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Physiology

Antibody to intercellular adhesion molecule 1 protects the kidney against ischemic injury

(acute renal failure/ischemia–reperfusion injury/adhesion molecule receptors/integrins)

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ABSTRACT **The pathophysiology of ischemic acute renal failure is complex, and the role of leukocyte adhesion in this process is not well defined. A monoclonal antibody (mAb) against intracellular adhesion molecule 1 (anti-ICAM-1), administered at the time of bilateral renal ischemia in the rat, prevented both functional impairment and histologic changes of acute renal failure. Plasma creatinine measured (mg/dl) 24 hr after 30 min of ischemia was 0.61 ± 0.05 in the anti-ICAM-1-treated animals compared with 2.4 ± 0.14 ($P < 0.0001$) in the vehicle-treated ischemic group. Forty-eight hours after ischemia, creatinine values were 0.46 ± 0.05 and 2.03 ± 0.22 ($P < 0.0001$) in anti-ICAM-1 and vehicle-treated groups, respectively. A low dose of anti-ICAM-1 that was itself nonprotective, when given with partially protective doses of a mAb against lymphocyte function-associated antigen-1 (anti-LFA-1), acted synergistically to prevent renal failure. Anti-ICAM-1 mAb also protected the kidney when administered 0.5 or 2 hr but not 8 hr after restoration of blood flow and when the ischemic period was extended to 40 min. Ischemia-induced increases in tissue myeloperoxidase, a marker of neutrophil infiltration, were mitigated with anti-ICAM-1 treatment. Thus, anti-ICAM-1 mAb protected the kidney against ischemic renal failure, even when the antibody was administered after the ischemic period. These results suggest a critical role for leukocytes and adhesion molecules in the pathophysiology of ischemic injury and may have important therapeutic implications.**

The pathophysiology of acute ischemic renal failure is poorly understood. Therapeutic interventions designed to inhibit cellular injury have frequently been either ineffective or equivocal in their effectiveness in both experimental animals and man, and rarely has an agent been effective when administered after the ischemic insult (1).

We hypothesized that leukocyte adhesion plays a critical role in renal ischemia–reperfusion injury. Outer medullary vascular congestion that occurs in ischemic acute renal failure (2) may result from leukocyte–endothelial cell interactions with obstruction of the vasa recta or leukocyte-mediated increases in endothelial permeability leading to erythrocyte aggregation (3). This would sustain ischemia to the outer medulla, even if total renal blood flow were restored.

Leukocyte adhesion to various cell types is mediated in large part by the three β_2 integrins: CD11a/CD18 [lymphocyte function-associated antigen 1 (LFA-1)], CD11b/CD18 (Mac-1), and CD11c/CD18. The intercellular adhesion molecule 1 (ICAM-1, CD54) is a ligand for CD11a/CD18 and CD11b/CD18. The CD54–CD11/CD18 interactions are important determinants of leukocyte-endothelial cell adhesion (4). The purpose of our studies was to evaluate the effects of a monoclonal antibody (mAb) directed against ICAM-1 on ischemic acute renal failure in the rat.

Ischemia results in increased tissue levels of proinflammatory mediators, including cytokines (5, 6) and products of arachidonic acid metabolism (7), which upregulate CD11/CD18 expression on leukocytes, increase ICAM-1 expression on endothelial cells, or increase CD11/CD18 avidity for ICAM-1 (4). As a result there is increased adherence of leukocytes to endothelial cells (8) and activation of leukocytes, likely increasing local production of additional inflammatory mediators and enhancing tissue damage. Antibody directed against ICAM-1 inhibits the adherence of activated neutrophils to endothelial cells *in vivo* (9) and *in vitro* (8, 10).

METHODS

Animal Protocols. Male Sprague–Dawley rats weighing 160–190 grams (Charles River Breeding Laboratories) were fasted for 12 hr prior to surgery. After sodium pentobarbital (65 mg/kg) anesthetic, and 6 ml of 0.9% NaCl were administered ip, the renal artery and vein were occluded bilaterally for 30 or 40 min with microaneurysm clamps. Mouse mAb against rat ICAM-1, rat LFA-1, monkey CD3 (control isotype-matched mAb), or vehicle (0.9% NaCl) was administered via the external jugular vein, which was then flushed with 1 ml of saline. All experiments were blinded. Blood samples (0.15 ml) for urea nitrogen (BUN) and creatinine determination were obtained from the tail vein at 0, 24, 48 and, in some cases, 72 hr after reperfusion. BUN and creatinine were measured by standard urease assay/conductivity and picric acid reactions, respectively.

mAbs. Hybridoma cell lines producing mouse IgG1 anti-rat ICAM-1 (anti-CD54) mAb (IA29) (11) and mouse IgG2 anti-rat LFA-1 (anti-CD11a) mAb (WT.1) (12) were provided by M. Miyasaka of the Tokyo Metropolitan Institute of Medical Science, Japan. Mouse IgG1 anti-monkey CD3 mAb was used as a control mAb. In some experiments, the ascitic supernatant was purified with a protein A-Sepharose CL-4B (Pharmacia) column. Experiments performed with unpurified ascites yielded results equivalent to those obtained with purified mAb; thus, the results were combined.

Experimental Groups. A total of 1 mg of mAb (or 2 mg of total protein in the case of ascites) in 500 μl of vehicle (0.9% NaCl) was administered except where specified (Table 1). Animals in group I were subjected to surgery and isolation of the renal pedicle but no ischemia. Group II received vehicle alone just after application of the vascular clamp. Group III received anti-CD3 mAb. Results in groups II and III were equivalent; thus, vehicle-treated animals were used as controls in most experiments. Group IV received anti-ICAM-1 mAb at the time of ischemia. Groups V, VI, and VII received anti-ICAM-1 mAb 30, 120, and 480 min, respectively, after ischemia. Groups VIII, IX, X, and XI received progressively lower doses (higher dilutions) of anti-ICAM-1 mAb to estab-

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Abbreviations: ICAM-1, intercellular adhesion molecule 1; mAb, monoclonal antibody; LFA-1, lymphocyte function-associated antigen; MPO, myeloperoxidase; BUN, blood urea nitrogen.

Table 1. Experimental groups and mortality rates

Group	Treatment: Antibody/ischemia length, min	Treatment: Time mAb given, min after ischemia	Mortality rate, % (*n*)
I	Sham surgery/0	0†	0 (12)‡
II	Vehicle/30	0	16 (32)
III	Anti-CD3/30	0	25 (4)
IV	Anti-ICAM/30	0	0 (18)‡
V	Anti-ICAM/30	30	25 (4)
VI	Anti-ICAM/30	120	0 (4)‡
VII	Anti-ICAM/30	480	20 (5)
VIII	Anti-ICAM, 1:2/30	0	0 (5)‡
IX	Anti-ICAM, 1:4/30	0	0 (5)‡
X	Anti-ICAM, 1:5/30	0	0 (4)‡
XI	Anti-ICAM, 1:8/30	0	33 (12)
XII	Anti-LFA-1/30	0	0 (4)‡
XIII	Anti-LFA-1/ anti-ICAM, 1:8/30	0	0 (4)‡
XIV	Anti-ICAM/40	0	0 (10)‡
XV	Vehicle/40	0	82 (12)

*Mortality rates in *n* rats that died or were followed for at least 48 hr after ischemia.
†No ischemia.
‡$P < 0.01$ compared with vehicle-treated postischemic animals.

lish a dose–response relationship. An equivalent volume (vehicle ± mAb) was administered to each group. Group XII received anti-LFA-1 mAb only. Group XIII received 1 mg of anti-LFA-1 mAb and one-eighth of that dose—0.13 mg—of anti-ICAM-1 mAb. Group XIV received a full dose (1 mg) of anti-ICAM-1 mAb with the ischemic period extended to 40 min.

Light Microscopy and Immunocytochemistry. Forty-eight hours after ischemia or sham surgery, kidneys were fixed in formalin and stained with hematoxylin and eosin. The percent of tubules in the outer medulla that showed epithelial necrosis or necrotic debris was quantitated (0 = none; 1+ = <10%; 2+ = 10–25%; 3+ = 26–75%; 4+ = >75%). To localize mAb, frozen sections from kidneys taken 1, 24, and 48 hr after ischemia or sham surgery were incubated with biotinylated horse anti-mouse IgG (1:500), followed by avidin–biotin–peroxidase [ABC method (13)]. Staining was graded (0 = none, 2 = very intense) on coded sections.

Myeloperoxidase (MPO) Activity. MPO activity, used as an indicator of neutrophil infiltration, was measured (14) in coded kidney tissue after 30 min of ischemia and 4, 24, 48, or 72 hr of reperfusion (or sham surgery). Aliquots (0.2 ml) of 40,000 × *g* supernatants of kidney homogenates, prepared as described by Bradley *et al.* (14), were added to 0.8 ml of reaction mixture containing 60 mM potassium phosphate buffer (pH 6.0), 0.20 mg of *o*-dianisidine dihydrochloride per ml, and 0.0006% H_2O_2. Absorbance was measured at 460 nm. Assay linearity was confirmed. MPO activity, normalized to protein content of the supernatant, was expressed as the percent of levels in kidneys subjected to sham surgery.

Statistics. Creatinine and BUN values are expressed as the mean ± 1 SEM. Differences among mean values of BUN, creatinine, and MPO levels were evaluated by analysis of variance. Student's *t* test was used for comparisons between groups. χ^2 was used to evaluate differences in mortality rates; $P < 0.05$ was considered significant.

RESULTS

Effects of Anti-ICAM-1 mAb on Renal Function After Ischemia. Plasma creatinine increased ≈5-fold over baseline 24 hr after 30 min of ischemia in vehicle-treated control animals (group II) (Fig. 1 *Upper*). By 72 hr after ischemia, creatinine had returned to values close to baseline. The control mAb (group III) had no effect on creatinine. Administration of anti-ICAM-1 mAb (group IV) at the time of ischemia protected against renal dysfunction (Fig. 1 *Upper*). Mean creatinine 24 hr after reperfusion in the anti-ICAM-1-treated group (group IV) was 0.61 ± 0.05 mg/dl vs. 2.4 ± 0.14 mg/dl in the vehicle-treated ischemic group (group II) ($P < 0.0001$). Creatinine values were 0.46 ± 0.05 and 2.03 ± 0.22 ($P < 0.0001$) 48 hr after ischemia in anti-ICAM-1- and vehicle-treated groups, respectively. In anti-ICAM-1 mAb-treated animals, mean creatinine at 24, 48, and 72 hr of reperfusion was not statistically different from baseline (time = 0 hr) values or values in sham-operated (group I) animals. BUN values in these (Fig. 1 *Lower*) and all subsequent experiments (not shown) paralleled those of creatinine.

Leukocyte counts (9500 ± 1100 vs. 7800 ± 1100), hematocrit (37.3 ± 0.3% vs. 38.5 ± 0.4%; $n = 7$), and leukocyte differential counts (data not shown), measured 24 hr after

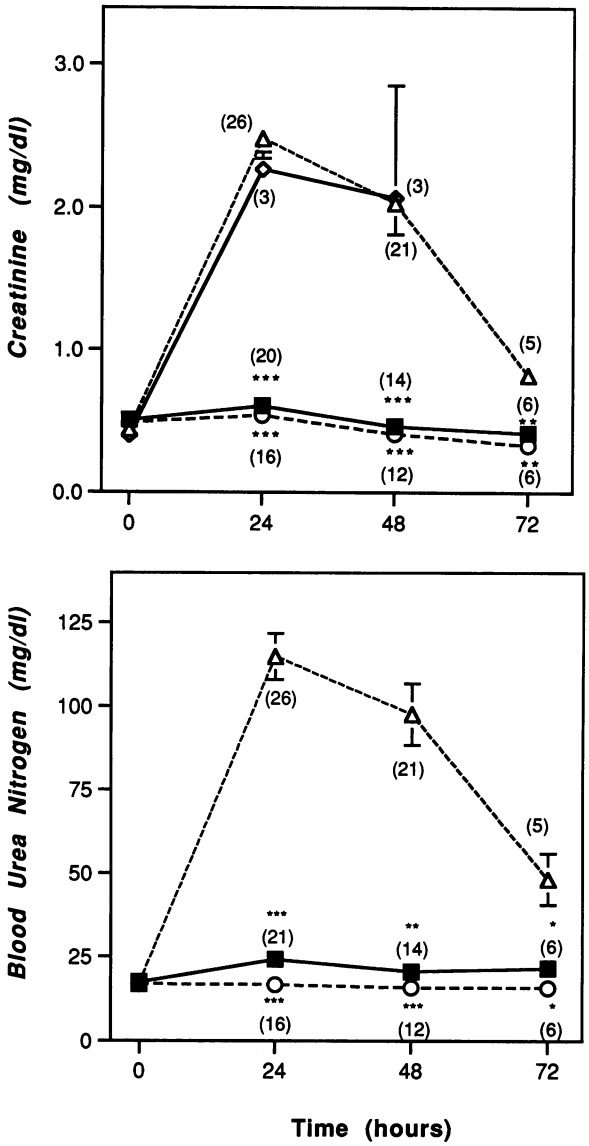

FIG. 1. Effect of anti-ICAM antibody on the course of ischemic acute renal failure. Animals were subjected to sham surgery (group I) (○), administered vehicle (group II) (△), control antibody (group III) (◇), or full-strength anti-ICAM-1 antibody (group IV) (■). Plasma creatinine (*Upper*) and urea nitrogen (*Lower*) were measured 0, 24, 48, and 72 hr after 30 min of bilateral renal ischemia or sham surgery. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.0001$ compared with the vehicle-treated group. Data are expressed as means ± 1 SEM. Numbers in parentheses represent the number of animals studied.

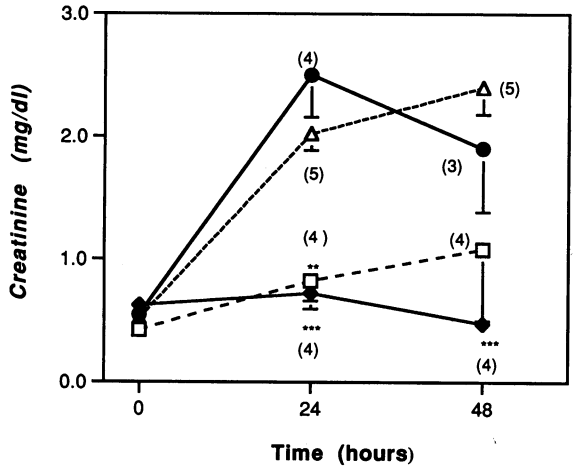

FIG. 2. Course of acute renal failure after administration of anti-ICAM-1 30 min (□), 2 hr (◆), or 8 hr (●) after 30 min of renal ischemia. Plasma creatinine values 0, 24, and 48 hr after ischemia are presented for groups V, VI, and VII and compared with values in animals treated with vehicle alone (group II) (△). **, $P < 0.01$; ***, $P < 0.0001$.

ischemia were not significantly different in anti-ICAM-1 mAb (full strength)-treated vs. vehicle-treated animals.

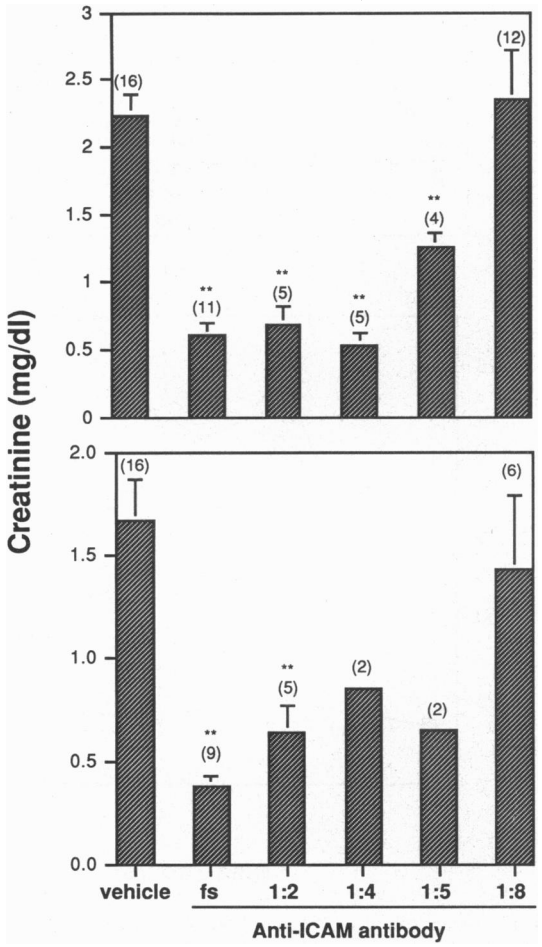

FIG. 3. Effects of administration of various dilutions of anti-ICAM-1 mAb on the course of acute renal failure. Equal volumes of full-strength ("fs") anti-ICAM-1 mAb (group IV) or anti-ICAM-1 mAb diluted 1:2 (group VIII), 1:4 (group IX), 1:5 (group X), or 1:8 (group XI) or vehicle (group II) were administered during the ischemic period, and serum creatinine levels were determined 24 hr (*Upper*) and 48 hr (*Lower*) after reperfusion. **, $P < 0.01$ compared to vehicle group.

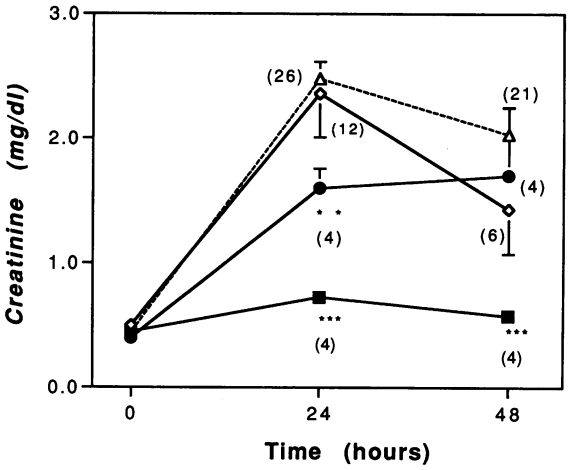

FIG. 4. Effects of administration of anti-LFA-1 mAb alone (group XII) (●) or together with 1:8 diluted anti-ICAM-1 mAb (group XIII) (■) on the course of acute renal failure. △, Vehicle; ◇, 1:8 diluted anti-ICAM-1 mAb alone. Plasma creatinine values ○, 24, and 48 hr after reperfusion are presented. **, $P < 0.05$; ***, $P < 0.0001$ compared to vehicle group.

Anti-ICAM-1 mAb also protected against renal failure when administered 30 (group V) or 120 (group VI) min but not 8 hr (group VII) after reperfusion (Fig. 2). Lower doses of anti-ICAM-1 mAb, diluted 1:2 (group VIII), 1:4 (group IX) or 1:5 (group X), were also protective, but a 1:8 dilution (group XI) was ineffective (Fig. 3).

Effects of Anti-LFA-1 mAb on Renal Function After Ischemia. mAb directed against LFA-1 (group XII) provided modest protection against ischemic injury (Fig. 4). Plasma creatinine in this group was statistically lower ($P < 0.02$) than that of control ischemic animals 24 but not 48 hr after ischemia. However, in animals treated with anti-LFA-1 mAb together with a nonprotective (1:8 dilution) dose of anti-ICAM-1 mAb (group XIII), plasma creatinine values 24 and 48 hr after ischemia were no different from values in sham-operated or postischemic animals treated with full-strength anti-ICAM-1 mAb (Fig. 1).

Effects of Anti-ICAM-1 and Anti-LFA-1 Antibodies on Mortality After Renal Ischemia. The mortality rate in the group treated with anti-ICAM-1 (group IV) mAb (0%; $n = 18$) was significantly lower ($P < 0.01$) than that of the group (group II) treated with vehicle (16%; $n = 32$) (Table 1). Mortality rates in groups VI, VIII, IX, X, XII, XIII, and XIV, in which there was protection from renal failure, were significantly ($P < 0.01$) lower than those in vehicle-treated ischemic animals. Mortality rates were no different from vehicle-treated animals when anti-ICAM-1 was administered at a low dose (group XI) or at 8 hr after ischemia (group VII), groups in which there was no protection from ischemic renal failure.

Effects of Anti-ICAM-1 mAb on Renal Function After 40 min of Ischemia. At 24 hr and 48 hr after 40 min of ischemia (group XIV), creatinine values were much lower in the anti-ICAM-1-treated animals than in the vehicle-treated controls (Fig. 5). BUN values at 48 hr were 41 ± 11 mg/dl and 190 ± 14 mg/dl ($P < 0.0001$) in the anti-ICAM-1- and vehicle-treated groups, respectively. All of the anti-ICAM-1 animals were alive at 72 hr, whereas 10 of 12 control ischemic animals had expired (Table 1).

Light Microscopy and Immunocytochemistry. Forty-eight hours after ischemia, there was moderate to severe necrosis (3+) in the outer medulla with frequent mitoses in vehicle-treated kidneys (group II) and anti-CD3-treated kidneys (group III) (Fig. 6 *Left*). Necrosis was mild to moderate (1+) with few mitoses in anti-ICAM-1-treated animals (group IV) (Fig. 6 *Center*). No necrosis or mitotic forms were seen in

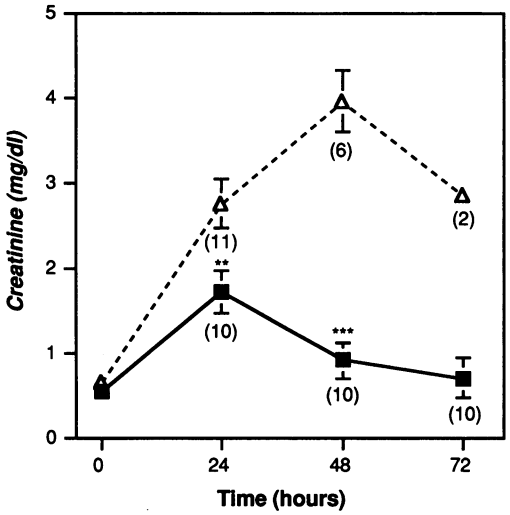

FIG. 5. Effects of anti-ICAM-1 mAb on the course of renal failure after 40 min of renal ischemia. Plasma creatinine values 0, 24, 48, and 72 hr after reflow in animals treated with anti-ICAM-1 mAb (group XIV) (■) or vehicle (group XV) (△) are presented. **, $P < 0.01$; ***, $P < 0.0001$ compared to vehicle group.

sham-operated kidneys (group I). Anti-mouse IgG, administered to localize anti-ICAM-1 mAb (group IV), stained prominently (2+) the vasa rectae in the outer medulla 1 hr after reperfusion (Fig. 6 *Right*). There was less intense staining in the cortical peritubular, glomerular capillary, and arteriolar endothelium. The staining was present in the same areas 24 and 48 hr after ischemia but was less intense (1+). There was no staining in animals treated with anti-CD3 or vehicle 1, 24, or 48 hr after ischemia.

MPO Activity. MPO activity was measured in kidney homogenates as an index of neutrophil infiltration (Fig. 7). There were significant differences in MPO activities between vehicle-treated (group II) and anti-ICAM-1 (group IV)-treated animals 4, 24, and 72 hr after ischemia. MPO levels in kidneys of animals treated with anti-CD3 mAb (group III) were indistinguishable from those of vehicle-treated animals (data not shown).

DISCUSSION

Acute renal failure is an important clinical problem with high morbidity and mortality (15). Therapeutic interventions for acute renal failure in general have been directed toward optimizing hydration status, increasing renal blood flow, increasing tubular flow to decrease tubular obstruction, or preventing tubular cell necrosis (1). The extent of protection afforded by anti-ICAM-1 mAb and its efficacy when administered up to 2 hr after reperfusion distinguishes anti-ICAM-1 therapy among various substances tested as putative protective agents in models of clamp-induced ischemia/reperfusion. These data suggest an important role for leukocyte–endothelial adhesion involving ICAM-1 in the pathophysiology of ischemic acute renal failure.

Neutrophils accumulate in various organs (16, 17) including the kidney (3) after ischemia/reperfusion. Neutropenia decreases renal ischemia/reperfusion injury in some studies (3, 7) but not in others (18, 19). In addition, reperfusion of isolated perfused kidneys with activated neutrophils can accentuate postischemic functional renal impairment (20).

Several mechanisms may contribute to leukocyte-mediated reperfusion injury involving ICAM-1. Activated leukocytes release cytokines, reactive oxygen species, proteases, elastases, myeloperoxidase, and other enzymes. These substances upregulate adhesion receptors, are chemotactic, increase vascular permeability to other inflammatory cells, damage tissue directly (21), and impair endothelial cell function (21, 22). Neutrophils increase injury to endothelial cells subjected to anoxia and reoxygenation (21). Endothelial damage results in a decreased vasodilatory response to hypoxia and acetylcholine (23), which may result in further ischemic injury. Activated neutrophils are more adherent and, along with aggregated platelets and erythrocytes, can physically obstruct capillaries. Activated neutrophils produce vasoconstrictive arachidonic acid metabolites, which may potentiate vascular obstruction (24). In the setting of leukocyte activation and vasoconstriction, we propose that ischemia enhances ICAM-1 expression in the vasa recta, which further increases

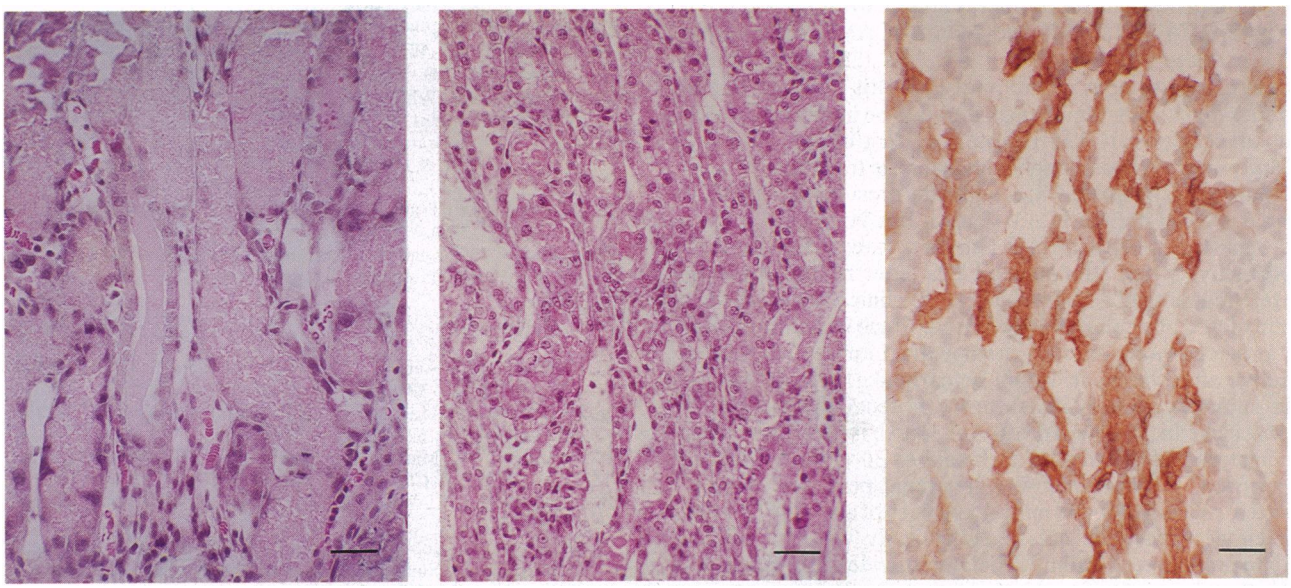

FIG. 6. Effects of anti-ICAM-1 mAb on histopathology after renal ischemia shown by light microscopy of kidney outer medulla 48 hr after 30 min of renal ischemia (*Left* and *Center*). (*Left*) In the control (anti-CD-3) antibody-treated (group III) animal, extensive necrosis (4+) of the tubules of the outer zone of the medulla is present, with regenerative changes of basophilia and mitosis. Tubules are filled with cell debris. (Bar = 50 μm.) (*Center*) In anti-ICAM-1-treated (group IV) animals, minimal individual cell necrosis (1+) of the outer medulla is present. Tubules are generally patent. (Bar = 50 μm.) (*Right*) Immunoperoxidase localization of mouse IgG to the vasa rectae of the outer stripe of the outer medulla in an anti-ICAM-1-treated animal (group IV) studied 1 hr after reperfusion. (Bar = 30 μm.)

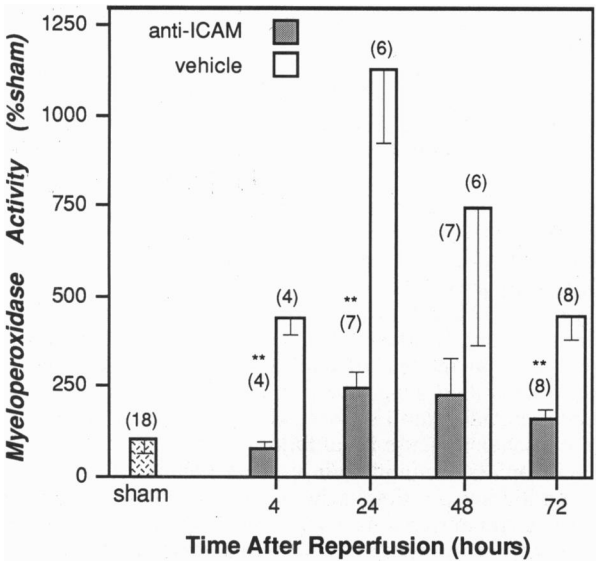

FIG. 7. MPO activity in kidneys exposed to 30 min of ischemia and removed 4, 24, 48, or 72 hr after reperfusion. MPO activity in vehicle-treated (group II) and anti-ICAM-1-treated (group IV) post-ischemic animals (*n* in parentheses) is presented as a percentage of the activity present at the same time in other animals after sham surgery (group I). **, $P < 0.01$ compared to vehicle group.

the adhesion of leukocytes to the endothelium of these vessels. Resultant leukocyte plugging or erythrocyte aggregation, facilitated by enhanced capillary leakage, may then account for sustained outer medullary ischemia, even after the renal artery clamp is removed. The kidney may be particularly susceptible to consequences of ICAM-mediated obstruction of small vessels because there is a tenuous balance between outer medullary oxygenation and energy demand (25). Anti-ICAM-1 mAb has been used in models of ischemia in other organs with varying results (e.g., refs. 10 and 26).

Synergy between anti-LFA-1 and nonprotective doses of anti-ICAM-1 mAbs (Fig. 4) may be explained by the inability of anti-LFA-1 alone to completely block leukocyte adherence. Stimulated neutrophils likely adhere to endothelial cells via both LFA-1–ICAM-1 and Mac-1–ICAM-1 interactions (27). In other injury models [vasculitis (28) or anti-GBM (glomerular basement membrane) nephritis (29)], mAbs directed against LFA-1 have been found to be ineffective, while antibodies against ICAM-1 protect against injury.

Our results may have implications for the treatment of acute ischemic renal failure in man and the preservation of renal allografts. Anti-human ICAM-1 mAb has been used in man after renal transplantation (13). Our data showing that the mAb is protective when administered 2 hr after reflow in the rat suggest that anti-ICAM-1 mAb may be clinically useful when administered after an ischemic period. Since ischemia is often not predictable in man, a therapeutic intervention that is effective after the insult would be particularly attractive.

In summary, anti-ICAM-1 mAb protects the rat kidney against ischemic injury. Anti-ICAM-1 mAb is protective when administered at the time of ischemia or 30 or 120 min after reperfusion. It is protective when renal perfusion is interrupted for 30 min or for 40 min. A low dose of anti-ICAM-1 that is itself nonprotective acts synergistically with anti-LFA-1 mAb to prevent renal failure. Histologic evidence of cell injury and ischemia-induced increases in tissue myeloperoxidase are markedly reduced in animals treated with anti-ICAM-1 mAb. These data suggest a critical role for leukocytes and adhesion molecules in the pathophysiology of ischemic acute renal failure and may have important therapeutic implications for the treatment of acute renal failure in man.

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1. Bonventre, J. V. (1993) *Kidney Int.* **43**, 1160–1178.
2. Mason, J., Joeris, B., Welsch, J. & Kriz, W. (1989) *Mineral Electrolyte Metab.* **15**, 114–124.
3. Hellberg, P. O. A., Källskog, Ö. T., Öjteg, G. & Wolgast, M. (1990) *Am. J. Physiol.* **258**, F1018–F1025.
4. Springer, T. (1990) *Nature (London)* **346**, 425–434.
5. Coletti, L. M., Remick, D. G., Burtch, G. D., Kunkel, S. L., Strieter, R. M. & Campbell, D. A. (1990) *J. Clin. Invest.* **85**, 1936–1943.
6. Minami, M., Kuraishi, Y., Yabuuchi, K., Yamazaki, A. & Satoh, M. (1992) *J. Neurochem.* **58**, 390–392.
7. Klausner, J. M., Paterson, I. S., Goldman, G., Kobzik, L., Rodzen, C., Lawrence, R., Valeri, C. R., Shepro, D. & Hechtman, H. B. (1989) *Am. J. Physiol.* **256**, F794–F802.
8. Yoshida, N., Granger, D. N., Anderson, D. C., Rothlein, R., Lane, C. & Kvietys, P. R. (1992) *Am. J. Physiol.* **262**, H1891–H1898.
9. Argenbright, L. W., Letts, L. F. & Rothlein, R. (1991) *J. Leuk. Biol.* **49**, 253–257.
10. Ma, X.-L., Lefer, D. J., Lefer, A. M. & Rothlein, R. (1992) *Circulation* **86**, 937–946.
11. Tamatani, T. & Miyasaka, M. (1990) *Int. Immunol.* **2**, 165–171.
12. Tamatani, T., Kotani, M. & Miyasaka, M. (1991) *Eur. J. Immunol.* **21**, 627–633.
13. Haug, C. E., Colvin, R. B., Delmonico, F. L., Auchincloss, H., Tolkoff-Rubin, N., Preffer, F. I., Rothlein, R., Norris, S., Scharschmidt, L. & Cosimi, A. B. (1993) *Transplantation* **55**, 766–773.
14. Bradley, P. P., Priebat, D. A., Christensen, R. D. & Rothstein, G. (1982) *J. Invest. Dermatol.* **78**, 206–209.
15. Corwin, H. L. & Bonventre, J. V. (1989) *Sem. Dialysis* **2**, 220–225.
16. Hallenbeck, J. M., Dutka, A. J., Tanishima, T., Kochanek, P. M., Kumaroo, K. K., Thompson, C. B., Obrenovitch, T. P. & Contreras, T. J. (1986) *Stroke* **17**, 246–253.
17. Komatsu, H., Koo, A., Ghadishah, E., Zeng, H., Kuhlenkamp, J. F., Inoue, M., Guth, P. H. & Kaplowitz, N. (1992) *Am. J. Physiol.* **262**, G669–G676.
18. Paller, M. S. (1989) *J. Lab. Clin. Med.* **113**, 379–386.
19. Thornton, M., Winn, R., Alpers, C. & Zager, R. (1989) *Am. J. Pathol.* **135**, 509–515.
20. Linas, S. L., Whittenburg, D., Parsons, P. E. & Repine, J. E. (1992) *Kidney Int.* **42**, 610–616.
21. Inauen, W., Granger, D. N., Meininger, C. J., Schelling, M. E., Granger, H. J. & Kvietys, P. R. (1990) *Am. J. Physiol.* **259**, G134–G139.
22. Lefer, A. M., Tsao, P. S., Lefer, D. J. & Ma, X.-L. (1991) *FASEB J.* **5**, 2029–2034.
23. Malis, C. D., Leaf, A., Varadarajan, G. S., Newell, J. B., Weber, P. C., Force, T. & Bonventre, J. V. (1991) *Circulation* **84**, 1393–1401.
24. Forman, M. B., Virmani, R. & Puett, D. W. (1990) *Circulation* **81**, 69–78.
25. Brezis, M., Rosen, S., Silva, P. & Epstein, F. H. (1984) *J. Clin. Invest.* **73**, 182–190.
26. Clark, W. M., Madden, K. P., Rothlein, R. & Zivin, J. A. (1991) *J. Neurosurg.* **75**, 623–627.
27. Smith, C. W., Marlin, S. D., Rothlein, R., Toman, C. & Anderson, D. C. (1989) *J. Clin. Invest.* **83**, 2008–2017.
28. Argenbright, L. W. & Barton, R. W. (1992) *J. Clin. Invest.* **89**, 259–272.
29. Mulligan, M., Johnson, K., Todd, R., Issekutz, T., Miyasaka, M., Tamatani, T., Smith, C., Anderson, D. & Ward, P. (1993) *J. Clin. Invest.* **91**, 577–587.