Differential Expression of the Cell Adhesion Molecules ICAM-1, VCAM-1, and E-Selectin in Normal and Posttransplantation Myocardium

Cell Adhesion Molecule Expression in Human Cardiac Allografts

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Background Cell adhesion molecules (CAMs) have been implicated in cardiac allograft rejection. However, previous studies have used qualitative analysis of immunohistochemical data and did not exclude patients with infection or malignancy.

Methods and Results We analyzed 40 endomyocardial biopsy specimens from 25 cardiac transplant patients and 8 specimens from patients undergoing cardiac surgery. Patients with evidence of infection or malignancy were excluded. Specimens were stained with monoclonal antibodies against ICAM-1, E-selectin, VCAM-1, and PECAM-1 (which labels all vessels). ICAM-1 expression was assessed by counting ICAM-1–positive vessels and dividing by the total number of vessels (measured by PECAM staining). Specimens were scored as positive or negative for VCAM-1 and E-selectin. We also determined whether serum-soluble ICAM-1 levels (sICAM) correlated with rejection by evaluating 145 serum specimens from 48 cardiac transplant patients and 8 specimens from patients undergoing diagnostic cardiac catheterization. ICAM-1 was present on 50% to 60% of vessels in normal and nonrejecting specimens. Specimens with histologically significant rejection (focal moderate, moderate, or severe) had an increased percentage of ICAM-1–positive vessels: focal moderate, 77%; moderate/severe, 92% (P<.01). E-selectin expression did not differ between groups. VCAM-1 frequently was not present on rejecting specimens. No correlation was noted between sICAM levels and the presence or absence of rejection.

Conclusions (1) ICAM-1 expression is strongly correlated with histologically significant cardiac allograft rejection. (2) The use of PECAM-1 staining as a vascular marker permits quantitative analysis of ICAM-1 expression. (3) VCAM-1 and E-selectin are not consistently increased during cardiac allograft rejection. (4) sICAM levels do not accurately reflect endomyocardial biopsy results. (Circulation. 1994;89:1760-1768.)

Key Words • transplantation • molecules, cell adhesion • ICAM-1 • myocardium

Cardiac transplantation has become widely accepted as therapy for end-stage heart disease. Despite major advances in the field of transplant immunology over the past decade, attempts to design safe, effective, and highly specific immunosuppressive agents remain hampered by incomplete understanding of the pathophysiology of allograft rejection. Over the past decade, it has become clear that an important component of any inflammatory response is the ability of leukocytes to effectively interact both with other leukocytes and with the vascular endothelium. These interactions are now known to be mediated by specific cell surface receptors called cellular adhesion molecules (CAMs).1-6 It is hypothesized that sets of CAMs function in a programmed and sequential fashion, allowing effective and precise cell-cell interactions. For example, leukocyte-endothelial interactions are postulated to begin with the transition from rapid flow of leukocytes over the vascular endothelium to leukocyte “rolling,” mediated by members of the selectin family present on leukocytes (L-selectin) and endothelial cells (P-selectin and E-selectin). After this initial “tethering,” firm adhesion is established by binding of leukocyte integrins, such as LFA-1, Mac-1 (“CD18 integrins”), and VLA-4 (α4β1), to members of the immunoglobulin superfamily (ICAM-1, ICAM-2, and VCAM-1), which are expressed on endothelial cells, and, in the case of ICAM-1 and -2, on other leukocytes.7 This interaction between leukocyte and endothelial cell CAMs is a prerequisite for the migration of white blood cells into areas of inflammation. Similarly, leukocyte-leukocyte interactions mediated by CAMs are required for antigen presentation and T and B cell interactions.2

Because the process of transplant rejection involves migration of recipient leukocytes into graft tissue, it is not surprising that a number of recent studies have suggested a relation between CAMs and cardiac allograft rejection.8-16 Although these studies are both provocative and interesting, a number of questions remain. First, the degree of rejection has not been optimally categorized or quantitated; most previous investigations have labeled biopsy specimens as either “−rejection” or “+rejection” for comparison.9,11-13,15,16 It is unclear how these two simple categories account for other histopathological appearances noted in cardiac allografts or the gradations in severity of rejection. Second, some previous studies have not systematically
excluded specimens from patients with concurrent infections or malignancy. This is an important consideration, because expression of some CAMs, such as ICAM-1, have been shown to be affected by cytokines such as interleukin-1, tumor necrosis factor-α, and interferon-γ, which are elaborated during infection as well as rejection.17 Third, previous reports have not, in general, been able to assess CAM expression in a rigorously quantitative fashion. Early reports evaluated specimens in a semiquantitative manner, describing, for example, an increase in ICAM-1 staining in specimens with histological evidence of rejection based on a scale of 0 to 1.10 In addition, there has been some disagreement among previous studies as to which CAMs show enhanced expression during rejection. For example, several investigators have noted upregulation of E-selectin (previously known as ELAM-1) during rejection,5,10,16 whereas others have not detected immunohistochemical evidence of E-selectin in any specimens.15 Recent data indicate that serum-soluble ICAM-1 (sICAM) may be elevated in hepatic and renal allograft rejection.18,19 This is of great interest, as a reliable noninvasive means of diagnosing transplant rejection could obviate the need for routine endomyocardial biopsy, which is at present the only reliable method for detecting cardiac transplant rejection. One report of sICAM levels in cardiac transplant recipients has been published in abstract form, but patients were not screened for evidence of infection or malignancy.20 The present study was designed to evaluate the expression of CAMs in human cardiac allograft rejection. We had three primary aims: (1) to quantitatively evaluate the expression of the endothelial CAMs—ICAM-1, VCAM-1, and E-selectin—in native myocardium as well as cardiac allografts, (2) to determine if increased CAM expression correlates with more severe degrees of rejection, and (3) to evaluate whether sICAM levels from transplant recipients correlate with histological evidence of rejection on simultaneously obtained endomyocardial biopsy specimens.

Methods

Immunohistochemical Studies

Subjects and Specimens

We studied 40 endomyocardial biopsy specimens from 25 cardiac transplant patients as well as 8 cardiac biopsies from patients without clinical evidence of congestive heart failure who were undergoing open heart surgery. (Patients with heart failure were excluded from the control group in light of recent data showing that patients with chronic congestive heart failure may have elevated serum levels of tumor necrosis factor.)21 The mean age of the transplant patients was 53.8 years (range, 21 to 65 years), and the mean age of nontransplant patients was 64 years (range, 32 to 76 years). All transplant patients were treated with immunosuppressive therapy, including cyclosporine A, azathioprine, and prednisone, as is customary at our institution. Allograft biopsies were obtained according to a routine posttransplant schedule (every week for the first 4 to 6 weeks, every 2 to 3 weeks for the next 2 months, every month until 1 year after transplantation, and then four times per year) or when patients developed clinical signs and symptoms suggestive of rejection. Episodes of significant rejection (biopsies demonstrating focal moderate, moderate, or severe rejection) were treated with high doses of methylprednisolone, OKT3, or antithymocyte globulin, as described elsewhere.22 Biopsy specimens for the study were not obtained from patients with clinically detectable infection or malignancies at the time of endomyocardial biopsy. Infection was defined by the presence of positive bacterial cultures of blood or other sites, serological evidence of viral infections such as cytomegalovirus or Epstein-Barr virus, or clinical evidence of infection such as fever, pneumonia, or cellulitis. In addition, patients' charts were retrospectively reviewed, and specimens from patients who subsequently were found to have developed infection or malignancy at the time of biopsy were excluded from the study. With these criteria, nine biopsy specimens were excluded. Specimens were also excluded if malignancy was present. Criteria for malignancy included the presence of known tumors or of lymphadenopathy of unknown etiology. Again, retrospective chart review would be conducted to rule out subsequent development of malignancy. No biopsies were excluded for malignancy because none of the transplant patients had this.

Specimens were also excluded if when sectioned they had a cross-sectional area of less than 0.25 mm² or if the corresponding hematoxylin and eosin (HE)-stained specimen demonstrated evidence of fibrosis from previous biopsy artifact or Quilty effect, because these processes conceivably could be associated with changes in CAM expression.

Control specimens consisted of tissue taken at the time of cardiopulmonary bypass from patients undergoing cardiac surgery. All tissue-procurement protocols were approved by the Institutional Human Studies Committee at The University of Pennsylvania.

Immunohistochemistry

Endomyocardial biopsy specimens (two per patient at the time of each biopsy) were embedded in OCT compound (Tissue Tek, Miles Diagnostics, Elkhart, Ind), snap-frozen in liquid nitrogen, and stored at −70°C until sectioned. Additional biopsy tissue was Formalin fixed, paraffin embedded, sectioned, and stained with HE for conventional histological evaluation. Thin (6 μm) sections were cut from the frozen specimens, fixed for 1 minute in cold acetone, and stored at −70°C until use. For staining, sections were blocked with 5% horse serum, washed in phosphate-buffered saline (PBS), and treated with primary antibodies for 1 hour at room temperature. Each antibody was titered for optimal reactivity. After being washed in PBS, the bound primary antibody was detected with the Vectastain ABC Elite kit (Vector Laboratories, Burlingame, Calif) with AEC as chromagen. No counterstaining was used to increase sensitivity.

The following mouse monoclonal antibodies were used: RR-1, anti-human ICAM-1 provided by Dr Robert Rothlein (Boehringer-Ingelheim Pharmaceuticals) (1 μg/mL);23 8E4, anti-human E-selectin provided by Dr Barry Woltzky (Hoffman-LaRoche Inc, Nutley, NJ) 10 μg/mL; E16, anti-human VCAM-1 provided by Dr Michael Bevilacqua (University of California, San Diego) 1:5 dilution of hybridoma supernatant; and 56E, anti-human PECAM-1 (CD31) purchased from AMAC, Inc (Westbrook, Me) 1 μg/mL. For negative control slides, PBS/0.4% bovine serum albumin (BSA)/0.02% azide was substituted for the primary antibody.

To allow comparisons between the results of immunohistochemical staining and the degree of rejection as assessed by conventional histological criteria, additional biopsy tissue was Formalin fixed, paraffin embedded, sectioned, and HE-stained in the usual manner. The frozen biopsy specimens used for immunohistochemistry also were stained with HE and showed findings similar to the Formalin-fixed specimens. Immunohistochemistry was determined from HE of the fixed specimens and of the frozen specimens. Specimens were evaluated according to a modification of theBillingham criteria:24 negative, grade 0; minimal/mild lymphocytic infiltrates, grades 1A and 1B; focal moderate rejection, grade 2; and moderate/severe rejection, grades 3A, 3B, and 4.
Evaluation of Staining

Each tissue section was evaluated by an observer without knowledge of the status of the patient. To allow more quantitative assessment of expression of endothelial cell CAMs, specimens were initially graded not on the intensity of staining but rather on the percentage of vessels expressing a particular CAM, as previously described:26

\[
\text{No. of Vessels Stained with RR1, 8E4, or E16} \times 100 \\
\text{Total No. of Vessels}
\]

Previous investigations have identified anti-PECAM-1 antibodies as highly reliable in staining all human vascular beds. Therefore, to assess the total number of vessels per specimen area, we recorded and averaged the number of vessels stained with the anti-human PECAM-1 monoclonal antibody 5.6E for three randomly selected fields of 0.25 mm\(^2\) (×10). A similar procedure was followed to evaluate the number of vessels per unit area staining with each of the above-named antibodies. To assess interobserver variation, the one observer repeated assessments of CAM expressions on the same specimens, and the results were compared. To assess interobserver variability, a second investigator assessed CAM expression on the same specimens, and these results were compared with those of the first observer.

Statistical Analysis

For ICAM-1 staining, intergroup differences were determined by ANOVA followed by the modified Tukey's test. Fisher's exact test was used to analyze the results of staining for VCAM-1 and E-selectin. For intraobserver and interobserver variability, the correlation coefficient for assessments of ICAM-1 staining was determined.

siCAM

Subjects and Specimens

One hundred forty-five specimens from 48 cardiac transplant patients undergoing routine follow-up endomyocardial biopsy and 8 specimens from patients undergoing cardiac catheterization to rule out coronary artery disease were evaluated. The mean age of the transplant patients was 54.8 years (range, 19 to 67 years). Exclusionary criteria were as noted above. As a result of infection, 27 additional specimens were excluded, whereas none were excluded for malignancy. Blood samples were obtained from each patient and spun at 3000 g for 10 minutes. The serum was removed and stored at −70°C until use. Endomyocardial biopsy specimens (transplant patients only) were stained with HE and scored as above. Cardiac histology of control patients was assumed to be within normal limits.

ELISA

Serum levels of ICAM-1 were assessed using a double-antibody ELISA kit (ICAM-1 Test Kit, T Cell Diagnostics). Briefly, the kit provided 96-well ELISA plates coated with murine anti-human ICAM-1 antibody, as well as standards containing known concentrations of ICAM-1, and all necessary solutions. Wells (except those used as blanks) were overlaid with 25 μL of standards containing a known concentration of ICAM-1 (0 to 8.94 ng/mL) or 25 μL of samples that were diluted 1:100 with BSA/thimerosal. Then, 75 μL of horseradish peroxidase–conjugated anti-human ICAM antibody was added to all wells except blanks. Plates were gently agitated for 15 seconds and incubated at room temperature for 2 hours on a rotator at 150 rpm. Wells were aspirated and then washed three times with 350 μL of PBS/surfactant. Chromogen (100 mL) was added to each well. Chromogen was produced by adding four chromogen tablets to 20 mL of urea/peroxide/thimerosal.) Plates were incubated uncovered for 30 minutes at room temperature. To end the reaction, μL of stop solution (2 N H\(_2\)SO\(_4\)) was added to each well. OD\(_{490}\) was read for each well, and a standard curve was derived from known concentrations of sICAM. All specimens were analyzed in duplicate and averaged, and the estimate of sICAM in experimental specimens was derived from comparison with the standard curve values. To determine the variability between kits as well as the effect of storage on sICAM-1 levels in serum, 42 specimens were assayed again using a second kit 2 months after the first assay.

Statistical Analysis

Differences among groups were determined by ANOVA. Results of repeat assays performed on 42 specimens were compared with the initial assay results using correlation coefficients.

Results

Immunohistochemical Studies

Specimens were grouped according to histological appearance of the HE specimens (normal, negative, mild, focal moderate, or moderate/severe).

PECAM-1

The vessels within each biopsy section were identified using the anti-PECAM-1 antibody, 5.6E (Figs 1A and 1C). There was strong staining of the microvessels running adjacent to each myofibril as well as the larger venules and arterioles. This was found in all specimens, regardless of the presence or absence of rejection.

ICAM-1

Fig 1B shows an example of ICAM-1 staining in a specimen without evidence of rejection (specimen 8). This can be compared with Fig 1D, which shows ICAM-1 staining in a specimen with moderate cellular rejection (specimen 34). ICAM-1 staining was noted in 58% of the vessels present in specimen 8 and 95% of the vessels in specimen 34. In general, ICAM-1 was found to be present on perimyocytic capillaries and larger vessels. No staining of the myocardium was noted in any specimen.

The degree of ICAM-1 staining for each group of biopsies was summarized in Table 1 and Fig 2. The following intergroup differences were noted. Specimens with evidence of moderate or severe rejection demonstrated significantly greater ICAM-1 expression than normal specimens, transplant specimens without rejection, and transplant specimens with mild rejection (all \(P<.01\)). Specimens with focal moderate rejection showed significantly greater ICAM-1 expression than normal specimens and specimens with mild rejection (both \(P<.05\)). Most important, when specimens with histologically significant rejection (focal moderate or moderate/severe) were compared with specimens without significant rejection (negative or mild rejection), there was a marked difference in ICAM-1 expression (\(P<.01\)). Assessment of ICAM-1 expression was reproducible with excellent intraobserver (\(r=.96\)) and interobserver (\(r=.92\)) variability.

VCAM-1 and E-Selectin

In contrast to the typical pattern of ICAM-1 expression, staining for VCAM-1 and E-selectin was patchy and infrequent. These CAMs were seen exclusively on noncapillary vessels, with the exception of one specimen with evidence of severe rejection that demonstrated trace capillary staining with the anti-E-selectin anti-
body. Because of the small number of stained vessels per specimen, we decided that a quantitative assessment of VCAM-1 and E-selectin expression as described above would be invalid. We therefore scored biopsies for the presence or absence of VCAM-1 and E-selectin staining anywhere within the specimen. These results are summarized in Table 2. (Specimens with mild rejection were not stained for VCAM-1 and E-selectin.) Results of Fisher’s exact test were as follows. Specimens with focal moderate rejection were more likely to express VCAM-1 than normal specimens ($P=.029$). In addition, when grouped together, specimens with histologically significant rejection were more likely to express VCAM-1 than nontransplant specimens ($P=.021$). No other intergroup differences in VCAM-1 expression were found to be significant, and there also were no differences in E-selectin expression between any of the groups.

**sICAM**

Mean sICAM in normal control blood samples was $402.91 \pm 51.8$ ng/mL (±SEM). These values were compared with those of blood specimens taken from transplant patients and grouped according to the histological appearance of the simultaneously obtained biopsy specimens (Table 1). As seen in Fig 3, there was a wide range of values within each group. ANOVA failed to reveal any significant intergroup differences. Comparison of the results of a second assay of sICAM-1 on 42 specimens with the results of the first assay performed 2 months earlier showed a close correlation between

### Table 1. ICAM-1 Expression as a Function of Degree of Rejection

<table>
<thead>
<tr>
<th>Biopsy Results</th>
<th>Immunohistological Study, % Vessels Expressing for ICAM-1</th>
<th>sICAM Study, sICAM (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean</td>
</tr>
<tr>
<td>Normal</td>
<td>8</td>
<td>53</td>
</tr>
<tr>
<td>Negative</td>
<td>10</td>
<td>58</td>
</tr>
<tr>
<td>Mild rejection</td>
<td>10</td>
<td>53</td>
</tr>
<tr>
<td>Focal moderate rejection</td>
<td>10</td>
<td>77*††</td>
</tr>
<tr>
<td>Moderate/severe rejection</td>
<td>10</td>
<td>92‡‡‡</td>
</tr>
</tbody>
</table>

* $P<.05$ vs normal.
† $P<.05$ vs mild rejection.
‡ $P<.01$, histologically significant rejection (focal moderate plus moderate/severe) vs no significant rejection (negative plus mild rejection).
§ $P<.01$ vs normal.
| $P<.01$ vs negative.‡‡‡
| $P<.01$ vs mild rejection.
Fig 2. Scatterplot of relation between ICAM-1 expression and biopsy results. Lines through groups represent mean value for the group. ICAM-1 expression correlates well with significant cardiac allograft rejection. CAM indicates cell adhesion molecule.

results \(r = .97\). This confirms the reliability of this ELISA over time.

It was possible that basal levels of sICAM-1 differed between individuals. We therefore hypothesized that during an episode of rejection, sICAM could be elevated relative to the nonrejecting sICAM “baseline” for that particular patient but not necessarily higher than the average sICAM “baseline” of a population of transplant patients. For this reason, we selected six individuals from our database who had at least five values for sICAM measured longitudinally over several months. For each of these patients, we examined the relation between sICAM and biopsy results over time (Fig 4). However, although sICAM values from some patients (1 and 2) tended to parallel histological appearance, those from other patients (3 through 6) appeared to be unrelated to the severity or the presence of rejection on biopsy.

Discussion

The present study examined a well-defined group of patients with various degrees of allograft rejection by using a quantitative method for evaluating ICAM-1 expression in endomyocardial biopsy specimens. The total number of vessels per unit specimen area was defined using anti–PECAM-1 antibodies and was compared with the number of vessels per unit area stained by RR-1, an anti-human ICAM-1 antibody. Values obtained were reproducible with subsequent restaining of specimens and had excellent intraobserver and interobserver variability (96% and 92%, respectively). Using this method of analysis, we found that specimens with evidence of moderate-to-severe rejection showed higher levels of ICAM-1 expression than nontransplanted specimens from healthy individuals and allograft specimens without significant rejection. Specimens with focal moderate rejection (grade 2) also showed increased ICAM-1 expression compared to with specimens from healthy individuals and specimens with mild rejection (grades 0, 1A, and 1B). Interestingly, if specimens with mild rejection were eliminated from this comparison, the difference between specimens with focal moderate rejection and specimens with no rejection (grade 0 alone) was not significant. This was likely due to one “outlying” specimen in the negative group; specimen 3 had 77% ICAM-1-positive vessels (this frozen-section specimen from which the assessment of ICAM-1 staining was made did not show more inflammation than the Formalin-fixed specimens obtained at the time of biopsy or than specimens from other biopsies in the negative group). Of note, no differences were seen between nontransplanted specimens and specimens with no rejection or with mild rejection. Most significantly, ICAM-1 expression appears to be a fairly accurate marker of clinically significant rejection, as specimens with significant rejection showed substantially greater ICAM-1 expression than transplant specimens without significant rejection (negative or mild rejection). In fact, a value for ICAM-1–positive vessels of \(\geq 80\%\) predicts significant rejection (necessitating treatment) with a sensitivity and specificity of 85% and 95%, respectively.

In contrast to our findings with ICAM-1, the expression of the two other CAMs examined (VCAM-1 and E-selectin) was much more limited and generally was restricted to fewer than five vessels per biopsy. In the present study, we found no difference between groups with respect to E-selectin expression. VCAM-1 was more likely to be present in specimens with significant rejection than in specimens from healthy individuals; however, this CAM was not seen in all specimens with rejection. These limited expression patterns may be due to the fact that VCAM-1 and E-selectin tended not to be expressed on capillary vessels but rather on larger

TABLE 2. VCAM-1 and E-Selectin Expression as a Function of Degree of Rejection

<table>
<thead>
<tr>
<th>Biopsy Results (Except Normals)</th>
<th>No. of Specimens Expressing VCAM-1</th>
<th>No. of Specimens Expressing E-Selectin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0/8</td>
<td>0/8</td>
</tr>
<tr>
<td>Negative</td>
<td>1/10</td>
<td>1/10</td>
</tr>
<tr>
<td>Mild rejection</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Focal moderate rejection</td>
<td>5/10*†</td>
<td>1/10</td>
</tr>
<tr>
<td>Moderate/severe rejection</td>
<td>3/10*</td>
<td>2/10</td>
</tr>
</tbody>
</table>

*P = .021 histologically significant rejection (focal moderate plus moderate/severe) vs normal.
†P = .029 vs normal.
venules, which were not always present in the tiny biopsy specimens.

The expression of endothelial cell CAMs has been examined in several types of allograft tissue, including kidney, liver, and pancreas. Rejecting renal allografts have increased expression of ICAM-1 on tubular epithelial cells and vascular endothelium. Brockmeyer et al. recently demonstrated that VCAM-1 is induced on peritubular capillaries and upregulated on the tubular epithelium during renal transplant rejection. Rejecting hepatic allografts show upregulation of ICAM-1 on bile duct epithelium, hepatocytes, and vascular endothelium, which are the primary targets of the rejection process. VCAM-1 staining has been noted on some endothelial cells as well as a population of dendritic-like cells in rejecting hepatic and pancreatic allografts.

Our results can be compared with those of previous studies that have assessed the relation between cardiac allograft rejection and CAM expression (summarized in Table 3). This comparison is somewhat difficult because most researchers did not stratify specimens according to degree of rejection, nor did they note whether specimens demonstrated evidence of Quilty effect or previous biopsy artifact. Degree of staining was assessed qualitatively or as counts of stained structures per unit specimen area. Importantly, the presence of infection or malignancy was an exclusion criteria in our study so that causes of increased CAM expression other than rejection were eliminated. Our results are consistent with the majority of preexisting data, showing that ICAM-1 expression is upregulated during cardiac allograft rejection. Unlike Taylor et al., we failed to find any evidence of ICAM-1 expression on myocytes.

Several previous studies have found an upregulation in E-selectin expression during cardiac allograft rejection. However, in agreement with Qiao et al. and Briscoe et al., we were unable to detect a significant difference in expression of this CAM during rejection. These differences may be due to the fact that E-selectin expression is relatively transient. Ferran et al. studied sequential weekly biopsies and found that although E-selectin often was noted on biopsies taken 1 to 2 weeks before an episode of rejection, expression of this CAM disappeared by the time histological evidence of rejection was manifest. Like previous studies, our data show a trend toward increases in VCAM-1 expression in specimens with histological evidence of rejection, but the limited nature of VCAM-1 expression when compared with ICAM-1 makes its role in transplant rejection somewhat questionable.

Endomyocardial biopsy is the only reliable means available for diagnosing cardiac allograft rejection, but it is costly and invasive. Therefore, a simple, noninvasive technique such as a blood test would be highly desirable. Because of the clear upregulation of ICAM noted on biopsy specimens from patients with histological evidence of rejection, it was of interest to determine whether allograft rejection was associated with elevated levels of circulating soluble ICAM-1. sICAM levels have been previously noted to be elevated in numerous inflammatory and malignant conditions, such as idiopathic pulmonary fibrosis, malignancies with liver metastases, acute Kawasaki's disease, and psoriasis. Adams et al. found evidence of elevated circulating sICAM levels during liver transplant rejection, although increases were also seen with various inflammatory but
TABLE 3. Previous Studies of CAM Expression in Human Cardiac Allografts

<table>
<thead>
<tr>
<th>Study</th>
<th>Population Description</th>
<th>Quantification of Staining</th>
<th>ICAM-1</th>
<th>VCAM-1</th>
<th>E-Selectin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Briscoe et al, 10</td>
<td>15 Specimens total 3 Groups with varying degrees of WBC infiltrate</td>
<td>Scale, 0 to 4</td>
<td>Increased with increasing degrees of WBC infiltrate</td>
<td>Variable but overall increased with increasing degrees of WBC infiltrates</td>
<td>Not seen on any specimens</td>
</tr>
<tr>
<td>Steinhoff et al, 9</td>
<td>10 Specimens from &quot;normal&quot; undergoing open heart surgery</td>
<td>Scale, &quot;-&quot; to &quot;++&quot;</td>
<td>Increased during acute and resolving rejection</td>
<td>Not studied</td>
<td>Not studied</td>
</tr>
<tr>
<td>Rose et al, 12</td>
<td>10 Specimens from donor heart 10 Posttransplant specimens with rejection</td>
<td>Cell counts per unit specimen area</td>
<td>Increased in posttransplant specimens</td>
<td>Not studied</td>
<td>Not studied</td>
</tr>
<tr>
<td>Allen et al, 11</td>
<td>90 Posttransplant specimens, with and without rejection</td>
<td>Graded as &quot;++&quot; or &quot;--&quot;</td>
<td>Seen in both rejecting and nonrejecting specimens</td>
<td>Seen only on specimens with rejection</td>
<td>Not studied</td>
</tr>
<tr>
<td>Qiao et al, 8</td>
<td>28 Specimens from patients with dilated cardiomyopathy 55 Posttransplant specimens with grades of rejection</td>
<td>Scale, 0 to 3+</td>
<td>Increased in cellular and humoral rejection</td>
<td>Not studied</td>
<td>Not seen in most specimens</td>
</tr>
<tr>
<td>Taylor et al, 13</td>
<td>15 Specimens from donor heart 15 Posttransplant specimens with rejection</td>
<td>Cell counts per unit specimen area</td>
<td>Increased in rejecting specimens</td>
<td>Increased in rejecting specimens</td>
<td>Increased in rejecting specimens</td>
</tr>
<tr>
<td>Carlos et al, 14</td>
<td>99 Posttransplant specimens 5 Specimens from explanted hearts</td>
<td>Graded as &quot;++&quot; or &quot;--&quot;</td>
<td>Seen on most specimens, regardless of rejection grade</td>
<td>Seen only in rejecting specimens, but not all of them</td>
<td>Not studied</td>
</tr>
<tr>
<td>Allen et al, 15</td>
<td>190 Posttransplant specimens with and without rejection and specimens from explanted hearts</td>
<td>?</td>
<td>Not studied</td>
<td>Not studied</td>
<td>Significantly more common on specimens with rejection</td>
</tr>
<tr>
<td>Ferran et al, 16</td>
<td>Weekly specimens from 16 post-transplant patients</td>
<td>Scale, 0 to 2</td>
<td>Present in specimens without rejection but increased with rejection</td>
<td>Seen in specimens with active or resolving rejection, not seen in nonrejecting specimens</td>
<td>Seen on a few specimens taken just before onset of rejection</td>
</tr>
</tbody>
</table>

nonrejection complications, including cholangitis and hepatitis. Stockenhuber et al 19 demonstrated an association between sICAM and acute renal allograft rejection, although the sample size was fairly small. With regard to cardiac rejection, Ballantyne et al 20 failed to find a correlation between sICAM and histological grade. Patients with infection or malignancy were not excluded in that study. Our data also suggest a poor correlation between sICAM levels and severity of cardiac allograft rejection defined histologically. No significant increases in sICAM levels were noted at the time of rejection compared with levels at times when rejection was not present. This could be due to several factors. First, basal levels of sICAM may vary between patients, necessitating intrapatient rather than interpopulation analyses of correlation. However, our preliminary data suggest that even for individual patients, sICAM often does not accurately reflect the presence of rejection as judged by biopsy. Second, the amount of ICAM-1 that is shed from the cell surface into the serum may not be proportional to the level of cell surface expression. A third possibility is that other inflammatory processes, such as occult infection, may have affected sICAM levels. Of note, four serum samples excluded because of infection underwent ELISA for sICAM. Interestingly, the mean (±SEM) sICAM for these samples was 728±84, which was statistically elevated (P<.001) compared with the mean values for other groups of samples. These data indicate that infection may affect sICAM-1 levels and thus confound the usefulness of sICAM-1 ELISA for the noninvasive detection of cardiac transplant rejection.

An upregulation of CAM expression in rejecting cardiac allografts seems logical in light of current understanding of the pathophysiology of transplant rejection. This is likely due to the release of inflammatory
mediators such as interleukin-1, tumor necrosis factor-α, and interferon-γ by immune cells infiltrating in the allograft. These factors lead to an upregulation of CAMs on various cells of the graft, a process that facilitates leukocyte transmigration and a full-scale immune assault on the foreign tissue. Leukocyte adhesion to explanted, rejecting murine cardiac allograft tissue has been shown to be impaired by anti-CAM antibodies, thus suggesting that increased CAM expression in allograft tissues is of critical significance.38

Two of the most important goals of transplant immunology research remain improved diagnostic and therapeutic strategies for rejection. Unfortunately, given their unreliability, it appears that sICAM levels are not useful as a means of detecting rejection. However, the pattern of myocardial vascular CAM expression in rejection suggests other interesting possibilities, such as the use of radiolabeled anti-CAM antibodies to noninvasively detect rejection.39 Of even greater importance are the potential therapeutic modalities. Several studies have shown monoclonal antibodies directed against various CAMs prolong graft survival and prevent rejection.40-42 Anti-CAM-1 antibodies are being used in clinical trials of patients who received renal transplantation.

**Study Limitations**

The present study demonstrates a potentially useful quantitative approach for the determination of ICAM-1 expression on vascular endothelium of cardiac transplant patients as a function of the severity of rejection. Samples were obtained from patients with various degrees of rejection. Vigorous attempts were made to prospectively and retrospectively exclude samples from patients with infection or malignancy. Occult infection without signs, symptoms, and clinical parameters indicative of infection may have confounded the results of this study to some degree, but follow-up of the patients longitudinally after transplant has not revealed the development of any infection in the patients included in this study. This study did not sequentially study numerous samples from individual patients to determine the changes of CAMs over time. It is possible that some CAMs may be expressed before histological evidence of rejection by HE is present. However, at the time of focal moderate rejection, which likely precedes moderate or severe rejection, only ICAM-1 expression is elevated. Although the other CAMs may play a role in the very early stages of rejection, their expression does not appear enhanced with mild or focal moderate rejection from the results of our study.

**Conclusions**

In summary, our study supports a relation between ICAM-1 expression and cardiac allograft rejection. The role played by other CAMs, such as VCAM-1 and especially E-selectin, is less certain. Serum ICAM-1 levels do not appear to reflect tissue ICAM-1 expression in a straightforward way. Further understanding of the importance of CAM expression in the pathophysiology of allograft rejection may lead to novel diagnostic and therapeutic strategies.

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