Expression of Vascular Cell Adhesion Molecule-1 in Human Renal Allografts

David M. Briscoe, Jordan S. Pober, William E. Harmon, and Ramzi S. Cotran

ABSTRACT

The expression of vascular cell adhesion molecule-1 (VCAM-1) in 11 human renal allograft biopsies and 3 normal kidney specimens was investigated by immunocytochemistry. VCAM-1 expression was correlated with the degree of CD3+ T cell infiltration and the clinical-pathologic diagnosis of acute rejection. CD3+ infiltrates were seen in all biopsies with rejection, but not in normal biopsies or one with acute tubular necrosis, and were accompanied by CD68+ monocyte/macrophage infiltrates. In normal biopsies, VCAM-1 was present on occasional tubules, where its expression was patchy and restricted to the basolateral surface of cells with slight cytoplasmic staining. The total number of tubules expressing VCAM-1 significantly increased in specimens infiltrated with CD3+ T cells. Moreover, in these infiltrated biopsy specimens, VCAM-1 was present throughout the cytoplasm of tubular cells concentrated on the basolateral surface. VCAM-1 was also observed on vascular endothelial cells where its expression correlated with the degree of CD3+ infiltrate. Mean scores (0 to 3+) for endothelial VCAM-1 expression increased from 0 (CD3+ score, 0) to a mean score of 2.25 in association with CD3+ T cell infiltrates (CD3+ score, 3). Endothelial VCAM-1 was predominantly on vessels in areas of infiltrate, including peritubular capillaries, venules, and arterioles, but was notably absent on glomerular endothelium. VCAM-1 also stained mesangial cells in an occasional CD3+ infiltrated specimen. It was concluded that the expression of VCAM-1 is increased on renal tubules and renovascular endothelium in rejecting renal allografts in association with CD3+ infiltrates. These findings are consistent with the hypothesis that VCAM-1 may contribute to the recruitment of lymphocytes and monocytes into, and accumulation within, the interstitium of renal allografts in rejection.

Key Words: Renal allografts, transplantation, vascular cell adhesion molecule-1, endothelium, renal tubules

Acute allograft rejection is a cell-mediated immune reaction characterized by the infiltration of inflammatory cells, principally lymphocytes and monocytes, into the graft (1–3). In the earliest stages of this reaction, inflammatory cells must interact with the vascular endothelium, specifically the endothelium lining postcapillary venules (4,5). Several cytokine-inducible adhesion molecules expressed on endothelial cells can contribute to this process (6–8). Endothelial-leukocyte adhesion molecule-1 (ELAM-1) (9), also known as E-selectin, has been shown to be a ligand for granulocytes, monocytes, and a subset of memory T cells via interactions with certain sialylated oligosaccharides (9–11). Intercellular adhesion molecule-1 (ICAM-1) is a ligand for all leukocytes via interactions with the leukocyte β2 integrins LFA-1 (CD11a CD18) (12) and Mac-1 (CD11b CD18) (13). Vascular cell adhesion molecule-1 (VCAM-1, also known as INCAM-110) (14,15), is a ligand for lymphocytes, monocytes, and eosinophils, via interactions with the leukocyte α4β1 integrin VLA-4 (CD49d CD29) (16).

During rejection, ICAM-1 is diffusely increased on renovascular endothelium and tubular epithelium (17–19). Antibodies to ICAM-1 have been shown to delay the onset of rejection in primates (20) and, in combination with antibodies to LFA-1, induce trans-
plant tolerance in a rodent allograft model (21). Because ICAM-1 is functional in antigen presentation as well as in the costimulation of lymphocytes (22–24), anti–ICAM-1 antibodies may block other immune functions in addition to ICAM-1–mediated endothelial-leukocyte adhesion. VCAM-1 is also expressed on the vascular endothelium of rejecting cardiac allografts. Unlike ICAM-1, which is present basally on endothelial cells, most VCAM-1 is induced de novo predominantly on postcapillary venules at sites of T-cell infiltration (25); antibodies to VLA-4, the leukocyte counter-receptor for VCAM-1, prevent lymphocyte and monocyte infiltration in cell-mediated immune reactions (26,27). These findings support a role for a VCAM-1-dependent adhesive mechanism in cell-mediated immune reactions, such as allograft rejection. We therefore wished to evaluate the distribution of this molecule in human renal allograft rejection.

METHODS

Renal allograft biopsy tissue obtained for diagnostic purposes after renal transplantation was used in this study. The pathologic diagnosis of the biopsies included cellular rejection (N = 8), ATN (N = 1), or normal (N = 2). In addition, normal renal tissue obtained from kidneys excised for neoplasia was also examined (N = 3). A portion of each specimen was embedded in OCT (Tissue Tek; Miles Diagnostics, Elkhart, IN), snap-frozen in isopentane/liquid nitrogen, and stored at −70° until use. The remaining tissue was Formalin fixed and paraffin embedded for diagnostic evaluation. Cryostat 4-micron-thick sections were then examined by immunohistochemistry by a single- or double-labeling technique as described (28). Monoclonal antibodies used were: E1/6 (mouse anti-human VCAM-1) (15,29), kindly provided by M. Bevilacqua, mouse anti-human CD3 and CD68 (Dako Corporation, Carpenteria, CA), rabbit anti-human keratin (Dako), rabbit anti-human von Willebrand factor (vWF) (Dako), and K16/16 (nonspecific mouse immunoglobulin), kindly provided by D. Mendrick, as a control. Briefly, primary antibodies made in different species (mouse and rabbit) were applied either alone or, for double labeling, mixed together for 60 min. Subsequently, peroxidase-conjugated or/and alkaline phosphatase-labeled goat anti-mouse and goat anti-rabbit immunoglobulins (Jackson Immunoresearch, Westgrove, PA), selected for minimal species cross-reactivity, were applied alone or mixed together. The specimens were then incubated in 0.1 M sodium acetate buffer (pH 5.2) and 0.25 mg/mL of amino-ethyl carbazole in 2% N,N dimethylformamide-0.1 M sodium acetate buffer with 0.03% hydrogen peroxide and, for double labeling, were subsequently incubated in 240 mg/L of Fast Blue RR salt in 0.01% Napthol AS-MX phosphate solution containing 0.6 mg/mL of levamisole. Finally, specimens were counterstained in Gills hematoxylin or methyl green and mounted in glycerol gelatin.

A semiquantitative scoring system, designed to assess the intensity and distribution of staining, was used to evaluate the results. Expression of VCAM-1 was scored on vascular endothelium as 0 = absent, 1 = occasional vessel only, 2 = focal intense or weak diffuse staining of vessels, and 3 = intense staining of most vessels and on renal tubules as 0 = absent, 1 = occasional tubule, 2 = focal staining, and 3 = diffuse staining of renal tubules. The degree of CD3+ T cell infiltrates were scored as follows: 0 = absent, 1 = occasional isolated cells, 2 = focal infiltrate, and 3 = diffuse infiltrates. Biopsy specimens were scored independently by two observers (D.M. Briscoe and R.S. Cotran) in a blinded manner, and any minor differences were subsequently resolved by conference.

RESULTS

Specimens were initially stained for the presence of CD3+ T cells, and findings were then correlated with the clinicopathologic diagnosis. CD3+ T cells were present diffusely throughout specimens with cellular rejection and, except for occasional isolated cells, were not found in normal kidneys, in normal renal allografts, or in the specimen with ATN (Table 1). CD3+ infiltrates were localized in the renal interstitium and around tubules and typically spared the glomerulus. CD68+ monocyte/macrophage infiltrates were always present along with CD3+ T cell infiltrates. Biopsy specimens were taken from kidneys excised for neoplasia was also included cellular rejection (N = 1), or normal (N = 3). A portion of each specimen was embedded in OCT (Tissue Tek; Miles Diagnostics, Elkhart, IN), snap-frozen in isopentane/liquid nitrogen, and stored at −70° until use. The remaining tissue was Formalin fixed and paraffin embedded for diagnostic evaluation. Cryostat 4-micron-thick sections were then examined by immunohistochemistry by a single- or double-labeling technique as described (28). Monoclonal antibodies used were: E1/6 (mouse anti-human VCAM-1) (15,29), kindly provided by M. Bevilacqua, mouse anti-human CD3 and CD68 (Dako Corporation, Carpenteria, CA), rabbit anti-human keratin (Dako), rabbit anti-human von Willebrand factor (vWF) (Dako), and K16/16 (nonspecific mouse immunoglobulin), kindly provided by D. Mendrick, as a control. Briefly, primary antibodies made in different species (mouse and rabbit) were applied either alone or, for double labeling, mixed together for 60 min. Subsequently, peroxidase-conjugated or/and alkaline phosphatase-labeled goat anti-mouse and goat anti-rabbit immunoglobulins (Jackson Immunoresearch, Westgrove, PA), selected for minimal species cross-reactivity, were applied alone or mixed together. The specimens were then incubated in 0.1 M sodium acetate buffer (pH 5.2) and 0.25 mg/mL of amino-ethyl carbazole in 2% N,N dimethylformamide-0.1 M sodium acetate buffer with 0.03% hydrogen peroxide and, for double labeling, were subsequently incubated in 240 mg/L of Fast Blue RR salt in 0.01% Napthol AS-MX phosphate solution containing 0.6 mg/mL of levamisole. Finally, specimens were counterstained in Gills hematoxylin or methyl green and mounted in glycerol gelatin.

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### TABLE 1. Lymphocytic infiltrates and VCAM-1 expression in human renal allografts

<table>
<thead>
<tr>
<th>CD3+ T cells</th>
<th>VCAM-1</th>
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<tr>
<td></td>
<td>Endothelium</td>
</tr>
<tr>
<td>Cellular Rejection (N = 8)</td>
<td>2.5</td>
</tr>
<tr>
<td>ATN (N = 1)</td>
<td>1</td>
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<tr>
<td>Normal Allograft (N = 2)</td>
<td>0.5</td>
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<tr>
<td>Normal Kidney (N = 3)</td>
<td>0.3</td>
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*Mean scores (from 0 to 3+).
Figure 1. Photomicrograph of renal allograft biopsies by a single-labeling (panels A through C) or double-labeling (panels D through F) technique. (A) Normal kidney showing VCAM-1 on an occasional proximal tubule (magnification, ×200); (B) CD3+ infiltrated biopsy showing increased VCAM-1 expression on renal tubules and on vascular epithelium (arrows) (×400); (C) a glomerulus in a CD3+ infiltrated specimen showing VCAM-1 on mesangial cells and Bowman's capsule, but not on glomerular endothelium (×1,000); (D) antikeratin antibody (blue) combined with control K16/16 antibody (brown) showing staining of renal tubules in a CD3+ infiltrated specimen. Note no K16/16 staining is seen (×400); (E) antikeratin antibody (blue) combined with anti-VCAM-1 antibody (brown). In the same specimen as that shown in panel D, showing VCAM-1 staining of peritubular capillaries (arrows) and double staining of tubules (T) (×400); (F) endothelial VCAM-1 expression at sites of leukocytic infiltrates (methyl green counterstain) (×2,000).
infiltrates, accounting in some areas for approximately 50% of the mononuclear cell population.

In normal biopsies, VCAM-1 was expressed on 10 to 30% of tubular profiles (Figure 1A). Within each tubule, the stain was often patchy and concentrated typically towards the basolateral surface of cells. VCAM-1 was also present on Bowman's capsule and on the endothelium of an occasional vessel in these normal tissues. In CD3+ infiltrated biopsies showing evidence of acute rejection, there was a significant increase in both the number of VCAM-1-expressing tubules and the cellular distribution of VCAM-1 staining (Table 1; Figure 2). VCAM-1 was expressed on more than 50% of tubular profiles/specimen in biopsies with evidence of cellular rejection; VCAM-1 was seen throughout the cytoplasm of tubular cells concentrated on the basolateral surface (Figure 1B). VCAM-1 was induced on renal tubules in areas of inflammation as well as in sites with slight infiltrate. Most of the definable VCAM-1-expressing tubules appeared to be proximal tubules, although staining of distal tubules could not be excluded.

We next evaluated the expression of endothelial VCAM-1 in these specimens. A double-labeling technique was used to avoid any difficulties in the differentiation of VCAM-1 staining of tubules from VCAM-1 staining of vascular endothelium. Two approaches were used: an anti-vWF antibody, which specifically binds to endothelial cells, was combined with anti-VCAM-1 antibody, or alternatively, an antikeratin antibody, which stains most tubules but not endothelium (Figure 1D), was combined with anti-VCAM-1 antibody. As judged by double staining, VCAM-1 was clearly present on vWF-positive endothelial cells and keratin-positive renal tubular cells (Figure 1E). The expression of endothelial VCAM-1 was absent or occasional in histologically normal allografts and in the case of ATN. In contrast, all eight specimens with cellular infiltrates and rejection showed endothelial VCAM-1 staining (Figure 1E and F). Mean scores for endothelial VCAM-1 induction increased from 0.5 in normal allografts to 2.1 in those with cellular rejection (Table 1). Endothelial VCAM-1 expression appeared to be related to the degree of CD3+ T cell infiltration (Figure 2), being increased from a mean score of 0 (CD3+ score, 0) to 0.7 (CD3+ score, 1) to 2 and 2.25 (CD3+ scores, 2 and 3, respectively). Moreover, endothelial VCAM-1 localized predominantly to vessels in areas of infiltrate (Figure 1F), on peritubular capillaries and large vessels including venules and arterioles. There was no demonstrable staining of glomerular endothelium.

VCAM-1 was present on other cells in most rejecting allografts, including mesangial cells (Figure 1C) and occasional vascular smooth cells. The intensity and distribution of VCAM-1 staining on Bowman's capsule did not appreciably change in biopsies with allograft rejection.

**DISCUSSION**

In this report, we show that VCAM-1 is expressed on the endothelium of peritubular capillaries and large vessels in association with CD3+ infiltrates and acute rejection in renal allografts. This is in agreement with our previous findings in rejecting cardiac allografts (25). Lymphocytes may bind to endothelial cells by ELAM-1, ICAM-1, and VCAM-1-dependent mechanisms. ELAM-1 was not found in any of these biopsies (not shown), consistent with our previous observations. The expression of ICAM-1 is increased in allograft rejection, and antibodies to ICAM-1 prevent rejection in animal allograft models (20,21). However, because humans who lack CD18, the leucocyte counter-receptor for ICAM-1, mount cell-mediated immune responses in *vivo* (30), it is likely that additional endothelial cell adhesion molecules, such as VCAM-1, are involved in lymphocyte recruitment. Indeed, we have found that VCAM-1 is expressed on vascular endothelium in allograft rejection. Moreover, CD68+ monocyte/macrophage infiltrates accompanied the CD3+ infiltrates in biopsies with rejection. Because VCAM-1 also binds monocytes *in vitro* (31), induction of endothelial VCAM-1 may also be involved in the recruitment of monocytes into rejecting allografts.

Renal tubular expression of VCAM-1 was increased in specimens infiltrated with CD3+ T cells. Although VCAM-1 is present on the basolateral surface of isolated renal tubules in normal kidneys,
Increases in VCAM-1 expression in CD3+ infiltrated specimens were seen throughout the biopsy on most tubules. Proximal tubule VCAM-1 has also been shown to be increased in a variety of glomerulonephritis associated with interstitial infiltrates but, curiously, in the absence of endothelial VCAM-1 induction (32). These findings, together with the ability of cytokine-stimulated cultured proximal tubular cells expressing VCAM-1 to support monocyte adhesion (33), are consistent with the potential for tubular cells to interact with leukocytes in vivo.

In summary, we found that the expression of VCAM-1 is increased on renal tubules and renovascular endothelium in rejecting renal allografts in association with CD3+ infiltrates. This is consistent with the hypothesis that VCAM-1 is involved in the interstitial accumulation of lymphocytes and monocytes in rejecting allografts.

ACKNOWLEDGMENTS

We thank Drs. M. Bevilacqua, T. Springer, and D. Mendrick for kindly providing reagents and George Stavrakis for technical assistance. D.M. Briscoe is a Paul Dudley White fellow of the American Heart Association, Massachusetts Affiliate, Inc., and is also supported by a Farley Fellowship Award, Children’s Hospital, Boston.

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