Adhesion Molecules in Multiple Sclerosis
Relation to Subtypes of Disease and Methylprednisolone Therapy

Irina Elovaara, MD, PhD; Maritta Ukkonen, MD; Minna Leppäkynnas, MD; Terho Lehtimaki, MD, PhD; Mari Luomala, MSc; Jukka Peltola, MD; Prasun Dastidar, MD

Objectives: To determine levels of adhesion molecules in blood and cerebrospinal fluid (CSF) samples from patients with different subtypes and activities of multiple sclerosis (MS) and to assess the effect of intravenous methylprednisolone sodium succinate treatment on the levels of soluble adhesion molecules.

Design: The expressions of very late activation antigen 4 (VLA-4), lymphocyte function associated antigen 1 (LFA-1), vascular cell adhesion molecule 1 (VCAM-1), and intercellular adhesion molecule 1 (ICAM-1) were determined immunocytochemically, and levels of soluble VCAM-1, ICAM-1, and E-selectin, by means of enzyme immunoassay technique. The volumes of T2- and T1-weighted MS plaques and brain atrophy were determined by means of the semi-automatic magnetic resonance imaging (MRI) segmentation technique.

Setting: A university hospital in Finland.

Patients: One hundred subjects (71 patients with MS and 29 healthy control subjects). The subtypes of MS were relapsing-remitting (RRMS [n = 26]), secondary progressive (SPMS [n = 20]), and primary progressive (PPMS [n = 25]).

Results: In patients with RRMS and SPMS, the expressions of VLA-4 and LFA-1 on immune cells from blood were at least 1.5- to 3-fold higher than in controls (RRMS, P = .002 and P < .001, respectively; SPMS, P = .03 and P = .001, respectively). In RRMS, LFA-1 and ICAM-1 expression in blood was more up-regulated than in SPMS (P = .03 and P = .01, respectively). The expressions of adhesion molecules on CSF lymphocytes in RRMS and SPMS were of similar magnitude, but the proportions of CSF VLA-4- and LFA-1-expressing lymphocytes were 3- to 4-fold higher than in controls (P = .04 and P = .008, respectively). The levels of serum soluble VCAM-1 were higher in SPMS than in RRMS (P = .005) or PPMS (P = .04). Intravenous methylprednisolone treatment of patients with RRMS in exacerbation caused a significant reduction in the serum levels of soluble VCAM-1 and E-selectin (P < .001). In SPMS, the volumes of T2-weighted plaques correlated with the serum level of soluble ICAM-1 (r = 0.64; P = .03).

Conclusions: Up-regulated adhesion molecules in blood and CSF indicate sustained potential for inflammation in the CNS throughout the clinical spectrum of MS. Therapies interfering with cell adhesion may be of key importance in suppressing MS.

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Multiple sclerosis (MS) is an autoimmune neurological disease characterized by multifocal areas of inflammatory demyelination within the central nervous system (CNS). The binding of circulating autoreactive T cells and macrophages to the CNS endothelial cells and subsequent migration through the blood-brain barrier is an essential step in the initiation of brain inflammation. This step of immune cell entry into the site of inflammation is mediated by adhesion molecules. In the CNS, T cells are exposed to the sensitization antigen that triggers inflammation by cytokines released during immunorecognition. Adhesion molecules induced by such cytokines on brain cells and immune cells promote interaction between these cells, leading to damage of myelin and oligodendrocytes with subsequent demyelination. Two adhesion molecule pathways have been well defined: the intercellular adhesion molecule 1 (ICAM-1, CD54) on endothelial cells and leukocytes together with its ligand on leukocytes, the lymphocyte function associated antigen 1 (LFA-1, CD11a), and the vascular cell adhesion molecule 1 (VCAM-1, CD106) on endothelial cell and macrophages and its ligand on monocytes and lymphocytes, the very late activation antigen 4 (VLA-4, CDw49d). Increased expression of various cell adhesion molecules has been
found in the CNS in MS and its model disease, experimental allergic encephalomyelitis. The adhesion molecules on the surface of white blood cells are increased during the clinically stable phase of secondary MS according to the criteria of Poser et al., and no evidence of organic CNS disease. The remaining controls were healthy volunteers who were staff members of Tampere University Hospital, Tampere, Finland.

Blood and CSF samples for analysis were obtained by routine procedures, with the informed consent of the patients. Blood samples were acquired at the time of our studies, the patients with SPMS did not experience symptoms of acute neurological worsening and had not received any immunosuppressive or other therapy because of MS for 8 weeks before the study. The clinical condition of the patients with PPMS at the time of neurological examinations was stable. Eleven controls were studied because of variable neurological symptoms, and they presented no evidence of organic CNS disease. The remaining controls were healthy volunteers who were staff members of Tampere University Hospital, Tampere, Finland.

Blood and CSF samples for analysis were obtained by routine procedures, with the informed consent of the patients, as described elsewhere. The study was approved by the Ethical Committee of Tampere University Hospital. The concentrations of circulating adhesion molecules were measured in frozen specimens stored at −70°C.

NEURORADIOLOGICAL EXAMINATION

All patients underwent studies on the 0.5-tesla magnetic resonance imaging (MRI) unit (Vector GE; General Electric, Milwaukee, Wis), using a standard head coil. Routinely used sagittal T1-weighted, axial T2-weighted, proton density-weighted, fluid-attenuated inversion recovery–weighted, and gadolinium-pentetic acid–enhanced T1-weighted images were obtained. In addition, the imaging protocol included axial T1-weighted fast spin echo images (repetition time [TR], 300 milliseconds; echo time [TE], 20 milliseconds; number of excitations, 5; field of view, 22 cm; matrix size, 160/256; echo train length, 5; and slice thickness, 5 mm with 2-mm gap) and axial 3-dimensional T2-weighted fast spin echo images (TR, 2000 milliseconds; TE, 150 milliseconds; number of excitations, 1; field of view, 22 cm; matrix size, 192/224; echo train length, 16; and slice thickness, 2 mm with no intervening gap) for segmentation and volumetric analysis. Segmentation and volumetric analysis were performed as described previously.

DETERMINATION OF SOLUBLE ADHESION MOLECULES

The concentrations of soluble VCAM-1 (sVCAM-1), sICAM-1, and sE-selectin were measured using commercially available sandwich enzyme immunoassay kits (BBE 3, BBE IB, and BBE 2B, respectively; R&D Systems, Minneapolis, Minn). Serum or CSF samples diluted 1:50 for sVCAM-1 and 1:20 for sICAM-1 and sE-selectin were added in duplicate to microtiter wells and assayed according to routine procedures. The signals obtained from standards of known concentration were used for development of a standard curve. The concentrations in patient samples were calculated using a 4-variable logistic curve fit.

DETERMINATION OF ADHESION MOLECULE EXPRESSION

Unless otherwise indicated, data are given as mean ± SEM. For phenotyping the mononuclear cells, the 3-layer indirect immunoperoxidase technique was applied. The primary antibodies used were LFA-1 (CD11a), VLA-4 (CDw49d), ICAM-1 (CD54), or VCAM-1 (CD106) (0157, 0764, 0544, and 1244, respectively; Immunotech, Marseille, France). The dilution used for the CSF specimens was 1:100; for blood specimens, 1:200. The secondary antibody was a peroxidase-conjugated rabbit anti–mouse used in dilution of 1:10 (P0161; Dako, Glostrup, Denmark) and the third was a peroxidase-conjugated goat anti–rabbit antibody used in dilution of 1:20 (L42007; Caltag, San Francisco, Calif). The specimens were analyzed at ×100 magnification. In blood, a total of 400 cells, and in CSF, a mean of 132 ± 55 mononuclear cells were analyzed for each cell surface marker. The results were expressed as the percentage of positively stained lymphocytes in the total number of lymphocytes counted and the percentage of positively stained monocytes in the total number of monocytes counted.

STATISTICAL METHODS

Independent 2-tailed t test was used in the group mean comparisons with normal distribution. Mann-Whitney test was used in cases of skewed distribution. In correlation analyses, Spearman rank order correlation coefficients were calculated. All statistical analyses were made on a microcomputer using a commercially available program package (Statistica for Windows; Statsoft, Inc, Tulsa, Okla).
progressive MS (SPMS), which would indicate a sustained potential for inflammation throughout the spectrum of MS disease, regardless of its activity. We also determined the concentrations of soluble adhesion molecules in different subtypes of MS and the effect of IV methylprednisolone treatment on the levels of these proteins.

RESULTS

EXPRESSION OF ADHESION MOLECULES IN DIFFERENT SUBTYPES OF MS

The proportions of the adhesion molecule expression on blood lymphocytes and monocytes are presented in Table 2. In patients with RRMS in exacerbation, the expressions of VLA-4 and LFA-1 in blood were 1.3- to 3.5-fold higher than in controls (VLA-4 lymphocytes, 41.3 ± 3.6 vs 13.1 ± 3.2 [P<.001]; LFA-1 lymphocytes, 45.9 ± 3.7 vs 13.0 ± 2.3 [P<.001]; LFA-1 monocytes, 56.6 ± 5.6 vs 24.4 ± 3.9 [P = .002]). In SPMS, the proportions of blood lymphocytes expressing these adhesion molecules were likewise at least 3-fold higher than in controls (VLA-4, 36.8 ± 12.0 vs 7.3 ± 2.5 [P = .001]; LFA-1, 42.4 ± 5.6 vs 12.0 ± 2.3 [P = .005]), but no statistically significant differences were found between RRMS and SPMS.

Comparison between the levels of soluble adhesion molecules in different MS subtypes revealed higher sVCAM-1 levels in RRMS than in SPMS (Table 3) (P = .005) or PPMS (P = .04), whereas the difference between SPMS and controls was not significant. However, in RRMS and PPMS, the levels of sVCAM-1 were even lower than in controls (P = .005 and P = .04, respectively). No differences were seen between both MS subtypes. No clear differences were detected in the levels of sICAM-1 among the MS subtypes or between these and control samples.

LEVELS OF SOLUBLE ADHESION MOLECULES

Comparison between the levels of soluble adhesion molecules in different MS subtypes revealed higher sVCAM-1 levels in SPMS than in RRMS (Table 3) (P = .005) or PPMS (P = .04), whereas the difference between SPMS and controls was not significant. However, in RRMS and PPMS, the levels of sVCAM-1 were even lower than in controls (P = .005 and P = .04, respectively). No differences were seen between both MS subtypes. No clear differences were detected in the levels of sICAM-1 among the MS subtypes or between these and control samples.
In PPMS, the level of sE-selectin tended to be higher than in SPMS, but the difference did not reach statistical significance (Table 3).

The levels of soluble adhesion molecules in the CSF of patients with PPMS are shown in Table 4.

**Table 3. Concentrations of Soluble Adhesion Molecules in Serum Samples**

<table>
<thead>
<tr>
<th>Soluble Adhesion Molecule</th>
<th>RRMS (n = 26)</th>
<th>SPMS (n = 13)</th>
<th>PPMS (n = 25)</th>
<th>Controls (n = 29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VCAM-1</td>
<td>466 ± 124‡‡</td>
<td>614 ± 139§§</td>
<td>495 ± 89∥∥</td>
<td>589 ± 123</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>229 ± 69¶</td>
<td>265 ± 37</td>
<td>273 ± 95</td>
<td>283 ± 55</td>
</tr>
<tr>
<td>E-selectin</td>
<td>47 ± 15</td>
<td>35 ± 14‡</td>
<td>53 ± 29</td>
<td>47 ± 19</td>
</tr>
</tbody>
</table>

*Abbreviations are given in the first footnotes to Tables 1 and 2. Data are given as mean ± SD nanograms per milliliter.

†P = .005, vs SPMS.
‡‡P = .003, vs controls.
§§P = .04, vs PPMS.
¶P = .04, vs controls.
∥∥P = .046, vs controls.
¶P = .10, vs PPMS.

**Table 4. Levels of Soluble Adhesion Molecules in Cerebrospinal Fluid From 20 Patients With PPMS**

<table>
<thead>
<tr>
<th>Soluble Adhesion Molecule</th>
<th>Mean ± SD Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>VCAM-1</td>
<td>34.8 ± 16.5 15.8-76.6</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>6.4 ± 1.8 3.7-10.9</td>
</tr>
<tr>
<td>E-selectin†</td>
<td>0.2 ± 0.2 0.0-1.0</td>
</tr>
</tbody>
</table>

*Abbreviations are given in the first footnotes to Tables 1 and 2. 
†Could be measured from the cerebrospinal fluid of 11 patients with a concentration higher than 0 ng/mL.

**Table 5. Levels of Soluble Adhesion Molecules in the Serum Samples of 16 Patients With RRMS Treated With High-Dose Intravenous Methylprednisolone**

<table>
<thead>
<tr>
<th>Soluble Adhesion Molecule</th>
<th>Before Methylprednisolone</th>
<th>After Methylprednisolone</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>VCAM-1</td>
<td>423 ± 116</td>
<td>398 ± 97</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>217 ± 64</td>
<td>181 ± 53</td>
<td>.06</td>
</tr>
<tr>
<td>E-selectin</td>
<td>40 ± 13</td>
<td>31 ± 11</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

*Abbreviations are given in the first footnotes to Tables 1 and 2. Therapy consisted of methylprednisolone sodium succinate.

Elevated levels of soluble adhesion molecules in MS have been reported by many investigators, but much less information is available on the cell surface expression of these proteins in CSF and blood. In our study, we found increased proportions of VLA-4– and LFA-1–positive immune cells in blood of patients with RRMS and SPMS compared with healthy controls. Although the expressions of adhesion molecules were higher in RRMS in exacerbation, up-regulated VLA-4 and LFA-1 were also detected in the clinically stable phase of SPMS, which indicates leukocyte-endothelial activation in acute and stable MS. Since the ability of activated immune cells to bind to their receptors on activated endothelium facilitates their migration through the blood-brain barrier, our results imply that the potential for brain inflammation is sustained throughout the course of the disease regardless of its activity.

Previous reports on the soluble endothelial adhesion molecules in the serum of patients with MS have been conflicting, showing elevated levels of sVCAM-1, sICAM-1, and sE-selectin in exacerbation compared with baseline and normal levels. This indicates that serum levels of adhesion molecules do not consistently reflect the activity of MS disease. Elevated levels of sICAM-1 or sVCAM-1 in the CSF during MS relapse seem to be more closely related to disease activity. Our results showed the level of serum sVCAM-1 in patients with SPMS to be higher than...
in patients with RRMS in relapse or with PPMS. Although these results were obtained in serum samples, they are consistent with the observation of Cannella and Raine on the predomiance of VCAM-1 and VLA-4 in chronic active MS. Other investigators have reported higher values for serum sICAM-1 and sE-selectin in PPMS compared with RRMS, although the activity of the disease has not always been clear. The partial discrepancies between our results and previous data may be explained by differences in methods or timing of sampling in relation to disease activity. The differences between chronically progressive types of MS and RRMS in exacerbation suggest that the cellular source of adhesion molecules and the kinetics of cleavage of endothelial cell surface molecules to their soluble form may differ in relapsing and chronically progressive disease courses.

We observed that methylprednisolone reduced the concentrations of serum sVCAM-1 and sE-selectin. A lowering of sE-selectin levels recently was reported by Droogan et al. These observations are in harmony with the current knowledge that corticosteroids suppress many important events in the inflammatory cascade. More specifically, our results suggest a down-regulating effect of corticosteroids on the expression of adhesion molecules on circulating immune cells and the cerebral endothelium or cells within the CNS.

The correlation between the expression of adhesion molecules and MS plaques or brain atrophy in patients with SPMS showed association only between the level of serum sICAM-1 and the volumes of T2-weighted plaques. This is not surprising, since previous studies on the association of biological markers, including adhesion molecules, and MS-related MRI or EDSS changes have been mostly disappointing. The lack of clear-cut associations in this study may be explained by the lack of clinical and MRI activity during examinations. In a previous study from our laboratory on RRMS in exacerbation, significant correlations were found between the numbers of T2-weighted lesions and expressions of VLA-4 and LFA-1 on mononuclear cells from blood.

Our results imply that therapies interfering with cell adhesion should be considered as a potential objective in MS. The importance of such therapies has been demonstrated by the blockade of the VLA-4–VCAM-1 pathway to prevent and reverse experimental allergic encephalomyelitis, a T-cell–mediated neuroinflammatory model for MS. Therapies modulating adhesion molecules with resultant restriction of inflammatory cell invasion of the CNS may provide an important tool in the effort to suppress MS.

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Reprints: Irina Elovaara, MD, PhD, Neuroimmunology Unit, Tampere University Hospital, Teiskontie 35, 33520 Tampere, Finland (e-mail: Ieloavaara@tays.fi).

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