0041-1337/93/5501-0117$03.00/0
TRANSPLANTATION

Vol. 55, 117–123, No. 1, January 1993
Printed in U.S.A.

VARIATION IN EXPRESSION OF ENDOTHELIAL ADHESION MOLECULES IN PRETRANSPLANT AND TRANSPLANTED KIDNEYS—CORRELATION WITH INTRAGRAFT EVENTS[1]

SUSAN V. FUGGLE,[2,3] JEREMY B. SANDERSON,[3] DEREK W. R. GRAY,[3] ARTHUR RICHARDSON,[4] AND PETER J. MORRIS[3]

Nuffield Department of Surgery, University of Oxford, John Radcliffe Hospital, Headington, Oxford, OX3 9DU, United Kingdom

Endothelial adhesion molecules are directly involved in the localization and migration of leukocytes from the circulation into tissues at sites of inflammation. We have compared the expression of PECAM-1 (CD31), ELAM-1, ICAM-1 (CD54), and VCAM-1 in pretransplant (n=20) and needle-core biopsies from renal transplants obtained during different clinical circumstances (n=42). PECAM-1 was consistently expressed on all endothelium in both pretransplant and transplant biopsies. In contrast, there was variation in endothelial expression of ELAM-1 and in proximal tubular expression of ICAM-1 and VCAM-1 between pretransplant biopsies. After transplantation induced expression of endothelial ELAM-1 and VCAM-1 and tubular induction of ICAM-1 and VCAM-1 was detected. Induced adhesion molecule expression was frequently associated with focal leukocyte infiltration, and there was a significantly higher level of CD45 and CD25 positive cell infiltration in biopsies with induced adhesion molecule expression. The induction of adhesion molecule expression is evidence of endothelial activation in these transplant biopsies. Comparison of adhesion molecule expression and HLA-class II antigen expression revealed that induced tubular class II antigens may be detected in the absence of induced adhesion molecule expression.

Acute rejection of a renal allograft is usually accompanied by high levels of interstitial leukocyte infiltration (*1–3*). The initial stage of leukocyte migration into tissues at the site of an inflammatory response is mediated by the interaction of endothelial adhesion molecules with their respective ligands on the leukocyte cell surface (*4*). Inflammatory cytokines can induce changes in the phenotype of the endothelium, termed "endothelial activation," that promote the adherence of leukocytes to the vascular endothelium (*5*). Three cytokine inducible adhesion molecules, intercellular adhesion molecule-1, CD54 (ICAM-1)* (*6, 7*); endothelial leukocyte adhesion molecule-1 (ELAM-1) (*8, 9*); and vascular cell adhesion molecule-1 (VCAM-1) (*10, 11*) have been described, whereas other endothelial adhesion molecules, e.g., platelet endothelial cell adhesion molecule-1, CD31 (PECAM-1) are constitutively expressed on endothelium (*12, 13*). The expression of ELAM-1 appears to be restricted to endothelium (*8, 14*), whereas ICAM-1 (*7*), VCAM-1 (*15*), and PECAM-1 (*16*) are more widely distributed.

IL-1 and TNF-α stimulate expression of ELAM-1, VCAM-1, and upregulation of ICAM-1 from basal levels on cultured endothelium (*17, 18*). ICAM-1 expression is also upregulated by IFN-γ (*17*). The kinetics of induction are different for the three molecules, suggesting involvement at distinct stages during the evolution of an inflammatory response. Peak expression of ELAM-1 occurs rapidly after stimulation and is transient in nature, whereas VCAM-1 and ICAM-1 demonstrate sustained expression (*4*). In an inflammatory response such as that to an allograft, the tissue will be subjected not to the action of single cytokines alone but to combinations of cytokines that may either act synergistically to raise the level of induced product, or may have a regulatory, inhibitory effect (*19, 20*).

Lymphocytes and macrophages are the major components of the leukocyte infiltration in a rejecting renal allograft (*2, 3*). Lymphocytes (*21*) and monocytes (*22*) bind to endothelial ICAM-1 via the ligands LFA-1 and Mac-1 (*18, 21, 23, 24*), and VCAM-1 interacts with lymphocytes and monocytes via the ligand VLA-4 (*25*). ELAM-1 was thought to be primarily involved in the interaction of neutrophils and monocytes with activated endothelium (*9, 26, 27*) via carbohydrate ligands (*28, 29*), but recent evidence shows that ELAM-1 is involved in the interactions between memory T lymphocytes and the endothelium (*30, 31*). Thus ICAM-1, VCAM-1, and ELAM-1 endothelial molecules are potentially all involved in the entry of leukocytes into an allograft as part of the alloresponse.

Adhesion molecules are not only important in the initial phase of an alloresponse in mediating the adhesion of leukocytes to the endothelium but also in the ensuing cellular interactions in the effector phase of the response. The aim of this study was to document the distribution of adhesion molecules in pretransplant biopsies and compare this with the expression in biopsies of the renal graft after transplantation. Increased levels of ELAM-1, VCAM-1, and ICAM-1 were detected in the transplant biopsies and have been related to the clinical events after transplantation, the level of leukocyte infiltration within the graft, and the expression of HLA-class II antigens on the graft parenchyma.

[1] This work was supported by grants from the Medical Research Council and National Kidney Research Fund of the U.K.

[2] Address correspondence to: Dr. Susan V. Fuggle, Nuffield Department of Surgery, John Radcliffe Hospital, Headington, Oxford, OX3 9DU, UK.

[3] Nuffield Department of Surgery, University of Oxford, John Radcliffe Hospital, Headington, Oxford, OX3 9DU, UK.

[4] Current address: Westmead Hospital, New South Wales 2145, Australia.

* Abbreviations: AEC, 3-amino-9-ethylcarbazole; DAB, 3,3′-diaminobenzidine tetrahydrochloride; ELAM-1, endothelial leukocyte adhesion molecule-1; ICAM-1, intercellular adhesion molecule-1; PECAM-1, platelet endothelial cell adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1.

MATERIALS AND METHODS

Patients and biopsy material. Wedge biopsies (n=20) were obtained from cadaveric donor kidneys after nephrectomy, perfusion, and storage, but before implantation of the graft. Routine needle-core transplant biopsies (n=42) were obtained from 24 patients (range, days 5–35; mean day 16.2). All patients were immunosuppressed with triple therapy (low-dose cyclosporine, azathioprine, and steroids). Biopsy material was immediately snap-frozen in liquid nitrogen. Clinical rejection was treated with daily intravenous boluses of methylprednisolone (0.5 g for 3 days) or a course of rabbit antithymocyte globulin (Fresenius: 2–4 mg/kg for 7–10 days) if there was no response to steroids.

Monoclonal antibodies and staining technique. Seven-μ cryostat tissue sections were stained with the following monoclonal antibodies: BBA 3 (anti-ICAM-1 [CD54]); BBA 7 (anti-PECAM-1 [CD31]); BBA 5 (anti-VCAM-1 [INCAM-110]); BBA 1 (anti-ELAM-1) all obtained from British Bio-Technology Ltd., Cowley, Oxford, L203 (anti-HLA-DR [*32, 33*]; Tu22 (anti-HLA-DQ [*34*]); B7/21 (anti-HLA-DP [*35*]); F10.89.4 (anti-CD45 [*36*]); F3. 20.7 (anti-dog Thy-1 [*37*]); and a cocktail of antibodies against CD25 (L54, L61, L62, B-B10, B-G3 [*38*]). The staining was performed using an immunoperoxidase staining technique as previously described (*39*). Briefly, antibody bound on the tissue sections was detected using peroxidase conjugated rabbit-anti-mouse Ig (DAKO Ltd., High Wycombe, Buckinghamshire). The reaction was developed using a substrate of 3,3′-diaminobenzidine tetrahydrochloride (DAB) and H_2O_2, counterstained with Harris's hematoxylin, dehydrated, and mounted in DPX (Merck Ltd., Poole, Dorset). Staining for the weakly expressed ELAM-1 and CD25 antigens was enhanced using a second peroxidase conjugated antibody, swine-anti-rabbit Ig (DAKO Ltd.). CD25 staining was performed using a substrate of 3-amino-9-ethylcarbazole (AEC) and H_2O_2, counterstained with Mayer's hematoxylin, and mounted in aquamount (Merck Ltd.).

Assessment of staining. The percentage area of positive CD45 and CD25 staining was quantitated using a morphometric point counting technique as previously described (*3*). The expression of ICAM-1, PECAM-1, ELAM-1, VCAM-1, and the HLA-class II antigens was assessed on glomeruli, intertubular structures, large vessel endothelium, and tubules on a semiquantitative basis. The analyses were performed independently by 2 observers without knowledge of the patient's clinical status. In control biopsies taken before transplantation, variability in tubular expression of ICAM-1 and VCAM-1 was graded on a scale 0–2 (0: predominantly negative, 1: positive, 2: strongly positive). Endothelial staining for ELAM-1 and VCAM-1 was graded: 0: negative, 1: occasional vessel very weakly stained, 2: more extensive, but weak staining. In transplant biopsies additional grades: 3: focal induction and 4: generalized induction of staining were also applied. ELAM-1 staining of pretransplant biopsies was also quantitated by morphometry and expressed as the percentage area of the biopsy occupied by ELAM-1 positive structures.

Evaluation of renal function. A rejection episode was defined by 3 senior clinicians involved in the management of the patients using all relevant biochemical and clinical data but in the absence of information from biopsy material.

Statistical analyses. Statistical analyses were performed using the Mann-Whitney *U* test, chi-square test, or Fisher's exact test as appropriate, and indicated in the *Results* section.

RESULTS

Pretransplant biopsies. In all 20 pretransplant control biopsies, PECAM-1 was strongly expressed on the endothelium of glomeruli, intertubular capillaries, and of larger arteries and veins, but renal tubules were negative (Fig. 1A). Thus PECAM-1 provides a good marker for the presence of endothelium in the transplant biopsies.

There was variability in ELAM-1 expression between control biopsies ranging from negative or an occasional intertubular capillary positively stained to 2.7% of the biopsy positively stained (mean ±SEM: 1.7±0.16). This staining was mainly confined to the intertubular capillaries (Fig. 1B), but an occasional larger vessel was weakly positive.

ICAM-1 was intensely expressed on all vascular endothelium but in addition expressed on the glomerular mesangium and variably on the proximal tubules. In 13 of 20 biopsies (65%) ICAM-1 was strongly expressed throughout the tubular cytoplasm (Fig. 1C) while in the remaining 35% of pretransplant biopsies the proximal tubules were weakly positively stained (Fig. 1D).

VCAM-1 was strongly expressed on the Bowman's capsule, while the glomerular endothelium and mesangium were negative. In some biopsies there was weak staining of an occasional intertubular structure and endothelium of veins or venules. The distal tubules were always negative, but the biopsies could be subdivided into 3 categories based on differences in the proximal tubular VCAM-1 staining. In 40% of biopsies there was strong cytoplasmic staining of >60% of the proximal tubules (Fig. 1E), but in 35% the tubules were predominantly negative with a strongly stained focal area associated with one of the epithelial cell nuclei (Fig. 1F). The remaining biopsies had an intermediate staining pattern, the tubules being weakly positive often with a more intensely stained focal area associated with the nucleus.

Transplant biopsies. The distribution of PECAM-1 staining provided a means of assessing the presence of capillary endothelium in the transplant biopsies (Fig. 2A). There was some irregular staining in the intertubular areas of 7 of 42 (17%) of biopsies, suggestive of focally abnormal microvasculature. PECAM-1 was not detected on the renal parenchyma, but within areas of focal leukocyte infiltration a small proportion of the leukocytes themselves were weakly positive.

It was difficult to detect differences in ICAM-1 expression on the vasculature since capillaries and large vessels alike were so intensely positive in pretransplant biopsies. Nevertheless, upregulation of ICAM-1 was detected in the proximal tubules of 29% of transplant biopsies, with the molecule being expressed throughout the cytoplasm but very intensely at the brush border (Fig. 2B). The induction was generally focal in nature and associated with leukocyte infiltration. Many of the leukocytes infiltrating the allografts expressed ICAM-1.

Increased levels of ELAM-1 and VCAM-1 expression were detected both on large vessels and capillaries of 29% and 33% of transplant biopsies, respectively (Fig. 2C and D). Intertubular capillaries and larger vessels including arterioles, venules, arteries, and veins became intensely stained, usually in close proximity to leukocyte infiltration. Induced ELAM-1 was not detected on the renal parenchyma, but increased levels of VCAM-1 were detected in the proximal tubules. VCAM-1 was also weakly present on some of the infiltrating leukocytes.

Relationship between adhesion molecule expression and clinical rejection. Biopsies were subdivided according to clinical status. The "no rejection" group included biopsies from patients with no clinical rejection episodes and biopsies obtained more than 7 days before or after a clinical rejection episode. The "rejection" group of biopsies was subdivided into biopsies obtained less than 7 days before, during and less than 7 days after a clinical rejection episode (Fig. 3). Increased levels of ELAM-1, VCAM-1, and ICAM-1 were detected in 17% of biopsies in

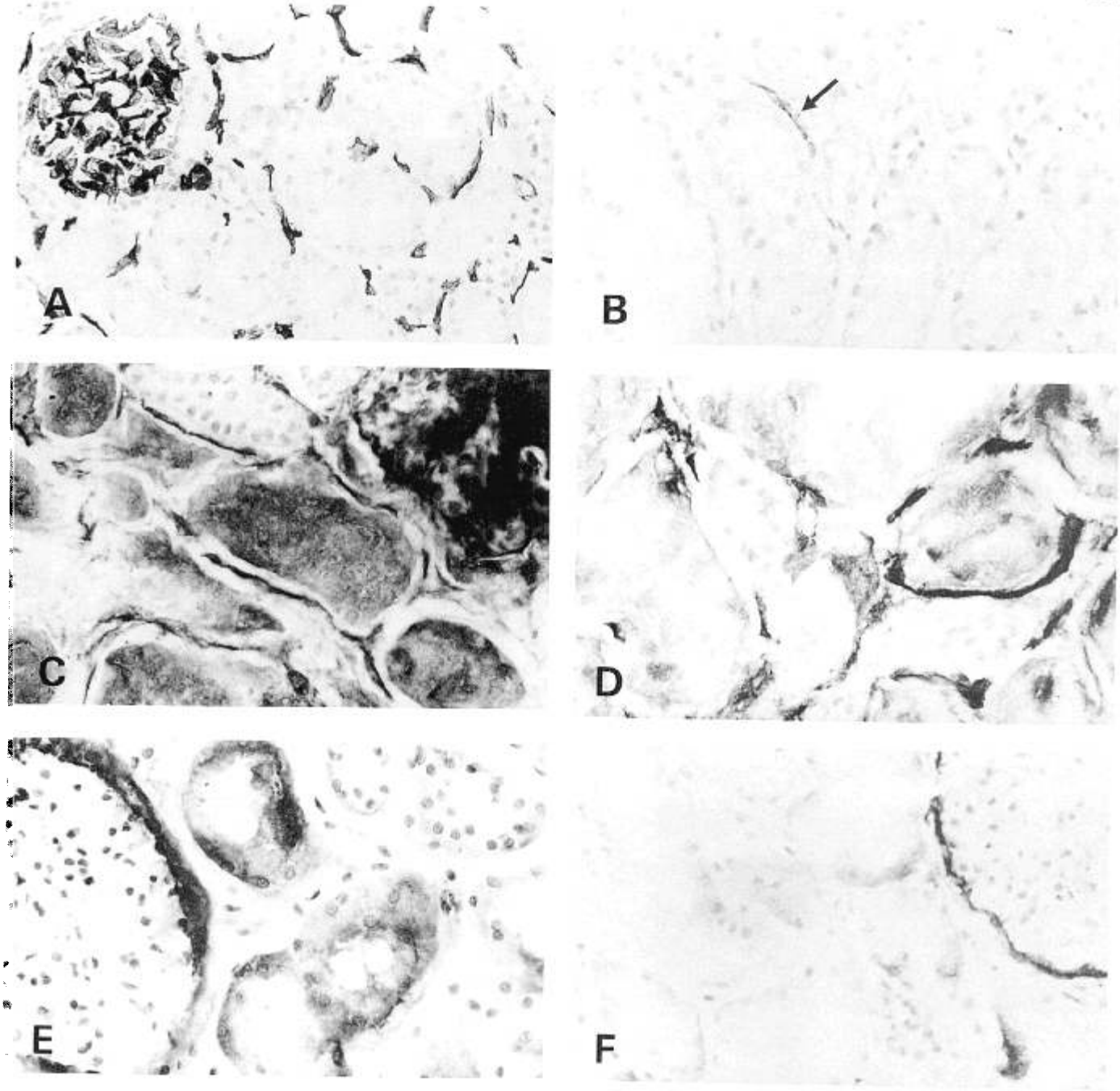

FIGURE 1. Adhesion molecule expression in pretransplant renal biopsies. Cryostat tissue sections were stained with monoclonal antibodies using an indirect immunoperoxidase technique. The reaction was developed with DAB substrate and the nuclei counterstained with hematoxylin. (A) PECAM-1 (CD31) staining of glomerular and intertubular capillaries (original magnification ×250). (B) ELAM-1 staining showing an isolated intertubular capillary (arrowed) (original magnification ×400). (C) ICAM-1 expression showing intensely stained glomerulus and intertubular capillaries and distinct cytoplasmic staining of the proximal tubules (original magnification ×400). (D) ICAM-1 staining illustrating positively stained intertubular capillaries but weak proximal tubular ICAM-1 expression (original magnification ×400). (E) VCAM-1 staining showing positive staining of the Bowman's capsule and strong staining of the proximal tubules (original magnification ×400); and (F) focal VCAM-1 staining of one area of the tubular epithelium (original magnification ×400).

the no rejection group. The induction of these markers was significantly linked (P=0.015, Fisher's exact test). There was a significant increase in VCAM-1 and ICAM-1 but not ELAM-1 expression in biopsies obtained in the 7 days preceding allograft rejection when compared with the "no rejection" group (VCAM-1 and ICAM-1 17% vs. 83% P<0.01; ELAM-1 17% vs. 50% NS. [Fisher's exact test]). The expression of adhesion molecules in biopsies obtained during and less than 7 days after rejection was not significantly different from the "no rejection" group.

Association between adhesion molecule expression and leukocyte infiltration (Fig. 4). There was a significantly greater level of leukocyte infiltration (CD45 staining) in biopsies with in-

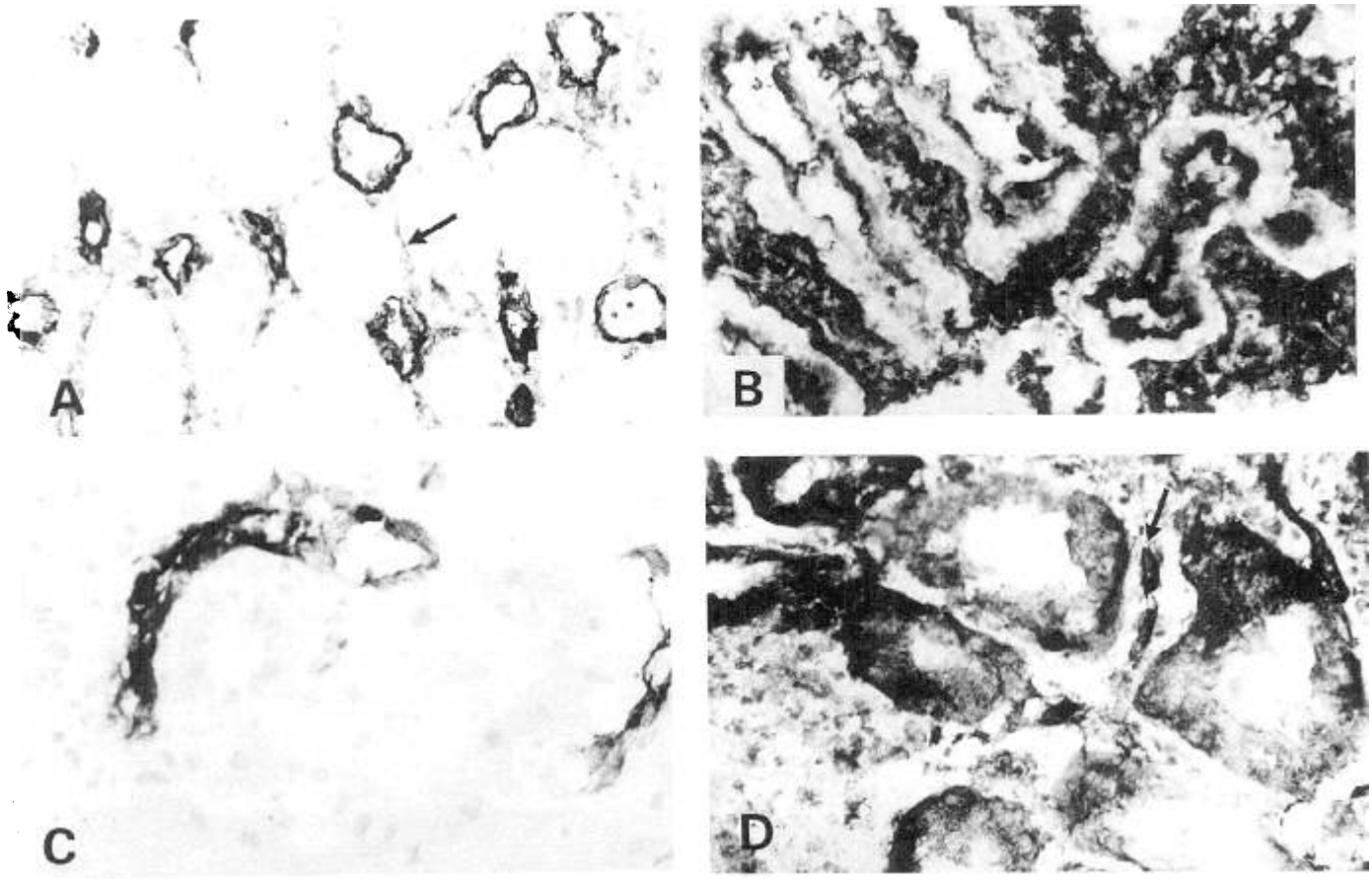

FIGURE 2. Adhesion molecule expression in renal transplant biopsies. (A) PECAM-1 staining identifying the vascular endothelium that is easily distinguished from the weak positive staining of some of the infiltrating leukocytes (arrowed). (B) ICAM-1 induction in the proximal tubules; many of the infiltrating leukocytes are positively stained. (C) ELAM-1 induction in a small venule. (D) VCAM-1 induction in the proximal tubules and endothelium of small vessels (arrowed) (×400).

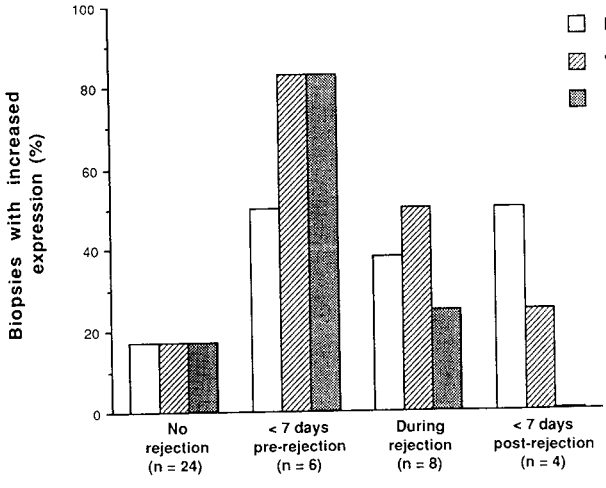

FIGURE 3. Relationship between clinical status and adhesion molecule expression (ELAM-1, VCAM-1, and ICAM-1) in biopsies from patients receiving triple-therapy immunosuppression.

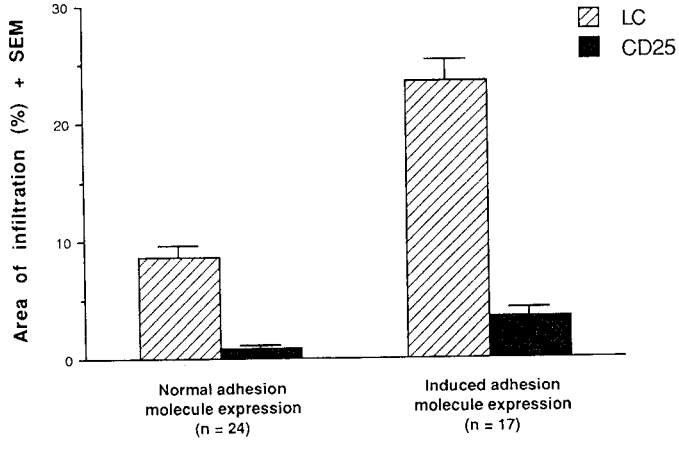

FIGURE 4. Leukocyte infiltration in renal transplant biopsies with normal and induced adhesion molecule expression.

creased levels of adhesion molecule expression when compared with those with normal levels (mean ±SEM: 23.4±1.9% vs. 8.6±1.0%, P=<0.0001, Mann-Whitney U test). The level of CD25 positive cells was also significantly increased in these biopsies (3.5±0.7% vs. 0.9±0.2%; P=0.0001).

Relationship between expression of adhesion molecules and HLA-class II antigens. The patterns of normal and induced expression of HLA-DR, DQ, and DP antigens in renal biopsies have been described in detail (*39*, *40*). For the purposes of comparison with adhesion molecule expression, the transplant biopsies in this study were additionally stained for the HLA-DR, DQ, and DP antigens. Induction of class II antigens was

TABLE 1. Relationship between patterns of expression of endothelial adhesion molecules and HLA-class II antigens in renal transplant biopsies

Adhesion molecule expression	HLA-class II expression	
	Normal	Induced
ELAM-1		
Normal	18	9
Induced	0	9
VCAM-1		
Normal	17	9
Induced	1	9
ICAM-1		
Normal	17	10
Induced	1	8

detected on the tubules and, where present, on large vessel endothelium in 18/36 (50%) of biopsies. The relationship between induction of adhesion molecules and class II antigens is shown in Table 1. In biopsies with a normal pattern of class II antigen expression adhesion molecule expression is generally normal, whereas the pattern of expression of ELAM-1 and VCAM-1 is normal in 50% and ICAM-1 in 60% of biopsies where induction of class II antigens is detected.

DISCUSSION

In this study we have demonstrated both variation in expression of adhesion molecules in renal biopsies taken from cadaver kidneys immediately before implantation (but after perfusion and storage) and upregulation of these molecules in biopsies obtained after transplantation. The expression of adhesion molecules in an allograft is likely to be important not only in the initial stage in mediating the adherence of leukocytes to the endothelium but also at a later stage when coexpression of adhesion molecules and MHC antigens on endothelium or parenchymal cells will strengthen the interaction between allospecific effector cells and these targets.

The variation in the basal level of expression of ICAM-1, VCAM-1, and ELAM-1 between kidneys at the time of transplantation is analogous to the previously described variation in expression of the cytokine inducible HLA-class II molecules (*41*). The variability in the expression of ELAM-1 and VCAM-1 on endothelium and ICAM-1 and VCAM-1 on the proximal tubules occurs on precisely the same structures in which induction occurs in the transplant biopsies. In this study ICAM-1 was consistently strongly expressed on the glomerulus and intertubular capillaries but variably expressed on the proximal tubules, where 65% of biopsies strongly expressed ICAM-1 while in the remaining 35% the expression was weaker. In previous immunohistological studies proximal tubular ICAM-1 was infrequently detected in pretransplant renal biopsies (*7, 42, 43*), but surface ICAM-1 was detected in primary cultures of renal tubules in the absence of stimulation by cytokines (*42, 44*).

VCAM-1 has a restricted distribution in kidney, being expressed on Bowman's capsule, weakly on the occasional venule or intertubular structure and variably on the proximal tubules, ranging from strong positive cytoplasmic staining of more than 60% of the proximal tubules (40% of biopsies) to predominantly negative with a strong focal area associated with an epithelial cell nucleus (35% of biopsies). VCAM-1 has previously been reported on the parietal epithelial cells of the Bowman's capsule and focally on about 10% of tubules (*15*). This is not inconsistent with our findings; the differences may be attributable to the larger sample size in our study.

PECAM-1 consistently stained endothelium throughout the pretransplant biopsies while ELAM-1 staining was variable, and where present, was restricted to veins, venules, and intertubular structures. ELAM-1 was not found in normal kidney in a previous study (*8*), but this difference may be attributable to the more sensitive staining technique we used for ELAM-1 detection.

The variation in ICAM-1, VCAM-1, and ELAM-1 expression, together with the generally higher levels of expression of tubular ICAM-1 and VCAM-1 and endothelial ELAM-1 in our pretransplant biopsies compared with previous studies may be explained on a technical basis or may be the result of either ischemic damage or perfusion injury. A larger study is currently in progress to evaluate the effect of these and other donor-related parameters on the variable expression of adhesion molecules and to determine their possible influence on the early posttransplant events.

Our data show induced ICAM-1, VCAM-1, and ELAM-1 in 26%, 33%, and 29%, respectively, of the posttransplant biopsies studied. Induced ELAM-1 was detected on vascular endothelium, VCAM-1 on vascular endothelium and proximal tubules, and ICAM-1 on proximal tubules. It was not possible to detect upregulation of ICAM-1 expression on vascular endothelium because of the high basal level of expression of this molecule; 17% of biopsies showed evidence of focal microvascular destruction as assessed by PECAM-1 staining. Despite the presence of PECAM-1 on some of the infiltrating cells, PECAM-1 remains a useful marker of endothelium that can be distinguished from the weaker leukocyte staining.

The presence of induced proximal tubular ICAM-1 has been reported in renal allografts showing histological signs of rejection (*42, 43*), whereas ELAM-1 induction was not previously found in rejecting renal allografts (*8*). VCAM-1 induction has not been studied in renal allografts. In a preliminary study of human cardiac allograft biopsies, induced ICAM-1 and VCAM-1, but not ELAM-1, were demonstrated in the presence of a $CD3^+$ leukocyte infiltration. It was postulated that since ELAM-1 is expressed early in an inflammatory response, the grafts may have been biopsied after the level of ELAM-1 had declined (*45*). Alternatively, it is possible that ELAM-1 may have been detected if a more sensitive staining technique was used.

There was a significant association between high levels of leukocyte infiltration and induced adhesion molecules in the renal allografts. This is consistent with the involvement of cytokines released from activated leukocytes in the upregulation of these molecules. Induction was usually focal in nature in association with cellular infiltration. The magnitude of induction will depend not only on the concentration and type of cytokines present but also on the susceptibility of cells to the particular cytokines, e.g., proximal tubules appear to be more susceptible than distal tubules. Induced endothelial ELAM-1 expression has been demonstrated in vessels surrounded by an inflammatory infiltrate in appendicitis and in other conditions with an inflammatory component, e.g., active inflammatory dermatitis and acute granulomatous lymphadenitis (*8*). VCAM-1 has been detected together with ELAM-1 in acute inflammatory conditions, and in the absence of ELAM-1 in chronic inflammatory conditions (*15*). There were 7 biopsies in our

data where there was not concommitant induction of all 3 markers, but there was no unifying factor to account for the differences on the basis of the kinetics of induction or decay.

While there is a clear relationship between the induction of adhesion molecules and the presence of high levels of leukocyte infiltration, the relationship between adhesion molecule status and clinical rejection in this study is not straightforward. Significantly increased levels of VCAM-1 and ICAM-1 were detected in the biopsies obtained in the seven days preceeding rejection, whereas there were no significant differences from the control biopsies in the small numbers of biopsies obtained during or less than 7 days after clinical rejection. However, of the 8 biopsies obtained during a clinical rejection episode, one graft had already suffered extensive damage and microvascular destruction, another had no histological signs of rejection, and 2 were borderline rejection episodes by histological criteria. There was significant infiltration in the remaining 4 biopsies accompanied by induced VCAM-1, induced ELAM-1 in 3, and ICAM-1 in 2 of these biopsies.

Our data demonstrate that induced adhesion molecule expression is rarely detected in the transplanted kidney in the absence of induced class II antigens. In contrast, normal ELAM-1 and VCAM-1 expression was found in 50% of biopsies and ICAM-1 in 60% of biopsies with induced class II antigen expression. Induced class II and ICAM-1 expression were generally seen together in a previous study of renal allograft biopsies. Nevertheless, class II induction was detected in the absence of induced ICAM-1 in 3 of 19 (15.8%) biopsies (*43*). In vitro, ICAM-1 is maximally induced on renal tubules before peak induction of class II antigens (*44*, *46*, *47*), and so the immunohistological findings cannot be explained on the basis of the kinetics of induction. However, it is possible that class II antigen expression may remain elevated after the adhesion molecules have declined to basal levels. Alternatively, our data may be explained in terms of the sensitivity of detection of the induced product. Low levels of induced HLA-DQ and DP antigens are easily detected because these antigens are not generally found in the tubular cytoplasm of the normal kidney, whereas there is often a basal level of ICAM-1.

In conclusion, upregulated expression of endothelial adhesion molecules in an allograft is evidence of endothelial activation and probably facilitates the entry of leukocytes into the graft. Furthermore, the induced adhesion molecules, together with HLA-class II antigens, may render the graft more susceptible to damage mediated by allospecific cytotoxic T lymphocytes. The presence of upregulated adhesion molecules in a biopsy is unlikely to provide a useful tool in the diagnosis of rejection because induction may be evident in the absence of rejection and vice versa. Nevertheless, the adhesion molecules remain a possible target for therapeutic intervention in organ transplantation.

REFERENCES

1. Platt JL, LeBien TW, Michael AFJ. Interstitial mononuclear cell populations in renal graft rejection: identification by monoclonal antibodies in tissue sections. J Exp Med 1982; 155: 17.
2. Hall BM, Bishop GA, Farnsworth A, et al. Identification of the cellular subpopulations infiltrating rejecting cadaver renal allografts: preponderance of the T4 subset of T cells. Transplantation 1984; 37: 564.
3. McWhinnie DL, Thompson JF, Taylor HM, et al. Morphometric analysis of cellular infiltration assessed by monoclonal antibody labelling in sequential human renal allograft biopsies. Transplantation 1986; 42: 352.
4. Pober JS, Cotran RS. The role of endothelial cells in inflammation. Transplantation 1990; 50: 537.
5. Pober JS. Cytokine-mediated activation of vascular endothelium: physiology and pathology. Am J Pathol 1988; 133: 426.
6. Rothlein R, Dustin ML, Marlin SD, Springer TA. A human intercellular adhesion molecule (ICAM-1) distinct from LFA-1. J Immunol 1986; 137: 1270.
7. Dustin ML, Rothlein R, Bhan AK, Dinarello CA, Springer TA. Induction by IL-1 and interferon gamma: tissue distribution, biochemistry, and function of a natural adherence molecule (ICAM-1). J Immunol 1986; 137: 245.
8. Cotran RS, Gimbrone MA Jr, Bevilacqua MP, Mendrick DL, Pober JS. Induction and detection of a human endothelial activation antigen in vivo. J Exp Med 1986; 164: 661.
9. Bevilacqua MP, Pober JS, Mendrick DL, Cotran RS, Gimbrone MA Jr. Identification of an inducible endothelial leucocyte adhesion molecule. Proc Natl Acad Sci USA 1987; 84: 9238.
10. Rice GE, Bevilacqua MP. An inducible endothelial cell surface glycoprotein mediates melanoma adhesion. Science 1989; 246: 1303.
11. Osborn L, Hession R, Tizard R, et al. Direct expression and cloning of vascular cell adhesion molecule 1, a cytokine-induced endothelial protein that binds lymphocytes. Cell 1989; 59: 1203.
12. van Mourik JA, Leeksma OC, Reinders JH, deGroot PG, Zandenbergen-Spaargaren J. Vascular endothelial cells synthesize a plasma membrane protein indistinguishable from the platelet membrane protein IIa. J Biol Chem 1985; 260: 11300.
13. Newman PJ, Berndt MC, Gorsky J, White GC, Paddock LS, Muller WA. PECAM-1 (CD 31): cloning and relation to adhesion molecules of the immunoglobulin gene superfamily. Science 1990; 247: 1219.
14. Redl H, Dinges HP, Buurman WA, et al. Expression of endothelial leukocyte adhesion molecule-1 in septic but not traumatic/hypervolemic shock in the baboon. Am J Pathol 1991; 139: 461.
15. Rice GE, Munro JM, Corless C, Bevilacqua MP. Vascular and nonvascular expression of INCAM-110. Am J Pathol 1991; 138: 385.
16. von dem Borne AEGKr, Modderman PW, Admiraal LG, Nieuwenhuis HK. Joint report of the platelet section. In: Knapp W, ed. Leukocyte typing IV: white cell differentiation antigens. New York: Oxford University Press, 1989: 951.
17. Pober JS, Gimbrone MA Jr, Lapierre LA, et al. Overlapping patterns of activation of human endothelial cells by interleukin 1, tumour necrosis factor, and immune interferon. J Immunol 1986; 137: 1893.
18. Rice GE, Munro JM, Bevilacqua MP. Inducible cell adhesion molecule 110 (INCAM-110) is an endothelial receptor for lymphocytes: a CD11/CD18-independent adhesion mechanism. J Exp Med 1990; 171: 1369.
19. Thornhill MH, Haskard DO. IL-4 regulates endothelial cell activation by IL-1, tumor necrosis factor, or IFN-γ. J Immunol 1990; 145: 865.
20. Thornhill MH, Wellicome SM, Mahiouz DL, Lanchbury JSS, Kyan-Aung U, Haskard DO. Tumour necrosis factor combines with IL-4 or IFN-γ to selectively enhance endothelial cell adhesiveness for T cells: the contribution of vascular cell adhesion molecule-1–dependent and –independent binding mechanisms. J Immunol 1991; 146: 592.
21. Dustin ML, Springer TA. Lymphocyte function–associated antigen-1 interaction is one of at least three mechanisms for lymphocyte adhesion to cultured endothelial cells. J Cell Biol 1988; 107: 321.
22. Arnaout MA, Lanier LL, Faller DV. The relative contribution of the leukocyte molecules Mo-1, LFA-1 and p150,95 (Leu M5) in adhesion of granulocytes and monocytes to vascular endothelium is tissue- and stimulus-specific. J Cell Physiol 1988; 137: 305.

23. Marlin SD, Springer TA. Purified intercellular adhesion molecule-1 (ICAM-1) is a ligand for lymphocyte function–associated antigen-1 (LFA-1). Cell 1987; 51: 813.
24. Makgoba MW, Sanders ME, Ginther Luce GE, et al. ICAM-1 a ligand for LFA-1–dependent adhesion of B, T, and myeloid cells. Nature 1988; 331: 86.
25. Elices MJ, Osborn L, Takada Y, et al. VCAM-1 on activated endothelium interacts with the leukocyte integrin VLA-4 at a site distinct from the VLA-4/fibronectin binding site. Cell 1990; 60: 577.
26. Bevilacqua MP, Stengelin S, Gimbrone MA Jr, Seed B. Endothelial-leukocyte adhesion molecule 1: an inducible receptor for neutrophils related to complement regulatory proteins and lectins. Science 1989; 243: 1160.
27. Lucinskas FW, Brock AF, Arnaout A, Gimbrone MA Jr. Endothelial-leucocyte adhesion molecule-1 dependent and leukocyte [CD11/CD18]-dependent mechanisms contribute to polymorphonuclear leukocyte adhesion to cytokine activated human vascular endothelium. J Immunol 1989; 142: 2257.
28. Phillips ML, Nudelman E, Gaeta FCA, et al. ELAM-1 mediates cell adhesion by recognition of a carbohydrate ligand, Sialyl-LeX. Science 1990; 250: 1130.
29. Brandley BK, Sweidler SJ, Robbins PW. Carbohydrate ligands of the LEC adhesion molecules. Cell 1990; 63: 861.
30. Picker LJ, Kishimoto TK, Smith CW, Warnock RA, Butcher EC. ELAM-1 is an adhesion molecule for skin-homing T cells. Nature 1991; 349: 796.
31. Shimizu Y, Shaw S, Graber N, et al. Activation-independent binding of human memory T cells to adhesion molecule ELAM-1. Nature 1991; 349: 799.
32. Lampson LA, Levy R. Two populations of Ia-like molecules on a B cell line. J Immunol 1980; 125: 293.
33. Hurley CK, Nunez R, Winchester R, Finn OJ, Levy R, Capra JD. The human HLA-DR antigens are encoded by multiple beta chain loci. J Immunol 1982; 129: 2103.
34. Zeigler A, Heinig J, Moller C, et al. Analysis by sequential immunoprecipitation of the specificities of the monoclonal antibodies Tu22, 34, 35, 36, 37, 39, 43, 58, and YD1/63. HLK directed against human HLA-class II antigens. Immunobiology 1986; 171: 77.
35. Watson AJ, DeMars R, Trowbridge IS, Bach FH. Detection of a novel class II HLA antigen. Nature 1983; 3: 358.
36. Dalchau R, Kirkley J, Fabre JW. Monoclonal antibody to a human leukocyte specific membrane glycoprotein probably homologous to the leucocyte-common (L-C) antigen of the rat. Eur J Immunol 1980; 10: 737.
37. McKenzie JL, Fabre JW. Studies with a monoclonal antibody on the distribution of Thy-1 in the lymphoid and connective tissues of the dog. Transplantation 1981; 31: 275.
38. Stein H, Schwarting R, Neidobitek G, Dallenbach F. Activation section report. In: Knapp W, ed. Leukocyte typing IV: white cell differentiation antigens. New York: Oxford University Press, 1989: 387.
39. Fuggle SV, McWhinnie DL, Chapman JR, Taylor HM, Morris PJ. Sequential analysis of HLA-class II antigen expression in human renal allografts: induction of tubular class II antigens and correlation with clinical parameters. Transplantation 1986; 42: 144.
40. Fuggle SV, McWhinnie DL, Morris PJ. Precise specificity of induced tubular HLA-class II antigens in renal allografts. Transplantation 1987; 44: 214.
41. Fuggle SV, Errasti P, Daar AS, Fabre JW, Ting A, Morris PJ. Localization of major histocompatibility complex (HLA-ABC and DR) antigens in 46 kidneys: differences in HLA-DR staining of tubules among kidneys. Transplantation 1983; 35: 385.
42. Bishop GA, Hall BM. Expression of leucocyte and lymphocyte adhesion molecules in the human kidney. Kidney Int 1989; 36: 1078.
43. Faull RJ, Russ GR. Tubular expression of intertubular adhesion molecule-1 during renal allograft rejection. Transplantation 1989; 48: 226.
44. Ishikura H, Takahashi C, Kanagawa K, Hirata H, Imai K, Yoshiki T. Cytokine regulation of ICAM-1 expression on human renal tubular cells in vitro. Transplantation 1991; 51: 1272.
45. Briscoe DM, Schoen FJ, Rice GE, Bevilacqua MP, Ganz P, Pober JS. Induced expression of endothelial-leukocyte adhesion molecules in human cardiac allografts. Transplantation 1991; 51: 537.
46. Miltenberg AMM, Meijer-Paape ME, Daha MR, et al. Donor-specific lysis of human kidney proximal tubular epithelial cells by renal allograft–infiltrating leucocytes. Transplantation 1989; 48: 296.
47. Jevnikar AM, Wuthrich RP, Takei F, et al. Differing regulation of ICAM-1 and class II antigens on renal tubular cells. Kidney Int 1990; 38: 417.

Received 29 February 1992.
Accepted 22 April 1992.