Soluble E-selectin in multiple sclerosis: raised concentrations in patients with primary progressive disease


Abstract

Objective—To determine whether concentrations of soluble E-selectin (sE-selectin), an immunological marker of endothelial activation, were correlated with gadolinium-DPTA enhancement on MRI in patients with multiple sclerosis.

Methods—Serial sE-selectin concentrations were measured in 28 patients with multiple sclerosis undergoing monthly gadolinium (Gd) enhanced MRI of the brain and spinal cord, and in 10 control subjects. C reactive protein (CRP), von Willebrand factor (vWF), and tumour necrosis factor-α (TNFα) were also determined.

Results—Primary progressive patients had significantly increased sE-selectin concentrations compared with the relapsing remitting and secondary progressive patients who had normal sE-selectin concentrations (22.2 (SD 6.1) ng/ml vs 9.8 (SD 2.1) ng/ml and 7.7 (SD 2.7) ng/ml, respectively, P = 0.03). This difference was attributable to five of the 10 primary progressive patients who had persistently raised sE-selectin concentrations, with relatively inactive MRI studies. No correlation could be found between sE-selectin concentrations and Gd enhancement on MRI, but a close correlation existed between mean concentrations of sE-selectin and TNFα (r = 0.71, P < 0.001).

Despite raised sE-selectin and TNFα concentrations, primary progressive patients had normal CRP concentrations (1.03 (SD 1.4) mg/l), which were significantly lower than the relapsing remitting (3.16 (SD 2.54) mg/l) and secondary progressive patients (2.28 (SD 2.1) mg/l, P = 0.03). Raised CRP concentrations did correlate with infectious episodes, clinical relapse, and Gd enhancement, and were significantly raised when no MRI activity was found. Concentrations of vWF were normal in all patient groups.

Conclusions—The results further highlight the differences between patients with primary progressive and those with relapsing remitting/secondary progressive multiple sclerosis.

(J Neurol Neurosurg Psychiatry 1996;60:20–26)

Keywords: multiple sclerosis; magnetic resonance imaging; gadolinium enhancement; E-selectin

On serial MRI of patients with relapsing remitting or secondary progressive multiple sclerosis, an early finding in most new lesions is gadolinium (Gd) DTPA enhancement.1 Evidence from chronic relapsing experimental allergic encephalomyelitis2 and multiple sclerosis3 suggests that enhancement in these disorders indicates the presence of perivascular inflammation in which macrophages and T lymphocytes predominate.4 Invasion of these macrophages and T lymphocytes into the perivascular space of the CNS is a complex process.5 A model of T cell/endothelium interaction suggests that initial adhesion involves molecules such as the selectins and CD44, which are expressed at increased concentrations by migratory memory T cells. E-selectin, the most endothelial specific of the adhesion molecules, is produced by activated endothelium and its expression is induced by interleukin-1 (IL1) and tumour necrosis factor-α (TNFα), and is augmented by interferon-γ (INF).6 E-selectin is a member of the selectin family with a lectin-like N-terminal domain. It exists either as a membrane bound receptor that recognises the tetrasaccharide siacyl-Lewisx or siacyl-Lewisx ligands of monocytes and granulocytes, or as a smaller soluble form (sE-selectin) that may function as a circulating inhibitor of cell adhesion.7 As E-selectin is produced predominantly by activated endothelium, the concentration of sE-selectin represents the degree of endothelial activation. Raised concentrations have been found in many infectious and inflammatory disease processes, including malaria, rheumatoid arthritis, primary biliary cirrhosis, polyarteritis nodosa, giant cell arteritis, scleroderma, systemic lupus erythematosus, Gram positive and Gram negative infections, and in patients with septic shock.8 Recently increased concentrations have been reported in two neurological conditions—Guillain-Barré syndrome and chronic inflammatory demyelinating polyneuropathy.9,10

We hypothesised that sE-selectin concentrations, from activated endothelial cells, may correlate with Gd enhancement on MRI. A secondary hypothesis was that primary progressive multiple sclerosis, with characteristically little Gd enhancement would differ from relapsing remitting and secondary progressive disease by having low concentrations of sE-selectin. To test these hypotheses we measured sE-selectin concentrations on various groups of patients with multiple sclerosis undergoing serial Gd enhanced MRI studies.
of the brain and spinal cord. We chose a longitudinal rather than a cross sectional study to control for any intradividual differences and to see if sE-selectin would be of any use as a potential surrogate marker of disease activity. We also correlated concentrations of sE-selectin with circulating concentrations of TNFα.

As sE-selectin is increased by many disease processes, including infections, we measured C reactive protein (CRP) as a marker of the systemic acute phase response. We also measured von Willebrand factor (vWF), a multimeric protein synthesised mainly by endothelium as a marker of endothelial damage. Experimentally induced endothelial damage causes an increase in circulating concentrations of vWF,12 and several vasculitic diseases13 and diabetic microangiopathy14 are associated with increased concentrations. If perivascular inflammation in multiple sclerosis causes endothelial damage vWF concentrations should be raised.

Methods

PATIENTS

Twenty-eight patients undergoing monthly MRI studies at the Institute of Neurology, London, with clinically definite or laboratory supported definite multiple sclerosis,15 were included in the study. All these patients had been recruited for a separate, independent serial MRI study, which was approved by the ethics committee of the National Hospital for Neurology and Neurosurgery. All patients had already given written informed consent. Ten patients had primary progressive multiple sclerosis, nine secondary progressive multiple sclerosis, and nine relapsing remitting disease. The diagnostic criteria of these clinical subtypes and the definitions used for defining a relapse and the period of remission in this study have been previously described.15 16 Each patient was assessed neurologically on entry into the study and at the time of each MRI study. Disability was measured with Kurtzke’s expanded disability status scale (EDSS).17 The rate of neurological deterioration for each patient was assessed by two indices; firstly the progression index,18 which is the ratio of the baseline EDSS to the duration of the disease in years, and secondly the change in EDSS per year over the study period. Any relapses, infections, or other intercurrent medical illnesses were noted as well as the use of corticosteroids or immunosuppressive agents. During each MRI study, blood was taken from an antecubital vein before giving Gd.

CONTROLS

Serial serum samples were collected from 10 healthy volunteers, at weekly intervals for a period of two months. Control samples taken during a symptomatic infection were not included in the establishment of the normal ranges. The rationale for doing serial controls was to check for any physiological fluctuations in sE-selectin, TNFα, CRP, and vWF concentrations.

MAGNETIC RESONANCE IMAGING

All MRI studies were performed on a 1.5 Tesla Signa system (IGE Medical Systems, Milwaukee, WI). Brain MRI was carried out with a standard quadrature headcoil. Pilot images were obtained in axial, coronal, and sagittal planes to ensure adequate repositioning. Axial dual echo (proton density and T2 weighted) fast spin echo (FSE500/10,90 echo train length 8) and, five minutes after the administration of Gd-DTPA (Magnevist 0.1 mmol/kg, Schering), T1 weighted spin echo (SE170/19) images were obtained. For both T1 and T2 weighted images contiguous interleaved 4 mm slices, one excitation, with a 192 × 256 matrix with a 24 cm field of view were used. Spinal imaging, with a spinal multiarray receiver coil, was carried out immediately after brain MRI. Sagittal T1 weighted spin echo images (SE 500/19, 4 mm contiguous interleaved slices, 256 × 512 matrix, 24 × 48 cm rectangular field of view, two excitations) were first obtained, about 10–15 minutes after Gd-DTPA injection. Then two sets of sagittal fast spin echo images with different degrees of T2 weighting were obtained; the parameters used were FSE500/3, and FSE500/102 echo train length 16, 3 mm contiguous interleaved slices, 512 × 256 matrix, 24 × 48 cm field of view, two excitations. The brain images were examined by one neuroradiologist (IFM) and, those of the spinal cords by another (BEK); both were unaware of the patients’ clinical details. The number of individual lesions on the initial T2 weighted images were counted and graded for size to give a baseline total CNS lesion load. On subsequent examinations all contrast enhancing lesions were counted. An overall MRI activity index was calculated for each patient by dividing the total number of contrast enhancing lesions seen during the study period by the number of examinations performed.

ASSAYS

The serum and plasma samples were separated as soon as possible, coded, frozen, and stored at −20°C. All samples were assayed within 12 months. Repeated freeze-thaw cycles were avoided. Aliquots of serum were thawed just before use, and all assays were performed blind.

SOLUBLE E-SELECTIN

Concentrations of sE-selectin were measured by a sandwich enzyme linked immunosorbent assay (ELISA).19 The capture antibody was a mouse monoclonal anti-human E-selectin/P-selectin (BBA 1, R and D Systems Europe). The detector antibody was a biotinylated mouse monoclonal antihuman E-selectin (BBA8, R and D Systems Europe) and was conjugated to streptavidin-biotin horseradish peroxidase (HRP) complex (Amersham) before the addition of o-phenylenediamine (Sigma) as the enzyme substrate. The assay was calibrated primarily with purified human E-selectin (BBE 2, R and D Systems Europe) and secondarily with pooled serum from patients with active rheumatoid arthritis. The
assay had a lower limit of detection of 0.6 ng/ml, an intra-assay coefficient of variation of 4-6%, an interassay coefficient of variation of 12-6%, and a mean recovery of 103 (range 83-112%).

C REACTIVE PROTEIN
An ELISA had been developed to measure concentrations of CRP less than 2 mg/l. The capture antibody used was a polyclonal rabbit antihuman CRP (PC044, The Binding Site) and the detector antibody was the same HRP conjugated polyclonal rabbit anti-human CRP (PP044, The Binding Site). O-Phenylene diamine (Sigma) was also used as the enzyme substrate. Purified human CRP (GP044-L, The Binding Site) and Calibrator 7 (84403, Atlantic Antibodies) were used to calibrate the assay. The assay had a lower limit of detection of 150 ng/ml, an intra-assay coefficient of variation of 6-1%, an interassay coefficient of variation of 7-9%, and a mean recovery of 98 (range 92-106%). A commercial radial immunodiffusion assay (Nanorid, The Binding Site) was used to determine CRP concentrations greater than 2 mg/l.

VON WILLEBRAND FACTOR
A vWF sandwich enzyme linked immunosorbent assay (ELISA) was developed using a polyclonal rabbit antihuman vWF (PC054, The Binding Site). HRP conjugated polyclonal rabbit antihuman vWF (P226, Dako), o-phenylene diamine as the enzyme substrate and purified human vWF (GP054, Binding Site) as the calibrant. The assay had a lower limit of detection of 0.004 IU/ml, an intra-assay CV of 3-4%, an interassay CV of 3-1, and a mean recovery of 95-5 (range = 87-102%).

TUMOUR NECROSIS FACTOR-α
Tumour necrosis factor-α was measured by ELISA using a previously described assay, with a lower limit of detection of 6-8 pg/ml. Due to a shortage of samples from several patients recruited early in the course of the study TNFα concentrations could only be assessed on 164 of the 222 samples (74%). Only plasma collected with EDTA as the anticoagulant was used for the determination of TNFα concentrations, as EDTA has been shown to prevent the in vitro production of TNFα which may occur before the separation of the samples.

STATISTICS
Data involving serial measurements on subjects were computed as subject means using all readings available for that particular subject. Bartlett's test for homogeneity of variance was used to test for normal distribution of data, which, if normally distributed, were compared by one way analysis of variance (ANOVA) and if not by a Kruskal-Wallis one way ANOVA. Soluble E-selectin, CRP, and TNFα data were normalised by log transformation; therefore all statistical results reported refer to the relevant log transformations. Non-parametric data were compared by the Yates’ corrected χ² test and if an expected value was less than 5 Fisher’s exact test was used. Simple linear regression was used to correlate concentrations of sE-selectin and TNFα. A P value < 0.05 was considered statistically significant.

Results
BASELINE CLINICAL AND MRI DATA (TABLE 1)
Relapsing remitting patients were significantly younger than either the secondary progressive or primary progressive patients (P < 0.001). Sex ratios of the groups differed significantly with nine of nine relapsing remitting, five of nine secondary progressive, and one of 10 primary progressive patients being female (P < 0.001). The age of disease onset of the groups did not differ statistically. The disease duration of the relapsing remitting patients was significantly shorter than that for the secondary progressive and primary progressive patients (P = 0.009). The relapsing remitting patients were less disabled on entry into the study, with a median EDSS of 2-5 compared with 6 and 5-5 for the secondary progressive and primary progressive patients respectively (P < 0.001); no relapsing remitting patients had an EDSS > 5-5 compared with 78% of the secondary progressive and 50% of the primary progressive patients (P = 0.001). There was no significant difference between the progression index of the three groups. The baseline MRI CNS lesion load seemed lower in the primary progressive patients than the other two groups but the difference was not significant.

The average study duration and the number of clinical and MRI assessments were similar for all groups of patients. Clinically, 13 relapses occurred during the study period: 10 in six relapsing remitting patients of whom three had one relapse, two patients two
Soluble E-selectin in multiple sclerosis: raised concentrations in patients with primary progressive disease

Figure 1 Scatter plots of serial data for relapsing remitting (n = 9), secondary progressive (n = 9), and primary progressive (n = 10) patients with multiple sclerosis. For comparative purposes each patient's position on the horizontal axis is maintained from figure IA to IC; solid vertical lines link all serial data points for a particular patient. Horizontal dashed lines represent 3 SDs above the mean of the controls and the shaded area the normal range. (A) Number of gadolinium-enhancing lesions per MRI study of the brain and spinal cord. (B) Soluble E-selectin (ng/ml). (C) C reactive protein (mg/l).

The three relapses in the secondary progressive group occurred in two patients. Five of the relapsing remitting patients were recruited during a relapse and improved clinically during the study accounting for the lower median exit EDSS of 2 compared with 2.5 at entry. None of the patients with relapses were treated with corticosteroids. The clinical onset of the 13 relapses occurred with a median of seven (range 2–42) days before the initial or follow up clinical and MRI examination. One of the primary progressive patients, with normal sE-selectin concentrations and no MRI activity, was on a continuous low dose of oral prednisone (10 to 40 mg per day) for the duration of the study period. No other patient received immunosuppressive treatment. Six symptomatic infective episodes occurred in five patients in the week preceding or at the time of an MRI assessment. These occurred in one secondary progressive and four relapsing remitting patients. Two of the infections involved the urinary tract and the remainder the upper respiratory tract. No primary progressive patients had symptomatic infections during the period defined above. Activity on MRI assessed by Gd enhancement was detected at some time during the study period in seven of nine relapsing remitting, six of nine secondary progressive, and in only two of 10 primary progressive patients (P = 0.03, fig 1A). The mean number of active lesions per scan was similar in relapsing remitting and secondary progressive patients but significantly less in the primary progressive group (P = 0.04). Nine of the 13 (69%) clinical relapses were associated with MRI activity compared with only 37 (27%) out of the other 139 follow up examinations (P = 0.03).

SERUM MARKERS (TABLE 2)

Soluble E-selectin concentrations were significantly higher in primary progressive compared with relapsing remitting or secondary progressive patients (P = 0.03, fig 1B). This increase was moderate compared with mean sE-selectin concentrations of 86 (range 45–147) ng/ml in 11 patients with active rheumatoid arthritis, measured with our assay. The increase in the primary progressive group could be attributed to five patients with persistently raised sE-selectin concentrations throughout the study period. These five patients seemed to be clinically more progressive during the study period, deteriorating on average 1.4 EDSS points/year compared with an average of 0.4 EDSS points/year in patients with low sE-selectin concentrations. This was not statistically significant. Mean concentrations of sE-selectin did not correlate with MRI activity, clinical relapse, or infective episodes. There was no difference in MRI activity between the five primary progressive patients with raised sE-selectin concentrations (> 3 SD

Table 2 Follow up clinical and MRI data

<table>
<thead>
<tr>
<th></th>
<th>Relapsing remitting</th>
<th>Secondary progressive</th>
<th>Primary progressive</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean duration of study period (days)</td>
<td>185 (35) (126–244)</td>
<td>208 (135) (66–412)</td>
<td>201 (166) (35–436)</td>
<td>NS</td>
</tr>
<tr>
<td>Mean No of scans/patient</td>
<td>6–9 (1–4) (5–9)</td>
<td>5–2 (1–8) (3–8)</td>
<td>5–0 (2–1) (2–8)</td>
<td>NS</td>
</tr>
<tr>
<td>Mean EDSS</td>
<td>0–2–5</td>
<td>56%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>3–5–5</td>
<td>44%</td>
<td>11%</td>
<td>30%</td>
<td>0.001</td>
</tr>
<tr>
<td>&gt; 5–5</td>
<td>0%</td>
<td>89%</td>
<td>70%</td>
<td>NS</td>
</tr>
<tr>
<td>No of relapses</td>
<td>10</td>
<td>3</td>
<td>0</td>
<td>0.003</td>
</tr>
<tr>
<td>No of infections</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Mean No Gd enhancing lesions/scan</td>
<td>1–7 (2–2) (0–6–7)</td>
<td>1–03 (1–7) (0–5)</td>
<td>0–28 (0–79) (0–2–5)</td>
<td>0–04</td>
</tr>
<tr>
<td>Activity (No Gd enhancing lesions/scan)</td>
<td>0</td>
<td>22%</td>
<td>33%</td>
<td>80%</td>
</tr>
<tr>
<td>0–1</td>
<td>34%</td>
<td>45%</td>
<td>10%</td>
<td>NS</td>
</tr>
<tr>
<td>1–1</td>
<td>22%</td>
<td>0%</td>
<td>0%</td>
<td>NS</td>
</tr>
<tr>
<td>2–2</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>NS</td>
</tr>
<tr>
<td>3–3</td>
<td>11%</td>
<td>11%</td>
<td>0%</td>
<td>NS</td>
</tr>
<tr>
<td>4</td>
<td>11%</td>
<td>11%</td>
<td>0%</td>
<td>NS</td>
</tr>
<tr>
<td>Mean soluble E-selectin (normal = 9 (3–4) ng/ml)</td>
<td>9–8 (2–0) (6–12–2)</td>
<td>7–7 (2–7) (3–6–10–8)</td>
<td>22–2 (1–6) (5–3–5–3)</td>
<td>0–03</td>
</tr>
<tr>
<td>Mean C reactive protein (normal = 0–78 (0–65) mg/l)</td>
<td>3–16 (2–54) (0–13–7–11)</td>
<td>2–28 (2–21) (0–48–7–7)</td>
<td>1–03 (1–14) (0–14–3–9)</td>
<td>0–03</td>
</tr>
<tr>
<td>Mean von Willebrand factor (normal = 1–26 (0–41) IU/ml)</td>
<td>1–10 (0–29) (0–55–2–87)</td>
<td>1–44 (0–25) (1–09–1–8)</td>
<td>1–33 (0–72) (0–55–2–87)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values in parentheses are (SD) (range).
above the norm) and the primary progressive patients with normal sE-selectin concentrations (data not shown).

By contrast CRP was intermittently raised relative to our normal range in several relapsing remitting and secondary progressive patients during the study (fig 1C), resulting in the means of the individual relapsing remitting or secondary progressive patients being significantly higher than the primary progressive patients (P = 0·03). With the caveat that individual CRP measurements may not be independent of each other, episodes of raised CRP were commonly associated with infectious episodes, clinical relapse, and MRI activity. The mean CRP of the normal controls was 0·8 (SD 0·65) mg/l, and this was significantly lower than the concentrations found during infectious episodes (5·59 (SD 4·16) mg/l, P < 0·001), during a clinical relapse (4·02 (SD 6·39) mg/l, P = 0·004) and those associated with disease activity on MRI (3·37 (SD 6·23) mg/l, P < 0·001). The CRP measurements associated with active MRI studies were significantly higher than the concentrations associated with inactive scans (3·37 (SD 6·23) mg/l v 1·47 (SD 1·74) mg/l, P = 0·04). The CRP concentrations of these patients with inactive studies without infections or clinical relapse were significantly higher than the normal controls (1·47 (SD 1·74) mg/l v 0·78 (SD 0·65) mg/l, P = 0·004). Despite the above differences, CRP concentrations had a very poor predictive value of disease activity, with only 39% of clinical relapses and 22% of active MRI studies being associated with a raised CRP (> 3 SD above the norm). From figs 1B and 1C it is apparent that patients with episodes of raised CRP concentrations had associated low sE-selectin concentrations, and only one patient with raised sE-selectin concentrations (primary progressive patient 9, fig 1C) had a slightly raised mean CRP concentration.

Serum vWF tended to be slightly higher in secondary progressive patients, but it did not reach a statistically significant value in any of the patient groups. Concentrations of vWF did not correlate with clinical relapse, MRI activity, or sE-selectin concentrations.

Plasma TNFα concentrations were not available for one primary progressive and three secondary progressive patients. Primary progressive patients tended to have higher plasma TNFα concentrations than the relapsing remitting and secondary progressive patients, but this did not reach statistical significance. However, mean plasma concentrations of TNFα did correlate with mean serum concentrations of sE-selectin over the study period (n = 34, r = 0·71 and P < 0·001; fig 2). Concentrations of TNFα did not correlate with MRI activity, clinical relapse, disease progression, CRP concentration, or vWF concentrations (data not shown).

Discussion
The baseline characteristics of the patients in our study confirm the different clinical and radiological features of the previously described clinical subtypes of multiple sclerosis.15

Multiple sclerosis lacks a practical and reliable immunological marker of disease activity, and MRI is currently the most widely used technique for monitoring disease activity.22 Our results suggest that for patients with relapsing remitting or secondary progressive disease, sE-selectin is not a useful marker of disease activity; sE-selectin concentrations in these patients correlated with neither clinical relapse or MRI activity. However, in the smaller subgroup of patients with primary progressive multiple sclerosis, sE-selectin concentrations were raised. These findings are in agreement with the those of Dore-Duffy and colleagues,23 who found significantly raised sE-selectin concentrations in chronic progressive patients compared with those with stable or relapsing remitting courses. This suggests that sE-selectin may be a useful indicator of activity in some patients with progressive multiple sclerosis, a group in whom MRI is much less useful because of the characteristically low frequency with which new lesion formation and blood-brain barrier leakage are seen. Furthermore, these findings add to the growing body of evidence from genetic,24 epidemiological,25 radiological,26 and pathological27 studies, which point to a major difference in the pathophysiology of primary progressive multiple sclerosis in comparison with relapsing remitting forms of the disease.

Rieckmann et al in a serial study of 29 patients with multiple sclerosis followed up over 12 months40 and Mohner et al in a cross sectional study including a subgroup of patients with multiple sclerosis,31 report similar findings to ours, with relapsing remitting patients having normal sE-selectin concentrations, despite evidence of disease activity. This finding of normal sE-selectin in the presence of perivascular inflammation may imply that insufficient endothelium is activated or that it is possibly activated for too short a time to release sufficient sE-selectin into the circulation. A possible threshold surface area of endothelium may need to be activated to result in a raised sE-selectin concentration. Also the window of E-selectin expression in stimulated endothelial monolayer cultures is from 24–48 hours,7 and this may be too short to be detected in vivo. Another aspect that needs consideration is the natural ligands of sE-selectin, sialyl-Lewisx and sialyl-Lewisx, on circulating leukocytes,28 as any increase in their expression would decrease the sE-
Soluble E-selectin in multiple sclerosis: raised concentrations in patients with primary progressive disease

selectin concentration proportionately. Another possibility is the inhibition of endothelial sE-selectin expression despite the presence of proinflammatory cytokines known to induce sE-selectin production. Transforming growth factor-β (TGFβ), a potent inhibitor of E-selectin expression, is increased in patients with multiple sclerosis during regression of a relapse and increases in TGFβ producing lymphocytes are found in the CSF and circulation of patients with relapsing remitting multiple sclerosis. An increased expression of TGFβ in and around blood vessels of acute multiple sclerosis plaques has also been described. Thus TGFβ may be inhibiting E-selectin expression in this group of patients. An interesting finding was the dichotomy in the primary progressive patients, with half the patients having persistently raised concentrations of sE-selectin. This may suggest that these primary progressive patients have more diffuse or ongoing endothelial activation, or that they produce insufficient quantities of TGFβ to inhibit E-selectin expression. A functional lack of TGFβ may explain the progressive non-remitting course of the primary progressive patients as TGFβ seems to be important in inducing remission. The factor has also been shown to improve acute and chronic relapsing experimental allergic encephalomyelitis. The close correlation between sE-selectin and TNFα concentrations is not surprising, as TNFα induces E-selectin expression, and this correlation has been described in Kawasaki’s disease and malaria.

The finding of modestly raised CRP concentrations during some relapses and in association with MRI activity confirms earlier reports of raised CRP concentrations in patients with multiple sclerosis during relapse. Another interesting finding was the significantly raised CRP concentrations in patients with inactive MRI compared with controls that could not be explained by infection, implying the presence of active inflammation that is either not associated with Gd enhancement or is below the spatial resolution of the MRI protocol used in this study. This finding supports a pathological study in which the number of histologically active lesions far exceeded the number detected by postmortem T2 weighted MRI. Reactive protein is synthesised by the liver in response to IL-6, and augmented by TNF-α, IL-1, and leukaemia inhibitory factor (LIF). The primary progressive patients did not have raised CRP concentrations despite half of them having evidence of endothelial activation due to IL-1 and TNFα, as well as detectable concentrations of circulating TNFα. This implies focal activity of these cytokines without the hepatic endocrine effects that result in the acute phase response. Interestingly TGFβ has a facilitatory role on the systemic acute phase response induced by IL-1 and TNFα, despite inhibiting their effects on endothelial expression of E-selectin.

The finding of normal vWF concentrations implies that significant endothelial damage is unlikely to occur in multiple sclerosis. This is by contrast with idiopathic uveoretinitis, an inflammatory disease of the eye (an immune privileged site similar to the CNS in multiple sclerosis), in which raised concentrations of vWF have been described. The lack of correlation between vWF and sE-selectin concentrations is not surprising, as the release of vWF by endothelial cells, unlike sE-selectin, is not primarily induced by proinflammatory cytokines, and requires additional stimuli—for example, thrombin.

In summary, sE-selectin concentrations did not correlate with lesion enhancement on MRI and were therefore not increased in patients with relapsing remitting or secondary progressive multiple sclerosis. Surprisingly, half the primary progressive patients who tended to have more progressive disease without lesion enhancement on MRI, had raised concentrations which were closely correlated with plasma concentrations of TNFα. By contrast, an acute phase response, as measured by CRP, was present in relapsing remitting and secondary progressive but not primary progressive patients, and was associated with MRI activity and clinical relapse. This highlights immunological differences between patient groups, and provides some clue to the possible nature of progressive disease in patients with primary progressive multiple sclerosis. In addition to confirmation of our findings, studies need to be performed to further elucidate the nature of these differences. Insights obtained from these studies would allow a rational approach to the treatment of patients with progressive multiple sclerosis.

This work was supported by a grant from the Multiple Sclerosis Society of Great Britain and Northern Ireland.

14 Porta M, Townsend C, Clover GM, et al. Evidence of functional endothelial cell damage in early diabetic retinopa-


