CIRCULATING SOLUBLE ADHESION MOLECULES IN PATIENTS WITH CLASSICAL POLYARTERITIS NODOSA

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SUMMARY

The objective was to evaluate whether changes in circulating soluble adhesion molecule levels reflect disease activity in patients with systemic polyarteritis nodosa (PAN). A sandwich ELISA was used to measure soluble (s) intercellular adhesion molecule 1 (sICAM-1), vascular cell adhesion molecule 1 (sVCAM-1), E-selectin, L-selectin and P-selectin in sera and plasma from 22 patients with active PAN, in sera from 13 of these patients taken serially during follow-up, and in sera from 13 healthy controls. At the time of diagnosis, sICAM-1, sVCAM-1 and sE-selectin levels (488.5 ± 201.3, 1176.5 ± 514.1 and 60.6 ± 27 ng/ml, respectively) were significantly higher in patients than in controls (P < 0.0001, P = 0.001 and P = 0.003, respectively). In contrast, sL-selectin levels tended to be lower in patients than in controls. Within the first 7 days after starting treatment, there was a significant increase in sICAM-1 concentrations, which fell thereafter, but did not completely reach normal levels when patients achieved clinical remission. sE-selectin also remained elevated during follow-up. Only sVCAM-1 decreased, tending to reach normal values in inactive disease. Increased levels of sICAM-1, sVCAM-1 and sE-selectin, and decreased levels of sL-selectin, in active PAN suggest immune and endothelial stimulation during disease activity. Abnormal levels of soluble adhesion molecules in clinically inactive patients might reflect persistence of immune activation and/or endothelial cell exposure to a remaining inflammatory microenvironment.

KEY WORDS: Polyarteritis nodosa, Adhesion molecules, Inflammation.

LONG-TERM follow-up of patients with systemic vasculitis has demonstrated that while immunosuppressive therapy has been life saving for most patients, it fails to induce a sustained remission in a remarkable proportion of individuals [1–3]. Subsequent relapses increase therapeutic requirements and, consequently, treatment-related morbidity and mortality. Clinical or laboratory findings identifying patients at high risk of relapse have not been recognized. In addition, our understanding of how immunosuppressive therapy influences vascular inflammation, injury and repair is still very limited, and accurate parameters discriminating persistent subclinical inflammatory activity from true remission have not been identified.

Over the past few years, cell–cell interactions critically involved in the development of inflammatory lesions have been discovered. Regardless of the primary immunopathogenic mechanisms, the development of vascular inflammatory infiltrates requires dynamic interactions between leucocyte surface receptors and their ligands on the endothelial cell surface. In order to infiltrate tissues, circulating leucocytes roll over the endothelial cell membrane through interactions between carbohydrates and selectins (L-selectin on leucocytes, P-selectin and E-selectin on the endothelial cells). Additional stimuli (chemokines and other co-stimulatory signals) activate leucocyte integrins, which bind tightly to their endothelial counter-receptors of the immunoglobulin superfamily: intercellular adhesion molecule 1 (ICAM-1), ICAM-2 and vascular cell adhesion molecule 1 (VCAM-1). These interactions are also involved in leucocyte transmigration through the endothelial layer [4–7].

Circulating forms of selectins and immunoglobulin superfamily members have been detected on human serum and plasma. These molecules are shed from the cell membrane or directly generated as splice variants lacking the transmembrane and cytoplasmic domain. Increased levels of circulating adhesion molecules have been detected in disorders where leucocyte/endothelial cell interactions play a significant role, namely infections, neoplasms and chronic inflammatory diseases [8–12]. In vitro studies have shown that these soluble forms appear in the supernatant of activated leucocytes or cytokine-stimulated endothelial cells [8, 13]. For that reason, elevated circulating levels of soluble adhesion molecules have been considered a consequence of endothelial and/or immune activation.

In this study, we measured circulating levels of soluble (s) ICAM-1, VCAM-1, E-selectin, L-selectin and P-selectin in patients with systemic polyarteritis nodosa (PAN), both at the time of diagnosis and during follow-up, in order to evaluate whether changes in soluble adhesion molecule levels may reflect disease activity.

PATIENTS AND METHODS

Patients

Twenty-two patients were included in the study. They all had a muscle and/or nerve biopsy showing
vasculitis in medium-sized vessels and fulfilled the ACR classification criteria for PAN [14]. Twenty-one patients were HBsAg negative. The remaining patient was not tested. None of these patients had microscopic polyangiitis as defined by the Chapel Hill International Consensus Conference [15]. Clinical features are summarized in Table I. Serum from 18 patients and plasma from four patients with active PAN were obtained at the time of diagnosis, frozen at −70 °C, and stored until analysis. None of these patients had received immunosuppressive therapy for more than 7 days. Sera from 10 and plasma from three of these patients were subsequently obtained at different times during follow-up (Table II). Whenever samples were drawn, the presence of any other disease, such as infection or neoplasia, was excluded. All these patients but one (who was treated with prednisone, 1 mg/kg/day) were or had been treated with prednisone (0.5–1 mg/kg/day) and cyclophosphamide (1–2 mg/kg/day), and followed during a median period of 15 months (range 1–36). None of these patients experienced relapses during the follow-up period. PAN was considered active when patients were clinically symptomatic and evaluated previously within the first week after starting treatment. We considered a patient to be in remission when he/she was not treated anymore; (b) there were no new signs or symptoms attributable to PAN; (c) biological markers of disease activity (erythrocyte sedimentation rate, C-reactive protein and platelet count) were within the normal range. Persistent sequelae due to vasculitic lesions as defined by Bacon et al. [16] did not exclude remission. Accordingly, patients in groups 1 and 2 were considered active; patients in group 3 were judged as having low-grade clinical activity; and groups 4 and 5 were considered to be in remission.

All samples were obtained with the previous consent of the patients.

Control serum samples were obtained from 13 healthy volunteers, seven men and six women, aged 43–77 (mean 63.2).

### Quantitation of soluble adhesion molecules

sICAM-1, sVCAM-1, sE-selectin, sL-selectin and sP-selectin levels were determined by a sandwich ELISA technique. We used commercially available kits from British Bio-technology Products, Abingdon, for sICAM-1, sVCAM-1 and sE-selectin, and kits from Bender MedSystems, Vienna, Austria, for sL-selectin and sP-selectin. The procedure was performed according to the manufacturer’s instructions. Briefly, a horseradish peroxidase-conjugated monoclonal antibody against sICAM-1, -VCAM-1, -selectin, sL-selectin or sP-selectin was added to microtitre plates coated with a murine monoclonal IgG antibody recognizing a different epitope of the corresponding molecule. After incubation with patient and control samples or standards in appropriate dilution, the colour reaction was developed with tetramethylbenzidine, and the plates were read on an automated multiscanner at 450 nm wavelength and at 600 nm wavelength to correct for background signal. All measurements were performed in duplicate.

The calculated overall intra-assay coefficient of variation was 5.5% for sL-selectin and 2.8% for sP-selectin (Bender MedSystems). For sE-selectin, sICAM-1 and sVCAM-1, the intra-assay coefficient of variation was 4.8, 4.4 and 5%, respectively (R&D Systems).

The interassay coefficient of variation was 7.6% for sL-selectin, 6% for sP-selectin, 7.4% for sE-selectin, 7.4% for sICAM-1 and 9.2% for sVCAM-1.

### Statistical analysis

Data presented are means ± S.D. For comparison between active patients and controls, Student’s t-test was used. For comparison among groups in the longitudinal study, ANOVA was used. Bonferroni’s method was applied to correct for multiple comparisons [17]. P values (two-tailed) <0.05 were considered significant.

## RESULTS

### Circulating adhesion molecules in the control group

Mean ± S.D. values of control serum samples were as follows: sICAM-1 246.8 ± 65.8 ng/ml, sVCAM-1 717.3 ± 175 ng/ml, sE-selectin 34.1 ± 14.2 ng/ml, sL-selectin 847.9 ± 220.9 ng/ml and sP-selectin 314.8 ± 155.7 ng/ml.

### TABLE I

<table>
<thead>
<tr>
<th>Clinical features of patients included</th>
</tr>
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<tbody>
<tr>
<td><strong>Sex (male/female)</strong></td>
</tr>
<tr>
<td>11/11 (50/50)</td>
</tr>
<tr>
<td><strong>Age (yr; mean, range)</strong></td>
</tr>
<tr>
<td>63 (43–77)</td>
</tr>
<tr>
<td><strong>Previous treatment (&lt; 1 week)</strong></td>
</tr>
<tr>
<td>3 (14)</td>
</tr>
<tr>
<td><strong>Clinical manifestations</strong></td>
</tr>
<tr>
<td>General*</td>
</tr>
<tr>
<td>20 (91)</td>
</tr>
<tr>
<td>Neuromuscular</td>
</tr>
<tr>
<td>16 (73)</td>
</tr>
<tr>
<td>Other†</td>
</tr>
<tr>
<td>5 (23)</td>
</tr>
</tbody>
</table>

*Includes malaise, anorexia, weight loss (>10% of initial weight) and/or fever.
†Includes abdominal pain, arthralgias and/or livedo reticularis.

### TABLE II

**Clinical status of the patients at the time when samples were obtained during follow-up**

<table>
<thead>
<tr>
<th>Group</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Active untreated patients</td>
</tr>
<tr>
<td>2</td>
<td>Patients at the beginning of treatment (1–7 days of treatment)</td>
</tr>
<tr>
<td>3</td>
<td>Patients with partial clinical remission between the first week and the first 3 months of treatment</td>
</tr>
<tr>
<td>4</td>
<td>Patients in remission still receiving immunosuppressive therapy</td>
</tr>
<tr>
<td>5</td>
<td>Patients in remission no longer receiving immunosuppressive therapy</td>
</tr>
</tbody>
</table>
Soluble adhesion molecule levels in active PAN patients

At the time of maximal disease activity, sICAM-1 (488.5 ± 201.3 ng/ml), sVCAM-1 (1176.5 ± 514.1 ng/ml) and sE-selectin levels (60.6 ± 27 ng/ml) were significantly higher than in controls (P < 0.0001, P = 0.001 and P = 0.003, respectively). sL-selectin levels (743.4 ± 182.5 ng/ml) were lower than in controls, but the difference was not statistically significant (P = 0.139). sP-selectin levels (275.4 ± 148.3 ng/ml) were not statistically different from those in controls (P = 0.461) (Fig. 1).

No differences were found between serum and plasma levels of sICAM-1, sVCAM-1, sL-selectin and sP-selectin (P = 0.226, 0.709, 0.406 and 0.572, respectively). sE-selectin was not tested in plasma samples.

Soluble adhesion molecules during follow-up (Fig. 2)

sICAM-1. At the time of maximal disease activity (group 1), sICAM-1 levels were significantly higher than in controls (P < 0.0001). At the beginning of treatment (group 2), a large increase in sICAM-1 was observed (P = 0.0004), which decreased thereafter (from group 3 on), although it never reached normal values (differences between group 5 and controls were still statistically significant; P = 0.011).

sVCAM-1. In active patients, sVCAM-1 levels were significantly higher than in controls (P = 0.004). After the beginning of treatment, there was a trend to a slight increase, and then levels tended to be similar to normal controls. Differences between samples obtained from patients in remission and controls were not statistically significant (P = 0.536).

sE-selectin. In active patients, E-selectin levels were higher than in controls and than in all the other groups (P = 0.005). Subsequently, they decreased but remained elevated, without reaching normal values (P < 0.01).

sL-selectin. In all groups, sL-selectin levels were lower than in controls. Although this difference did not reach statistical significance at the time of

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Fig. 1.—Box plots indicating range (error bars), 25–75% interval and median value (horizontal line) of levels of soluble adhesion molecules (sICAM-1, sVCAM-1, sE-selectin, sL-selectin and sP-selectin) in our patients at the time of maximal disease activity (1) and in 13 healthy controls (0).
maximal disease activity \( (P = 0.327) \), it became significant when remission began (from group 3 on; \( P = 0.01 \)).

**sP-selectin.** A significant peak in sP-selectin concentration was observed in patients treated for <1 week \( (P = 0.006) \), but none of the other groups differed significantly from controls.

**DISCUSSION**

Our results show that in patients with active PAN, circulating sICAM-1, sVCAM-1 and sE-selectin levels are elevated compared with those found in healthy matched controls. Given the widespread constitutive endothelial expression of ICAM-1 and its potential induction in a variety of cells under appropriate stimuli, the mechanisms leading to sICAM-1 increase are probably complex and heterogeneous. Elevated sICAM-1 may reflect both immune and endothelial activation [8]. Conversely, VCAM-1 has a more restricted distribution, mainly on the endothelium, and E-selectin is exclusively expressed by endothelial cells. Both VCAM-1 and E-selectin have a low or absent constitutive endothelial expression and are induced by proinflammatory cytokines [4,18]. Accordingly, it is likely that the major source of these adhesion molecules is activated endothelial cells from inflamed vessels. Increased levels of circulating ICAM-1, VCAM-1 and E-selectin have been detected previously in other vasculitides. Most of these clinical studies, restricted to a single molecule, include miscellaneous patients with different vasculitis syndromes and, therefore, the results are difficult to interpret [9,10,19,20]. Attempts to study the pattern of various circulating adhesion molecules in homogeneous groups of patients have found elevated concentrations of sICAM-1 and sVCAM-1 in Wegener’s granulomatosis [11,12], particularly in patients with extensive organ involvement compared with those with limited disease [11]. Increased levels of sICAM-1 and sE-selectin have also been shown in Kawasaki
disease [21, 22], with higher sICAM-1 concentrations in patients with coronary artery lesions [21]. Raised levels of E-selectin were also found in a previous study performed on giant cell arteritis and polyarteritis nodosa patients [23]. While sICAM-1 and sVCAM-1 seem to be elevated in all the vasculitis studied, two independent groups have failed to demonstrate increased sE-selectin levels in Wegener’s granulomatosis [9, 11], and another one found elevated levels of sICAM-1, but not sE-selectin, in rheumatoid vasculitis [24]. These observations suggest a possible preferential use of particular adhesion pathways in different vasculitis syndromes.

Circulating P-selectin and L-selectin have not been thoroughly studied in vasculitis. In our patients, P-selectin levels were not significantly elevated. In vitro studies have shown that although P-selectin synthesis can be enhanced by cytokines [25], its translocation to the cell membrane from the granules where it is stored is a rapid process driven by acute inflammatory mediators [25, 26]. Consequently, P-selectin probably plays a role at the very initial steps of leucocyte/endothelial cell interactions and, at the time our patients were diagnosed, they all had well-established lesions of several weeks duration.

Interestingly, circulating L-selectin, which is shed from the cell membrane upon leucocyte activation [25, 26], tended to decrease in PAN patients. This apparent paradox has also been observed in Kawasaki disease [27]. Critically ill patients with low sL-selectin levels are at higher risk of developing adult respiratory distress syndrome [28]. It has been postulated that circulating L-selectin binds to diffusely activated endothelium in these conditions.

Curiously, in patients followed longitudinally, a transient increase in sICAM-1 and sP-selectin concentrations was observed during the first few days after starting therapy. This phenomenon might indicate a release of bound soluble adhesion molecules and suggests that treatment might modulate receptor/ligand affinity. Little is known about how corticosteroid or immunosuppressive therapy influences adhesion molecule expression or function. Corticosteroids decrease cytokine production [29] which, in turn, may reduce adhesion molecule production. In addition, in vivo studies, Cronstein et al. [30] have demonstrated that corticosteroids directly decrease endothelial ICAM-1 and E-selectin expression induced by lipopolysaccharide or interleukin-1. However, these potentially downregulatory effects of corticosteroids on adhesion molecule synthesis were not observed in our patients with PAN. In patients followed longitudinally, ICAM-1 and E-selectin remained elevated in spite of an appropriate clinical response to corticosteroid and immunosuppressive therapy. Only VCAM-1 tended eventually to normalize. Although long-term follow-up studies are still not available, other authors have also noticed elevated levels of adhesion molecules in vasculitis patients in remission [11, 23]. Corticosteroid and immunosuppressive agents often induce clinical remission in patients with PAN. The tendency of systemic vasculitis to relapse, and the histopathological evidence of persistent inflammatory lesions demonstrated in some treated patients [1–3, 31], suggest that some components of the disease are highly responsive to current treatments, whereas other components are more refractory. Consequently, elevated levels of soluble adhesion molecules in patients apparently in remission might reflect a persistent exposure of endothelial cells to a mild remaining inflammatory microenvironment. Whether adhesion molecule detection may help in discriminating low-grade subclinical activity from true remission deserves further investigation in large, prospective, long-term follow-up studies.

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REFERENCES

12. Mrowka C, Sieberth HG. Circulating adhesion molecules ICAM-1, VCAM-1 and E-selectin in systemic


