Leukocyte Adhesion Molecule Expression in Scleritis

Virender S. Sangwan, MD; Amyna Merchant, MD; Maite Sainz de la Maza, MD, PhD; C. Stephen Foster, MD

Objective: To analyze the expression and cellular distribution of intercellular adhesion molecule 1, E-selectin (endothelial leukocyte adhesion molecule), vascular cell adhesion molecule 1, very late antigen 4, and lymphocyte function associated antigen 1 (LFA-1) in diseased and normal human sclera.

Methods: Monoclonal antibodies to vascular cell adhesion molecule 1, very late antigen 4, intercellular adhesion molecule 1, LFA-1, and E-selectin were used to perform immunohistochemical staining on frozen sections of 16 cryopreserved human sclera specimens and 5 conjunctival specimens.

Results: The normal human sclera did not express any of the adhesion molecules. The expression of LFA-1 was dramatic in all the scleral and conjunctival specimens on the inflammatory cells. Intercellular adhesion molecule 1, the ligand for LFA-1, was expressed in 7 of 12 scleral specimens. Furthermore, the expression of LFA-1 and intercellular adhesion molecule 1 were focally present in areas of inflammatory infiltrate. E-selectin expression was detected on the vascular endothelial cells in 8 of 12 patients. There was variable expression of vascular cell adhesion molecule 1 and very late antigen 4 in the inflamed sclera and conjunctiva.

Conclusions: Our results demonstrate the presence of LFA-1 in the sclera and in the conjunctiva of patients with scleritis. Variable expression of other leukocyte adhesion molecules was noted in the sclera and the conjunctiva of these patients.


ECROTIZING scleritis is the most severe and destructive form of scleritis, and it is often associated with systemic vasculitic disease, occult or overt. Indeed, patients with necrotizing scleritis associated with rheumatoid arthritis are at substantial risk of a lethal vasculitic event within 5 years of onset of necrotizing scleritis if not treated with immunosuppressive chemotherapy.1-4 The development of scleritis probably entails the interaction of genetically controlled mechanisms with environmental factors (infectious agents) or endogenous substances. This interaction gives rise to a putative autoimmune process that damages the episcleral and scleral perforating capillary and postcapillary venules (inflammatory microangiopathy) through immune complex deposition in vessels, subsequent complement activation, and neutrophil enzyme release (type 3 hypersensitivity).5 Persistent immunological injury leads to a chronic granulomatous response (type 4 hypersensitivity) mediated by macrophages, epithelioid cells, multinucleated giant cells, and T lymphocytes.5

In recent years, a great deal of information has emerged about the mechanisms that control extravasation of inflammatory cells at sites of inflammation. These mechanisms are controlled, at least in part, through the up-regulated expression of adhesion molecules on inflammatory cells and vascular endothelium. Considerable advances in delineating the characteristics of cell-surface adhesion molecules have recently been described.6-19 Adhesion receptors play a vital role in a myriad of immunological and inflammatory reactions, and inhibition of adhesion receptors may provide a useful treatment strategy for a variety of diseases.20,21

Up-regulated expression of cell adhesion molecules has been demonstrated in various ocular tissues, including the endothelial cells of retinal and choroidal blood vessels, the retinal pigment epithelium, glial cells, the ciliary body epithelium, cornea, and conjunctiva.22-25 The molecular basis for the selective migration and accumula-
MATERIAL AND METHODS

TISSUES

Scleral and/or conjunctival specimens were harvested after informed consent was obtained from patients with scleritis. Sclera was obtained from 12 patients (10 with necrotizing scleritis and 2 with nodular scleritis); conjunctiva from 4 of these patients was also studied. In 1 patient with necrotizing scleritis, only conjunctiva was obtained (Table 1). Normal sclera was obtained from 4 eyes supplied by the New England Eye Bank, Boston, Mass, after corneal harvesting for corneal transplantation, all within 8 hours of death, and normal human conjunctiva was taken after informed consent was obtained at the time of cataract surgery; these tissues served as control tissue. The tissue was immediately snap frozen in liquid nitrogen, embedded in Tissue Tek OCT (optimal cutting temperature) cryostat embedding compound (Ames Co, Division of Miles Laboratory, Elkhart, Ind), and stored at −70°C until sectioning and analysis.

ANTIBODIES

Mouse monoclonal antibodies to adhesion molecules were used for immunohistochemical staining of the tissue specimens. The specificity, dilutions, and sources of the monoclonal antibodies are shown in Table 1.

IMMUNOSTAINING

The specimens (6-μm sections) were cut in a microtome cryostat (International Equipment Co, Needham Heights, Mass), mounted on gelatin-coated slides, and stored at −70°C. The sections were then stained with the panel of monoclonal antibodies listed in Table 2 by means of a 4-step immunoperoxidase method as previously described.26 Briefly, serial cryostat sections were air dried for 20 minutes, fixed in acetone, and incubated for 20 minutes with normal serum from species in which biotinylated antibody was raised (1:50 dilution). After the excess serum was drained off, all the sections were incubated for 60 minutes with an appropriate dilution of the primary antibodies (Table 2). After rinsing with phosphate-buffered saline, all the sections were incubated for 30 minutes with biotinylated horse anti–mouse (1:200) antibody. After final incubation for 30 minutes with peroxidase-conjugated streptavidin (Jackson Immunoresearch Laboratories, West Grove, Pa) (1:500 dilution), the reactions at sites of antibody binding were developed in peroxidase substrate containing 3-amino-9-ethylcarbazole and hydrogen peroxide in 0.1-mol/L acetate buffer. The specimens were then fixed in formalin, counterstained with Gill 3 hematoxylin, and coverslipped with a glass cover (Corning, Fisher Scientific, Springfield, NJ). Negative controls were tissue sections incubated without primary antibodies. The positive controls were tissue sections incubated for 60 minutes with primary antibody (rabbit anti–human IgG, 1:5000) against collagen IV. The second antibody used for collagen IV was biotin-conjugated, affinity-purified mouse anti–rabbit Ig G (1:500), and the rest of the procedure was the same as described above.

The vascular endothelial expression of cell adhesion molecules was evaluated by means of a scoring system27 based on a scale of 0 to 3+ (0, no staining; 0.5, minimal staining; 1+, definite staining; 2+, marked staining; and 3+, very marked staining) for intensity of staining of individual endothelial cells as well as the number of blood vessels stained. Similarly, the inflammatory cell and epithelial staining was evaluated by means of a scoring system based on a scale of 0 to + (0, no staining; 1+, staining of less than 25% cells; 2+, 25%-50% of cells positive; 3+, 50%-75% of cells positive; and 4+, 100% of cells positive). All the scoring was done in a masked fashion by 2 independent experienced observers (A.M. and C.S.F.).

RESULTS

Negative controls prepared with omission of primary antibodies showed no specific staining.

There was no expression of any of the adhesion molecules studied in the control sclera or in control conjunctiva.

There was a marked presence of LFA-1 in all the patient scleral specimens. It was expressed focally on the inflammatory cells and on the perivascular cellular infiltrate. The ligand for LFA-1, ICAM-1, was also impressively present on the inflammatory cells in 7 of the 12 patients and on the vascular endothelial cells in 4 of 12 patients (Table 3). E-selectin was expressed in 8 of the 12 patients (Table 3) and in 2 patients it was also detected on the inflammatory cells. There was variable expression of VCAM-1 and its ligand VLA-4 in 5 of 12 patients (Table 3).

We also studied the expression of these adhesion molecules in the conjunctiva from 5 patients with scleritis (Table 4). In 1 patient (Table 4, patient 2), only conjunctiva was available; in the rest of these patients the sclera was also studied (Table 3, patients 1, 5, 10, and 12). Expression of LFA-1 was consistently marked on the stromal inflammatory cells in all the conjunctival specimens. However, ICAM-1, the ligand for LFA-1, was present in only 2 specimens, and the rest of the adhesion molecules were variably expressed (Table 4).
We studied the expression of leukocyte adhesion molecules in healthy sclera and conjunctiva and in sclera from patients with scleritis. In 5 patients with scleritis we also studied the expression of these adhesion molecules.

In 5 patients with scleritis we studied the expression of these adhesion molecules in healthy sclera and conjunctiva and in sclera. Healthy sclera and conjunctiva did not express any of the adhesion molecules studied. Inflamed sclera shows immune complex deposition in vessels, leading to complement activation and neutrophil enzyme release as well as a marked increase of macrophages and T lymphocytes.\(^1,^2\)

E-selectin, which is exclusively expressed on vascular endothelial cells stimulated by interleukin 1\(\alpha\), interleukin 1\(\beta\), and tumor necrosis factor \(\alpha\), participates in the initial capture of neutrophils from circulation onto the vascular endothelium.\(^{28,29}\) Persistent E-selectin expression has been found in vivo experimental studies\(^{30,31}\) and in chronic inflammatory diseases.\(^{23,32}\)

We found expression of E-selectin on vascular endothelial cells in 8 of 12 scleral specimens (Figure 1) and in 3 of the 5 conjunctival specimens. These results are in concert with other reports in which E-selectin was found on endothelial cells of venules in other chronically inflamed tissues (eg, in synovia of patients with rheumatoid arthritis and osteoarthritis,\(^{32}\) in chronically inflamed skin,\(^3^3\) and in conjunctiva of patients with vernal keratoconjunctivitis\(^{27}\)). In a mouse model of endotoxin-induced uveitis,\(^{24}\) blocking of both E-selectin and P-selectin resulted in a significant decrease in endotoxin-induced intraocular inflammation.

### Table 1. Associated Systemic or Ocular Disease, Medical Treatment, and Duration of Scleritis Before Biopsy

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Disease</th>
<th>Therapy at Time of Biopsy</th>
<th>Duration of Scleritis, mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Crohn disease</td>
<td>Naproxen sodium; prednisone</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>Rheumatoid arthritis</td>
<td>Prednisone; topical corticosteroid</td>
<td>17</td>
</tr>
<tr>
<td>3</td>
<td>Previous scleral buckle</td>
<td>Aspirin; topical corticosteroid</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>Systemic lupus erythematos</td>
<td>Indomethacin sodium; prednisone</td>
<td>12</td>
</tr>
<tr>
<td>5</td>
<td>Wegner granulomatosis</td>
<td>Prednisone</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>Ulcerative colitis</td>
<td>Sulfasalazine; prednisone</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>Rheumatoid arthritis</td>
<td>Gold; prednisone</td>
<td>8</td>
</tr>
<tr>
<td>8</td>
<td>Relapsing polychondritis</td>
<td>Naphroxx; dapsone</td>
<td>7</td>
</tr>
<tr>
<td>9</td>
<td>Rheumatoid arthritis</td>
<td>Prednisone</td>
<td>10</td>
</tr>
<tr>
<td>10</td>
<td>Idiopathic</td>
<td>Indomethacin</td>
<td>15</td>
</tr>
<tr>
<td>11</td>
<td>Systemic lupus erythematos</td>
<td>Piroxicam</td>
<td>24</td>
</tr>
<tr>
<td>12</td>
<td>Previous zoster ophthalmicus</td>
<td>Tetracycline hydrochloride; prednisone</td>
<td>5</td>
</tr>
</tbody>
</table>

### Table 2. Monoclonal Antibodies (Primary) Used in the Study

<table>
<thead>
<tr>
<th>Antigen</th>
<th>CD</th>
<th>Monoclonal Antibody</th>
<th>Working Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>VCAM-1</td>
<td>CD106</td>
<td>R&amp;D System, Minneapolis, Minn, BBA5, lot 10231</td>
<td>1:1000</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>CD54</td>
<td>R&amp;D System, BBA3, lot 9544</td>
<td>1:3000</td>
</tr>
<tr>
<td>E-selectin</td>
<td>CD11a/CD18</td>
<td>R&amp;D System, BBA16, lot 9383</td>
<td>1:1000</td>
</tr>
<tr>
<td>LFA-1</td>
<td>CD49d/CD29</td>
<td>Pharmingen, San Diego, Calif, catalog No. 3147A, lot M014078</td>
<td>1:1000</td>
</tr>
</tbody>
</table>

*VCAM-1 indicates vascular cell adhesion molecule 1; ICAM-1, intercellular adhesion molecule 1; LFA-1, leukocyte function associated antigen 1; VLA-4, very late antigen 4; and CD, cluster of differentiation.

### Table 3. Adhesion Molecule Expression in the Sclera of Patients With Scleritis

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>VCAM-1</th>
<th>VLA-4</th>
<th>ICAM-1</th>
<th>LFA-1</th>
<th>E-Selectin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stroma</td>
<td>BV</td>
<td>Stroma</td>
<td>BV</td>
<td>Stroma</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>3+</td>
<td>0</td>
<td>1+</td>
<td>2+</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3+</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3+</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*VCAM-1 indicates vascular cell adhesion molecule 1; BV, blood vessel; VLA-4, very late antigen 4; ICAM-1, intercellular adhesion molecule 1; and LFA-1, leukocyte function associated antigen 1. See the “Immunostaining” subsection of the “Materials and Methods” section for an explanation of scoring.

### Table 4. Adhesion Molecules in the Conjunctiva of the Patients With Scleritis

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Epi</th>
<th>Stroma</th>
<th>BV</th>
<th>Epi</th>
<th>Stroma</th>
<th>BV</th>
<th>Epi</th>
<th>Stroma</th>
<th>BV</th>
<th>Epi</th>
<th>Stroma</th>
<th>BV</th>
<th>Epi</th>
<th>Stroma</th>
<th>BV</th>
<th>Epi</th>
<th>Stroma</th>
<th>BV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>1+</td>
<td>2+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1+</td>
<td>2+</td>
<td>3+</td>
<td>0</td>
<td>1+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>1+</td>
<td>1+</td>
<td>0</td>
<td>1+</td>
<td>0</td>
<td>0</td>
<td>2+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
<td>2+</td>
<td>0</td>
<td>3+</td>
<td>4+</td>
<td>0</td>
<td>0</td>
<td>3+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*VCAM-1 indicates vascular cell adhesion molecule 1; Epi, epithelium; BV, blood vessels; VLA-4, very late antigen 4; ICAM-1, intercellular adhesion molecule 1; and LFA-1, leukocyte function associated antigen 1. See the “Immunostaining” subsection of the “Materials and Methods” section for an explanation of scoring.
The possible explanation for not detecting E-selectin in the rest of our specimens may come from differences in inflammatory activity or may be secondary to a different phase of inflammation, since the biopsies were performed at different times. Surprisingly, in 2 scleral specimens, E-selectin was detected on the inflammatory cells. The significance of this finding is not clear.

After the initial E-selectin–mediated adhesion, interactions between the immunoglobulin superfamily adhesion molecules (ICAM-1 and VCAM-1) and their ligands (integrins such as LFA-1 and VLA-4) establish the firm adhesion of leukocytes to vascular endothelium and regulate the emigration of the leukocytes into tissue. The LFA-1 is expressed on a variety of cell types, including neutrophils, monocytes, macrophages, and T lymphocytes. In scleritis, immune complex vessel deposition on postcapillary venules leads to neutrophil activation. Furthermore, extravasation of monocytes and T lymphocytes could be initiated by binding of those inflammatory cells to endothelial cells; this binding could be controlled through the up-regulated expression of adhesion molecules such as LFA-1 (monocytes and T lymphocytes) and ICAM-1 (endothelial cells).

Our findings of very marked expression of LFA-1 (Figure 2) and the localization of LFA-1 and ICAM-1 in the inflammatory cell foci implicates the importance of this pathway in the pathogenesis of necrotizing scleritis. Expression of ICAM-1, the ligand for LFA-1, was impressive on the inflammatory cells in 7 of the 12 scleral specimens (Figure 3) and on the vascular endothelial cells in 4 of the 12 specimens (Table 3). Similarly, the conjunctiva from all the patients (Table 4) showed LFA-1 on the stromal inflammatory cells. The pattern of expression of LFA-1 was similar to that of ICAM-1, but LFA-1 was expressed more consistently and markedly in all the tissue specimens (both conjunctiva and sclera). Both of these adhesion molecules were detected focally in the inflammatory infiltrate around the blood vessels, and ICAM-1 also was present on the vascular endothelial cells. The ICAM-1 and LFA-1 also are present in the inflamed conjunctiva from patients with cicatrizine conjunctivitis (V. S. S., Panayotis Zafirakis, MD, Blanca Rojas, MD, et al, unpublished data, July 1998), and ICAM-1 is expressed in the conjunctiva from patients with Behçet disease and from patients with vernal keratoconjunctivitis, in corneal inflammations, and in corneal graft rejection.

In a rat model of endotoxin-induced uveitis, monoclonal antibodies to ICAM-1 and to LFA-1 were effective in suppressing the inflammation, indicating that, in ocular diseases in which adhesion molecules participate, blockade of adhesion molecule activity may be a promising therapeutic strategy.

The VLA-4 is expressed on a variety of cell types, including monocytes and T lymphocytes; in scleritis, binding of monocytes and T lymphocytes to endothelial cells could be controlled through the up-regulated expression of adhesion molecules such as VLA-4 (monocytes and T lymphocytes) and VCAM-1 (endothelial cells). The VCAM-1 selectively mediates the adhesion of monocytes and lymphocytes, particularly of memory T cells, to vascular endothelial cells, and VCAM-1 expression—like that of ICAM-1—characterizes later phases of inflammation. Greater presence of ICAM-1 and LFA-1 (neutrophil activation and monocyte and T-lymphocyte extravasation) vs VCA-M-1 and VLA-4 (monocyte and T-lymphocyte extravasation) could be explained because of neutrophil activation.

We demonstrated, in the present immunohistochemical study, the presence of leukocyte adhesion mol-
molecules in inflamed human sclera from patients with scleritis. These data suggest that ICAM-1 and LFA-1 may be the predominant pathway involved in mediating the selective recruitment of different leukocyte populations in scleritis. E-selectin may be crucial for the initial phase of the inflammation. Because the adhesion of leukocytes to vascular endothelial cells is a key event in the development of an inflammatory reaction,36 interfering with such adhesive interactions (with humanized monoclonal antibodies or antisense oligonucleotides) to adhesion molecules may provide new therapeutic modalities for the treatment of scleritis.

Accepted for publication June 4, 1998.

Reprints are not available from the authors.

REFERENCES


A look at the past...

Remarks.—Haab's electro-magnet, newest construction, is a powerful and most efficient instrument; connected with the constant street current of 120 volts, it extracted foreign bodies in my cases cite et juacente. . . . The great superiority of Haab's instrument seems to me evident, being so much more powerful, and, in fact, never failing in its work; and to those who do not use Asmus's sideroscope it proves an excellent help for the detection of metallic intraocular foreign bodies, for Haab's magnet acts in a way as a sideroscope. Having made numerous experiments with very minute metallic fragments, introduced near the posterior pole of a hog's eye, I feel certain that the smallest foreign body which might penetrate into the human eye could be detected, in doubtful cases, by the Haab instrument, and extracted if present, in the vitreous.

Reference: Barkan R. Four cases of iron foreign bodies removed from the interior of the eye with Haab's electromagnet. Arch Ophthalmol. 1898;27:42.