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UPREGULATION OF TUBULAR CELL ICAM-1 MEDIATES INFLAMMATION IN CHRONIC OBSTRUCTIVE UROPATHY (COU). SB Shappell*, G. Kukiella*, T Gurpinar*, CW Smith, W Suki, L Truong. Depts. of Pathology, Pediatrics and Medicine, Baylor College of Medicine, Houston, TX. The roles of adhesion molecules in COU, especially the interstitial inflammation, have not been clarified. COU was induced in rats by unilateral ureteral ligation. Immunohistochemistry of sections from the ligated (L) and contralateral (C) kidneys was performed to evaluate inflammatory cell phenotype, and the possible upregulation of adhesion molecules. Interstitial, and to a lesser extent, tubular inflammatory cells with a predominance of T cells and macrophages were first noted in the renal medulla at day 3, gradually increased, and involved the whole kidney starting from day 15 to the end of the experiment. ICAM-1 and VCAM-1 expression was evident in peritubular capillaries of control kidneys, without obvious changes during the 90 day evolution of COU. ICAM-1 showed no change on day 1, increased on the lumenal aspect of proximal and dilated distal tubules on the L but not the C kidneys, on days 3, 6, 9, 15, and 25, with diminished expression at day 31 and thereafter. On day 6, ICAM-1 expression appeared maximal, with approximately 80% of proximal tubule cross sections showing ICAM-1 expression on the L kidneys vs. rare (<10%) positive tubules on C kidneys. De novo ICAM-1 expression was also evident in dilated distal nephron segments. Northern blot analysis showed increased ICAM-1 mRNA in tissue from L kidneys. No expression of VCAM-1 by tubular cells or alterations in E-selectin were evident. While increased tubular ICAM-1 expression may play a crucial role in interstitial inflammation in early phase of COU, sustaining this inflammatory infiltrate in late phase may require additional factors.

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Thomas E. Andreoli, M.D., Editor
Kidney International
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May 28, 1996

Dear Dr. Andreoli:

Please find enclosed our manuscript entitled "The Role of Adhesion Molecules in Chronic Obstructive Uropathy. Upregulation of Tubular Cell Intercellular Adhesion Molecule-1 (ICAM-1)", which we submit for your consideration for publication in *Kidney International*. All of the authors agree with the presentation and publication of the material as presented herein. This work has been partially presented in abstract form at the 1995 meeting of the American Society of Nephrology in San Diego, CA. It has not been submitted in manuscript form previously and is not under consideration for publication anywhere else. The original and four copies of the manuscript, five complete sets of glossy prints, and a diskette with the manuscript on it are included.

Thank you for your consideration. We look forward to hearing from you.

Sincerely,

Luan D. Truong, M.D.

The Role of Adhesion Molecules in Chronic Obstructive Uropathy. Upregulation of Tubular Cell Intercellular Adhesion Molecule-1 (ICAM-1)

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Short Title: Adhesion Molecules in Rat COU

ABSTRACT

The Role of Adhesion Molecules in Chronic Obstructive Uropathy.

Upregulation of Tubular Cell Intercellular Adhesion Molecule-1 (ICAM-1). Chronic obstructive uropathy (COU) induced by maintained unilateral ureter ligation in the rat is characterized morphologically by interstitial inflammation, interstitial fibrosis, and tubular atrophy, features similar to those seen in chronic tubulointerstitial nephritis in humans. Infiltrating mononuclear inflammatory cells, particularly T-lymphocytes and macrophages, may contribute to the progression of this lesion by mediating tubular injury and by the activation of interstitial fibroblasts, with resultant tubular atrophy and interstitial fibrosis, respectively. Altered expression and activation of adhesion molecules by leukocytes, vascular endothelial cells, and parenchymal cells likely contributes both to the infiltration of inflammatory cells into the tubulointerstitial compartment and to the interaction of activated inflammatory cells with parenchymal cells. In the current study, we examined changes in the expression of E-selectin, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) in a 90-day model of maintained unilateral ureter ligation in male Spraque-Dawley rats. Rat kidneys showed constitutive expression of ICAM-1 mRNA and constitutive immunostaining for ICAM-1 in peritubular capillaries, glomeruli, and a small percentage of cortical tubules. Ureter ligation resulted in a rapid increase in ICAM-1 mRNA, which was evident as early as three hours and which was maintained at a high level in the ligated vs. contralateral kidneys throughout the entire 90-day time course. There was a marked increase in ICAM-1 expression within tubular epithelium, with up to 80 % of both cortical and medullary tubule cross sections showing strong apical immunostaining from days 6 to 25, with a subsequent decrease at day 31 and the remainder of the experiment. Glomerular expression of ICAM-1 was decreased in the ligated compared to the contralateral kidneys throughout the entire experiment, whereas

there was a gradual increase in ICAM-1 immunostaining in the expanding interstitium in the ligated kidneys, perhaps reflecting expression by peritubular capillaries, interstitial fibroblasts, or interstitial inflammatory cells. There was a later and less striking increase in VCAM-1 mRNA in ligated vs. contralateral kidneys, which was first evident at two days and which was maintained throughout the entire time course. VCAM-1 immunostaining was most evident within the expanding interstitium in ligated vs. contralateral kidneys. There was less glomerular VCAM-1 expression in ligated vs. contralateral kidneys. In contrast to the strong tubular epithelial expression of ICAM-1, ureter ligation did not result in any detectable increase in tubule VCAM-1 expression. E-selectin immunostaining was detectable only focally in a few animals, including those with complication by acute pyelonephritis. Under the employed experimental conditions, significant alterations in E-selectin expression were not detected at time points examined. Increased ICAM-1 and VCAM-1 may contribute to the prominent inflammatory cell infiltration in the chronic tubulointerstitial nephritis accompanying maintained unilateral ligation. Tubule expression of ICAM-1, which occurs during a similar time course as previously documented for tubular cell proliferation and especially tubular cell apoptosis in this model, may contribute to injurious interactions of activated inflammatory cells with tubular epithelium.

INTRODUCTION

Increased expression and activation of leukocyte and endothelial adhesion molecules of the selectin, integrin, and immunoglobulin supergene families plays a crucial role in promoting inflammatory cell infiltration into diseased tissues (1). Such alterations are regulated by a diverse array of soluble chemotactic factors and cytokines at sites of inflammation. In general, selectins and their carbohydrate ligands on both leukocytes and vascular endothelium are crucial in mediating initial margination and rolling of leukocytes, whereas subsequent stronger adhesive interactions, leukocyte spreading, and transendothelial migration of inflammatory cells are mediated by leukocyte and endothelial proteins of integrin and immunoglobulin families (1-3). Such adhesive events can also contribute to endothelial cell injury. Furthermore, altered expression of integrins and the immunoglobulin molecules intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) on parenchymal cells may contribute to cytotoxic interactions with infiltrating inflammatory cells (4,5).

Altered expression of adhesion molecules in glomerular, tubular, and vascular compartments has been reported in a variety of human kidney diseases and in experimental animal models of renal disease (2,3,6). Increased E-selectin expression on glomerular endothelium has been described in acute glomerulonephritis, IgA nephropathy, and lupus nephritis (7). E-selectin is also expressed on interstitial vessel endothelium in these disorders as well as in allograft rejection (7). Increased glomerular expression of ICAM-1 has also been observed in a variety of human glomerulonephritides, and upregulation of ICAM-1 and VCAM-1 is seen on large vessel and peritubular capillary endothelial cells in a variety of glomerular and primary tubulointerstitial disorders (2,3,8). Presumably, increased expression of these molecules on glomerular and peritubular capillary endothelial cells facilitates influx of inflammatory cells into glomeruli and tubulointerstitial compartments. In addition, de

novo and increased expression of ICAM-1 on tubular epithelial cells has been described in human glomerulonephritides, allograft rejection, and cyclosporine nephrotoxicity and animal models of glomerulonephritis and tubulointerstitial nephritis (2,3,8). Tubular cell expression of VCAM-1 has also been described in many of these disorders (2,3,8). Tubular expression of these molecules has been correlated with areas of intense leukocytic infiltration (9), and may participate in leukocyte-tubular epithelial interactions in tubulointerstitial inflammation seen in both primary tubulointerstitial diseases and that which accompanies glomerular inflammatory injury.

Chronic obstructive uropathy (COU) is also characterized by interstitial inflammation. Infiltrating inflammatory cells may contribute to tubular injury and activation of interstitial fibroblasts, crucial processes leading to the tubular atrophy and interstitial fibrosis characteristic of advanced stages of this lesion (9,10). The possible role of altered adhesion molecule expression on vascular endothelium and tubular epithelium in mediating these inflammatory and injurious processes in obstructive uropathy has not been prevously explored.

We have created a model of COU by unilateral ureter ligation in rats. The renal lesion in this model is characterized by marked tubular atrophy, which appears to be mediated by apoptosis of tubular epithelial cells, expansion of the interstitial volume, and interstitial inflammation and fibrosis (11). Inflammation begins in the papillae and becomes progressively uniform throughout the kidney by day 15. The predominant inflammatory cells are T-lymphocytes (particularly CD4 cells) and macrophages, similar to the composition of interstitial inflammation in most examples of progressive human renal disease (9,10). In the current study, we have characterized the alterations of E-selectin, ICAM-1, and VCAM-1 in this model of COU.

MATERIALS AND METHODS

Experimental Model: Under pentobarbital anesthesia, male Sprague-Dawley rats weighing 150-200 grams were subjected to complete ligation of the left ureter at the ureteropelvic junction using a double silk suture (11). Animals were subsequently allowed free access to a regular diet and tap water and sacrificied in groups of three at days 1,6,9,15,25,34,43,60,75, and 90. A control group of sham operated rats were sacrificed at day 0,15,43, and 90. At the time of sacrifice, a catheter was placed in the infrarenal aorta and 0.9 % saline was infused until the renal vein effluent was clear. Ligated, contralateral, and control kidneys were harvested and portions were fixed in 10 % buffered formalin, frozen in OCT for frozen section preparation and snap frozen for subsequent RNA extraction.

Monoclonal antibodies: Anti-rat ICAM-1 monoclonal antibody (MAb) 1A29 (12) was obtained from Dr. M. Miyasaka (Dept. of Immunology, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan). Anti-rat VCAM-1 MAb 5F10 (IgG_{2a}) was obtained from Biogen Corp., Cambridge, MA, and was prepared by immunizing mice with COS cells transfected with rat VCAM-1 (13). MAbs CL3 (IgG_1) and CL37 (IgG_1) against E-selectin were prepared by immunizing mice with IL-1 stimulated human umbilical vein endothelial cells (14). Cross-reactivity of these MAbs for rat E-selectin was demonstrated by reactivity with E-selectin expressing tumor necrosis factor (TNF) alpha-stimulated rat pulmonary artery endothelial cells (14)and subsequently by immunohistochemical studies in a rat model of nephrotoxic nephritis (15).

Immunohistochemistry: Possible expression of ICAM-1, VCAM-1, and E-selectin in tubular, glomerular, vascular, and interstitial compartments of obstructed, contralateral, and control kidneys was examined by immunohistochemical staining of

frozen tissue sections with a modified avidin-biotin peroxidase technique, as previously described (16). Briefly, endogenous avidin binding activity was blocked by preincubation with avidin D followed by biotin solutions (Vector Laboratories, Burlingame, CA) according to the manufacturer's instructions. Sections were incubated with primary MAbs to rat ICAM-1 (1A29, 1 ug/ml), VCAM-1 (5F10, 1 ug/ml), and E-selectin (CL3 or CL37, 10 ng/ml) diluted in PBS with 1 % BSA at room temperature for one hour. After washing x 3 in PBS, sections were subsequently incubated with biotinylated horse anti-mouse Ig (Vector Laboratories) secondary antibody diluted in PBS with 20 % (by volume) normal rat serum at room temperature for one hour. Sections were then incubated for one hour with avidin-biotin complex (Vector Laboratories) and developed with a ten minute incubation in a 50 mg % solution of diaminobenzidine in Tris buffer (pH 7.5) activated with 1:1000 dilution of 30 % H_2O_2. Sections were counterstained with methyl green-alcian blue, dehydrated, and mounted.

Morphometry: Glomerular staining for adhesion molecules was assessed in a semi-quantitative manner, reflecting both the extent (percentage of individual glomerulus demonstrating immunoreactivity) and intensity (weak, moderate, or strong) of immunohistochemical staining. Fifteen to twenty glomeruli were assessed per section, and scored as follows: 0 = < 10 % of glomerulus showing only weak staining; 1 = 10- 25 % of glomerulus staining, generally weak to moderate in intensity; 2 = 25-50 % of glomerulus staining, with moderate intensity; 3 = staining in 50-75 % of glomerulus, moderate to strong intensity; 4 = 75-100 % of glomerulus staining.

The percentage of tubules expressing adhesion molecules was assessed by counting the number of positive and negative tubule cross sections in at least five high power fields of both cortex and medulla. At early time points (0-9 days) different tubular segments can be differentiated on the basis of morphology. However, generally after 9 days of maintained unilateral ureter ligation, different nephron segments cannot be

readily differentiated. Cortex and medulla (even at late time points) can be reliably recognized by reference to glomeruli and vessels. Hence, results for tubule expression are given for cortex and medulla instead of for individual nephron segments. Statistical analyses were made by analysis of variance and Dunnett's test for multiple comparisons or Student's *t*-test where appropriate.

Results for vascular and interstitial staining are reported descriptively. The reason for this approach is that although staining of peritubular capillary endothelium can be readily appreciated in control kidneys, with interstitial expansion and inflammation, distinction between capillary and inflammatory or other interstitial cell immunoreactivity cannot be reliably differentiated by light microscopy alone.

RNA extraction and Northern blot analysis: Rat RNA was isolated from ligated and contralateral kidney tissue using the single step acid guanidinium-phenol-chloroform procedure (17). RNA (12 ug/sample) was electrophoresed in 1 % agarose gels containing formaldehyde, then transferred to a nylon membrane (Genescreen Plus, New England Nuclear, Boston, MA) using standard proceedures (18). Loading of RNA was monitored by probing membranes with human glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Membranes were hybridized at 68 C for 2 hours with QuikHyb hybridization buffer (Stratagene Cloning Systems, LaJolla, CA), containing 1 x 10^6 cpm/ml random nanomer-primed ^{32}P-labeled ICAM-1 or VCAM-1 cDNA probes. The rat ICAM-1 cDNA probe consisited of a 600 bp fragment from the 3' untranslated region of rat ICAM-1 cDNA and the VCAM-1 probe consisted of a 1.1 kb fragment from the translated region of murine VCAM-1 cDNA (19). Filters were washed with 2xSSPE at 68 C for 10 min., with 1xSSPE with 1 % SDS at 68 C for 30 min., rinsed with 1xSSPE at room temperature, and exposed to Hyperfilm-MP (Amersham Corporation, Arlington Heights, IL) for 12-24 hours. Alternatively, analyses of radioactivity were performed on

a Phosphorimager (Molecular Dynamics, San Francisco, CA) by scanning the blots for 3 to 6 hours and subsequent densitometry analysis.

RESULTS

ICAM-1 expression in control, obstructed, and contralateral kidneys:

Tubules: The results of immunoperoxidase staining with anti-ICAM-1 and anti-VCAM-1 MAbs in various kidney compartments in sections from ligated or contralateral kidneys are demonstrated in Figure 1. The extent of tubule expression of ICAM-1 is summarized in Figure 2. In control kidney sections and sections from sham-operated animals, there was only focal ICAM-1 expression in proximal tubules, with approximately 20 % of tubule cross sections in the renal cortex showing weak immunostaining on the luminal aspect. In contrast, there was essentially no constitutive ICAM-1 expression in distal tubules and collecting ducts.

There was no detectable increase in proximal tubule or non-dilated distal tubule staining for ICAM-1 in kidney cortex and medulla sections from animals sacrificed one day after ligation (Day 1). There was luminal epithelial ICAM-1 staining evident in dilated distal collecting ducts in the papillary region of the medulla in the obstructed kidneys at day one. ICAM-1 expression was markedly increased in tubular epithelial cells in the cortex of the obstructed kidneys, with positive staining on the luminal surface evident in approximately 50 % of tubule cross sections at day 3 and of approximately 75 % of tubule cross sections at day 6 (Figures 1a, 1b, and 2). This level of increased ICAM-1 immunostaining was maintained in renal tubules of the ligated kidneys through day 25, with a modest reduction at day 31, and a subsequent reduction throughout the rest of the experimental period. In contrast, there was only a slight increase in tubule ICAM-1 expression within the tubules of the cortex of the contralateral kidneys, evident at day 15 (Figure 2). This increase was not maintained, with staining in the contralateral kidney throughout the experiment generally not different from control kidneys. The difference between ICAM-1 expression in obstructed and contralateral kidneys was even more

pronounced in the renal medulla. Whereas no medullary tubules expressed ICAM-1 in contralateral kidneys, between 65-80 % of tubular cross sections were positive on the ligated side between days 6 and 25, with a slight decrease at day 31 and reduction thereafter (Figures 1c, 1d, and 2).

Glomeruli: ICAM-1 was strongly expressed in a constituitive manner in glomeruli from kidneys of control animals. Strong staining was evident in all glomeruli, with each glomerulus generally showing staining in most or all lobules. Staining was also observed in the epithelial cells lining Bowman's capsule, with moderate staining seen in a complete or near complete circumferential manner. This pattern and intensity of ICAM-1 immunostaining remained unchanged in glomeruli in the contralateral kidneys (Figures 1e and 3). In contrast, there was a marked reduction in ICAM-1 immunostaining in glomeruli from the obstructed kidneys, with a readily evident decrease in both the glomerular area staining positively and in the intensity of positive staining (Figures 1f and 3). This decrease was apparent as early as six days following ureter obstruction and was clearly present at nine days. There appeared to be a further (and essentially maximal) reduction in glomerular ICAM-1 expression by 15 days, which was maintained until the end of the experiment (Figure 3). In contrast to the readily apparent reduction in ICAM-1 staining in glomerular capillary and/or mesangial regions, ICAM-1 remained present (and perhaps even slightly increased) in the epithelial lining of Bowman's capsule of the obstructed kidney.

Interstitium and vasculature: Immunohistochemical staining for ICAM-1 in kidneys at day 0 and in sham-operated control kidneys showed constituitive expression in peritubular capillaries. This pattern of staining remained essentially the same throughout the duration of the experiment in sections from the contralateral kidneys. In ligated kidneys, there was increased staining evident in the expanded interstitium as interstitial inflammation and fibrosis ensued (Figures 1g and 1h). Staining of inflammatory cells was readily observed, which increased throughout the duration of the experiment. It was

not possible to discern alterations in the level of staining in peritubular capillaries vs. that of infiltrating inflammatory cells or interstitial fibroblasts at the light microscopic level.

VCAM-1 expression in control, obstructed and contralateral kidneys:

Tubules: There was essentially no VCAM-1 immunohistochemical staining of tubular epithelial cells in control kidneys. Contralateral kidney sections did not show any appreciable tubule epithelial VCAM-1 expression throughout the duration of the experiment. In contrast to the prominent increase in tubule ICAM-1 expression, ligated kidneys did not show any significant increase in VCAM-1 expression (Figures 1k and 4). Occasional tubules in middle and late time points contained one or two weakly staining cells, which may also have represented intratubular immunoreactive inflammatory cells.

Glomeruli: Glomeruli in control kidneys showed little VCAM-1 immunoreactivity, with focal segmental staining, usually limited to isolated cells or small cell groups involving only a minor portion of the glomerular tuft and occasional cells of Bowman's capsule. Increased expression of VCAM-1 was evident in glomeruli of both the ligated and contralateral kidneys at day one (Figure 5). This increased glomerular expression was maintained in the contralateral kidneys, but was subsequently decreased in the ligated kidneys, with clear reduction of VCAM-1 immunoreactivity at day 15 that was maintained at this low level throughout the remainder of the experimental course (Figures 1j, 1k, and 5).

Interstitium and vasculature: Control kidneys showed constituitive VCAM-1 expression in peritubular capillaries and in the intima of small (e.g., interlobular and arcuate) arteries. This pattern of vascular staining was maintained in both contralateral and ligated kidneys throughout the experiment, without appreciable changes in peritubular capillary or arterial intimal staining. As the lesion progressed in the ligated kidneys, there was prominent interstitial immunoreactivity, with staining of the majority of cells, that blended with and partially obscured the staining of intertubular capillaries (Figure 1k).

E-selectin expression in control, obstructed and contralateral kidneys:

Glomeruli: No appreciable E-selectin immunostaining was seen in glomerular endothelium in control kidneys using two different monoclonal antibodies against rat E-selectin. Glomerular immunoreactivity was not detected in ligated or contralateral kidneys at any time studied during the course of the experiment.

Interstitium and vasculature: There was no constituitive E-selectin immunoreactivity in arterial, venous, or peritubular capillary endothelium. No significant expression was detected in arterial endothelium in ligated or contralateral kidneys throughout the experiment. Scattered positive capillaries were seen in the renal papilla on the ligated side on day one and rare scattered positive isolated capillaries and/or small vessels were seen in prominently inflamed medullary regions in sections from the ligated side on days 9, 15, 31, and 60, with generally similar results with the two different MAbs. Occasional positive cells were also seen in the inflamed atrophic subcapsular cortical area on day 60.

Changes in ICAM-1 mRNA in obstructed and contralateral kidneys: The marked increase in ICAM-1 expression in ligated kidneys as assessed by immunohistochemistry was supported by an examination of ICAM-1 mRNA in ligated and contralateral kidneys. Consistent with the low level of constitituitive ICAM-1 protein expression in vessels and some tubules by immunohistochemistry, ICAM-1 mRNA was detectable in control kidneys (Figure 6). Unilateral ureter ligation was accompanied by a marked increase in ICAM-1 mRNA in the ligated vs. the contralateral kidney. This increase in ICAM-1 mRNA was evident as early as three hours, was marked and essentially maximal at six hours and was maintained at a high level throughout the ninety day experimental duration (Figure 6).

Changes in VCAM-1 mRNA in obstructed and contralateral kidneys:

Unilateral ureter ligation was accompanied by an increase in VCAM-1 mRNA in the ligated vs. the contralateral kidney (Figure 7). This increase was not as pronounced as that for ICAM-1 mRNA. Increased VCAM-1 message in the ligated vs. contralateral kidney was evident at two days, was pronounced at 5 and 13 days, and was maintained throughout the remainder of the 90 day experimental period (Figure 7).

DISCUSSION

The altered expression or activation of specific adhesion molecules on circulating leukocytes, vascular endothelial cells, and parenchymal cells is crucial in inflammatory kidney disease (2,3,6). Such changes function not only in recruiting inflammatory cells into the diseased kidney, but likely also in the execution of certain potentially harmful effector functions during leukocyte adhesion to endothelium, interstitial cells and matrix, and tubular epithelial cells (2,3,6). In this study we have addressed possible alterations in the expression of endothelial and parenchymal cell adhesion molecules during the evolution of COU in the rat. Using immunohistochemistry in conjunction with careful morphometry, we have clearly demonstrated for the first time that COU in the rat is associated with distinct changes in tissue expression of adhesion molecules. In summary, these changes were increased tubular ICAM-1, decreased glomerular ICAM-1, increased interstitial ICAM-1, unchanged tubular VCAM-1, decreased glomerular VCAM-1, increased interstitial VCAM-1, and unchanged E-selectin. These immunohistochemical results were confirmed by noting a concordant alteration in the mRNA for ICAM-1 and VCAM-1.

The mediators responsible for the increase in ICAM-1 and VCAM-1 in COU remain to be established. ICAM-1 and VCAM-1 expression is increased in cultured vascular endothelial cells and other cell types, including proximal tubule epithelial cells, by cytokines, including TNF-alpha, interleukin -1, and interferon-gamma (20-22). These cytokines are known to be products of the various types of inflammatory cells which progressively infiltrate the tubulointerstitium in COU. Although cytokines derived from inflammatory cells can potentially induce overexpression of adhesion molecules in advanced COU, their role in early COU is dubious since marked increase in ICAM-1 mRNA was noted as early as three hours after ureter ligation, at which time increased renal inflammatory cells are not noted (23-25). The mechanisms accounting for this

early upregulation of ICAM-1 in COU are not clear, but may be related to changes known to occur virtually immediately after ureter ligation, including increased renal blood flow, activation of the intrarenal renin-angiotensin system, and increased intratubular pressure (23-25). Damaged tubular cells themselves may be the source of a large variety of cytokines (26).

Whether increased expression of adhesion molecules represents a cause of the renal lesions observed in COU remains to be elucidated. Similar to chronic tubulointerstitial disease in humans, COU in the rat is characterized by marked tubular atrophy, interstitial inflammation, and interstitial fibrosis. Infiltrating inflammatory cells, particularly lymphocytes and monocytes, may contribute to this lesion by activating interstitial fibroblasts and by directly interacting with tubular epithelial cells (9,10). Using this model of rat COU, we have recently characterized the inflammatory infiltrate over the same time course as employed in the current study. The interstitial inflammatory infiltrate is composed predominantly of T-lymphocytes, particularly CD4-positive cells, and monocyte/macrophages. In addition, tubulitis, characterized by the presence of inflammatory cells within the tubular epithelium, is a readily observable feature (manuscript in preparation). Tubule expression of specific adhesion molecules likely functions in these adhesive interactions. This mode of tubule injury has been postulated in other examples of tubule inflammation, including allograft rejection and animal models of primary tubulointerstitial nephritis (2,3,6,8,21,27,28). Interestingly, in most other reports where tubule expression of both ICAM-1 and VCAM-1 are examined or by comparing studies where they have been looked at separately in the same models or conditions, ICAM-1 and VCAM-1 upregulation are generally seen together (2,3,6,8,27). As in the current study, ICAM-1 expression is generally seen at the apical aspect of the tubules, whereas VCAM-1 is more uniformly distributed or concentrated at the basolateral aspect of the cells. Possible differential expression of ICAM-1 vs. VCAM-1 has been reported in cyclosporine nephrotoxicity in renal allografts (29). The

significance of expression of these specific molecules with regard to leukocyte interactions remains uncertain. Lymphocytes and monocytes express both $beta_2$ and $beta_1$ integrins capable of adhesive interactions with ICAM-1 and VCAM-1, respectively (1). Although both types of interactions appear capable of mediating transendothelial migration of leukocytes under appropriate conditions, differential functional significance in cytotoxic interactions with parenchymal cells remains possible. Antibodies to LFA-1 and ICAM-1 have been shown to reduce lymphocyte toxicity towards tubular epithelial cells *in vitro* (4). Differential activation of cytotoxicity by $beta_2$ vs. $beta_1$ integrins has been observed during monocyte-endothelial interactions (30).

Altered expression of adhesion molecules may be related to tubular cell apoptosis. The major mechanism of tubular injury and atrophy in this model of COU appears to be apoptosis of the tubular epithelium, which is preceded by an increase in tubular epithelial cell proliferation (11). ICAM-1 is expressed in the tubules during the same time periods as this surge in proliferation and especially the subsequent surge in apoptosis. Although this temporal synchrony invites speculation on the possible roles of altered intercellular adhesion in disrupting tubular cell cycles, whether and how adhesion molecule ligation can potentially induce apoptosis remain to be clarified. However, leukocyte products such as TNF-alpha, reactive oxygen species, perforin, and Fas ligand have been implicated in apoptosis in other systems (31,32), and integrin-mediated adhesion (including integrin-ICAM-1 interactions) is known to lead to the release of some of these and other mediators (5,33).

In contrast to the marked increase in tubule ICAM-1 expression and ICAM-1 and VCAM-1 immunostaining in the interstitium in the obstructed kidneys in this study, there was a significantly decreased expression of glomerular ICAM-1 and VCAM-1 in the ligated vs. the contralateral kidneys. The significance of this novel observation is uncertain. It has been well documented that glomeruli in kidneys with COU show little

change, even in advanced disease when there is severe tubulointerstitial damage. The mechanisms responsible for this structural integrity of glomeruli are not currently known, but the decreased glomerular expression of adhesion molecules clearly documented in our study may potentially antagonize leukocyte recruitment into glomeruli, thereby preventing glomerular damage. Although no attempt was made in the current study to correlate the number of accumulated leukocytes and expression of adhesion molecules in glomeruli, we have observed a progressively decreased number of leukocytes in glomeruli in rat kidneys with COU over a similar time course (manuscript in preparation). In addition, decreased glomerular macrophages following ureter ligation in the rat has been previously documented (34). Reduction of glomerular ICAM-1 and VCAM-1 at the same time as marked increases in tubule and interstitial expression of these same adhesion molecules in our study of COU suggests a complex and differential regulation of expression of these surface proteins in different kidney compartments, either due to differences in local release of stimulating vs. inhibiting mediators or different responsiveness of glomerular vs. tubule and interstitial cells during the evolution of inflammatory kidney injury. A recent immunohistochemical study of human kidney biopsies in a variety of glomerulonephritides demonstrated correlation of tubular and interstitial expression of ICAM-1 and VCAM-1 with the degree of chronic histologic damage (27). There was no such correlation with glomerular expression of adhesion molecules. These results, as with those of the current study in an experimental animal model of chronic tubulointerstitial injury, lend further support to the notion that upregulation of adhesion molecules in the tubulointerstitium is related to the evolution of interstitial fibrosis and tubular atrophy.

In summary, we have documented for the first time a distinct pattern of alteration of adhesion molecules in kidney tissue with COU. These alterations are implicated in the pathogenesis of the characteristic renal lesion observed in COU, including progressively severe chronic tubulointerstitial damage with little accompanying glomerular change.

The specific role of ICAM-1 or other adhesion molecules in COU will need to be further addressed in interventional studies employing blocking monoclonal antibodies or mice genetically deficient in adhesion molecules (35,36).

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FIGURE LEGENDS

FIG. 1: *Immunoperoxidase staining of ligated and contralateral rat kidney frozen sections with anti-rat ICAM-1 and anti-rat VCAM-1 MAbs.* A) Contralateral cortex, day 6, showing strong expression of ICAM-1 in glomerulus as well as ICAM-1 expression in peritubular capillaries and weak luminal expression in occasional proximal tubule cross sections (arrowhead) (original magnification 400x). B) Ligated cortex, day 6, showing strong ICAM-1 expression in luminal aspect of most proximal tubule cross sections (arrowhead) and ICAM-1 immunostaining in expanding interstitium, including peritubular capillaries (open arrow) (400x). C) Medulla of contralateral kidney, day 15, showing ICAM-1 immunostaining of capillary network (arrowhead), but lack of expression in tubular epithelium (400x). D) Medulla of ligated kidney, day 15, showing strong expression of ICAM-1 in epithelium of dilated and non-dilated tubules (arrowheads) (400x). E) Strong expression of ICAM-1 in glomerulus from contralateral kidney, day 15. Focal tubule immunostaining is also evident (400x). F) Decreased glomerular expression of ICAM-1 in ligated kidney, day 15. Strong staining of most tubules and peritubular capillary/interstitial staining is also evident (arrow) (200x). G) Anti-ICAM-1 MAb immunostaining of contralateral kidney cortex, day 15, showing staining only of peritubular capillaries (arrow) in normal interstitium (400x). H) Strong expression of ICAM-1 within expanded interstitium (arrow) in cortex of ligated kidney, day 15 (400x). J) Strong glomerular VCAM-1 expression in contralateral kidney, day 6. Some staining of structures suggestive peritubular capillaries is present (arrow) (400x). K) Reduced glomerular VCAM-1 expression and increased interstitial VCAM-1 expression (arrow) in ligated kidney cortex, day 9. Note lack of expression by tubular epithelial cells (400x).

FIG. 2: *Tubule ICAM-1 Expression in Rat Kidney with COU.* Expression of ICAM-1 by tubules in cortex (A) and medulla (B) of ligated kidneys (closed squares) vs. contralateral kidneys (closed circles). Data are given as percentage of tubule cross sections showing positive immunoperoxidase staining with anti-ICAM-1 MAb 1A29 (mean +/- s.d.; * $p < .01$, ** $p < .05$ in ligated vs. contralateral kidneys).

FIG. 3: *Glomerular ICAM-1 Expression in Rat Kidney with COU.* Glomerular expression of ICAM-1 in ligated kidneys (closed squares) vs. contralateral kidneys (closed circles) as assessed using immunoperoxidase staining of frozen sections with anti-rat ICAM-1 MAb 1A29. Mean +/- s.d., with staining quantitation as described in Methods. (* $p < .01$, ** $p < .05$ in ligated vs. contralateral kidneys).

FIG. 4: *Cortical Tubule VCAM-1 Expression in Rat Kidney with COU.* Expression of VCAM-1 in cortical tubules of ligated kidneys (closed squares) vs. contralateral kidneys (closed circles), as percentage of tubule cross sections showing positive immunoperoxidase staining with anti-rat VCAM-1 MAb 5F10. (Mean +/- s.d.).

FIG. 5: *Glomerular VCAM-1 Expression in Rat Kidney with COU.* Glomerular expression of VCAM-1 in ligated kidneys (closed squares) vs. contralateral kidneys (closed circles) as assessed using immunoperoxidase staining of frozen sections with anti-rat VCAM-1 MAb 5F10. Mean +/- s.d., with staining quantitation as described in Methods. (* $p < .01$ in ligated vs. contralateral kidneys).

FIG. 6: *Induction of ICAM-1 mRNA in Rat Kidney with COU.* Northern blot analysis of ICAM-1 mRNA (upper panel) from day 0 control animal kidney and ligated (L) and contralateral (C) kidneys from the time points indicated in hours and days. 12 ug total kidney RNA was loaded per lane. GAPDH mRNA signal for the identical blot is shown

in the bottom panel. Number above each lane represents the integrated area of the band in each lane as assessed by subsequent scanning of the blot with a Phosphorimager (Molecular Dynamics).

FIG. 7: *Induction of VCAM-1 mRNA in Rat Kidney with COU.* Northern blot analysis of VCAM-1 mRNA (upper panel) from day 0 control animal kidney and ligated (L) and contralateral (C) kidneys from the time points indicated in hours and days. 12 ug total kidney RNA was loaded per lane. GAPDH mRNA signal for the identical blot is shown in the bottom panel. Number above each lane represents the integrated area of the band in each lane as assessed by subsequent scanning of the blot with a Phosphorimager (Molecular Dynamics).

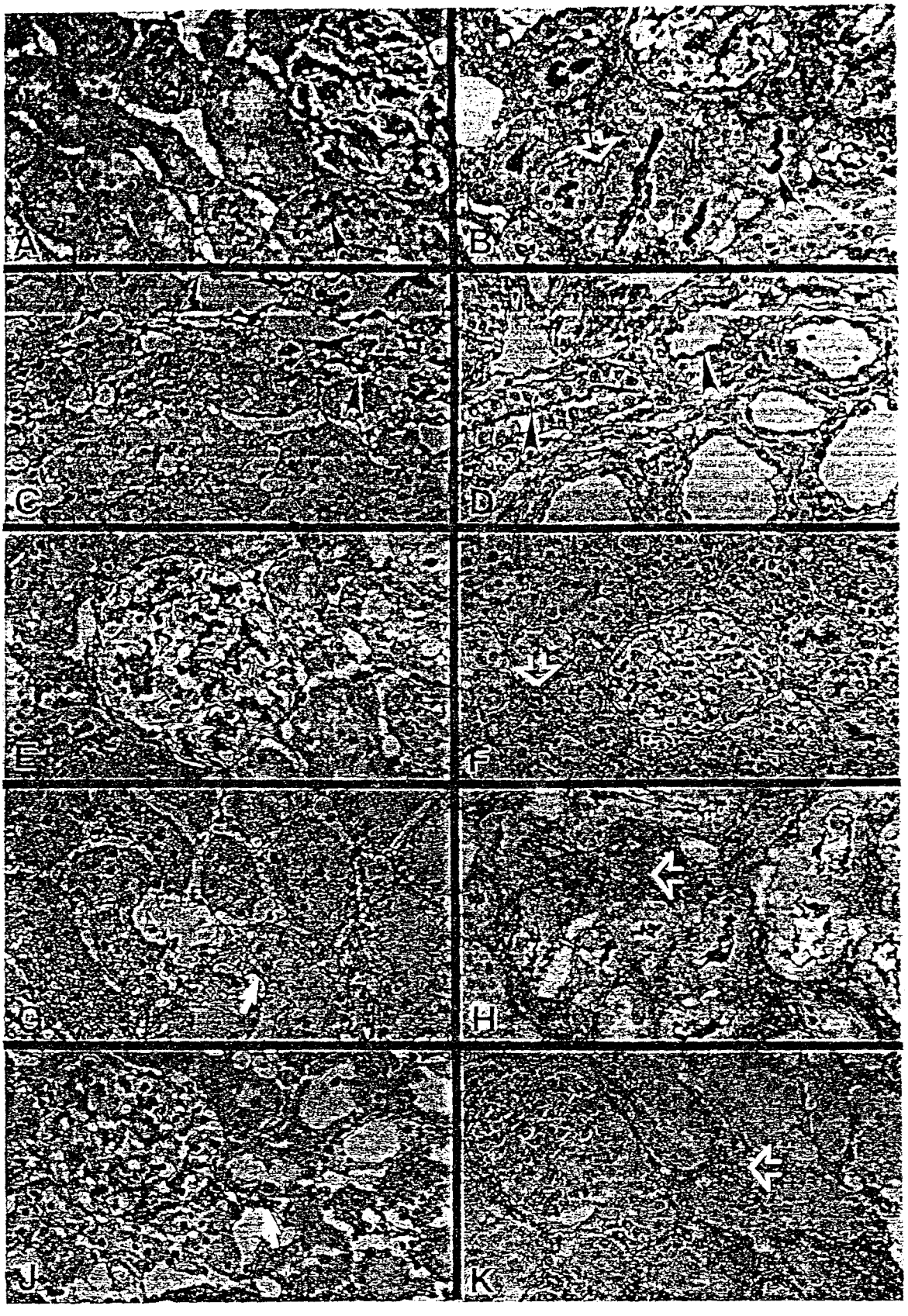

Fig 1

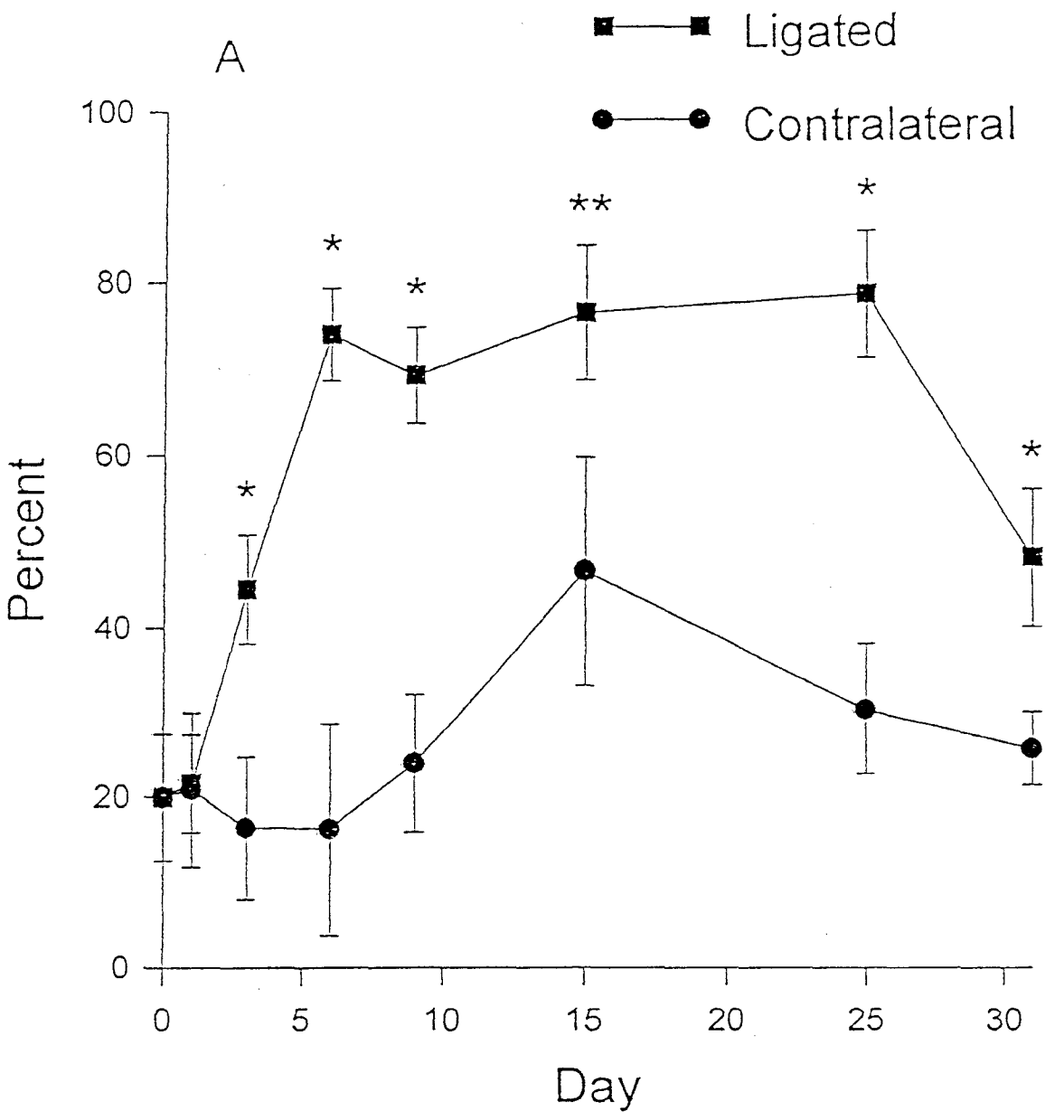
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Fig. 2a

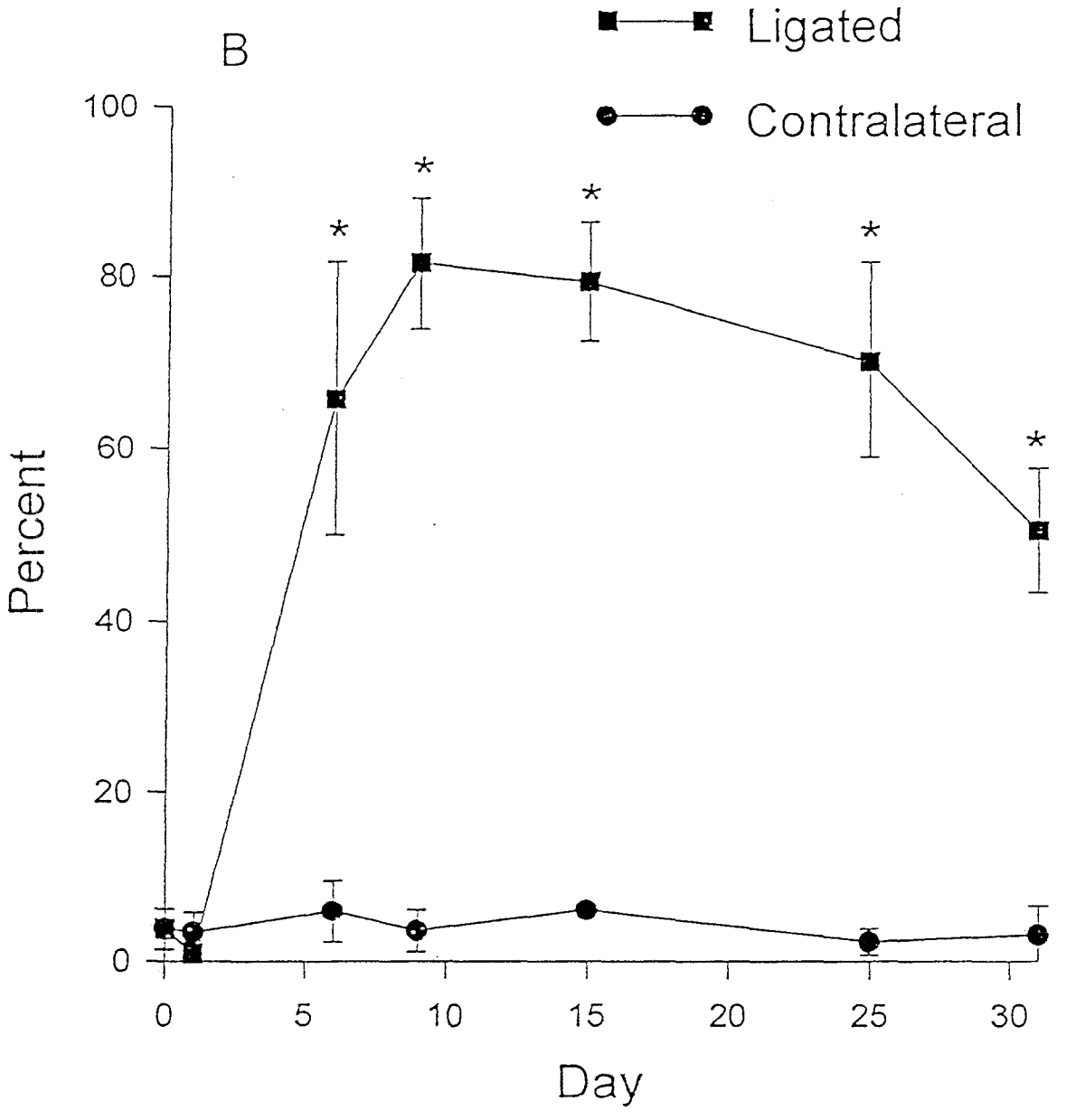
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Fig 2b

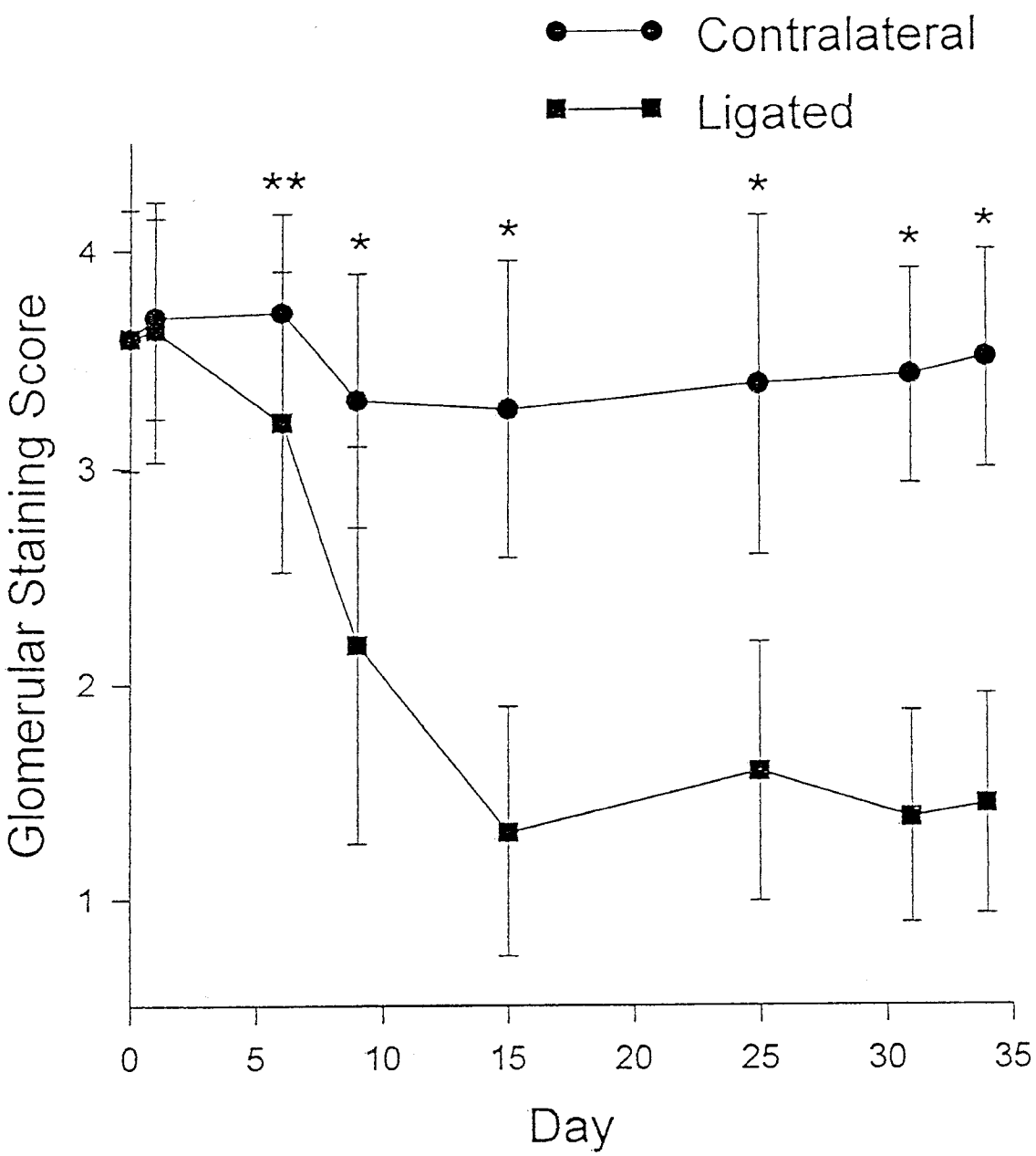
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Fig 3

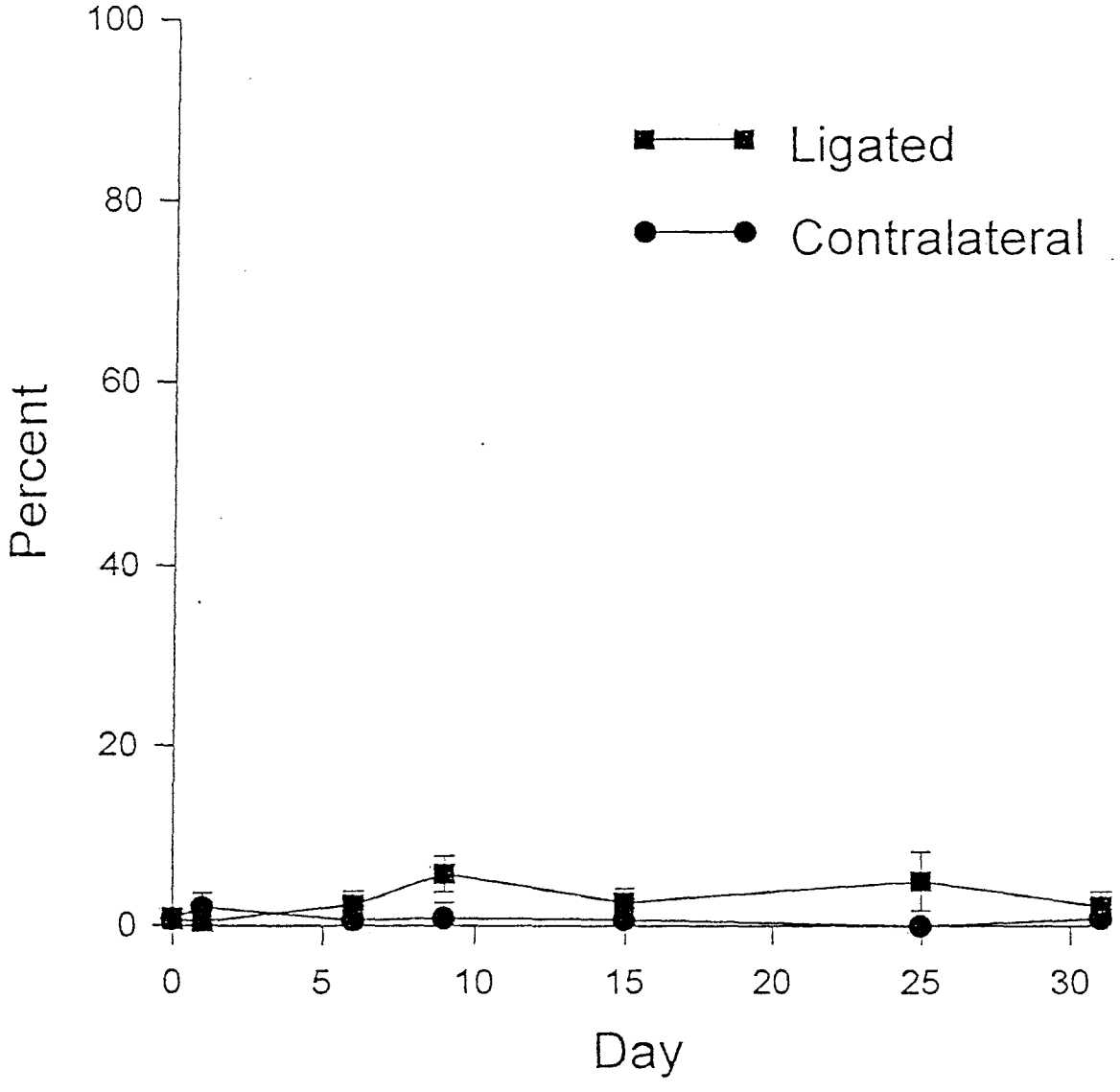
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Fig 4

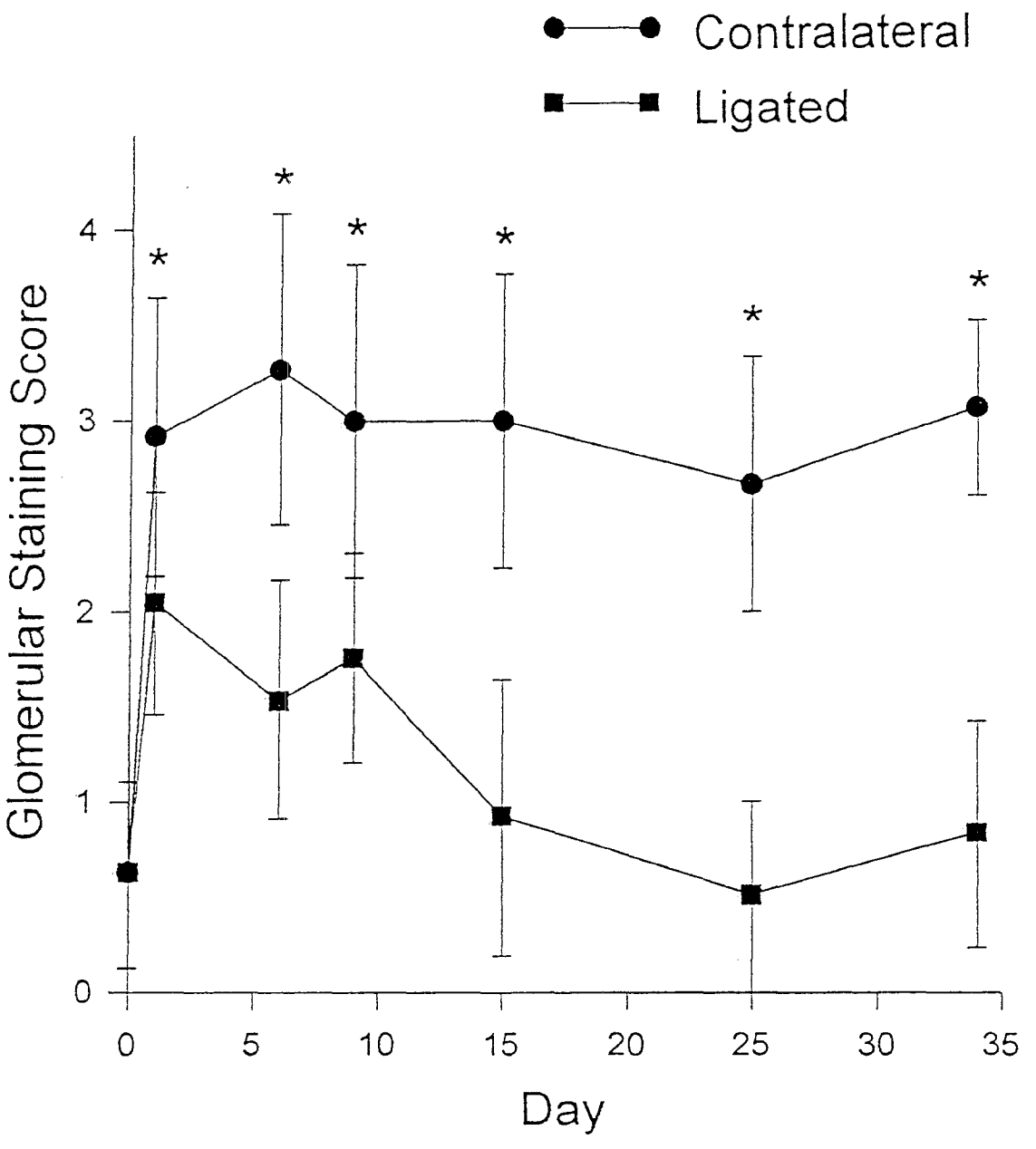
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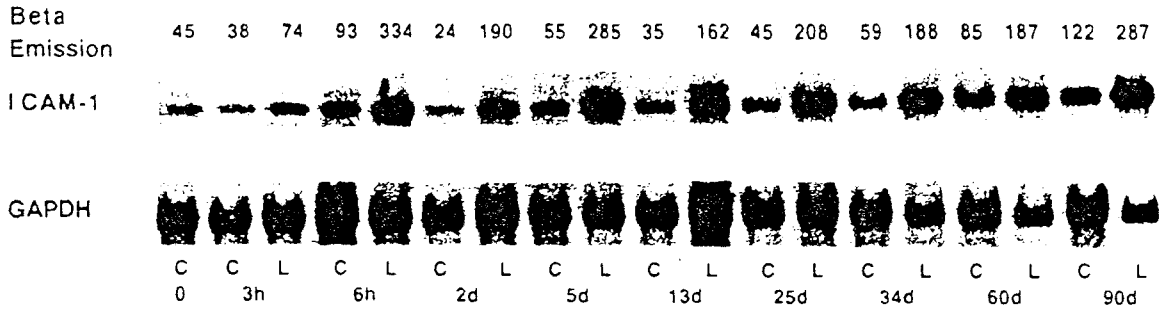
Fig. 5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GAPDH
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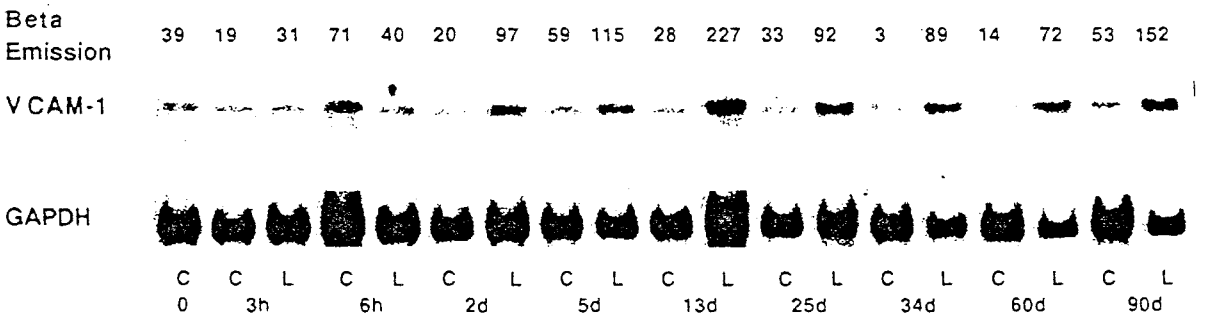
Fig 6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0 3h 6h 2d 5d 13d 25d 34d 60d 90d

Fig. 7