Protection from ischemia-reperfusion induced severe acute renal failure by blocking E-selectin

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Objective: Despite progress in renal replacement therapy and critical care medicine, acute renal failure (ARF) still carries a very high mortality rate. Neutrophil infiltration has been recognized as a hallmark in postischemic renal injury. Neutrophil recruitment requires adhesion molecules including E-selectin, which mediates leukocyte rolling and adhesion. This study aims to identify the role of E-selectin in ischemia-reperfusion-induced severe ARF.

Design: Prospective, controlled, experimental study.

Setting: University animal research laboratory.

Subjects: C57BL/6 wild-type mice or C57BL/6 mice gene-deficient for E-selectin.

Interventions: Mice underwent 32-min bilateral renal ischemia or identical sham operations. After 4, 12, 24, or 48 hrs, kidneys were harvested and blood samples were taken. A separate group of wild-type mice received either antineutrophil serum or control serum 18 hrs before ischemia. Another group of wild-type mice was injected with function-blocking monoclonal E-selectin antibody or with control antibody 10 mins after reperfusion. Blood samples were taken 24 hrs later.

Measurements and Main Results: Blood creatinine and urea nitrogen concentrations, as well as renal myeloperoxidase activity indicating neutrophil infiltration, were measured. Reducing neutrophil counts by antineutrophil serum showed that in this model, organ failure strongly depends on neutrophil counts at time of ischemia. E-selectin deficient mice showed lower creatinine and blood urea nitrogen concentrations than wild-type mice at 24 and 48 hrs (a reduction of 60% to 80%). Kidneys of E-selectin deficient mice also revealed a lower myeloperoxidase activity maximum (75% reduction) at 24 hrs. Western blot analysis showed maximum E-selectin expression 24 hrs after ischemia-reperfusion. Immunostaining localized E-selectin to the endothelium of the peritubular capillary plexus. Compared with control antibody, postischemic injection of anti-E-selectin antibody gave lower creatinine concentrations at 24 hrs, similar to that seen in E-selectin deficient mice.

Conclusions: In this model, blocking E-selectin even after onset of reperfusion protects from severe ARF, presumably by reducing postischemic neutrophil infiltration into the kidney. This suggests a new potential therapeutic perspective. (Crit Care Med 2000; 28:2507–2514)

KEY WORDS: acute renal failure; ischemia-reperfusion; E-selectin; kidney function; leukocytes; neutrophils; adhesion molecules; mice; gene targeting; antibody

In clinical practice, ischemia-reperfusion (I/R)-induced tissue injury accounts for a significant number of organ failures including those of the heart, intestine, brain, and kidney. Despite recent progress in renal replacement therapy and critical care medicine, the mortality rate of acute renal failure (ARF) still remains very high (1), especially in intensive care unit patients. Of all ARF episodes in intensive care patients, ~75% are caused by acute tubular necrosis to which those related to surgical interventions contribute a substantial amount (1). Although the pathophysiology of I/R-induced acute renal failures is not completely understood, several important events resulting in tissue damage and subsequently in kidney failure have been identified. Ischemia initially leads to vasoconstriction, tubular swelling, and desquamation as well as to endothelial activation and edema (2). Two other processes are believed to aggravate tissue damages in the postischemic or reperfusion period, no-reflow and reflow paradox (3, 4). No-reflow refers to individual capillary perfusion failure leading to focal tissue hypoxia and thus exacerbating tubular injury (5). Platelets, red blood cells, and leukocytes all have been postulated to account for this phenomenon (6). The reflow paradox describes postischemic injury caused by activated leukocytes, especially neutrophils, that generate and release cytotoxic compounds while adhering to vascular endothelium and infiltrating into the tissue (4).

Neutrophil infiltration into postischemic tissue has been identified as a hallmark of kidney injury in many experimental models (7–12). Neutrophil recruitment into inflamed tissues requires a complex sequence of events, including adhesion to the endothelium and transmigration (13, 14). Leukocyte and endothelial adhesion molecules are thought to mediate leukocyte recruitment in a cascade-like fashion. Capturing or tethering of flowing leukocytes is the initial step and can result in stable rolling of these cells along the endothelium. The selectin class of adhesion molecules

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largely mediates capture and rolling (14, 15). Rolling neutrophils are thought to become activated by chemokines and other chemoattractants, resulting in activation of integrins, firm adhesion, and transmigration (13, 14).

Some adhesion molecules participating in the leukocyte adhesion cascade have been studied in renal I/R injury (7–10, 12, 16–18). Inhibiting either β_2-integrins (16) or intercellular adhesion molecule-1 (ICAM-1) (7, 9, 12, 18) results in a significant reduction of kidney damage after I/R. In contrast, blocking of L-selectin function was not found to have a protective effect (10). E- and P-selectin have not been investigated so far.

Murine E-selectin is a 110 kD, type-1 transmembrane glycoprotein expressed only in endothelial cells and only after cytokine activation (19). E-selectin participates in leukocyte rolling and firm adhesion *in vitro* (20) and *in vivo* (21). Like L-and P-selectin, E-selectin contains an N-terminal lectin domain followed by a short domain homologous to epidermal growth factor and consensus repeats with homology to complement regulatory proteins (22). E-selectin is down-regulated by reinternalization and by shedding from the endothelial surface into plasma (23).

In several descriptive studies, E-selectin expression has been reported in kidney models of I/R (24–26). To date, there are two publications suggesting a distinct role for E-selectin in ischemia-reperfusion injury of other organs. In models of myocardial I/R (27) or splanchnic artery occlusion shock (28), preischemic application of polyclonal antibodies against human E-selectin reduced organ damage and lowered tissue myeloperoxidase activity. The role of E-selectin in I/R-induced acute renal failure has not been explored.

The present study was designed to identify the role of E-selectin in I/R-induced severe acute renal failure. To this end, we used E-selectin deficient mice and application of a blocking monoclonal antibody after reperfusion. To validate our model, we determined the impact of neutrophils on renal function and related the extent of kidney failure with the concentration of circulating neutrophils at the time of ischemia.

MATERIALS AND METHODS

Animals. After approval by the local Animal Care and Use Committee, experiments were conducted in adult (age, 2–5 months; body weight, 20–32 g) C57BL/6 wild-type mice and mice gene-targeted for a null mutation in the E-selectin gene (29). Mutant mice were back-crossed into a C57BL/6 background for ≥5 generations and maintained in colonies at the Center for Comparative Medicine University of Virginia.

Chemicals. If not stated otherwise, all chemicals were obtained from Sigma Chemical, St. Louis, MO.

Surgical Procedure. Mice were anesthetized with injections of ketamine (125 μg/g body weight ip) (Ketalar; Parke-Davis, Morris Plains, NJ), xylazine (12.5 μg/g body weight ip) (Phoenix Scientific, St. Joseph, MO), and atropine sulfate (0.025 μg/g body weight ip) (Elkins-Sinn, Cherry Hill, NJ) and were placed on a heating pad to maintain body temperature. Both renal pedicles were prepared using a median dorsal skin incision and bilateral paramedian opening of the retroperitoneal space. In animals undergoing I/R, both pedicles were clamped off for 32 mins with hemostatic microclips. This model has been shown to induce severe acute renal failure in untreated wild-type mice with a 50% mortality rate at 72 hrs (7). Kidneys were inspected for

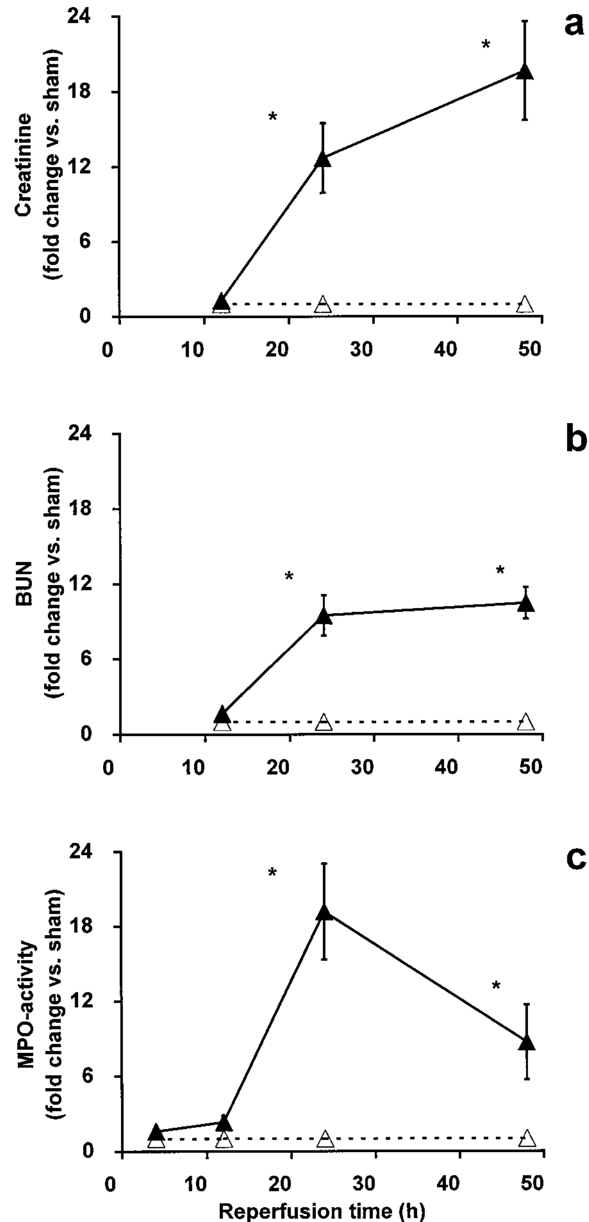


Figure 1. Effect of 32-min bilateral renal ischemia on kidney function and renal MPO activity in wild-type mice (*solid triangles,* ischemia-reperfusion; *open triangles*, sham). Creatinine (*a*) and blood urea nitrogen (*BUN*) (*b*) were measured at 12, 24, and 48 hrs after reperfusion or sham surgery. Myeloperoxidase activity (*MPO*) (*c*) was measured at 4, 12, 24, and 48 hrs after reperfusion or sham surgery. Data are given as mean ± SEM for groups of six mice each. $^*p < .05$ vs. sham.

immediate color change indicating successful clamping. After clamp removal, kidneys were checked for a change in color within 3 mins to ensure reperfusion. In 20 of 154 mice, these criteria were not fulfilled; these mice were excluded from this study. In 12 mice, kidneys showed signs of hemorrhagic infarction at the time of harvesting, indicating venous obstruction rather than arterial occlusion (30). Two mice revealed polycystic kidneys. These mice were also excluded. In animals subjected to sham operation, the surgical procedure was identical except that no clamps were applied. After surgery the wounds were covered with wet gauze. Incisions were closed in two layers, and animals were allowed to recover. Postoperative analgesia was provided by injections of buprenorphine (2 μg/g body weight sc) diluted with isotonic saline to provide sufficient volume substitution for 12 hrs. 4, 12, 24, and 48 hrs later, mice were euthanized, blood samples were taken by heart puncture, and kidneys were harvested.

Therapeutic Intervention Experiments. In two additional groups of wild-type mice (n = 6 each), 100 μg of either function-blocking monoclonal anti-mouse E-selectin antibody (9A9, described in reference 31 and kindly provided by B.A. Wolitzky, Hoffman-La Roche, Nutley, NJ) or isotype-matched control antibody (Pharmingen, San Diego, CA) were injected intraperitoneally 10 mins after reperfusion. This dose of 9A9 has previously been shown to completely block E-selectin-mediated leukocyte rolling (32). At 24 hrs after reperfusion, mice were killed and blood samples were taken.

Neutrophil Depletion Experiments. A separate group of wild-type mice was injected with 2 μl/g body weight intraperitoneally of either rabbit anti-mouse neutrophil serum or preimmune rabbit serum as recommended by the manufacturer (Inter-Cell Technologies, Hopewell NJ) 18 hrs before ischemia. In preliminary experiments, this was shown to sufficiently reduce neutrophil counts (data not shown). Blood samples for neutrophil counts were obtained at the time of onset of ischemia by tail bleeding. Neutrophils were counted by a blinded investigator using Kimura's stain. Animals were euthanized, blood samples were taken, and kidneys were harvested 24 hrs later.

Kidney Function. Whole blood samples were used to determine creatinine and blood urea nitrogen (BUN) concentrations (NOVA analyzer 16^+, NOVA Biomedical, Waltham, MA). Creatinine measurements were based on a three-step enzymatic assay (creatinine amidohydrolase, creatinine amidinohydrolase, and sarcosine oxidase), converting creatinine and water into formaldehyde, glycine, and H_2O_2. BUN was determined by the urease method, converting urea into ammonia and CO_2.

Myeloperoxidase Activity. Myeloperoxidase activity (MPO), indicative of neutrophil infiltration into tissue, was measured in equally sized samples of both kidneys. The assay used represents a modified combination of two previously published methods (33, 34). Briefly, samples were homogenized (1:20 w/v) in ice-cold 20 mM KPO_4 buffer (pH, 7.4). After removing supernatants (17,000 × *g*; 4°C [39°F], 30 mins), pellets were again resuspended in ice-cold 20 mM KPO_4 buffer (pH, 7.4) followed by two additional spins. Then, 0.5% (w/v) hexacyltrimethylamonium bromide-10 mM EDTA in 50 mM KPO_4 (pH, 6.0) was added to the remaining pellet (buffer:pellet 6:1). Suspensions were sonicated for 5 × 1 sec on ice, freeze-thawed three times, and incubated for 20 mins at 4°C (39°F). After final centrifugation (17,000 × *g*, 15 mins, 4°C [39°F]), supernatants were used for measuring MPO. In triplicates, assay buffer (0.2 mg/mL o-dianisidine and 158 μM H_2O_2 in 50 mM KPO_4, pH 6.0) was added to supernatant at a ratio of 4:1. Changes in absorbance were recorded at 460 nm over 5 mins. The linear part of the resulting curve was used for calculating MPO activity. One unit of activity is defined as a change in absorbance of 1.0 per minute at 25°C (77°F). Results are expressed as units of MPO per gram of protein of supernatant as determined by bichionic acid assay (Pierce Chemical, Rockford, IL). In preliminary experiments, kidney samples were spiked with known numbers of neutrophils and the myeloperoxidase activity was quantitatively recovered (data not shown).

Western Blotting. Kidneys were homogenized in ice-cold protein extraction buffer (50 mM Tris pH 7.5, 150 mM NaCl, 2 mM EDTA, 1% Triton X-100, 10 μg/mL phenylmethanesulfonyl fluoride, 1 μg/mL leupeptin, 1 μg/mL aprotinin). After 10 mins incubation, homogenate was centrifuged (10,000 × *g*, 5 mins, 4°C [39°F]). Supernatant was stored at −80°C (−112°F). Samples were loaded at 100 μg protein per lane (bichionic acid assay) and were run under reducing conditions on a SDS-polyacrylamide gel (4% stacking gel, 6% separating gel). Thereafter, gels were electroblotted on nitrocellulose membranes. Further processing was done according to a commercially available kit (Immun-Star Kit, Bio-Rad, Hercules, CA). Monoclonal anti-mouse E-selectin antibody (9A9, 1:500) was used as primary antibody (31). Alkaline phosphatase conjugated goat anti-rat IgG antibody (1:50000) (Pierce Chemical) was used as secondary antibody. Resulting E-selectin bands were quan-

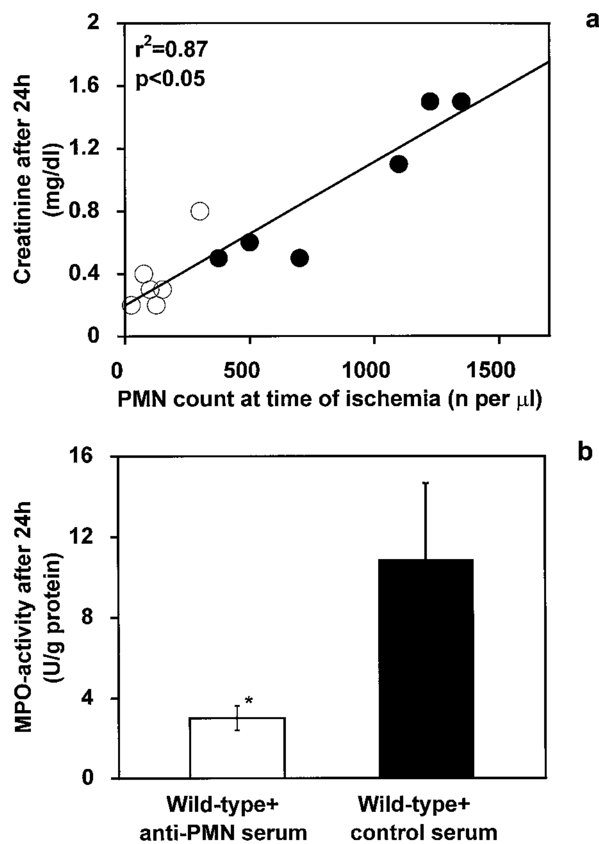


Figure 2. Impact of systemic neutrophil concentration at the time of ischemia on postischemic kidney function. *A*, linear regression of creatinine concentrations at 24 hrs after reperfusion against systemic neutrophil counts at the time of ischemia. Wild-type mice received either rabbit anti-mouse neutrophil serum (*open circles*) or preimmune rabbit serum (*solid circles*) as control 18 hrs before bilateral ischemia. Each data point represents one animal. *B*, myeloperoxidase activity (mean ± SEM) for the rabbit anti-mouse neutrophil serum-treated group (*open bar*) and the control group (*filled bar*). *$p < .05$ vs. control group.

tified using the Scion Image program (National Institutes of Health, Bethesda, MD).

Immunohistochemistry. Snap-frozen kidney sections (5 μm) were first incubated with rat anti-murine E-selectin antibody 10E9.6 (10 μg/mL, described in reference 35), followed by biotinylated secondary antibody (1:500) (Pharmingen, San Diego, CA) in 10% rabbit serum to reduce background staining, and finally by avidin-biotin-peroxidase (Vector Laboratories, Burlingame, CA).

Statistics. Statistical analysis was performed using analysis of variance followed by unpaired Student's *t*-test with Bonferroni correction when appropriate. Multiple and single linear regression were used for analyzing the relation between creatinine and systemic neutrophil concentration. Most data are given as fold change vs. corresponding sham group. All results are expressed as mean ± SEM for groups of six mice each. Statistical significance was set at $p < .05$.

RESULTS

Acute Renal Failure Induced by Ischemia-Reperfusion

After bilateral renal pedicle clamping for 32 mins followed by reperfusion, wild-type mice showed a dramatically elevated creatinine concentration at 24 hrs (13-fold over sham) and 48 hrs (19-fold over sham) after reperfusion (Fig. 1a). BUN (1) concentrations (Fig. 1b) in these mice revealed similar, although smaller rises at 24 hrs (ten-fold over sham) and 48 hrs (ten-fold over sham). Concomitant with the dramatic loss of kidney function, untreated wild-type mice that had undergone 32 mins ischemia and reperfusion showed a continuous increase in MPO up to 24 hrs after reperfusion to 17.9 ± 3.6 U/mg protein, a 19-fold increase above sham control (Fig. 1c). MPO did not increase any further (10.3 ± 3.5 U/g protein) at 48 hrs.

To directly demonstrate the decisive role of neutrophils in our model, we designed a neutrophil depletion experiment. Wild-type mice were injected either with anti-neutrophil serum or control serum 18 hrs before I/R. Pretreatment with either anti-neutrophil serum or control serum had no further effect independent of neutrophil counts ($p >$ .05). Neutrophil counts at the time of ischemia explained 87% of the variation in creatinine concentration after 24 hrs. Figure 2a demonstrates how 24-hr creatinine concentrations depended on ischemic neutrophil counts ($r^2 = 0.874$, $p <$ .00001). When taken as one group, kidneys from neutrophil depleted mice showed significantly lower MPO than mice pretreated with control serum (Fig. 2b).

Role of E-Selectin

Having shown that our model of ARF is severe, reproducible, and neutrophil-dependent, we next asked whether E-selectin played an important role in neutrophil influx and kidney failure. Gene-targeted mice deficient for E-selectin had a significantly smaller elevation in both creatinine (Fig. 3a) and BUN (Fig. 3b) concentrations when challenged with 32 mins of ischemia followed by 24 and 48

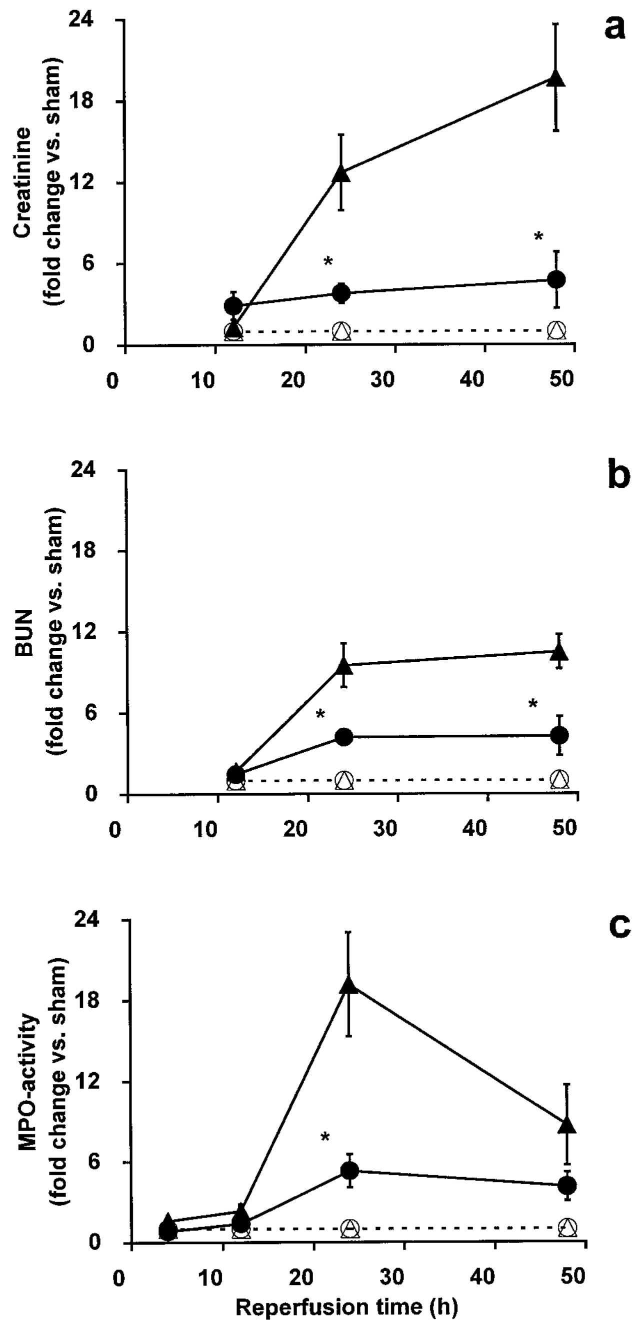


Figure 3. Effect of 32-min bilateral renal ischemia on kidney function and neutrophil accumulation in wt (*solid triangles*, ischemia-reperfusion; *open triangles*, sham) (same data as in Fig. 1) and E-selectin null mice (*solid circles*, ischemia-reperfusion; *open circles,* sham). Creatinine (*a*) and blood urea nitrogen (*BUN*) (*b*) were measured at 12, 24, and 48 hrs after reperfusion or sham surgery. Myeloperoxidase activity (*MPO*) (*c*) was measured at 4, 12, 24, and 48 hrs after reperfusion or sham surgery. *$p < .05$ vs. corresponding wt.

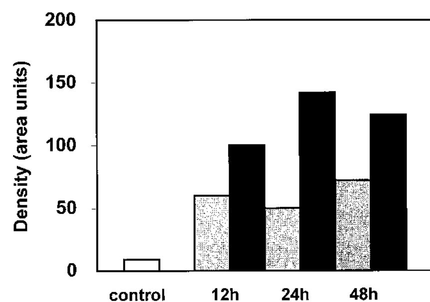
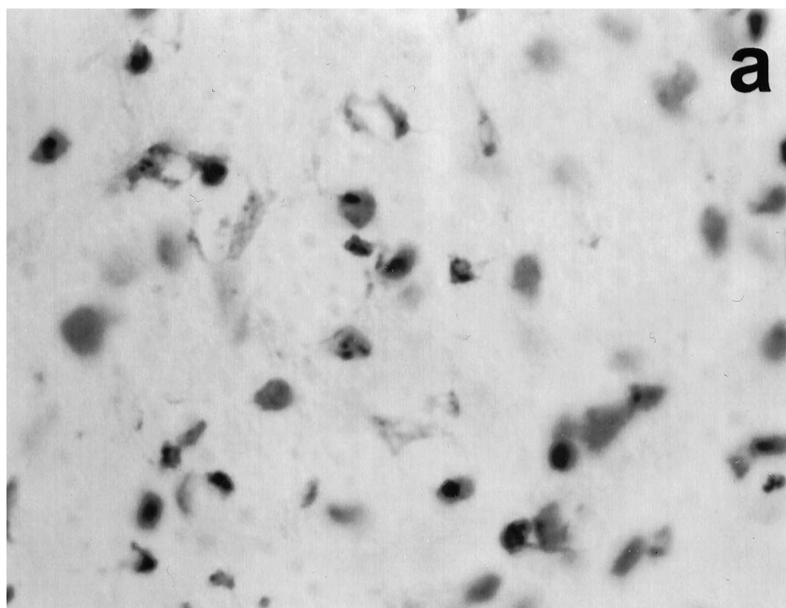


Figure 4. Effect of 32-min bilateral renal ischemia on E-selectin expression in the kidney. Densitometry results of E-selectin bands from a representative Western blot are shown. Kidneys from nonoperated (control, *white bar*), sham-operated (*gray bar*), and ischemia-reperfusion (*black bars*) wild-type mice at 12, 24, and 48 hrs after ischemia/reperfusion were analyzed.

hrs of reperfusion. In E-selectin deficient mice, the reduction in creatinine elevation appeared to be more pronounced than that seen in BUN, corresponding to 80% (creatinine) and 60% (BUN) protection of kidney function compared with wild-type mice. E-selectin deficient mice showed a qualitatively similar time course in MPO after I/R when compared to sham-operated mice lacking E-selectin; however, MPO only reached a shallow maximum at 24 hrs of 9.8 ± 2.3 U/g protein, corresponding to only a five-fold elevation above MPO levels in sham (Fig. 3c). This is a significant ($p < .05$) reduction of neutrophil influx by 70% compared with wild-type mice after I/R.

The MPO maximum coincided with peak expression of E-selectin in postischemic wild-type kidneys as demonstrated by densitometry tracing from a representative western blot (Fig. 4). Protein extracts from kidneys of nonoperated, sham-operated, and I/R wild-type mice were compared. Interestingly, not only postischemic kidneys expressed E-selectin, but kidneys from sham-operated mice were also found to express some E-selectin. However, no E-selectin expression could be seen in nonoperated control mice. This suggests that the sham procedure is sufficient to induce some E-selectin expression, but not sufficient to cause neutrophil recruitment and kidney damage.

To localize E-selectin expression within the kidney, immunohistochemistry was performed on frozen kidney sections from wild-type mice 24 hrs after reperfusion and from nonoperated mice serving as controls. No E-selectin expression was found in control kidneys (Fig. 5a), whereas 24 hrs after I/R, E-selectin was heterogeneously expressed in the endothelium of the peritubular capillary plexus (Fig. 5b).



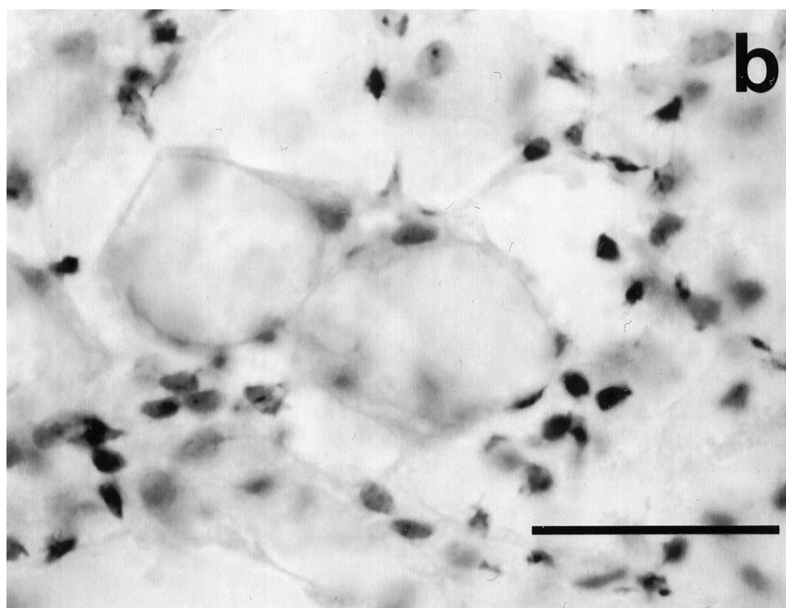


Figure 5. Localization of renal E-selectin expression in nonoperated control mice (*a*) and in wild-type mice 24 hrs after 32-min ischemia and reperfusion (*b*). No E-selectin expression at all could be detected in control animals (*a*). By contrast, 24 hrs after ischemia-reperfusion, E-selectin expression was found in the endothelium of the peritubular capillary plexus, but neither in larger vessels nor in glomeruli. 10E9.6 was used as primary antibody. Giemsa counter stain. Bar = 9μm.

Therapeutic Potential of Blocking E-selectin

To identify any therapeutic benefit from inhibiting E-selectin function, wild-type mice that had undergone 32-min bilateral ischemia received either function-blocking monoclonal antibody to E-selectin (9A9) or isotype matched control antibody 10 mins after clamp removal. Treatment with mAb 9A9 resulted in significant attenuation of creatinine increase 24 hrs after ischemia. Creatinine was 68% lower than in the control group (Fig. 6), similar to the protection seen in mice lacking the E-selectin gene (70% protection). These data show that blockade of E-selectin is therapeutically beneficial even after onset of reperfusion, as

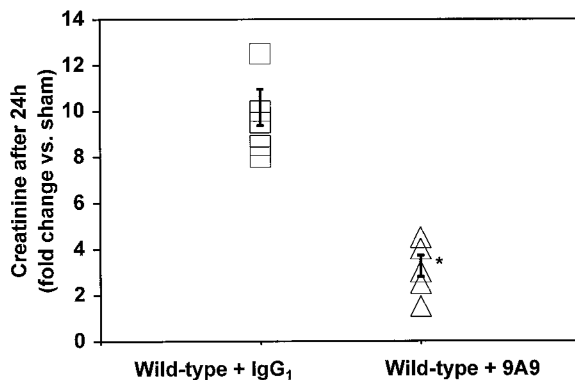


Figure 6. Therapeutic effect of postischemic E-selectin blockade on kidney function after ischemia-reperfusion. Wild-type-mice received either function-blocking monoclonal anti-E-selectin antibody (9A9) (*triangles*) or isotype-matched control antibody (*rectangles*) 10 mins after 32-min bilateral renal ischemia. Blood samples were taken 24 hrs later for determining creatinine concentrations. Data are given as mean ± SEM for groups of six mice each. *$p < .05$ vs. wild-type treated with control antibody.

reflected by the significantly better kidney function at 24 hrs.

DISCUSSION

These experiments demonstrate that E-selectin plays a fundamental role in I/R-induced acute renal failure by recruiting neutrophils into postischemic tissue. Importantly, we show that postischemic blocking of E-selectin function drastically preserves organ function at a clinically relevant level.

Whereas many studies have found neutrophil infiltration in postischemic renal tissues (6–10), the impact of neutrophils on kidney function after I/R has remained controversial. In some studies, neutrophil depletion before I/R protected against kidney failure (7, 36–38); in contrast, other studies failed to show such protection (17, 39). Aside from differences in model design, these conflicting results can probably be attributed to discrepancies in the degree of neutrophil depletion. Supporting this, a recent study has suggested that the contribution of neutrophils to ischemic injury depends on the duration of ischemia and the state of neutrophil activation (7). Those studies, which fail to show a protective effect of neutrophil depletion, have indicated that severe (very long) ischemia causes acute renal failure independently of reperfusion events such as neutrophil recruitment (17). However, in addition to comparing postischemic kidney function between two groups of mice (neutrophil depleted vs. normal), we also related individual neutrophil counts at the time of ischemia to corresponding creatinine concentrations at 24 hrs after reperfusion. This clearly showed that neutrophil concentration at time of ischemia predicts kidney function after I/R in this model. Thus, our model is valid to study the impact of adhesion molecules on leukocyte recruitment into postischemic kidneys.

E-selectin is expressed in endothelial cells activated by cytokines such as interleukin-1 and tumor necrosis factor-α (19). Renal I/R has been shown to produce increased serum concentration of these two cytokines shortly after reperfusion (7). Moreover, *in vitro* studies have demonstrated that both tumor necrosis factor-α (40) and interleukin-1 (41, 42) mediate E-selectin expression during anoxia/hypoxia-reoxygenation. These findings provide possible mechanisms by which E-selectin expression may be induced in our model.

E-selectin has been found to be expressed after I/R in organs such as the brain (43), muscle (44), liver (45), heart (46), and kidney (24). In our model, E-selectin-dependent neutrophil recruitment into the kidney was transient with a single peak 24h after reperfusion. This pattern is consistent with E-selectin expression patterns seen *in vitro* (19, 47) and with E-selectin messenger RNA expression data from renal I/R experiments in rats (24). Transient E-selectin expression appears to be necessary to recruit a relevant number of neutrophils into postischemic kidney tissue as indicated by our MPO data. Compared with wild-type mice, E-selectin deficient mice showed a 70% reduction in MPO activity 24 hrs after I/R. Supportive of our findings, Takada et al. (24) observed that renal E-selectin messenger RNA expression paralleled neutrophil accumulation in the postischemic kidney. Thus, the parallel time course of E-selectin expression and renal MPO activity, together with the decisive role of neutrophils in our model, suggests that E-selectin dependent neutrophil recruitment into the kidney is responsible for a large portion of I/R-induced renal failure.

E-selectin deficient mice are protected against ischemia-reperfusion induced severe acute renal failure. Postischemic blockade of E-selectin provides comparable protection to that seen in E-selectin deficient mice, suggesting that a blocking strategy directed at E-selectin may be of potential therapeutic value.

E-selectin expression was seen in the endothelium of the interbundle capillary plexus, which supplies the highly vulnerable outer medulla. Taken together with the parallel time course of MPO-activity and E-selectin expression, E-selectin mediated neutrophil recruitment appears to occur in a relatively small but very susceptible region and leads to profound organ dysfunction.

A potential preventive effect of E-selectin blockade has been suggested for myocardial I/R injury (27) and for splanchnic artery occlusion shock (28). No data exist for the role of E-selectin in I/R-induced acute renal failure. Our study is the first to demonstrate a distinct role for E-selectin in I/R-induced organ failure, and it is also the first showing a therapeutic effect of postischemic E-selectin blockade. In kidney I/R injury, this has been reported so far only for ICAM-1 blockade (9). In rats, application of monoclonal antibody against ICAM-1 2 hrs after a 30-min ischemia resulted in protection of kidney function and de-

creased renal myeloperoxidase levels (9). Similarly, ICAM-1 deficient mice are protected against I/R-induced renal failure (7) in the same model of renal ischemia as presented here. Because no data from sham-operated ICAM-1-deficient mice were reported in the previous study, it is not possible to directly compare the efficacy of blocking E-selectin with that of blocking ICAM-1.

Surprisingly, we found that sham surgery was sufficient to induce some renal E-selectin expression. Major trauma is known to cause large elevations in plasma concentrations of tumor necrosis factor-α and interleukin-1 (48). E-selectin can be expressed remote from the site of tissue injury (49). Thus, the observed E-selectin expression in sham-operated animals appears to be caused by the trauma associated with the surgical procedure itself. Moreover, in the absence of severe local (renal) injury, E-selectin expression is not sufficient to recruit neutrophils and mediate organ damage.

As an experimental study of acute renal failure in mice, the current data support the concept of neutrophil adhesion, specifically that E-selectin is crucial in mediating kidney damage in this model. However, it cannot be expected during ischemic insults that the kidney will equally benefit from blocking E-selectin. The causes of ischemic renal failure encompass many pathophysiological events. Specifically, the duration of the ischemic insult may be of major importance for the contribution of neutrophils. If the ischemic period is much longer than in the presented model, a beneficial effect of blocking E-selectin cannot necessarily be expected, because the ischemic injury will be neutrophil-independent (17).

In summary, E-selectin deficient mice are protected against ischemia-reperfusion induced severe acute renal failure. Postischemic blockade of E-selectin provides comparable protection to that seen in E-selectin deficient mice, suggesting that a blocking strategy directed at E-selectin may be of potential therapeutic value.

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