ORIGINAL ARTICLE

Serum levels of soluble intercellular adhesion molecule-1 (s-ICAM-1) and soluble endothelial leukocyte adhesion molecule-1 (s-ELAM-1) in amyotrophic lateral sclerosis

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Abstract

Immunological disturbances have been implicated in the pathogenesis of amyotrophic lateral sclerosis (ALS). Adhesion molecules are markers of activated endothelial cells up-regulated by action of cytokines. To investigate the activation or inactivation of the vascular cells in ALS, serum soluble intercellular adhesion molecule-1 (s-ICAM-1) and soluble E-selectin (s-ELAM-1) were evaluated (ELISA) in 16 patients with ALS, 30 patients with non-inflammatory neurological diseases (NINDS) and 15 healthy control subjects. Patients with ALS had no higher s-ICAM-1 levels compared with the NINDS patients and the control subjects (p > 0.31 and p > 0.21, respectively). s-ELAM levels were not statistically significant compared with the NINDS patients and healthy subjects (p > 0.21 and p > 0.24, respectively). We conclude that the low values of s-ICAM-1 and s-ELAM-1 in the serum of ALS patients do not exclude the presence of immunological abnormality in this disorder. Soluble E-selectin is a glycoprotein which is considered an exclusive marker of endothelial activation. Its low level in our study may suggest a neural rather than an endothelial s-ICAM origin in patients with ALS.

Key words: ICAM-1, ELAM-1, amyotrophic lateral sclerosis, immunological processes

Introduction

Amyotrophic lateral sclerosis (ALS) is a degenerative disease of unknown aetiology. There is now evidence that immunological factors may be involved in pathogenetic mechanisms of the disease. The existence of HLA-DR staining astrocytes in ALS lesions, the induction of immune models of motor neuron disease and the elevated circulating IgG immune complexes of the ALS sera (1–3) are some of these. An increased incidence of autoimmune disorders (4), high levels of paraproteinaemia and anti-gangliosidic antibodies (5–7), and antibodies binding to calcium channels have been reported (8,9). CSF IgG index, complement involvement and CSF High oligoclonal bands have also been reported (10).

The transendothelial migration of leukocytes is a key step in the inflammatory process of autoimmune disorders. Transmigration is governed by adhesion molecules (11) of which there are three groups: the selectin family including E-selectin (ELAM-1), the immunoglobulins superfamily including ICAM-1 and the integrins family. Selectin family members help circulating activated leukocytes to weakly attach to the endothelium. The other two family members stabilize the weak and transient interaction and prepare T-cells for migration through the endothelium. Recently an elevation of serum soluble E-selectin (s-E-selectin) associated with antibodies to sulfoglucuronyl paragloboside (SGPG) was reported in patients with ALS (12). Sulfoglucuronyl paragloboside (SGPG) was first found as a target antigen in sensory-motor neuropathy with IgM paraproteinaemia (13). Antibodies to myelin-associated glycoprotein (MAG)/SGPG have been reported in ALS patients (14) but their significance is still unknown. SGPG is localized not only in nervous tissue but also in vascular endothelial cells (15). Endothelial cells are an important structure of the blood-brain barrier (BBB), and SGPG may act as an adhesion molecule resulting in a greater attachment of leukocytes (16). Ikeda et al. (12) examined serum anti-SGPG antibodies in order to investigate their role in activation of endothelial cells in ALS patients. They also measured serum soluble E- and P-selectins which are markers of...
activated endothelial cells. They found that the mean s-E-selectin levels were higher in patients with anti-SGPG antibodies than in those without them. These results may suggest an endothelial cell activation in some ALS patients.

We examined serum values of soluble-intercellular adhesion molecule-1 (s-ICAM-1) and soluble-endothelial leukocyte adhesion molecule-1 (s-ELAM-1) in patients with ALS, other 'non-inflammatory' neurological diseases (NIND) and age- and gender-matched control subjects. The objective of our study is to support, or not support, the concept of isolated CNS or systemic inflammation in ALS patients by examining if there is activation of endothelial cells of the BBB in ALS patients resulting in recruitment of activated T-cells in the central nervous system.

Materials and methods

Patients

Serum samples were studied in 61 individuals comprising the following groups (Table I): 1) 16 patients with ALS (10 males and 6 females, age 41–75 years, mean 62 years); 2) 30 age-matched patients with other non-inflammatory neurological diseases (NIND) (19 males and 11 females, age 39–74 years, mean 59 years); 8 of these patients had cerebrovascular disease, 9 had tension headache, 5 had Parkinson’s disease, 2 had Lewy body disease, 3 had spinocerebellar ataxia and 3 had non-inflammatory neuropathies (1 with diabetic neuropathy, 1 with Charcot-Marie-Tooth type I and 1 with bilateral peroneal nerve paresis); 3) 15 age-matched patients with ALS (10 males and 6 females, age 41–77 years, mean 60 years) who were studied as a control group.

Ethical consent was obtained from all patients for studying CSF and serum. Serum samples were stored at –70°C until assayed. Cerebrospinal fluid (CSF) samples of all the patients with ALS were obtained by lumbar puncture in order to study BBB permeability by measuring albumin index. CSF was centrifuged to separate the cells and then frozen within 2 h in multiple aliquots at –70°C.

Table I. Clinical features of our patients.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALS</td>
<td>16</td>
</tr>
<tr>
<td>CBD</td>
<td>8</td>
</tr>
<tr>
<td>Tension headache</td>
<td>9</td>
</tr>
<tr>
<td>Parkinson’s disease</td>
<td>5</td>
</tr>
<tr>
<td>Lewy body disease</td>
<td>2</td>
</tr>
<tr>
<td>Spinocerebellar ataxia</td>
<td>3</td>
</tr>
<tr>
<td>Diabetic neuropathy</td>
<td>1</td>
</tr>
<tr>
<td>Charcot-Marie Tooth type I</td>
<td>1</td>
</tr>
<tr>
<td>Bilateral peroneal palsy</td>
<td>1</td>
</tr>
</tbody>
</table>

ALS: amyotrophic lateral sclerosis; CBD: cerebrovascular disease.

In the ALS group, all patients met the El Escorial WFN criteria (17) for possible, probable and definite ALS. Duration of symptoms at the time of examination was from 5 months to 3 years, mean 22 months.

s-ICAM-1, s-ELAM assay

Serum concentrations of circulating adhesion molecules (s-ICAM-1, s-ELAM-1) were measured by commercially available enzyme-like immunosorbent assay (ELISA) kits (R. and D. Systems). Serum samples diluted 1:20 were added in duplicate to microtitre wells and assayed according to manufacturer’s protocol. Optical densities were determined by means of a micro-ELISA reader (LP 400, Pasteur diagnostics). Values were calculated by comparison with a standard curve generated with s-ICAM and s-ELAM standards, respectively. The normal range for s-ICAM-1 and s-ELAM-1 was within the mean value±2 standard deviations obtained from NIND and control patients. The limits of detection were 0.35 ng/ml for s-ICAM-1 and 0.1 ng/ml for s-ELAM-1.

Statistical analysis

Results were expressed as mean ± standard deviation. The Student’s t-test and the Mann-Whitney U-test were used, as appropriate, to compare titres of s-ICAM and s-ELAM-1 between the examined study groups. All comparisons were two-sided, with a p-value of less than 0.05 used to indicate statistical significance. The effect of gender and age was examined by regression analysis. The statistical software used for this analysis was Statistica 6.0.

Results

Serum ICAM-1 levels were not significantly elevated in patients with ALS (n=16; mean value 237.3 ng/ml, 95% CI 117.3–303.2) compared with patients with NIND (n=30; mean value 194.4 ng/ml, 95% CI 156.9–221.5; p<0.21) and healthy subjects (n=15; mean value 193.3 ng/ml, 95% CI 135.3–251.3; p<0.31 (Figure 1, Table II). Serum ELAM-1 levels were not elevated in ALS patients (mean value: 37.4 ng/ml, 95% CI 26.8–57.5) compared with patients with NIND (mean value 28.1 ng/ml, 95% CI 22.1–38.5; p<0.21) and normal control subjects (mean value 38.6 ng/ml, 95% CI 20.2–52.9; p<0.24) (Figure 1, Table II). There was no difference between genders or age.

In two patients with ALS, s-ICAM-1 levels were higher than all measured levels of our study (550 and 525 ng/ml, respectively). These patients had a more protracted course of the disease (32 and 36 months, respectively). According to site of onset of the disease they had no difference compared to the other patients. Albumin index was normal (<10) in
all patients with ALS studied for BBB permeability disturbances.

**Discussion**

ICAM-1 is released from activated endothelial cells, lymphocytes, monocytes or microglia. Increased serum and CSF levels of ICAM-1 were measured in patients with inflammatory and demyelinating diseases of the central and peripheral nervous system (18,19).

ELAM-1 is expressed exclusively on endothelial cells, so may be a more useful marker for endothelial activation (12). Elevated serum levels of ELAM-1 have been reported in Guillain-Barre syndrome (GBS) and chronic inflammatory demyelinating polyneuropathy (CIDP) (20) and in MS (21). ELAM-1 was also detected in the epineural vessels of patients with CIDP (22).

We did not find increased levels of serum s-ICAM-1 and s-ELAM-1 in patients with ALS compared with patients with NIND and control subjects. This finding is in contrast to a previous study by Ikeda et al. which demonstrated increased levels of E-selectin reported in patients with ALS (12). The reason for this discrepancy is not clear, but may arise from different patient populations. Our study included more patients suffering from different non-inflammatory neurological disorders such as tension headache, cerebrovascular disease,
non-inflammatory polyneuropathies and Lewy body disease. We also used a third group of healthy subjects which was not included in the study of Ikeda et al.

Our results do not exclude the direct expression of ICAM in the microglia of the central nervous system without activation of endothelial cells. Microglial cells and astrocytes can be induced to express ICAM-1 by proinflammatory cytokines such as TNF, IFN-γ and IL-1 (23). This observation is supported by the finding that: 1) s-ELAM-1 (which is an indicator of exclusive endothelial activation) is not elevated in the serum of our ALS patients, and 2) the blood-brain barrier in the same patients is intact. BBB in our patients was intact as is evident from the normal albumin index. This prevents large molecules such as proteins or complement from passing passively through the BBB and entering the brain. Also, the non-statistically significant elevated levels of sELAM-1, compared to those of healthy subjects, does not suggest the active transport of inflammatory signals through activation of vascular cells.

Two of our patients (10%) with more protracted course of the disease had higher serum s-ICAM values than all the other individuals of our study. Duration of the disease could be a critical factor in the initiation of immune complex cellular and molecular interactions involved in progression of ALS and probably other neurodegenerative diseases.

References