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Expression of Vascular Cell Adhesion Molecule (VCAM-1) in Liver and Pancreas Allograft Rejection

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VCAM-1, a leukocyte adhesion molecule expressed by cytokine-activated endothelial cells in culture, may mediate mononuclear leukocyte infiltration in vessels and interstitium in solid organ allograft rejection. Using the avidin-biotin immunoperoxidase technique and two different antibodies—4B9, a murine monoclonal antibody, and rabbit polyclonal antisera to recombinant human VCAM (rVCAM Ab), which work in methacarn-fixed tissues—we studied the expression of this molecule in biopsies of transplanted liver and pancreas with and without features of rejection as well as nontransplant control tissues. The rVCAM Ab, but not 4B9, showed a population of reactive endothelial cells limited to sites of prominent subendothelial leukocytic cell infiltration in arteries and veins in rejecting allografts. VCAM-1 expression by sinusoidal endothelium in rejecting liver allografts was also observed. In addition, a population of cells (DC) with dendritic morphology was identified by rVCAM Ab within sites of lymphoid cell aggregations in both liver and pancreas allografts. Further evidence that these cells represent true DC was obtained by identification of VCAM-1 positive morphologically similar cells in both germinal centers and interfollicular areas of reactive lymph nodes; and by similar staining of these cells in allograft organs by a monoclonal antibody to nerve growth factor receptor, previously shown to recognize DC. DCs were generally not seen in normal control organs or portions of allografts uninvolved by lymphoid aggregates. This study provides evidence that 1) endothelial cell expression of VCAM-1 may be important in transplant rejection, 2) different epitopes of VCAM-1 may be preserved in tissue sections and recognized by different antibodies, and 3) there is probably a population of VCAM-1 expressing DC that participates in the cellular rejection progress. (Am J Pathol 1993, 142:579–591)

Acute rejection in liver and pancreas allografts, as in other solid organ allografts such as heart and kidney, is characterized by infiltration of the parenchyma by effector immunocompetent and inflammatory cells, principally lymphocytes and monocytes/macrophages.[1–6] In liver, the pathology of rejection seen in allograft biopsies most often displays features of a portal triaditis, inflammatory injury to interlobular bile ducts, and what has been called endothelialitis involving the portal or central veins.[1–7] In pancreas, the pathologic features of rejection typically include inflammatory infiltration of the interstitial tissue as well as the epithelium of the exocrine pancreas.[8–10]

In both liver and pancreas, a process of significant vascular injury can be identified in many cases as part of the rejection process. This injury may take the form of a vasculopathy involving muscular arteries, which can be manifest acutely as an endothelialitis with infiltration of the subendothelial intima by mononuclear inflammatory cells. This lesion, like that occurring in cardiac and renal allografts, may then progress to a chronic arteriopathy with smooth muscle migration into the intima and matrix deposition, leading to intimal sclerosis.[11–13] The microvasculature is also a common participant in solid organ allograft rejection, in which the microvascular endothelium apparently may serve either as an antigenic target to immunocompetent cells of the host[14] or may undergo phenotypic changes that enhance the

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transmigration of host leukocytes into interstitial tissues,[15,16] where they can encounter additional donor cell types against which a rejection response may be mounted.

It is known that these vascular rejection processes are not uniform and that rejection of solid organs is usually an irregularly distributed process. The mechanisms by which circulating leukocytes localize to only certain artery segments or areas of extravascular parenchyma are not known. Nonetheless, an intriguing mechanism that could explain in part a process of focal leukocyte recognition and attachment to portions of the vasculature corresponding to areas of injury involves the induction or up-regulation of specific leukocyte adhesion molecules on the surface of vascular endothelium. At present, four such endothelial proteins have been molecularly cloned[17–20] and shown to be involved in leukocyte adhesion in man[21–26]; these include endothelial leukocyte adhesion molecule-1 (ELAM-1, E-selectin), PADGEM (platelet activation-dependent granule-external membrane protein, GMP-140, CD62, P-selectin), intercellular adhesion molecule-1 (ICAM-1, CD54), and vascular cell adhesion molecule-1 (VCAM-1, INCAM 110). It seems likely that modulation of the expression or configuration of such adhesion proteins on the endothelium or similar modulation of the corresponding ligands on circulating leukocytes might be especially important in establishing the endothelial and subendothelial injury characteristic of vascular rejection in solid organ allografts, and might also be vital to localization of the intraparenchymal inflammatory infiltrates mediating interstitial rejection in such allografts.

Induction of VCAM-1 expression by microvascular endothelium in areas of leukocyte sequestration in cardiac transplant rejection has been demonstrated.[27,28] The interaction between VCAM-1 expressed on endothelium present in inflamed synovium in patients with rheumatoid arthritis and its counter-receptor on resting and activated T lymphocytes has also been shown to be involved in recruitment and localization of leukocytes to sites of immune injury occurring in disease settings other than transplantation.[26] In this study, we have used both a recently characterized monoclonal antibody (4B9) to VCAM-1, and a rabbit polyclonal antisera to recombinant human VCAM-1 (anti-rVCAM-1) to evaluate the expression of this molecule in the setting of liver and pancreas allograft rejection. Both antibodies recognize VCAM-1 epitopes that are preserved in tissue fixed in methyl-Carnoy's solution. In this study, we show that anti-rVCAM-1, but not 4B9, recognizes up-regulated expression of VCAM-1 on vascular endothelium at sites of active inflammatory infiltration characteristic of rejection, and that this VCAM-1 expression becomes undetectable as rejection episodes are successfully treated by increased immunosuppression. We also demonstrate that a population of dendritic cells with immunophenotypic features of both follicular and interdigitating dendritic cells of lymph nodes can be identified focally in the interstitium of occasional livers at the time of organ donation and within interstitial lymphoid aggregates present in acutely rejecting liver and pancreatic allografts.

Materials and Methods

Tissue Selection

A total of 38 hepatic and 11 pancreatic biopsies from 20 patients who underwent pancreas and liver transplants, respectively, were used in this study. All tissues were obtained as core needle biopsies. Eleven of the 15 patients undergoing liver transplantation had at least one episode of biopsy-proven rejection, as did all of the pancreatic transplant patients. Three patients had two consecutive allograft rejection biopsies available for study, for a total of 14 rejection biopsies. All the liver transplant patients had protocol biopsies obtained at the time of transplantation, and after episodes of rejection. The protocol baseline biopsy, biopsies showing rejection, and at least one follow-up biopsy in which features of rejection had resolved were studied in each liver transplant case, and biopsies showing rejection and subsequent resolution of rejection (three of five cases; one death; one persistent rejection) were studied in pancreas transplant cases. Eleven additional donor liver biopsies or normal liver obtained from uninvolved portions of hepatectomy specimens resected for localized malignancy were also studied.

Because VCAM-1 has been identified within dendritic cells in lymphoid tissues, the reactivity of the antisera used in this investigation was also studied in seven reactive lymph nodes and tonsils, surgically removed for lymphoid hyperplasia.

All tissues were fixed in methacarn fixative (60% methanol, 30% chloroform, 10% acetic acid) for at least 12 hours and then processed, paraffin embedded, and sectioned using conventional techniques.

Immunohistochemistry

Briefly, sections of methyl-Carnoy's fixed tissue were deparaffinized with xylene and graded ethanol, blocked with 3% hydrogen peroxide, and washed

with phosphate-buffered saline (PBS) (138 mmol/L NaCl, 2.7 mmol/L KCl, 3.2 mmol/L Na_2HPO_4, 1.5 mmol/L KH_2PO_4, pH 7.3). The tissue was then incubated with one of the primary murine monoclonal antibodies (see below) or rabbit polyclonal antisera and subsequently processed using an avidin-biotin immunoperoxidase method with 3,3′-diaminobenzidine (with nickel chloride enhancement) as the chromogen as previously described.[29,30] Sections were counterstained with methyl green or hematoxylin. For all samples, negative controls for the immunohistochemical procedures consisted of substitution of the primary antibody with both irrelevant murine monoclonal antibodies, or nonimmune rabbit sera, and PBS. Positive controls included concurrent staining of fixed human tonsil, a tissue with detectable constitutive expression of VCAM-1 on dendritic cells,[31] (Figure 1A) and fixed normal human kidney, a tissue with detectable constitutive expression of VCAM-1 on parietal epithelial cells.[31]

Antibodies

Vascular Cell Adhesion Molecule-1

Two sources of antibodies were utilized for this study. Murine monoclonal antibody 4B9 has been characterized previously and its specificity for VCAM-1 established through competitive binding inhibition studies.[25] Reactivity and specificity of this antibody in fixed tissues was established using methyl-Carnoy's fixed-cell aggregates of Chinese Hamster Ovary cells either transfected or untransfected (negative control) with VCAM cDNA (kindly provided by Dr. Margaret Rosa, Biogen, Cambridge, MA), which were then shown to express VCAM-1 at the cell surface by appropriate binding of leukocyte cell lines as well as binding inhibition assays.[32]

We also used a rabbit polyclonal antisera that was raised against a recombinant form of human VCAM-1 (rsVCAM) that was purified to homogeneity by immunoaffinity chromatography as previously described.[33] New Zealand White rabbits, 3 to 4 kg, were immunized with purified rsVCAM (1 mg), emulsified (1:1) in Freund's complete adjuvant (Difco Laboratories, Detroit, MI). The rabbits were boosted with rsVCAM (1 mg) in incomplete adjuvant, at monthly (3×) and then bimonthly intervals. Bleeds were taken 7 to 14 days after each boost. Rabbit antisera was affinity purified by passage over protein A, with the IgG fraction then passed over an affinity resin of human rsVCAM-1 immobilized on Affigel (8 mg of rsVCAM-1 per milliliter of resin). The antisera was eluted with buffer at pH 3.0, dialized into PBS, aliquoted, and stored at −80 C.

Nerve Growth Factor Receptor-5 (NGFR5)

NGFR5 is a monoclonal antibody originally developed to study the expression of nerve growth factor receptor in tumors and normal tissues.[34] Among normal tissues, in addition to expected neural immunostaining, NGFR5 has been demonstrated to react with several non-neural cell types, including lymphoid follicular dendritic cells.[34,35] In this study we used the NGFR5 as an independent confirmatory immunolocalization marker of dendritic cells in lymphoid and nonlymphoid tissue.

Leukocyte Markers

Immunophenotypic characterization of infiltrating leukocytes was performed as previously described.[13] Commercially available antibodies were used to identify populations of monocytes/macrophages (anti-CD 68, monoclonal antibody KP-1, Dako Corporation, Carpinteria, CA),[36] T lymphocytes (anti-CD 43, monoclonal antibody Leu 22, Dako Corporation),[37] and B lymphocytes (anti-CD20, monoclonal antibody L26, Dako Corporation).[38,39]

Endothelial Markers

Rabbit polyclonal anti-human factor VIII related antigen/von Willebrand factor (Dako Corporation) was used as previously described.[40]

Proliferating Cell Nuclear Antigen (PCNA)

19A2, a murine monoclonal antibody to PCNA/cyclin (Coulter Corporation, Hialeah, FL) was used as previously described.[41]

Absorption Assay

Chinese Hamster Ovary (CHO) cells transfected with VCAM-1, ICAM-1, and ELAM-1 and demonstrating surface expression of each of these molecules as well as untransfected CHO cells, were maintained in culture as previously described.[32] Culture plates were washed with PBS, scraped, and pelleted after centrifugation at 1200 rpm. Cell pellets were fixed in methyl-Carnoy's solution, and processed and embedded in paraffin for tissue immunohistochemistry using procedures detailed above. Both the rabbit polyclonal antisera to recombinant VCAM-1 and the murine monoclonal antibody 4B9 were demonstrated to be reactive with the cell surface of CHO cells transfected with VCAM-1, but not with untransfected cells or those transfected with ICAM-1 or ELAM-1 (data not shown).

Cell pellets, each containing approximately 25 × 10^6 total cells, of VCAM-1, ICAM-1, and untransfected CHO cells were also collected in serial dilutions of PBS and then incubated for 60 minutes at room temperature with aliquots of both rabbit polyclonal anti-VCAM-1 antisera and the monoclonal antibody 4B9. After incubation, the suspensions were again centrifuged at 1200 rpm and the supernatants collected for incubation on tissue sections of both the cell pellets as noted above and on methyl-Carnoy's fixed tissue sections of human tonsil. Identification of tissue binding was determined with the avidin-biotin immunoperoxidase technique detailed above, and tissues were counterstained with methyl green.

Results

Lymphoid Organs

The rabbit polyclonal anti-rs VCAM-1 showed prominent reactivity with follicular dendritic cells in the germinal centers of reactive lymph nodes, and to a less numerous population of interdigitating cells in the T-cell–dependent interfollicular areas of lymph nodes and tonsils (Figure 1A). The monoclonal antibody 4B9 showed a similar but less pronounced pattern of staining. Absorption of the polyclonal antisera with VCAM-1 expressing CHO cells, but not ICAM-1 transfected or untransfected CHO cells, completely blocked the reactivity of the antisera with dendritic cells (Figure 1B).

A second antibody, NGFR5, was also reactive to follicular, but not interdigitating, dendritic cells in lymphoid tissues as previously described.[34] The reactivity patterns of the NGFR5 and VCAM-1 antisera with follicular dendritic cells in germinal centers were concordant.

Allograft Tissue

Expression of VCAM-1 in both hepatic and pancreatic allograft biopsies is summarized in Table 1. The results shown and discussed below were obtained with the rabbit polyclonal antisera to recombinant human VCAM-1. Immunostaining with the 4B9 monoclonal antibody occasionally was similar to that obtained with the polyclonal antisera, but most often was entirely absent in liver and pancreatic tissue. There were no examples of cells reactive with the 4B9 antibody but unreactive with the rabbit antisera.

Liver

VCAM-1 expression was not detected in normal liver. No VCAM-1 expression was detected in 13 of 15 protocol baseline liver biopsies; 1 in 15 cases showed the presence of portal dendritic cells with VCAM-1 expression (see below) and 1 in 15 biopsies showed endothelial expression in a single hepatic artery segment but was otherwise negative. At times of rejection, diagnosed by established morphologic criteria,[1–6] focal expression of VCAM-1 was demonstrated in 12 of 14 biopsies (Figure 2). This expression was manifest in one of three ways. Focal staining of the endothelial lining of portal veins in areas of triaditis and endothelialitis (Figure 2E) was the most common finding identified in 10 in 12 of the rejection biopsies demonstrating VCAM-1 expression. Portal vein endothelial expression of VCAM-1 was always focally distributed and associated with either adherence of mononuclear leukocytes to/the luminal surface of the vessel (rarely seen), or more commonly associated with infiltration of the immediately adjacent tissues by mononuclear leukocytes (Figure 2, A to C). Immunophenotypic studies of these leukocytes showed that virtually all could be identified as

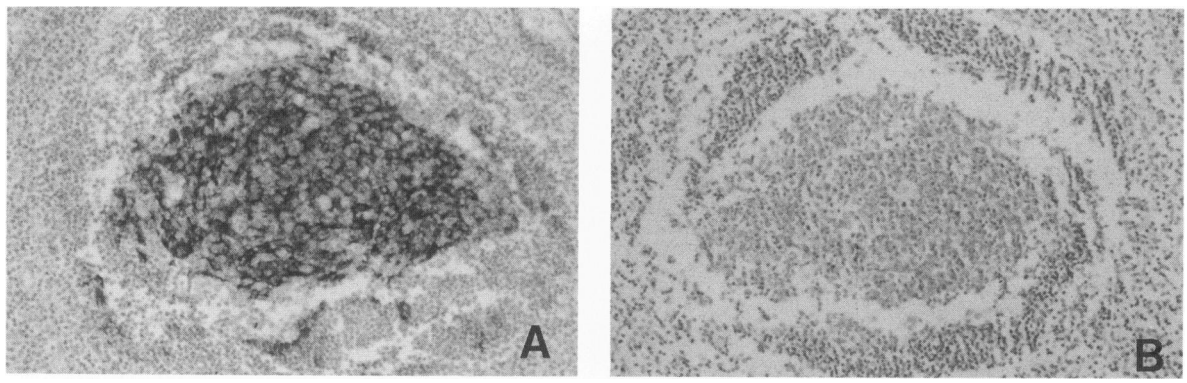

Figure 1. A: *Demonstration of prominent reactivity of tonsillar follicular dendritic cells with the rabbit polyclonal antibody raised against recombinant human VCAM-1.* B: *Reactivity of antiserum is abolished after serum is absorbed against VCAM-1 transfected CHO cells. Reactivity of the serum is not abolished after absorption against ICAM-1 or ELAM-1–transfected CHO cells or nontransfected CHO cells (data not shown). The Avidin-biotin immunoperoxidase technique was used, with methyl green counterstain. Magnification ×370.*

Table 1. *VCAM-1 in Solid Organ Allografts*

Diagnosis	*n*	VCAM-1 expression*			
		Sinusoidal endothelium	Portal vein endothelium	Dendritic cells (lymphoid aggregates)	Arteritis
Liver transplants					
Normal liver	2	–	–	–	–
Donor biopsies	17	–	–	–	–
Protocol biopsies (no rejection)	5	–	–	–	–
Acute rejection‡	14	8/14	10/14	4/14	–
Post rejection	13	–	–	3/14§	–
Pancreas transplants					
Acute rejection†	6			6	2/2
Chronic vascular rejection (autopsy)	1			–	–
No evidence of rejection	4			–	–

* Detectable by rabbit polyclonal antisera to recombinant human VCAM-1.
† Only 2 of 6 cases had arteritis.
‡ Two of 14 cases of acute rejection were completely negative for VCAM-1 expression.
§ One in three cases demonstrated *de novo* viral hepatitis.

belonging to T-lymphocyte ($CD43^+$) or monocyte/macrophage ($CD68^+$) lineage (Figure 2, B and C) but not B-lymphocyte ($CD20^+$) lineage. Further immunolabeling with the antibody to PCNA showed at times significant numbers of these infiltrating leukocytes actively proliferating (Figure 2D).

A second pattern of VCAM-1 expression in liver allografts was that of focal, irregularly distributed expression on sinusoidal endothelium, seen in 8 of 14 cases of allograft rejection (Figure 2F). Unlike the pattern seen with portal vein endothelium, areas of VCAM-1 expression were usually not associated with the infiltration of adjuvant tissues by leukocytes. However, although VCAM-1 expression at this site could apparently be disassociated from concomitant localized inflammatory injury, it is emphasized that this pattern of expression was encountered only in those allograft biopsies that had unequivocal features of rejection, and hence inflammatory cell infiltration, identified in other sites within the hepatic parenchyma.

The third pattern of VCAM-1 expression was the finding of a population of cells with dendritic morphology within portal triads that showed prominent expression of NGFR and somewhat less widespread expression of VCAM-1. By both morphologic appearance and this immunohistochemical characterization, these cells could be identified as dendritic cells similar to those found in both lymph nodes and tonsil. These cells were always found within prominent aggregates of lymphoid cells, which by immunophenotypic characterization could be shown to be predominately T cells ($CD43^+$) with only small numbers of B cells ($CD20^+$). The finding of dendritic cells amid the portal lymphocyte aggregates was present in 4 of 14 cases of rejection. VCAM-1 expressing dendritic cells was found in only a single prerejection liver biopsy obtained 1 hour after achieving anastomosis; these cells were identified in a portal tract with a prominent accumulation of leukocytes. Both the portal inflammation and presence of dendritic cells in this patient are of uncertain significance; in no other biopsy before rejection was this pattern identified.

In all but three biopsies obtained after clinical resolution of rejection, no residual expression of VCAM-1 could be identified. One of these three, obtained 3 months after the rejection episode, showed evidence of a newly diagnosed viral hepatitis. Two biopsies showed persistent portal lymphocytic infiltrates without other features diagnostic of rejection; within these infiltrates was a persistent population of $NGFR^+$, $VCAM\text{-}1^+$ dendritic cells.

No examples of acute or chronic arteritis were present in this series.

Pancreas

Of 10 pancreatic allograft biopsies from five patients, six showed features of acute rejection according to conventional criteria,[8–10] with two of these showing additional features of an acute arteritis. All six cases of acute rejection showed the presence of lymphoid aggregates within the interstitial tissue. As in the liver allografts, such infiltrates invariably could be characterized as predominately composed of T-lymphocytes ($CD43^+$) by immunohistochemical analysis, and within these aggregates a population of $NGFR^+$ and, less commonly, $VCAM\text{-}1,^+$ dendritic cells was present (Figure 3, A–D). Examples of arteritis, in which the persistent presence of the endothelial lining could be demonstrated by immunohistochemical localization of the endothelium specific marker factor VIII-related antigen/von Willebrand factor, showed unequivocal endothelial expression of VCAM-1 (Figure 3, E and F). The subendothelial space underlying this $VCAM\text{-}1^+$ endothelium contained numerous

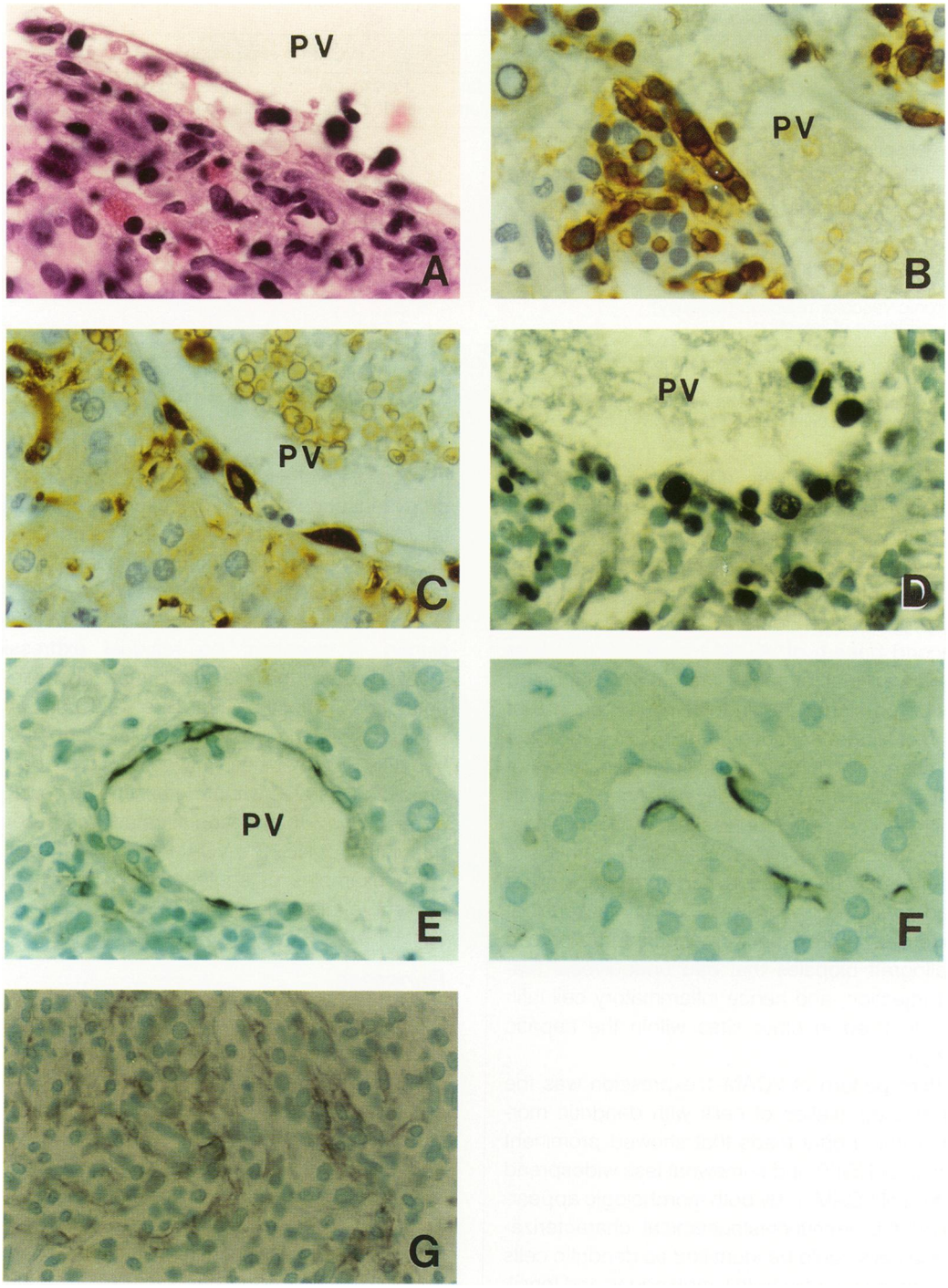

Figure 2. **A:** *Acute cellular rejection of liver. Florid endothelialitis is present in a branch of a portal vein (PV). (Hematoxylin-eosin; magnification ×720.)* **B:** *Acute cellular rejection of liver showing florid endothelialitis involving a branch of a portal vein (PV). There is subendothelial infiltration of T cells expressing CD43 antigen. (Magnification ×720.)* **C:** *Acute cellular rejection of liver showing florid endothelialitis involving portal vein (PV). Immunostaining of CD68-expressing monocytes/macrophages shows numerous cells in subendothelial location. (Magnification ×720.)* **D:** *Acute cellular rejection of liver. Presence of PCNA-positive immune cells in the wall of a portal vein (PV) branch with endothelialitis indicates active replication of these cells at sites of rejection injury. (Magnification ×720.)* **E:** *VCAM-1 expression by endothelial cells is present in a portal vein (PV) with endothelialitis. (Magnification ×370.)* **F:** *VCAM-1 expression by endothelial cells lining sinusoidal spaces in a case of acute cellular rejection involving the liver. (Magnification ×370.)* **G:** *VCAM-1 expression by dendritic cells within a lymphoid aggregate in a liver allograft with rejection. (Magnification ×370.) For* **B** *to* **G** *the avidin-biotin immunoperoxidase technique was used. For* **B** *and* **C**, *hematoxylin counterstain was used. For* **D** *to* **G**, *methyl green counterstain was used.*

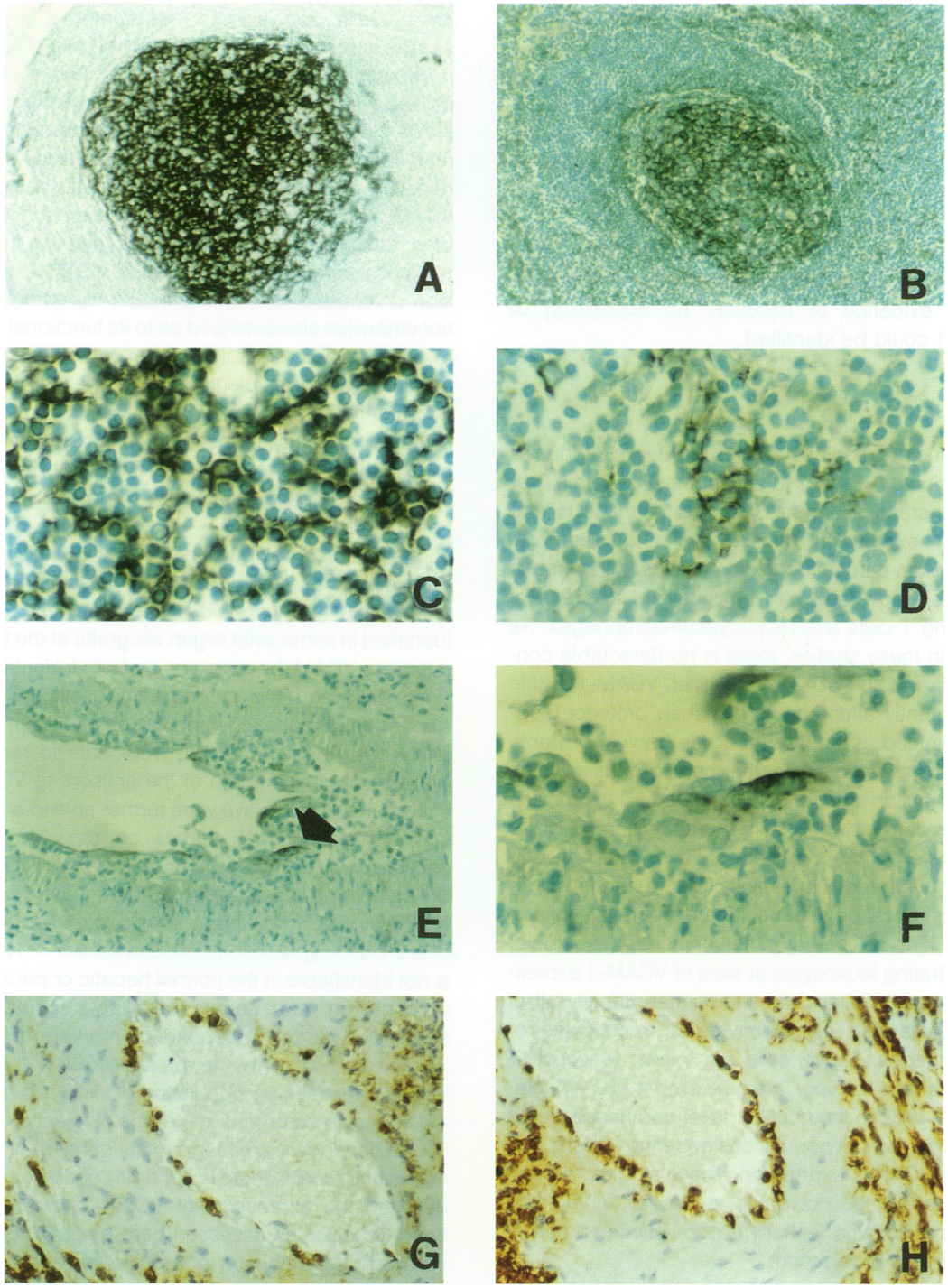

Figure 3. A: *Follicular dendritic cells in a germinal center of a tonsil are strongly reactive with a monoclonal antibody to nerve growth factor receptor (NGFR-5). (Magnification ×185.)* B: *VCAM-1 shows similar expression by follicular dendritic cells in germinal centers of tonsils. (Magnification ×185.)* C: *Lymphoid aggregate in a pancreatic allograft tissue where cellular rejection was present. Numerous dendritic cells are strongly immunostained by NGFR-5 monoclonal antibody. (Magnification × 720.)* D: *Lymphoid aggregate in the same pancreatic allograft tissue as illustrated in* Figure 1C *with dendritic cells expressing VCAM-1. There are fewer VCAM-1–expressing cells compared with cells expressing NGFR, but distribution of positive cells is similar. (Magnification × 720.)* E: *Medium-sized artery of a pancreatic allograft tissue with florid endothelialitis. Endothelial cells are expressing VCAM-1 in their luminal surface (arrow). (Magnification ×185.)* F: *Detail of the same field of the* Figure 1E. *Endothelial cells expressing VCAM-1. (Magnification × 720.)* G: *Pancreatic allograft tissue with vascular rejection (arteriopathy). Numerous lymphocytes in the subendothelial area and in the wall of the artery express the T-cell–associated marker CD43. (Magnification ×370.)* H: *Pancreatic allograft tissue with vascular rejection (arteriopathy). Numerous monocytes/macrophages expressing the CD68 antigen are present in a subendothelial location and in the wall of the artery. (Magnification ×370.) In all, avidin-biotin immunoperoxidase preparation was used. For* A *to* F, *methyl green counterstain was used. For* G *and* H, *hematoxylin counterstain was used.*

mononuclear leukocytes, which could be identified as T cells ($CD43^+$) and monocytes/macrophages ($CD68^+$) (Figure 3, G and H).

One pancreatic allograft studied at autopsy contained areas of chronic arteriopathy identical to the changes of chronic vascular rejection seen in some allograft kidneys[13]; VCAM-1 expression could not be identified in this case. The remaining parenchyma in this case was obliterated by neoplastic cells comprising a post-transplant lymphoproliferative disorder. In the four pancreatic allograft biopsies without evidence of rejection, no expression of VCAM-1 could be identified.

Discussion

Two principal findings of this study link induced expression of VCAM-1 to active rejection within liver and pancreas allografts. The first of these is that VCAM-1 expression on vascular and sinusoidal endothelium can be identified in tissue at the time of acute rejection, and can be localized to areas of infiltrating T cells and monocytes/macrophages. As shown in these studies, there is no detectable constitutive endothelial expression of VCAM-1 within subsequently affected organs when protocol biopsies obtained at the time of organ harvest or transplantation are studied. Furthermore, organs in which acute rejection and concomitant VCAM-1 expression can be demonstrated in general no longer show detectable VCAM-1 expression after rejection episodes have been successfully treated and follow-up biopsies reveal no residual features of active inflammatory injury. Our study of the immunophenotype of the infiltrating leukocytes at sites of VCAM-1 expression by endothelial cells shows that virtually all of these cells are T-lymphocytes or monocytes or macrophages. These cells are known to constitutively express the very late activation-4 (VLA-4) integrin (CD49d) on their cell surface, and hence would be among the cell populations adhering to cells with surface VCAM-1 expression. These studies strongly implicate, but do not prove, a role for VCAM-1 in attracting specific leukocyte populations to parenchymal sites of rejection.

However, to prove that VCAM-1 has a primary role in mediating rejection, it is necessary to exclude the alternate possibility that the up-regulated expression of VCAM-1 is a result of, rather than a contributory factor to, active rejection. In this scenario, it is the infiltrating leukocytes or damaged parenchymal cells that provide signals necessary to induce VCAM-1 expression. Distinction between these two possibilities cannot be accomplished by relatively static biopsy studies such as this one, but may become amenable to analysis like that employed in recent studies in primates and in mice on the role of ICAM-1 in promoting allograft rejection.[42,43] Those studies utilized the effect of functional blocking antibodies to ICAM-1 administered *in vivo* to help establish a primary role for this leukocyte adhesion molecule in the rejection process.[42,43] The functional activity of VCAM-1 has been studied in a murine model of cardiac transplantation. In that model, rejecting cardiac allografts demonstrated increased VCAM-1 expression on endothelium. When an anti-VCAM antibody, not otherwise characterized as to its functional ability to inhibit leukocyte adhesion to endothelium, was administered to recipients of cardiac allografts, leukocyte infiltration of the allografts was reportedly not inhibited.[44] Clearly, further studies will be required to assess the extent to which VCAM-1 may be necessary in mediating leukocytic injury solid organ transplantation.

The second principal finding is the identification of prominent numbers of VCAM-1 expressing dendritic cells within the lymphoid aggregates that can be identified in some solid organ allografts at the time of rejection. VCAM-1 expression on both follicular dendritic cells and the interdigitating cells in T-cell–dependent regions of lymph nodes has been previously described.[31,45,46] We were able to replicate these findings with each of the antisera to VCAM-1 utilized in this study. We were further able to show by immunohistochemical studies on replicate tissue sections of lymphoid tissues that the follicular dendritic cells that express VCAM-1 also express NGFR-5, which has been shown previously to be useful phenotypic marker of such cells.[34,35] NGFR-5 is not identifiable in the normal hepatic or pancreatic parenchyma, and so we were able to utilize its expression as an independent marker to further identify and confirm the presence of a transient VCAM-1 expressing population of dendritic cells in the transplanted organs in the time of rejection. In the B-cell–dependent areas (germinal centers) of lymph nodes and tonsils, there is generally good correlation between the expression of VCAM-1 and NGFR-5 dendritic cells, as assessed both by staining intensity and by distribution of cells reactive with these antisera. However, our studies of dendritic cells within allografted organ shows the population of NGFR-5–expressing cells within lymphoid aggregates to be somewhat greater than that of VCAM-1–expressing cells. Taken together, these data suggest that there is a population of follicular dendritic cells present within some rejecting solid organ allografts, but that only a subpopulation of these cells expresses detectable levels of VCAM-1.

Our studies of transplanted organ tissue both preceding and following episodes of rejection have not revealed a stable population of NGFR-5– or VCAM-1–expressing dendritic cells that are present in the absence of inflammatory injury. It would appear such a cell population either migrates from the host lymphoid tissues to the allograft at times of rejection, where these cells presumably help present antigen to immunocompetent cells as part of the rejection response, or, if constitutively present in donor organs, undergo phenotypic modulation from $NGFR^-$, $VCAM^-$ to a presumably more active state where these proteins are expressed. In either scenario, the finding of such cells in lymphoid aggregates and allografts, as in reactive lymphoid tissue, is indicative of a role for these cells in mediating the rejection response.

These findings are of interest in view of recent studies in the mouse that have demonstrated the migratory nature of dendritic cells and demonstrated their role in initiating an allostimulatory response.[47] These studies suggest that dendritic cells comprise at least part of a population of passenger leukocytes that are transplanted within the donor organ.[48] After transplantation the cells migrate out of the donated organ to central lymphoid organs of the host, such as the spleen, where they serve as the sensitizing stimulus for activation of host immunocompetent cells. The finding of dendritic cells at sites of organized lymphocytic responses in human organ allografts provides evidence that their role in recruitment and stimulation of activated lymphocytes is likely to take place, at least in part, locally within the graft rather than centrally within the host's lymphoid organs.

Studies by Freedman et al[45] have shown that VCAM-1 expression by follicular dendritic cells may promote adhesion of lymphoid cells, and hence may help further localize immunocompetent cells to sites of immune injury. It seems likely that the dendritic cell containing lymphoid follicles encountered in organ allografts are an *in vivo* correlate to these *ex vivo* studies. Finally, studies by several groups have demonstrated that VCAM-1 interactions with lymphocytes occurring via binding with the VLA-4 counter receptor may result not just in enhanced leukocyte adhesion, but may provide additional costimulatory activation of resting T cells beyond that provided by antigen and mixed histocompatibility complex (MHC) class II molecules and so may amplify or promote allograft rejection responses.[49–51] These studies suggest another mechanism by which $VCAM\text{-}1^+$ dendritic cells may contribute to liver and pancreas transplant rejection.

Previous studies of both human and experimental rat liver transplantation have suggested a role for dendritic cells in mediating the rejection response. In studies of arteries with features of chronic vascular rejection (alternately termed obliterative arteriopathy) in man, Oguma et al[11] have identified a population of dendritic cells recognized by their reactivity with antibodies to the S-100 protein. That study did not further characterize the nature of the dendritic cells, nor did it address whether dendritic cells were present in the hepatic parenchyma. Within the rat, populations of Ia^+ dendritic-appearing cells have been identified with hepatic and pancreatic interstitial tissue, but such cells have not been well characterized and their distinction from tissue macrophages is not well established.[52] Demetris et al[53] observed close temporal and spatial clustering of infiltrating T-lymphocytes and Ia^+ dendritic-shaped cells in early rat liver allograft rejection, and also noted the presence of donor Ia^+ cells in recipient spleen 1 day after transplantation. Their findings provide evidence of both central (host lymphoid system) and local (intragraft) mechanisms of sensitizing immune cells in this model of graft rejection. None of these studies have addressed issues of VCAM-1 expression by dendritic cells or that of phenotypic modulation by dendritic cells. Although we know of no data that addresses this last issue directly, we believe it is an important area for future study. Although we cannot currently exclude the possibility that a population of dendritic cells may normally exist within the human pancreas or liver, we and others[31,54] provide evidence that a population of $VCAM^+$ dendritic cells cannot typically be identified in the interstitial tissues of the normal liver, although expression by normal Kupffer cells has been identified in one study.[31]

Our findings also point to a peculiar paradox in the nature of the dendritic cells that we have identified within rejecting organ allografts. As revealed by immunohistochemical studies on human lymphoid tissue, VCAM-1 can be shown to be expressed on both the follicular dendritic cells present in B-cell–dependent portions of lymphoid tissues, as well as on the interdigitating dendritic cells that are present in T-cell–dependent areas. It is currently thought that despite somewhat similar terminology and morphologic appearance, these represent very distinct cell types. Follicular dendritic cells are thought to be of nonhematopoietic origin, bind B-lymphocytes, and promote B-lymphocyte proliferation.[46] Interdigitating dendritic cells are thought to be of hematopoietic origin, related to monocyte lineage and to Langerhans cells of the skin, and principally promote T-lymphocyte activation and proliferation. Immunohistochemical staining for the nerve growth factor

receptor shows that its expression is confined to the follicular dendritic cells of the B-cell–containing germinal centers.[34,35] Yet we have observed that within the lymphoid aggregates present in rejecting transplants, the dendritic cells identified strongly express nerve growth factor receptor, suggestive of a follicular dendritic cell phenotype. However, the surrounding lymphoid infiltrate immunophenotypes as a population of predominately T-lymphocytes rather B-lymphocytes. These findings are not explainable at the present time, given the still limited knowledge available about human dendritic cells. They do suggest that rigid characterization of interactions between specific types of dendritic cells and some classes of lymphocytes may be an unduly limiting conceptualization of how immune cells may be stimulated.

We must also point out certain findings in this study that limit our ability to assess the role of VCAM-1 in liver and pancreas rejection. The expression of this molecule by sinusoidal and venous endothelium was only seen within focal areas of the biopsies. In no case was VCAM-1 expression seen to be widespread. Although this may be due to the sensitivity of the antibodies employed, this finding might instead reflect the fact that VCAM-1 expression may be a dynamic and rather transient process that is only intermittently detectable by biopsy studies. It also seems likely that a number of cytokines and cell-stimulatory molecules may direct local endothelial expression of VCAM-1, and that further knowledge of these kinds of stimuli will be required before we can understand clearly the basis for the focality of both VCAM-1 expression and of the rejection response.

A second area of concern is the basis for the discrepancy in immunohistochemical findings between the rabbit polyclonal antisera and the murine monoclonal antibody, both of which are directed against human VCAM-1. Both antibodies show similar degrees of sensitivity in detecting dendritic cells and other structures known to express VCAM-1 (eg, kidney parietal epithelial cells) in control tissues fixed in a manner identical to the those of the transplant biopsies. Yet it is clear that the epitope recognized by the rabbit polyclonal antisera is not reliably detected by the 4B9 monoclonal antibody when the VCAM-1 molecule is expressed on endothelium. We believe the rabbit polyclonal antisera is a more sensitive reagent, and we are confident of its specificity for the following reasons: 1) the antisera was raised against purified recombinant human VCAM-1, 2) the antisera was affinity purified and retained all of its reactivity in transplant biopsies as described above, and 3) the specific staining patterns described were abolished when the antisera was absorbed against VCAM-1 expressing transfected cell lines, but not when the antisera was absorbed against similar cell lines either transfected with ICAM-1 or untransfected. It remains possible that the 4B9 monoclonal antibody recognizes an epitope of the VCAM-1 molecule inaccessible to the antibody in our tissue immunohistochemical studies. It is even possible that 4B9 recognizes a VCAM-1 epitope not biologically expressed in this transplantation setting. While speculative, this last consideration derives support from recent observations that alternatively spliced forms of VCAM-1 can be produced[55] that have different binding sites with respect to various VCAM-1 antibodies that have currently been developed (L. Osborn, personal communication).

Finally, although our study demonstrates that up-regulated VCAM-1 expression in specific tissue sites is associated with the allograft rejection response, it does not address the issue whether VCAM-1 mediated leukocyte adhesion is essential for such a response to develop. Other proinflammatory processes are certainly involved, and up-regulation of other leukocyte adhesion molecules, such ICAM-1, which has previously been demonstrated in hepatic allografts and inflammation, may also be critical to the infiltration of leukocytes into tissues in solid organ transplant rejection.[54,56,57]

In summary, while cognizant of the above caveats, we have demonstrated up-regulated expression of VCAM-1 within liver and pancreas organ allografts at times of rejection. The sequence and localization of VCAM-1 expression by endothelial and dendritic cells suggest that VCAM-1 has an important role in recruitment, localization, and activation of host immunocompetent cells at sites of the rejection response.

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