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ANTIBODIES TO ICAM-1 PROTECT KIDNEYS IN SEVERE ISCHEMIC REPERFUSION INJURY

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SUMMARY: ICAM-1 has been implicated in the pathophysiology of ischemic-reperfusion injury in a number of organs, but its role in mediating severe ischemic-reperfusion injury in the kidney has not been extensively studied. Uninephrectomized Sprague Dawley rats were pretreated with either control monoclonal antibody (mAb) or mAb to ICAM-1 and subjected to 60 min of renal artery occlusion. The serum creatinine, complete blood count and kidney histo-pathological damage scores (PDS) (Scale:0-4) were assessed prior to and 24 hours after ischemia. Mean serum creatinine (mg/dl) 24 hours after ischemia was significantly decreased in the anti-ICAM-1 group (1.38 ± 0.23, $p<0.001$) compared to control (2.87 ± 0.34). PDS was also reduced in anti-ICAM-1 (2.55 ± 0.20, $p<0.05$) group compared to control (3.35 ± 0.30). These data demonstrate that blocking ICAM-1 significantly mitigates severe ischemic acute renal failure, findings which may lead to improved therapy for this condition.

Renal injury after ischemia appears to be a consequence of not only tissue hypoxia from interrupted blood supply but also from the process of reperfusion which leads to an active inflammatory process (1). Infiltrating leukocytes are a potential source of reactive oxygen species, proteolytic enzymes and cytokines, which during reperfusion may play a detrimental role. Leukocytes have been found to accumulate in the outer and inner medulla post-ischemia in both humans and rats (2), and both functional and morphologic renal protection has been demonstrated in neutrophil-depleted rats (3). The role of the leukocyte remains controversial, however, as other studies in neutropenic rats have failed to show either a functional or histologic reduction of tubular necrosis after ischemia (4,5).

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Abbreviations: ICAM-1, Intercellular adhesion molecule-1; IRI, ischemic reperfusion injury; PDS, pathologic damage score; mAb, monoclonal antibody.

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It has been suggested that leukocyte recruitment to inflamed tissue involves a series of steps: 1) cell rolling on endothelium, 2) leukocyte activation, 3) firm adhesion to endothelium, and 4) migration out of the vascular space (6). Rolling is felt to be mediated by the selectins, while firm adhesion to the endothelium and migration through the vascular wall is thought to be mediated by the CD11/CD18 leukocyte adhesion molecules which bind to ICAM-1 on endothelial cells. We have previously demonstrated that blocking CD11a & CD11b partially attenuated severe renal ischemic reperfusion injury in a single kidney rat model with 60 min of renal artery clamping which produces extensive tubular necrosis (7). In the present study, we evaluated the role of ICAM-1 on IRI in a one-kidney rat model of severe (60 min) ischemic acute renal failure.

MATERIALS AND METHODS

Surgical procedure: Twenty male Sprague Dawley rats (Harlan, Wisconsin) weighing 250-380 grams were used in the experiments. All animal experimentation was conducted in accord with the NIH Guide for Care and Use of Laboratory Animals. All rats had free access to water and standard rat chow. The rats were anesthetized with sodium pentobarbital (60 mg/kg) intraperitoneally (IP), the abdominal region shaved, and the animal placed on a heating pad to maintain constant temperature. The abdominal area was prepared with betadine and sterile drapes were applied. Sterile surgery was performed as previously described in detail (7). A midline incision was made and the left kidney was harvested. The right renal artery was bluntly dissected and a non-traumatic vascular clamp (Roboz microaneurysm clamp, Roboz Surgical Instrument Co., Inc., Washington, D.C.) was applied across the artery for 60 minutes. Ischemia was visually confirmed by the blanching of the kidney. After releasing the clamp the abdominal wall was closed in 2 layers with 4-0 silk sutures. The animals received 50 ml/kg of normal saline at room temperature instilled into the abdominal cavity during the entire surgical procedure. The animals were then allowed to recover. Approximately 24 hours after ischemia, the animals were re-anesthetized, the abdominal wall reopened, aorta identified and cannulated. Blood was collected and the right kidney was perfused with 10% buffered formalin at a constant flow for 30 seconds, harvested and placed immediately in 10% buffered formalin.

Experimental groups: The rats were divided into two groups. Group 1 animals (10 rats) were given a control IgG1 mAb intravenously in 0.5 ml saline at the time of ischemia. Group 2 animals (10 rats) were given mAb to ICAM-1 intravenously at time of ischemia. 1.5 ml of blood was taken through an inferior vena cava puncture just prior to ischemia and 24 hours post-ischemia for determination of hematocrit, leukocyte count, differential, and serum creatinine determinations.

Monoclonal antibodies: The mAb 1A29 is an IgG1 mAb directed against rat ICAM-1, which was initially generated by immunization of mice with high endothelial venule cells of rat lymph nodes (8). The hybridoma clone was kindly provided to us (C.W.S.) by Dr. M. Miyasaka, Tokyo Metropolitan Institute of Medical Science, Japan. A non-adhesion molecule IgG1 murine mAb was used in the control group. Antibodies were administered intravenously at a dose of 2 mg/kg.

Pathological scoring: The formalin-fixed kidneys were cut coronally and imbedded in paraffin. Four micron sections were prepared. The sections were then stained with Hematoxylin and Eosin (H & E) and Leder stains, reviewed in a blinded fashion by a renal pathologist and nephrologist, and scored with a semiquantitative scale designed to evaluate the degree of tubular necrosis changes in the kidney 24 hours after IRI (3,7). Higher scores represented more severe damage (maximum score = 4), 0 = normal kidney, 1 = minimal necrosis, <5% involvement, 2= mild necrosis, 5-25% involvement, 3 = moderate necrosis, 25% - 75% involvement, and 4 = severe, > 75% involvement. Neutrophil infiltration was assessed by counting the neutrophils present in ten randomly selected glomeruli and ten randomly

selected high power fields in the interstitium, five in the cortical region and five in the corticomedullary junction in areas exhibiting the most ischemic changes.

Renal function: The renal function was gauged by the serum creatinine level, measured using the Jaffe reaction in an autoanalyzer (9). Multiple assays were initially performed with rat blood to ensure that small changes in creatinine concentration could be reliably assessed.

Statistical analysis: All values are presented as means ± standard error of the mean (S.E.M.) Statistical analysis comparing control and anti-ICAM-1 groups were performed by analysis of variance (ANOVA) and Fisher's LSD. Statistical significance was set at $P<0.05$.

RESULTS

Effect of mAbs to ICAM-1 on peripheral neutrophil counts. The mAb to ICAM-1 did not deplete peripheral white blood cell counts nor affect differentials, and in both groups, there was an increase in neutrophil counts 24 hours post-IRI (Table 1).

Effect of mAbs to ICAM-1 on serum creatinine. Serum creatinine (mg/dl) rose approximately 6-fold from baseline 24 hrs after the 60 min of ischemia in the control mAb treated group (2.87 ± 0.34). This was significantly reduced in the anti-ICAM-1 group (1.38 ± 0.23, $p<0.001$) (Figure 1).

Effect of mAbs to ICAM-1 on renal morphology after IRI. The pathologic damage score 24 hrs after IRI in the anti-ICAM-1 group (2.55 ± 0.20, $p<0.05$) was reduced when

TABLE 1

PERIPHERAL WHITE BLOOD COUNTS AND DIFFERENTIALS IN CONTROLS AND ANTI-ICAM-1 GROUPS (MEAN ± SEM)

TEST	CONTROL	ANTI-ICAM-1
WBC x 10^3		
0 hrs	8.60±0.41	7.81±0.66
24 hrs	8.16±0.36	6.96±0.47
NEUTROPHILS X 10^3		
0 hrs	0.69±0.13	0.71±0.11
24 hrs	2.45±0.25	1.69±0.17
LYMPHOCYTES x 10^3		
0 hrs	7.78±0.34	6.87±0.65
24 hrs	5.52±0.23	5.23±0.49

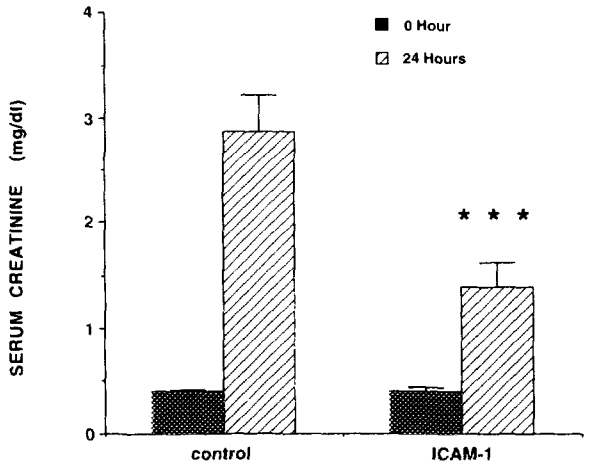

Figure 1. Serum creatinine (mg/dl) 0 and 24 hrs post-ischemia in rats given control mAbs and mAbs to ICAM-1. Serum creatinine was significantly decreased at 24 hrs in anti-ICAM-1 group (*** $p < 0.001$) exposed to ischemia when compared to the control mAb treated group.

compared to control (3.35 ± 0.30) (Figure 2). There was less intense tubular necrosis, and a patchy (versus diffuse) distribution to the tubular necrosis in the ICAM-1 group compared to control (Figure 3).

Effect of mAbs to ICAM-1 on renal tissue neutrophil counts. There were significantly less neutrophils in the interstitium of the anti-ICAM-1 treated animals compared to controls (Table 2).

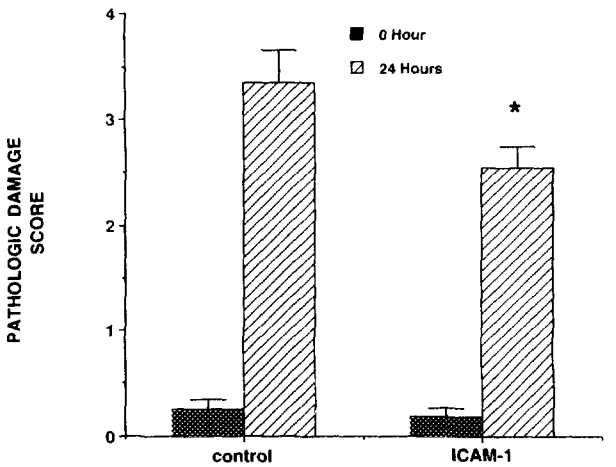

Figure 2. Renal pathologic damage score in control and anti-ICAM-1 rats. Rats treated with mAbs to ICAM-1 had significantly lower PDS (* $p < 0.05$) at 24 hrs compared to controls.

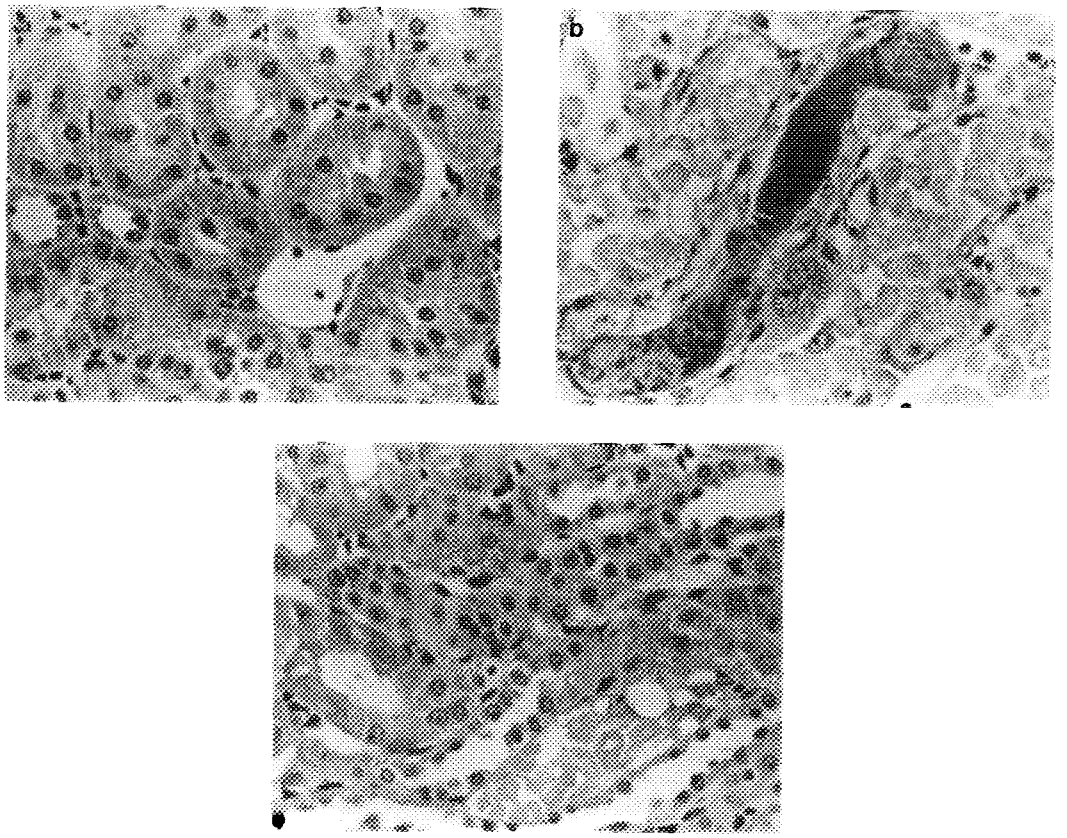

Figure 3. Light microscopy of renal tubular morphology in control and anti-ICAM-1 treated rats. a) Normal renal tubule in nephrectomized sample obtained prior to ischemia (H & E, X 300). b) Representative section from control mAb treated rats showing diffuse acute tubular necrosis with tubular damage, sloughing of epithelial cells, and tubular basement membrane disruption. A cast is evident in the center of the figure (H & E, X 300). c) Representative section from anti-ICAM-1 treated rats showing less severe tubular necrosis with areas of patchy damage (H&E, X 300).

DISCUSSION

The present study demonstrates that administration of blocking mAbs to ICAM-1 can mitigate the structural and functional renal damage in a single kidney rat model of severe reperfusion injury. These data are consistent with studies demonstrating a protective role of mAbs to ICAM-1 in reperfusion injury in the heart (10), and provide further evidence for a pathogenic role of leukocyte adhesion molecules in renal IRI.

ICAM-1 is perhaps the most well characterized endothelial adhesion molecule. A member of the immunoglobulin supergene family, it is a rhinovirus receptor and also a ligand for the ß2 integrins CD11/CD18 (11). ICAM-1 has been shown to be upregulated in a number of renal diseases such as glomerular focal sclerosis and transplant rejection (12). In addition, the use of mAbs to block ICAM-1 has attenuated renal transplant rejection in

TABLE 2

EFFECTS OF ANTI-ICAM-1 mABS ON KIDNEY NEUTROPHIL COUNTS IN 10 RANDOMLY SELECTED GLOMERULI AND 10 RANDOMLY SELECTED INTERSTITIAL HIGH POWER FIELDS (MEAN ± SEM)

TEST	CONTROL	ANTI-ICAM-1
GLOMERULI (WBC)		
0 hrs	1.20±0.55	1.90±0.42
24 hrs	3.00±0.70	3.30±0.82
INTERSTITIAL (WBC)		
0 hrs	1.60±0.60	2.40±0.65
24 hrs	61.4±7.20	*22.0±4.10

* $p < 0.05$ compared to control group.

primates (13) as well as models of glomerulonephritis (14,15). Animal studies focusing on many organs, especially the heart, have shown a pathogenic role of ICAM-1 in IRI (10,16). Our current results demonstrate that ICAM-1 mediates severe renal IRI in rats. Recently, studies by Kelly et al have also demonstrated a protective role of mAbs to ICAM-1 on the rise in serum creatinine after ischemia in a rat model of renal IRI (17). In their two-kidney, 30 min artery and vein clamp model, the serum creatine rise was dramatically reduced from 2.3 mg/dl to 0.62 mg/dl at 24 hours by use of mAb to ICAM-1. However, most cases of mild to moderate renal ischemia resolve on their own, and it is the severe forms that have substantial mortality and require dialysis treatment. In our experiments using 60 min of renal artery clamping of the single remnant kidney, we still obtained significant renal protection with mAb to ICAM-1, but considerably less than Kelly et al observed. It appears that as the ischemic insult becomes more severe, the mechanism of tissue injury is less ICAM-1-dependant.

It is essential in these types of studies that leukocytes are not depleted from the circulation by treatment with mAbs. MAb to ICAM-1 did not deplete peripheral blood leukocytes during the 24 hour experimental period. Upon examining the number of neutrophils in the renal parenchyma, the increased manually counted interstitial neutrophils seen after reperfusion injury in the control mAb group was significantly reduced in the anti-ICAM-1 treated group. This attenuation of neutrophil influx we observed is consistent with that noted by Kelly et al using a myeloperoxidase assay instead to asses PMN number (17).

This study on the role of ICAM-1 in severe ischemic acute renal failure and that of Kelly et al (17) are the first to demonstrate a pathogenetic role of these adhesion molecules in renal IRI. These results open up a potentially new direction in the search for improved therapies for renal ischemic reperfusion injury.

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REFERENCES

1. Bonventre, J.V. (1993) Kidney Int. 43, 1160-1178.
2. Solez, K., Kramer, E.C., Fox, J.A., and Hepinstall, R.H. (1974) Kidney Int. 6, 24-37.
3. Klausner, J.M., Paterson, I.S., Goldman, G., Kobzick, L., Rodzen, C., Lawrence, R., Valeri, C.R., Shepro, D., and Hechtman, H.B. (1989) Am. J. Physiol. 256, F794-802.
4. Paller, M.S., Hoidal, J.R., and Ferris, T.F. (1984). J. Clin. Invest. 74, 1156-1164.
5. Thornton, M.A., Winn, R., Alpers, C.E., and Zager, R.A. (1989) Am. J. Pathol. 135, 509-515.
6. Mulligan, M.S., Polley, M.J., Bayer, R.J., Nunn, M.F., Paulson, J.C., and Ward, P.A. (1992) J. Clin. Invest. 90, 1600-1607.
7. Rabb, H., Mendiola, C.C., Dietz, J., Saba, S.R., Issekutz, T.B., Abanilla, F., Bonventre, J.V., and Ramirez, G. (1994) Am. J. Physiol. 267, F1052-1058.
8. Tamatani, T., and Miyasaka, M. (1990) Intern. Immunol. 2,165-171.
9. Chasson, A.L., Grady, H.J., and Stanley, M.A. (1960) Tech. Bull. Regist. Med. Techn. 30, 207-212.
10. Yamazaki, T., Seko, Y, Tamatani, T., Miyasaka, M., Yagita, H., Ocumura, K., Nagai, R., and Yazaki, Y. (1993) Am. J. Pathol. 143, 410-418.
11. Springer, T.A. (1990) Nature. 346, 425-434.
12. Rabb, H. (1994) Am. J. Kidney Dis. 23,155-166.
13. Cosimi, A.B., Conti, D., Delmonico, F. L., Preffer, F., Wee, S.L., Rothlein,R., Faanes, R., and Colvin, R.B. (1990) J. Immunol. 144, 4604-4612.
14. Kawasaki, K., Yaoita, E., Yamamoto, T.,Tamatani, T., M. Miyasaka, M., and Kihara, I. (1993) J. Immunol. 150, 1074-1083.
15. Mulligan, M.S., Johnson, K.J., Todd, R.F., Issekutz, T.B., Miyasaka, M., Tamatani, T., Smith, C.W., Anderson, D.C., and Ward, P.A. (1993) J. Clin. Invest. 91, 577-587.
16. Harlan, J.M., Winn, R.K., Vedder, N.B., Doershuk, C.M. and Rice, C.L. (1 9 9 2) I n Adhesion: its role in inflammatory disease (J. M. Harlan and D. Y. Liu, eds) p p . 117-150. WH Freeman and Co, New York.
17. Kelly, K.J., Williams, W. W., Colvin, R.B., and Bonventre, J.V. (1994) Proc. Natl. Acad. Sci, USA. 91, 812-816.