICAM-1 index, Ig G index and Gd enhancement.

Results

The mean age of the patients was 30.28 ± 8.43 years and the mean disease duration was 5.09 ± 6.10 years. Mean relapse number was 2.23 ± 0.83 in the last 2 years. Serum levels of sICAM-1 (245.23 ± 92.88 ng/ml) were higher during relapse than those in remission (219.90 ± 110.94 ng/ml), but the difference was not statistically significant (Fig. 1). Likewise, in relapse periods CSF levels of sICAM-1 (1.304 ± 0.92 ng/ml) were higher than those in remission (1.06 ± 0.86 ng/ml), but not significantly (Fig. 2). However, during relapse periods the patients had significantly higher sICAM-1 index values (1.76 ± 0.60) than those found during remission periods.