Glomerular Inflammation Induces Resistance to Tubular Injury in the Rat
A Novel Form of Acquired, Heme Oxygenase–dependent Resistance to Renal Injury

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Abstract

Considerable attention is directed to a surprising biologic phenomenon wherein tissues exposed to one insult acquire resistance to another. We identify a novel example of acquired resistance to acute renal failure and a mechanism that contributes to such resistance. Nephrotoxic serum, administered to rats 24 h before the induction of glycerol-induced acute renal failure, reduces functional and structural injury that occurs in this model. Since heme oxygenase, the rate-limiting enzyme in heme degradation, protects against heme protein–induced renal injury, we questioned whether induction of heme oxygenase underlies the protection afforded by nephrotoxic serum. Kidney heme oxygenase (HO-1) mRNA was induced 6 h after nephrotoxic serum and renal tubules were identified as the site of expression of heme oxygenase protein. Induction of heme oxygenase was accompanied by increased renal content of ferritin but not by induction of other antioxidant enzymes. Inhibition of heme oxygenase prevented the protection afforded by nephrotoxic serum. Nephrotoxic serum did not protect against ischemic acute renal failure, a model in which heme oxygenase is not induced. Thus, nephrotoxic serum protects against glycerol-induced acute renal failure by inducing heme oxygenase in tubules. This study provides the first demonstration of resistance to tubular injury acquired from glomerular inflammation, uncovers a mechanism for such resistance, and exposes the dialogue that occurs between glomeruli and tubules. (*J. Clin. Invest.* 1996. 98:2139–2145.) Key words: nephrotoxic serum • heme oxygenase • acquired resistance • glycerol-induced acute renal failure

Introduction

Considerable attention is currently directed to a surprising, and seemingly universal, biologic phenomenon wherein the exposure of tissues to a given insult confers resistance to subsequent or different insults. For example, preconditioning the myocardium with transient periods of ischemia (1, 2) or increased temperature (3) reduces myocardial damage sustained after subsequent episodes of ischemia; the prior administration of endotoxin reduces hyperoxic lung injury (4); the transient exposure of pancreatic cells to increased temperatures reduces islet cell injury induced by nitric oxide and reactive oxygen species (5); and conditioning plant cells with the injurious oxidant, hydrogen peroxide, elicits in these cells the hypersensitive disease resistance response (6).

Resistance to injury has been recognized in studies involving the kidney that date to the early part of this century (7–11). In these early studies it was observed that the exposure of the kidney to a particular insult, uranyl nitrate, rendered the kidney resistant to subsequent challenges with the same nephrotoxin (9). Resistance to the same insult was thereafter noted with other, more clinically relevant insults including aminoglycoside antibiotics (12) and the glycerol model of acute renal failure (13, 14), the latter representing a model of rhabdomyolysis. Resistance to renal injury was also recognized when the kidney was conditioned by dissimilar insults; for example, rats recovering from tubular injury induced by hypertonic glycerol were resistant to tubular damage induced by the tubular toxin, mercuric chloride (15); rats treated with tubular toxins such as potassium dichromate (16) or mercuric chloride (17) exhibited reduced sensitivity to the nephrotoxic actions of gentamicin. These experimental findings were complemented by clinical observations which suggested that an analogous phenomenon occurred in patients recovering from renal insufficiency (18).

Little else is known about conditions that promote resistance to renal injury, and as is the case for resistance in other tissues, even less is understood about underlying mechanisms (19). While the type, timing, and severity of the insult are recognized as critical determinants and that certain insults, for example, ischemic injury (10, 11, 20), are less likely to be protected by other insults in vivo, the cellular basis for acquired renal resistance in such settings is unresolved (10, 11). Such possibilities as altered glomerular hemodynamic response, impaired renal concentration of nephrotoxins, less tubular backleak, reduced cast formation, and an inherent resistance of regenerating cells, are all speculative (10, 11). Resistance to injury is dictated, in part, by the presence of the appropriate defense mechanisms as exemplified by the relative susceptibility to oxidant injury in different parts of the nephron (21, 22). Quite recently, Zager and colleagues have provided significant contributions indicating that the acquisition of resistance is influenced by the setting, in vivo vs in vitro, in which it is explored (23, 24); that an inherent resistance to injury resides in plasma membrane of proximal tubules harvested from kidneys previously conditioned in vivo by ischemia (25, 26) or urinary tract obstruction (27); and, as emblematic of one of the few mechanistic explorations for acquired resistance, cellular resistance to ATP depletion is dependent on the disappearance/de-

phosphorylation of a 130-kD tyrosine-phosphorylated protein/protein complex (28).

We describe a novel example of acquired renal resistance wherein a disease model characterized by acute glomerular inflammation confers a profound resistance to tubular injury, findings representing the first description of cellular resistance exhibited in one renal compartment but acquired from events in another. We identify the induction of a particular enzyme, heme oxygenase, a key enzyme in the degradation of heme proteins, and one, to date, not recognized as induced in glomerular inflammation, as critical to such acquisition of resistance to injury.

Methods

Induction of nephrotoxic serum nephritis. Nephrotoxic serum was prepared as previously described from sheep injected with purified rat glomerular basement membrane extract (29, 30). Nephrotoxic serum (0.25 ml diluted in PBS to 0.5 ml) was administered intravenously to male specific pathogen free Sprague Dawley rats (body weight 200–225 grams). Control rats received an equivalent amount of nonimmunized sheep serum (10 mg sheep IgG) by intravenous injection. Kidneys were harvested from ether-anesthetized rats 6 or 24 h after injection of nephrotoxic serum or nonimmunized sheep serum. Kidneys were immediately imbedded in O.C.T. Compound (Miles Inc., Diagnostics Division, Elkhart, IN) and snap frozen in liquid nitrogen. To evaluate renal binding of nephrotoxic serum, four micron tissue sections were stained with rabbit anti–sheep IgG (Pel-Freez, Rogers, AR) and examined by routine immunofluorescence microscopy.

Glycerol-induced acute renal failure. The glycerol model of acute renal failure was induced as described previously (31). Specific pathogen-free male Sprague Dawley rats (275–300 grams; Harlan, Madison, WI) were deprived of water for 17 h with free access to rat chow (Ralston Purina Co., St. Louis, MO). Under ether anesthesia, rats were injected with 50% glycerol in water, 8 ml/kg body weight (BW), half of the dose injected into each anterior thigh muscle. Serum creatinine measurements were performed on tail vein blood samples using a Beckman Creatinine Analyzer II (Beckman Instruments, Inc., Fullerton, CA). To determine the functional effect of induction of heme oxygenase by nephrotoxic serum, studies were also performed in which nephrotoxic serum was administered 24 h before inducing the glycerol model of acute renal failure.

Morphometric studies. Morphometric studies for histologic injury were conducted in kidneys harvested from rats which were pretreated with nephrotoxic serum or nonimmunized sheep serum 24 h before glycerol-induced acute renal failure (32). Kidneys were perfusion-fixed 48 h after the administration of glycerol and processed for the morphometric determination of volume density of tubular necrosis and casts as previously described (32).

Evaluation of renal expression of heme oxygenase. Tissue sections from kidneys of rats killed 6 or 24 h after injection of nephrotoxic serum or nonimmunized sheep serum were prepared for immunofluorescence studies, the latter performed with an anti–rat heme oxygenase antibody as the primary antibody (33). RNA extraction, Northern analysis, and hybridization against cDNA probes were performed as previously described (31, 34). Autoradiograms were quantified by video densitometry and standardized by factoring the densitometric reading for heme oxygenase mRNA with the densitometric reading for the 18S rRNA, the latter obtained on a negative of the ethidium bromide–stained nylon membrane (34).

Inhibition of heme oxygenase activity. Tin protoporphyrin (Porphyrin Products, Inc., Logan, UT), a specific competitive inhibitor of heme oxygenase, was administered subcutaneously into the posterior neck tissue at a dose of 20 μmol/kg BW in 0.5 ml normal saline vehicle 3 h before, at 5 and at 21 hours after the administration of nephrotoxic serum or nonimmunized sheep serum (31, 34). The control

group for tin protoporphyrin received normal saline vehicle at the same three timepoints. Serum creatinine was determined in the baseline state and for two sequential days after the admininistration of hypertonic glycerol. In these studies we thus examined 3 groups of rats: rats in all groups received hypertonic glycerol; one group was pretreated with nephrotoxic serum while another was pretreated with nonimmunized sheep serum before the administration of hypertonic glycerol; the third group was pretreated with nephrotoxic serum prior to the administration of hypertonic glycerol but was also treated with tin protoporphyrin. Serum creatinine measurements obtained from all rats so treated according to one of these three groups were pooled and analyzed by ANOVA.

Determination of antioxidant enzyme activities. Kidney antioxidant enzyme activities were measured in a kidney cytosolic preparation 6 and 24 h after administration of nephrotoxic serum or nonimmunized sheep serum. Catalase activity was measured by the method of Aebi et al. (35) while glutathione peroxidase activity was determined by the method of Lawrence and Burk (36).

Kidney ferritin content. Kidney ferritin content was measured 8 h after the administration of nephrotoxic serum or nonimmunized sheep serum by an ELISA method using a primary antibody consisting of anti–rat ferritin, as previously described (31).

Ischemic acute renal failure. Ischemic acute renal failure was induced as previously described (37). Under methohexital anesthesia (5 mg/100 grams BW by intraperitoneal injection), rats (225–275 grams) underwent right nephrectomy and occlusion of the left renal artery for 45 min using a nontraumatic vascular clamp.

Urinalysis and quantitation of urinary content of hemoglobin and heme. To determine whether nephrotoxic serum induced hematuria, we analyzed urine for the presence of erythrocytes, hemoglobin, and heme. Rats were injected with nephrotoxic serum or nonimmunized sheep serum, and urine was collected in metabolic banks for 16 h after administration of the respective sera. Urine specimens were tested for the presence of blood by Hemastix (Ames Division, Miles Laboratories, Inc., Elkhart, IN) and microscopic urinalysis was performed on a centrifuged aliquot of urine. Urinary concentration of hemoglobin was determined spectrophotometrically as previously described (38, 39), while urinary concentration of heme was determined by the pyridine hemochromogen method (40) as previously described (39).

Statistics. Data are presented as mean±standard error of the mean. Student's unpaired *t* test was used for statistical comparisons involving two unpaired groups. For analyses involving three groups, ANOVA was used followed by the Student Neuman Keuls test.

Results

Effect of nephrotoxic serum on glycerol-induced acute renal failure. Prior administration of nephrotoxic serum conferred marked protection against renal injury induced by intramuscular hypertonic glycerol (Fig. 1). The mean serum creatinine in rats with glycerol-induced acute renal failure, pretreated with nonimmunized sheep serum, markedly increased by the second day after the administration of glycerol, attaining an 11-fold increase over basal values. In contrast, rats pretreated with nephrotoxic serum exhibited less severe renal insufficiency on each of the days following the administration of glycerol. Nephrotoxic serum did not affect serum creatinine in rats with intact, disease-free kidneys as shown by the comparison of baseline values in these groups (Fig. 1).

The administration of nephrotoxic serum reduced histologic damage in kidneys harvested 2 d after the onset of glycerol-induced acute renal failure. This timepoint was chosen in the glycerol model since structural injury is established at this stage and such injury can be quantified by morphometry (31). Morphometric scores for the volume density of tubular necrosis were significantly lower in glycerol-treated rats infused be-
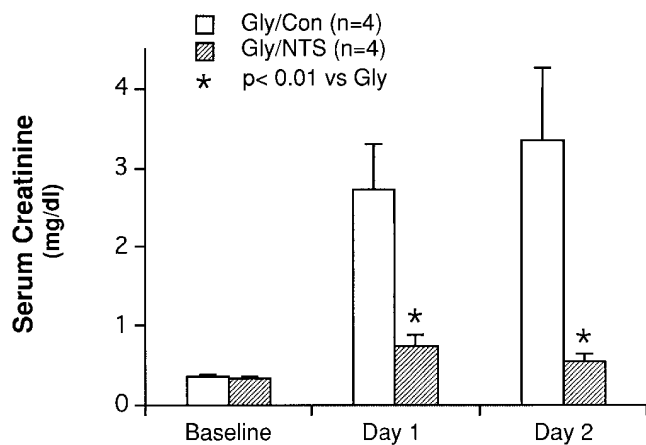
Figure 1. Effect of prior administration of nephrotoxic serum (*NTS*) or nonimmunized sheep serum (*Control*) on renal function as measured by serum creatinine on 2 sequential days after the administration of hypertonic glycerol (Gly, 8 ml/kg body weight administered intramuscularly). Baseline values were obtained the day after the administration of nephrotoxic serum or nonimmunized sheep serum after which hypertonic glycerol was administered to both groups.

forehand with nephrotoxic serum as compared to rats pretreated with nonimmunized sheep serum (10.8 ± 4.2 vs. $32.3\pm1.3\%$; $P < 0.01$); scores for the volume density of tubular casts were reduced in rats pretreated with nephrotoxic serum but this comparison did not achieve statistical significance (2.9 ± 0.8 vs. $4.6\pm0.7\%$, $P = $ ns). Thus, prior administration of nephrotoxic serum significantly attenuates glycerol-induced renal injury, the latter assessed both functionally and morphologically.

Effect of nephrotoxic serum in rats with intact, disease-free kidneys. The kidneys of rats injected with nephrotoxic serum 24 h previously showed intense linear glomerular basement membrane (GBM) staining by immunofluorescence indicating effective binding of nephrotoxic serum antibody to the glomerular basement membrane; these findings are similar to those in previously published studies using this antibody (29, 30). Well-defined glomerular basement membrane staining was not present in the control glomerulus. Tubular basement membrane staining was absent in the nephrotoxic serum-treated kidney, demonstrating the specificity of glomerular injury in this model. Renal function, as measured by serum creatinine, was not significantly altered 24 h after the administration of nephrotoxic serum (nephrotoxic serum-treated rats 0.3 ± 0.1 vs nonimmunized sheep serum-treated rats 0.4 ± 0.1 mg/dl; $P = $ ns).

Effect of nephrotoxic serum on renal expression of heme oxygenase. To determine whether the protective effects of nephrotoxic serum were dependent on heme oxygenase, an enzyme induced and protective in the glycerol model (31), we examined expression of this enzyme by immunofluorescence and Northern analysis. There was striking induction of heme oxygenase by immunofluorescence in kidneys of rats examined 6 h after the administration of nephrotoxic serum (Fig. 2). Induction of heme oxygenase was confined to the tubules and was not observed in glomeruli. Expression of heme oxygenase was also present in renal tubules 24 h after the administration of nephrotoxic serum but was less intense than that seen in kidneys examined at 6 h. In contrast, kidneys from rats injected with nonimmunized sheep serum failed to show expres-

sion of heme oxygenase either in the tubules or in the glomeruli at both the 6-h (Fig. 2) and 24-h (not shown) timepoints.

In addition, kidneys from rats treated with nephrotoxic serum displayed marked induction of heme oxygenase mRNA by Northern analysis at 6 h while such induction was absent in those rats treated with nonimmunized sheep serum (Fig. 3). The densitometric indices for the heme oxygenase mRNA factored for the 18S mRNA was increased approximately sixfold in rats treated with nephrotoxic serum compared with nonimmunized sheep serum (4.90 ± 1.16 vs 0.86 ± 0.11, $P < 0.01$).

To determine whether nephrotoxic serum increased heme oxygenase activity we measured such enzyme activity in kidneys of rats treated 8 h previously with nephrotoxic serum or nonimmunized sheep serum. Heme oxygenase activity in kidney was significantly increased in rats treated with nephrotoxic serum, 226.7 ± 17.8 vs 121.3 ± 12.4 pmol/mg protein per hr, $n = 6$ in each group, $P < 0.001$. Thus, increased heme oxygenase enzyme activity accompanies increased mRNA expression and increased amounts of protein detected by immunofluorescence.

Urinalysis and urinary content of hemoglobin and heme after the administration of nephrotoxic serum. To determine whether induction of heme oxygenase was linked to the presence of erythrocytes or heme proteins in urine after the administration of nephrotoxic serum, we performed urinalyses and measurements of hemoglobin and heme in urine. In none of the urine specimens was macroscopic hematuria observed. All urine specimens were negative for heme on testing by Hematix while urinalysis showed 0–2 red blood cells per high power field in all specimens in both groups. In the rats treated with nephrotoxic serum, concentrations of hemoglobin were in fact significantly lower than in rats treated with nonimmunized sheep serum (6.1 ± 0.6 vs 11.6 ± 2.3 μmol/liter, $P = 0.03$, $n = 6$) while the rate of hemoglobin excretion was not significantly different in the two groups (89.3 ± 6.5 vs 89.2 ± 10.7 nmol/16 h, $P = $ ns, $n = 6$). Similarly, in rats treated with nephrotoxic serum, concentrations of heme were significantly lower than in rats treated with nonimmunized sheep serum (0.30 ± 0.03 vs 0.49 ± 0.09 μmol/liter, $P = 0.04$, $n = 6$) while the rate of heme excretion was not significantly different (4.7 ± 0.8 vs 4.0 ± 0.6 nmol/16 h, $P = $ ns, $n = 6$). Thus this model of nephrotoxic serum nephritis fails to evince gross or microscopic hematuria and does not display increased urinary concentrations or increased urinary excretory rates for hemoglobin or heme.

Effect of nephrotoxic serum on kidney ferritin content and antioxidant enzymes. We measured kidney ferritin content in nephrotoxic serum-treated rats and nonimmunized sheep serum-treated rats. Ferritin synthesis is increased in circumstances in which heme oxygenase is induced (31); this iron-storage protein can bind iron that is released as heme oxygenase degrades heme thereby ensuring safe sequestration of iron (41, 42). Ferritin synthesis peaks after the maximum induction of heme oxygenase (41); thus, we measured ferritin content at 8 h after the administration of nephrotoxic serum. We found significant increments in kidney ferritin content in nephrotoxic serum-treated rats (19.7 ± 1.23 vs 14.3 ± 0.79 μg/mg protein, $P < 0.05$); thus, increased ferritin content accompanies induction of heme oxygenase activity.

The activities of other antioxidant enzymes, catalase and glutathione peroxidase, were not increased either at 6 h or 24 h after the administration of nephrotoxic or nonimmunized sheep sera (data not shown). Thus, other antioxidant enzymes,
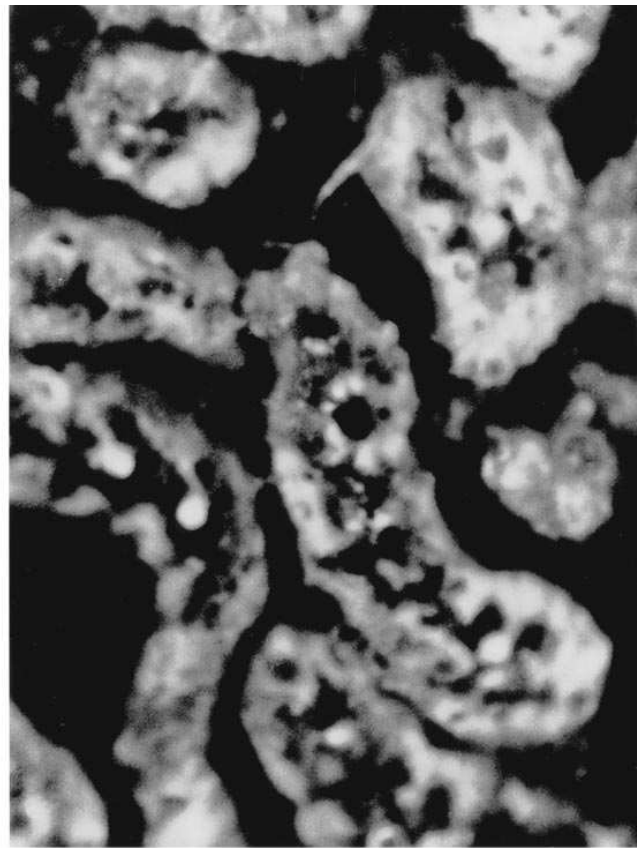
Control   NTS

Figure 2. Representative immunofluorescence studies of rat kidneys for the expression of heme oxygenase 6 h after the administration of non-immunized sheep serum (*Control*, *left*) or nephrotoxic serum (*NTS*, *right*).

relevant to the pathogenesis of renal injury in this model, are not induced in this model.

Effect of inhibiting heme oxygenase in rats treated with nephrotoxic serum prior to the administration of glycerol. To determine whether induction of heme oxygenase in renal tubules contributed to the protection observed after the administration of nephrotoxic serum, we studied the protective effects of nephrotoxic serum in the presence of tin protoporphyrin, a specific competitive inhibitor of heme oxygenase. The protec-

tion conferred by prior administration of nephrotoxic serum was prevented by the concomitant administration of tin proto-porphyrin (Fig. 4); tin protoporphyrin did not increase serum creatinine in rats treated with nephrotoxic serum (data not shown). Thus, the protective effect of nephrotoxic serum is dependent on the induction of heme oxygenase.

Effect of nephrotoxic serum on ischemia reperfusion injury. Prior administration of nephrotoxic serum provided no protection against ischemia reperfusion injury as reflected by
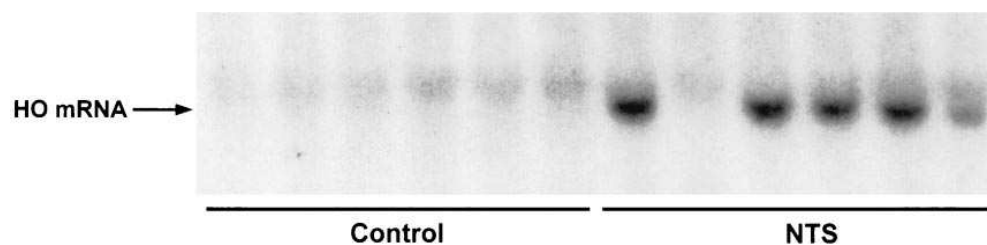

HO mRNA →

Control   NTS

Figure 3. Northern analysis of the expression of heme oxygenase (HO) mRNA 6 h after the administration of nonimmunized sheep serum (*Control*) or nephrotoxic serum (*NTS*). Each lane represents RNA extracted from a single kidney of an individual rat. The densitometric indices for the heme oxygenase mRNA factored for the 18S mRNA was increased approximately sixfold in rats treated with NTS compared with control (0.86 ± 0.11 vs 4.90 ± 1.16, $P < 0.01$).
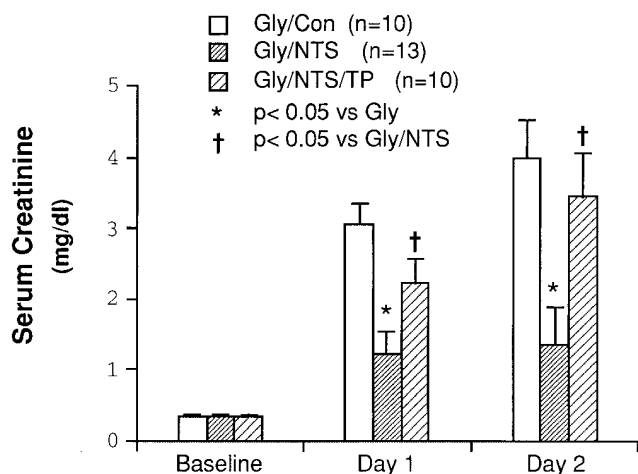
Figure 4. Effect of inhibition of heme oxygenase by tin protoporphyrin (*TP*) in rats pretreated with nephrotoxic serum (*NTS*) 1 d before the administration of hypertonic glycerol (*Gly, 8 ml/kg BW*). Renal function was assessed by serum creatinine on 2 sequential days after the administration of Gly. Rats in all groups received hypertonic glycerol; one group was pretreated with NTS while another was pretreated with nonimmunized sheep serum (*CON*) before the administration of hypertonic glycerol; the third group was pretreated with NTS before the administration of hypertonic glycerol but was also treated with TP.

serum creatinine determinations on two sequential days in the post ischemic phase (day 1: 1.6 ± 0.3 vs 1.7 ± 0.4, $P =$ ns; day 2: 0.9 ± 0.1 vs. 1.0 ± 0.2 mg/dl, $P =$ ns). Thus, the protective effects provided by nephrotoxic serum in the glycerol model are not conferred in another model of acute renal failure. In this latter model heme oxygenase is not induced nor does prior induction of heme oxygenase by hemoglobin protect against renal injury (31).

Discussion

Our findings provide a novel example of acquired renal resistance in which the administration of nephrotoxic serum protects against glycerol-induced acute renal failure. The specificity of such resistance was underscored by the failure of nephrotoxic serum to mitigate acute ischemic injury; these latter observations are consonant with prior observations by Zager et al. which demonstrate that other glomerulopathies such as passive Heymann nephritis or adriamycin nephrosis do not protect against ischemic renal failure (43). Our study is thus the first to demonstrate that a disease model, characterized and utilized as a model of glomerular inflammation, confers resistance to a model of acute tubular injury. Nephrotoxic serum nephritis is typefied by acute glomerular inflammation (29, 30). On the other hand, injury in the glycerol model is mainly confined to tubules, the latter exhibiting lethal and sublethal cell injury in conjunction with luminal cast formation; protection against such tubular injury would necessitate mechanisms of resistance instigated in, and centered on, the tubular compartment. Thus, while the inflammatory effects of nephrotoxic serum in the glomerulus are the more widely appreciated features of this model, it is the tubular effects that, in all likelihood, account for the resistance to glycerol-induced acute tubular injury.

It was with this latter consideration in mind that we explored the induction of heme oxygenase as a mechanism underlying resistance to glycerol-induced acute renal failure. In

this model, the kidney is exposed to a large burden of myoglobin and hemoglobin, and such heme proteins are incriminated in injury that ensues (31). Heme oxygenase is the rate-limiting enzyme in the catabolism of heme and is critical to the daily clearance of heme as senescent red blood cells are removed from the circulation (44); it is induced in the kidney after the systemic administration of heme proteins (31, 45). Additionally, heme oxygenase is upregulated in mammalian cells in response to diverse forms of oxidative stress (46, 47), and such induction of heme oxygenase is incriminated as a beneficial, antioxidant response (47, 48). The glycerol model is characterized by a rapid and robust expression of heme oxygenase that is a protective response (31): inhibition of heme oxygenase worsens renal function while induction of heme oxygenase in the kidney, before the onset of myolysis and hemolysis, is protective (31). The beneficial effect of upregulation of heme oxygenase was recently underscored by the resistance of endothelial cells to injury by hemoglobin and heme when these cells are transfected with a gene encoding the human heme oxygenase enzyme (49). Heme oxygenase protects against heme-mediated injury by degrading heme and by stimulating ferritin synthesis (31, 42); synthesis of ferritin, the major iron storage protein, is often induced in tandem with heme oxygenase, and this iron-storage protein sequesters iron released as the heme ring is degraded by heme oxygenase (31, 41, 42). The protective role of heme oxygenase likely extends beyond overt heme-mediated injury as recent evidence indicates a protective effect of this enzyme in cisplatin nephropathy (34). Our present studies extend this body of information by demonstrating upregulation of heme oxygenase, induced as it is in tubules by nephrotoxic serum, as responsible, at least in part, for a novel form of acquired renal resistance. The efficacy of the competitive inhibitor, tin protoporphyrin, in ablating the protective effect of nephrotoxic serum may encompass a direct adverse effect of the competitive inhibitor in the glycerol model. However, the stimulation of heme oxygenase enzyme activity by nephrotoxic serum in amounts comparable to that achieved by hypertonic glycerol in conjunction with the denial of nephrotoxic serum-induced protection by tin protoporphyrin underscores the involvement of heme oxygenase in mediating such acquired resistance to heme protein renal injury.

In view of the lack of deposition of antibody to IgG in the peritubular area in the kidney in nephrotoxic serum-treated rats, the induction of heme oxygenase in renal tubules likely derives from events instigated in the glomerular compartment. The induction of heme oxygenase in tubules cannot be ascribed to hematuria since erythrocytes were not detected in increased amounts in the urine of rats treated with nephrotoxic serum; nor did such urine demonstrate increased concentration or excretion of hemoglobin or heme. Thus it is unlikely that leakage of erythrocytes in the urinary space and tubular cell uptake of erythrocytes and hemoglobin underlie the induction of heme oxygenase in renal tubules. In this regard the recent studies of Mulligan et al. are of interest (30): these studies demonstrate that in response to nephrotoxic serum, TNF-α is upregulated in the glomerulus and contributes to glomerular injury (30). TNF-α and other cytokines are recognized as inducers of heme oxygenase (50, 51). Thus, TNF-α and other cytokines, elaborated in the glomerular and renal microcirculatory beds after the administration of nephrotoxic serum, and in turn delivered to the renal tubules via the urinary space or by peritubular uptake, may induce heme oxygenase in renal tu-

bules. Another possible mechanism that may contribute to the induction of heme oxygenase and ferritin in renal tubules in glomerulopathic conditions relates to the egress of transferrin into the urinary space as glomerular permselectivity is lost (52). Transferrin is taken up by renal tubules in such conditions and iron, released from intracellular transferrin, may drive the induction of heme oxygenase and ferritin by oxidative and other mechanisms.

Our findings may have relevance to certain clinical features of glomerulonephritides associated with hematuria. Acute renal dysfunction occurring at the time of presentation of acute glomerulonephritides or during exacerbation of these disorders may represent, at least in part, acute tubular injury resulting from the toxicity of heme proteins (53). Additionally several clinical studies have indicated that recurrent episodes of hematuria are associated with a more benign prognosis in progressive IgA nephropathy (54); it is possible that recurrent episodes of hematuria may upregulate heme oxygenase and ferritin in renal tubules which in turn may protect against renal injury in such states.

Our findings are relevant to a number of settings in which acquired resistance to injury is recognized. The prior administration of TNF-α, protects against endotoxin toxicity (55), experimental sepsis (56), and hyperoxic lung injury (57); conversely, the endotoxin-tolerant condition is protective against a lethal challenge of TNF-α (58) and reduces mortality arising from infected thermal insults (59). Such states as endotoxin toxicity, experimental sepsis and hyperoxic injury are all characterized by oxidative stress, and thus the elicitation of an antioxidant response, including one that involves heme oxygenase, may be protective in such settings.

The induction of heme oxygenase in renal tubules after a proinflammatory glomerular insult provides a novel instance of crosstalk between the glomerular and tubulointerstitial compartments. Such dialogue may be relevant to progressive renal disease that so commonly appears as an adverse consequence of glomerular inflammation (60). In all renal diseases studied to date, regardless of etiology or the renal compartment wherein the disease originates, tubulointerstitial changes, and not glomerular changes, correlate more closely with renal functional decline (60). The basis for such correlation of tubulointerstitial changes with renal functional decline in progressive glomerulopathies remains puzzling; however our findings, uncovering as they do major biochemical alterations in renal tubules in inflammatory glomerulonephritides, even when little or no histologic changes involve the tubulointerstitium, point out that very early in the course of glomerular disease significant changes are occurring pari passu with, or as a consequence of, changes in the glomerulus.

In summary, our findings uncover a new form of acquired resistance to renal injury: acute glomerular inflammation as an instigator of resistance to tubular injury. We identify the induction of heme oxygenase, an enzyme not previously recognized as induced in glomerular inflammation, as a critical contributor to such resistance, and we call attention to biochemical changes in tubules as an early accompaniment of acute glomerular inflammation.

Acknowledgments

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