Postischemic inflammatory syndrome: a critical mechanism of progression in diabetic nephropathy

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Kelly KJ, Burford JL, Dominguez JH. Postischemic inflammatory syndrome: a critical mechanism of progression in diabetic nephropathy. Am J Physiol Renal Physiol 297: F923–F931, 2009. First published August 5, 2009; doi:10.1152/ajprenal.00205.2009.—Diabetes is a major epidemic, and diabetic nephropathy is the most common cause of end-stage renal disease. Two critical components of diabetic nephropathy are persistent inflammation and chronic renal ischemia from widespread vasculopathy. Moreover, acute ischemic renal injury is common in diabetes, potentially causing chronic kidney disease or end-stage renal disease. Accordingly, we tested the hypothesis that acute renal ischemia accelerates nephropathy in diabetes by activating proinflammatory pathways. Lean and obese-diabetic ZS rats (F1 hybrids of spontaneously hypertensive heart failure and Zucker fatty diabetic rats) were subjected to bilateral renal ischemia or sham surgery before the onset of proteinuria. The postischemic state in rats with obesity-diabetes was characterized by progressive chronic renal failure, increased proteinuria, and renal expression of proinflammatory mediators. Leukocyte number in obese-diabetic rat kidney was markedly increased for months after ischemia. Intrarenal blood flow velocity was decreased after ischemia in lean control and obese-diabetic rats, although it recovered in lean rats. At 2 mo after ischemia, blood flow velocity decreased further in sham-surgery and postischemia obese-diabetic rats, so that RBC flow velocity was only 39% of control in the obese-diabetic rats after ischemia. In addition, microvascular density remained depressed at 2 mo in kidneys of obese-diabetic rats after ischemia. Abnormal microvascular permeability and increases in interstitial fibrosis and apoptotic renal cell death were also more pronounced after ischemia in obese-diabetic rats. These data support the hypothesis that acute renal ischemia in obesity-diabetes severely aggravates chronic inflammation and vasculopathy, creating a self-perpetuating postischemia inflammatory syndrome, which accelerates renal failure.

chronic kidney disease; acute kidney failure; renal fibrosis; apoptosis

METABOLIC SYNDROME (diabetes, dyslipidemia, obesity, and hypertension) afflicts an ever-expanding proportion of the world’s population, frequently leading to diabetic nephropathy and end-stage renal disease. An enlarging body of data supports the novel hypothesis that anomalous immunologic responses, triggered by metabolic derangements, are critical at every stage of diabetic nephropathy. Diabetic patients with renal disease have increased levels of inflammatory markers, including C-reactive protein, IL-6, and TNF-α (9, 10), as well as markedly abnormal leukocyte function (48). Serum TNF-α levels are correlated with urinary protein excretion in diabetic patients without or with overt nephropathy (39), and specific cytokine genotypes are associated with diabetic nephropathy (33). Renal infiltrates of inflammatory cells with concurrent renal upregulation of leukocyte adhesion receptors, including intercellular adhesion molecule-1 (ICAM-1), are found in human diabetic nephropathy (4). Moreover, the number of interstitial macrophages is strongly correlated with renal dysfunction, proteinuria, and fibrosis in renal biopsy specimens from diabetic patients (40). In addition, immune suppression is protective in models of diabetic nephropathy (11, 53), and, conversely, macrophages in adoptive transfer studies induce proteinuria and mesangial expansion in rat kidneys (21).

In humans, acute kidney injury (AKI) from renal ischemia is often superimposed on diabetic injury (20; unpublished observations). Furthermore, inflammation may contribute to AKI in humans, inasmuch as elevated urinary IL-6 and IL-8 in renal allograft recipients can predict AKI (32). In diabetic animals, greater vulnerability to renal ischemia has been shown (17, 56), and emerging data reveal that inflammation is likely an aggravating factor. For example, leukocyte-binding molecules ICAM-1 and oxidized LDL receptor-1 (LOX)-1 (28, 30) are increased after renal ischemia in rats, and increases in renal leukocytes, TNF-α, and IL-1 are seen after renal ischemia in mice (28). Thus we tested the hypothesis that, in obesity-diabetes, acute renal ischemia would further activate renal proinflammatory pathways and accelerate the progression of renal injury. We used the ZS model of the metabolic syndrome: lean and obese F1 hybrid rats derived from the Zucker diabetic and the spontaneously hypertensive heart failure rat. Obese ZS rats are known to develop albuminuria, glomerulosclerosis, interstitial fibrosis, and renal failure (13, 29). The lean ZS littermates served as normal controls. Acute renal ischemia in obese-diabetic rats evoked severe, progressive renal inflammation that persisted well after the acute ischemic insult. The inflammation occurred in conjunction with far more severe renal vasculopathy, fibrosis, apoptotic cell death, and organ failure. These findings indicate that a single episode of acute renal ischemia has long-term and self-sustained adverse renal consequences in obesity-diabetes.

MATERIALS AND METHODS

Animal protocols. All experiments were conducted in conformity with the “Guiding Principles for Research Involving Animals and Human Beings.” The investigations were approved by the Institutional Animal Care and Use Committee of Indiana University School of Medicine. Lean controls and obese-diabetic male ZSF1 (ZS) rats (Jackson Laboratories, Bar Harbor, ME), 8–32 wk old, were fed Purina diet 5008, which consists of 27% protein, 27% animal fat, and 56% carbohydrate. Body weights were recorded and sera plus urine samples were collected at biweekly intervals. Sera were analyzed for glucose, creatinine, and urea and urine was analyzed for protein and creatinine on a Beckman CX4CE system (11). The rats were anesthetized with pentobarbital sodium (50 mg/kg ip) and placed on a homeothermic table, which maintained core body temperature at ~37°C. After adequate anesthesia was ensured, renal ischemia was induced by occlusion of both renal pedicles for 25 min with microa-
neurysm clamps, as described elsewhere (27). This procedure results in a relatively mild acute functional insult. Mean blood urea nitrogen levels were 27 ± 1 mg/dl in the lean control rats 24 h after ischemia (vs. 12 ± 0.5 mg/dl in sham-surgery controls). Sham surgery was an identical surgical procedure in which both kidneys were exposed but renal ischemia was not induced. For intravitral imaging (see below), a small flank incision was made to expose the left kidney. Systolic blood pressure was measured by tail cuff or femoral artery catheter before imaging.

**Intravitral multiphoton fluorescence microscopy.** Intravitral imaging was performed with a confocal/multiphoton microscope (model MRC-1024MP, Bio-Rad, Hercules, CA) equipped with a titanium-sapphire laser (Spectraphysics, Mountain View, CA). Imaging was performed at 12, 20, and 32 wk of age (2, 8, and 12 wk after renal ischemia or sham surgery). The rats were placed on the heated (37°C) microscope stage and covered with a temperature-controlled pad. General anesthesia was accomplished with pentobarbital sodium (50 mg/kg ip) or thiobarbital (80 mg/kg ip). The left kidney was surgically exposed, placed in a cell culture dish with a glass bottom (Warner Instruments, Hamden, CT), and bathed in warm 0.9% NaCl (14). Hoechst 33342 (250 μg in 0.5 ml of 0.9% NaCl; Molecular Probes, Eugene, OR) was injected intravenously immediately before imaging to identify nuclei and the focal plane. Renal microvascular flow was visualized using FITC-conjugated 100-kDa (large) dextran (400 μg in 0.5 ml of 0.9% NaCl; Molecular Probes), which was injected intravenously immediately before imaging. A smaller (20-kDa) Texas Red-conjugated dextran (2 mg in 0.5 ml of 0.9% NaCl injected intravenously) was used to assess microvascular permeability. Excitation wavelength (800 nm), laser output (~30%), and photomultiplier settings were chosen on the basis of prior studies (14), so that the fluorescence intensity of nuclei was ~50% of maximum across different animals observed at different times.

**Immunohistochemistry.** Tissue was fixed in 3.8% paraformaldehyde and preserved in 30% sucrose before 10-μm frozen sections were obtained. Sections were incubated with rabbit anti-rat LOX-1 (12) and mouse anti-rat ICAM-1 (28) followed by a Texas Red-conjugated donkey anti-rabbit IgG and FITC-conjugated donkey anti-mouse IgG (Jackson ImmunoResearch, West Grove, PA) and the nuclear dye 4,6-diamidino-2-phenylindole (Molecular Probes). Images were collected with a Zeiss LSM 510 confocal microscope and analyzed with Zeiss LSM and MetaMorph software (Universal Imaging, Downingtown, PA). Renal collagen was rendered visible with second harmonic imaging microscopy, which was performed directly on noncentrosymmetric collagen with the two-photon microscope (7), without exogenous fluorophores. Standard trichrome and chloroacacetate esterase (Leder’s) stains were also used (12) to confirm collagen and leukocyte infiltration, respectively.

**Microvascular flow, permeability, and density.** Renal capillary RBC velocity (RBCV) was measured to estimate renal capillary blood flow rates. MetaMorph software was used to determine the displacement of intracapillary RBCs on sequential images, with correction for microvascular angle. Vascular leak was quantified by analysis of initial intravitral images in 4 × 4 grids, with each grid scored for the presence or absence of small and large dextran extravasations, expressed as fractions of total grid segments (47). Renal capillary density was quantified as the fractional area in each section representing intravascular high-molecular-weight dextran in the initial images obtained after dextran injection.

**Image scoring.** All quantification was performed on coded images. Intravascular leukocytes were identified as nucleated cells within the vasculature in intravitral images and classified as free-flowing (not adherent to vessel wall) or adherent (adherent to vessel wall for >10 s) leukocytes. RBC aggregates were identified as shadows of stacked RBCs moving in unison and were recorded as present or absent in each quadrant of the coded images. Fluorescence corresponding to immunoreactive LOX-1 and ICAM-1, as well as fibrosis, capillary area, and fraction of abnormal tubules, was quantified using MetaMorph software. Fibrosis is expressed as the fraction of the tissue area imaged as collagen. Tubules with areas of denudation, shrunken cells, or intraluminal casts were classified as abnormal. Apoptotic cells were identified as those with condensed, fragmented nuclei and expressed as the fraction of total nuclei in the image.

**Statistics.** Values are means ± SE. ANOVA was used to determine whether differences among mean values reached statistical significance. Tukey’s test was used to correct for multiple comparisons. Correlations were determined using nonparametric (Spearman’s) correlation coefficients. The null hypothesis was rejected at P < 0.05.

**RESULTS**

**Metabolic and renal functional parameters.** ZS rats were randomly divided into four study groups: lean sham controls (LS, n = 4), in which the kidneys were surgically exposed; lean ischemic (LI) rats (n = 4), in which the kidneys were exposed and both renal pedicles were clamped for 25 min; obese-diabetic sham-surgery (OS) rats (n = 3), in which the kidneys were exposed; and obese-diabetic ischemic (OI) rats (n = 7), in which the kidneys were surgically exposed and clamped for 25 min. The initial values for serum creatinine were similar in LS and OS rats at 8 wk of age, the point of entry to the study (2 wk before surgery). However, even at this early age, obese-diabetic rats were significantly heavier than their lean littermates, and their blood glucose levels were higher than those of their lean littermates (Fig. 1). These differences persisted throughout the study. Mean serum creatinine progressively increased in the OI rats from 16 to 24 wk of age, well after the acute ischemic insult. In contrast, serum creatinine remained unchanged in the other three groups (Fig. 1).

![Fig. 1. Effect of renal ischemia on progression of diabetic nephropathy. Metabolic and renal parameters, as well as mean weights and systolic blood pressure, in lean sham (LS), lean ischemic (LI), obese-diabetic sham (OS), and obese-diabetic ischemic (OI) ZS rats are presented. Weights and serum glucose were comparable between OS and OI rats. Mean serum creatinine, urinary albumin/creatinine, and systolic blood pressure were significantly higher (P < 0.05) by 24 wk of age in OI rats. BUN, blood urea nitrogen. *P < 0.05 vs. LS. §P < 0.05 vs. OS.](http://ajprenal.physiology.org/content/297/10/AJP-RenalPhysiol/fig-1)
1). Urinary protein excretion was elevated in OS and OI rats but increased to significantly higher levels in OI rats. Proteinuria at 12 wk was highly correlated with fibrosis at study termination ($r = 0.98$). Systolic blood pressure was also higher in OS and OI rats and increased to higher levels in OI rats (Fig. 1). Two OI rats died within 2 days of periodic intravital imaging at 12 and 32 wk of age. This outcome was unique to the OI group: all the other rats recovered from imaging and finished the study successfully ($P < 0.05$). Accordingly, direct comparisons among the four groups were only made at those time points when the OI group numbered four rats or more. An additional three OI rats died during the course of the study, whereas all LI rats completed the study.

Effect of renal ischemia and time on microvascular blood velocity in the diabetic kidney. Renal capillary blood flow and leukocyte dynamics were visualized in living rats using intravital, multiphoton fluorescence microscopy (Fig. 2). Peritubular capillary plasma flow was determined from direct measurements of RBCV. The initial set of RBCV values was obtained in 12-wk-old rats, 2 wk after surgery; the second and third sets of measurements were collected in the same rats when they had reached 20 and 32 wk of age. The initial RBCV values, at 12 wk of age, were similar in LS and OS rats and were depressed in LI and OI rats: $211 \pm 58$ and $209 \pm 39$ mm/s in LI and OI rats, respectively. At 20 and 32 wk of age, mean RBCV was significantly lower in OS than LS rats. RBCV was more severely depressed in the LI group at 20 wk, when serum creatinine begins to increase. RBCV decreased further with time in LI rats.

Effect of renal ischemia on microvascular density. The impaired renal capillary blood velocity in obese-diabetic rats was accompanied by renal microvascular attenuation (51). Accordingly, collected intravital two-photon renal images were used to measure the effect of ischemia on the extent of the renal microvascular network (Figs. 2 and 3). Specific intravascular fluorescence was quantified as the number of intravascular pixels per image and then expressed as a fraction of the total number of pixels in the same image, or fractional intravascular fluorescence. Renal ischemia caused an early attenuation of the microvasculature 2 wk after ischemia in control lean and obese-diabetic rats that worsened with time. The percentage of total tissue area attributable to intravascular fluorescence in kidneys at 32 wk of age was $10.4 \pm 0.3\%$ in LS rats and $8.5 \pm 0.2\%$ in OI rats.

**Fig. 2.** Effect of renal ischemia on leukocyte-endothelial adhesion and microvasculature in diabetes. Representative intravital images of renal peritubular capillaries of LS (A) and LI (B), OS (C), and OI (D) rats 2 mo after sham surgery or ischemia are shown. Renal vascular space is delineated by a large FITC-conjugated dextran, which leaks in OI rats (arrowhead in D). Leukocyte-endothelial adhesion in real-time images is significantly increased in OS and OI rats (arrows). Vascular density is markedly attenuated in OI rats. Nuclei are labeled with Hoescht (blue). Quantification of WBC and RBC aggregation is shown in Fig. 5, and RBC velocity (peritubular capillary plasma flow), vascular leak, and microvascular rarefaction are shown in Fig. 3.

**Fig. 3.** Effect of renal ischemia on RBC velocity and microvascular function and density in diabetes. Quantification of RBC velocity (peritubular capillary plasma flow), microvascular leak, and rarefaction in intravital images is presented. *$P < 0.05$ vs. LS. §$P < 0.05$ vs. OS.
0.2% in OI rats (P < 0.01; Fig. 3). The significant pruning of the renal peritubular vasculature was associated with extensive renal capillary leak (see below).

Effect of renal ischemia on microvascular integrity (permeability) in diabetes. Representative two-photon intravital images of kidneys from LS, LI, OS, and OI rats are shown in Figs. 2 and 4, and the data are summarized in Fig. 3. In preparation for imaging, rats were injected intravenously with three fluorescent dyes: Hoechst 33342, a blue fluorescing dye to label nuclei; a 20-kDa (small) Texas Red-conjugated dextran, a red fluorescing dye; and a 100-kDa (large) FITC-labeled dextran, a green fluorescing dye to label intravascular spaces. Sequential images were obtained beginning 20 min after injection of Hoechst 33342 and 2 min after injection of dextrans. Specific fluorescence intensities for small and large dextrans in the interstitial space were analyzed with MetaMorph software. The fraction of grids in each image with evidence of interstitial leaked dextran is shown in Fig. 3. At 2 wk after ischemia, interstitial fluorescence, indicating leak of the smaller Texas Red-dextran, was indistinguishable from background in LS rats and slightly elevated in LI rats (0.019 ± 0.01 and 0.11 ± 0.05, respectively). The dye leak values in LS and LI rats remained relatively constant throughout the study. Initial and subsequent dye leakage was high in OS rats and even more abnormal in OI rats (0.24 ± 0.03 and 0.63 ± 0.06, respectively; Figs. 3 and 4). The larger-dextran leakage was similarly distributed, albeit at a lower rate. Leaked interstitial fluorescence of the larger FITC-dextran was indistinguishable from background in LS and LI rats and remained relatively constant throughout the study. Microvascular permeability to the large dextran was elevated in OS rats and more markedly increased in OI rats (0.13 ± 0.03 and 0.40 ± 0.06, respectively) 2 wk after ischemia. Hence, these data show that generalized renal capillary leakage in obesity-diabetes was further aggravated by ischemia.

Effect of renal ischemia on leukocyte dynamics in the diabetic kidney. Renal intravascular leukocytes were identified and quantified by intravital microscopy and confirmed in postmortem renal sections. In vivo, total, free-flowing, and adherent leukocytes were quantified in intravital images and expressed as numbers of intravascular leukocytes in ×60 microscopic fields. Initially, few intravascular renal leukocytes per field were seen in kidneys from LS, LI, and OS rats. Leukocyte number was much higher in OI rats (13.7 ± 1.5, P < 0.0001 vs. OS; Fig. 5). Subsequently, capillary leukocyte number increased further in OI rats and to a lesser extent in OS and LI rats (Fig. 5). Furthermore, those leukocytes that adhered to vascular endothelium were identified and counted in vivo. Initially, there were far more adherent renal leukocytes in OI rats than in OS (47 ± 5% vs. 3 ± 3%, P < 0.0001 vs. OI), LI (3 ± 3%), or LS (0 ± 0%, P < 0.0001 by ANOVA) rats. Subsequently, leukocyte number remained higher in the OI rats than in the LS and LI rats (Fig. 5). Neutrophils were counted in postmortem renal sections stained with Leder’s stain and identified by their typical multilobed nuclei. Neutrophil numbers were higher in OS than LS rats (17.7 ± 1.0 vs. 0.2 ± 0.2, P < 0.0001), and after ischemia, the number of renal neutrophils was higher in OI than LI rats (25.8 ± 3.0 vs. 1.2 ± 0.4, P < 0.01). Total intravascular leukocyte number was highly correlated with serum creatinine (r = 0.83).

Effect of renal ischemia on erythrocyte aggregation in the diabetic kidney. Renal capillary blood flow in obesity-diabetes was distorted by increased numbers of circulating RBC aggrega-
gates. The adherent RBCs formed free-flowing microclusters, which are easily identified by intravital microscopy. RBC aggregates were virtually nonexistent in LS and OS rats (0.06 ± 0.06 and 0.23 ± 0.07 microaggregates per ×60 microscopic field, respectively, P < 0.07). Initially, the effect of ischemia on the number of RBC microaggregates was striking in LI and OI rats (0.65 ± 0.16 and 1.09 ± 0.15, respectively, P < 0.05). RBC aggregation resolved in LI rats but, subsequently, increased further in OS and OI rats (Fig. 5).

Effect of renal ischemia on expression of the proinflammatory receptors ICAM-1 and LOX-1 in the diabetic kidney. The renal proinflammatory state in obesity-diabetes is characterized not only by invading inflammatory cells, but also by induction of adhesion receptor molecules that anchor and retain leukocytes (11, 28, 35, 45). Two of these critical recognition molecules, LOX-1 and ICAM-1, have restricted expression in lean rats but are strongly expressed in renal tubules of obese-diabetic rats (29). Accordingly, these two recognition molecules were sought and identified in postmortem renal sections obtained at 32 wk (Fig. 6). Immunoreactive ICAM-1 and LOX-1 were barely detected in LS rats but increased markedly (2.01 ± 0.32 and 1.58 ± 0.14 fold, respectively) in LI rats (P < 0.05). LOX-1 and ICAM-1 were also strongly expressed (1.94 ± 0.38 and 4.53 ± 1.63 fold, respectively) in OS rats, consistent with previous results (29). Ischemia further enhanced LOX-1 and ICAM-1 expression (7.92 ± 0.95 and 8.98 ± 3.02 fold, respectively) in OI rats (both P < 0.01). ICAM-1 and LOX-1 expression was correlated with serum creatinine, proteinuria, and fibrosis (via trichrome stain; Table 1).

Table 1. Correlation of ICAM-1 and LOX-1 expression with fibrosis, proteinuria, and creatinine

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<th>Fibrosis</th>
<th>Proteinuria</th>
<th>Creatinine</th>
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<tr>
<td>ICAM-1</td>
<td>0.86</td>
<td>0.82</td>
<td>0.41</td>
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<tr>
<td>LOX-1</td>
<td>0.90</td>
<td>0.75</td>
<td>0.67</td>
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Values are correlation coefficients. ICAM-1, intercellular adhesion molecule-1; LOX-1, oxidized LDL receptor-1.

Effect of renal ischemia on fibrosis in the diabetic kidney. Renal fibrosis is a decisive morbid outcome in nephropathy, and it was followed in vivo by second harmonic two-photon imaging microscopy of renal noncentrosymmetric collagen. In addition, the final extent of renal fibrosis was measured in postmortem renal sections stained with Masson’s trichrome. In vivo, renal fibrosis was first noticeable in sham-operated 20-wk-old OS rats and was more extensive in OI rats (24.5 ± 1.7 and 29.8 ± 1.4% of tissue area, respectively, P < 0.05). In contrast, early renal fibrosis in LS and LI rats was not detectable (13.5 ± 2.1 and 15.2 ± 1.6%), although, later, fibrosis increased slightly in LI rats (Fig. 7).

Effect of renal ischemia on cell death in the diabetic kidney. Apoptotic cell death has been proposed as a cause of diabetic nephropathy, particularly tubular atrophy and tubulointerstitial fibrosis (31, 46, 55). Tubular apoptosis has been demonstrated
in renal biopsies of patients with early and advanced diabetic nephropathy and correlated with subsequent loss of function, as well as LDL levels and duration of diabetes (55). Multiple abnormalities in the metabolic syndrome, including hyperglycemia, inflammation, reactive oxygen species, and dyslipidemia, result in apoptosis in cultured cells (1, 42, 50, 54). The loss of cells in the kidney via apoptosis may be a significant contributor to loss of function and interstitial fibrosis. Therefore, we quantified apoptosis in sham and obese-diabetic rat kidneys after renal ischemia or sham surgery. Renal apoptosis was markedly increased in OS rats, as indicated by intravital microscopy (Fig. 8). Renal cell death was increased further after ischemia and escalated progressively in OI rats with time. This sustained increase in the rate of renal cell death induced by ischemia was still evident 22 wk after the single episode of ischemia (Fig. 8).
Diabetic patients and those with CKD are predisposed to acute ischemic renal injury (20). Thus we tested the hypothesis that ischemia would exacerbate injury in the diabetic kidney. We investigated the effect of a single episode of renal ischemia on function, structural abnormalities, inflammation, microvascular dysfunction, and cell death in an animal model of obesity-diabetes. Lean and obese-diabetic 10-wk-old rats were subjected to sham surgery or bilateral renal ischemia and followed for 22 wk. Months after renal ischemia, renal function, as well as fibrosis, inflammation, and apoptosis, was worse in OI rats. Over the long term, serum creatinine levels remained unchanged in LS, LI, and OS rats. In contrast, after an initial recovery, serum creatinine increased progressively in OI rats, reaching levels consistent with advanced renal failure (13). This supports the hypothesis that AKI exacerbates chronic renal injury. Conversely, proteinuria was progressive after ischemia, consistent with impaired recovery from an acute insult in CKD. Urine protein excretion was elevated in OI rats compared with LS, LI, and OS littermates.

Three critical determinants of nephropathy, renal vasculopathy, inflammation, and fibrosis, were monitored by direct intravital multiphoton fluorescence microscopy in the four groups of rats. The goal was to examine the modifying role of ischemia on these key effectors of nephropathy. Progressive inflammation, fibrosis, and microvascular dysfunction, as well as apoptotic cell death, in the diabetic kidney were exacerbated by ischemia.

Nephropathy in obese-diabetic rats is characterized by protracted inflammation and subsequent fibrosis (11), the end-point consequence to metabolic abnormalities and regional vascular hypoperfusion (12, 29). Hence, we investigated the potential magnifying role of inflammation on the progression of nephropathy in postischemic obese-diabetic rats. Although all models have limitations, CKD is the hallmark of human diabetic nephropathy and, as the Animal Models of Diabetic Complications Consortium has pointed out, “the major deficiency in [prior] animal models of diabetic nephropathy is the absence of kidney failure” (5, 6, 34). Although the pathognomonic change in early diabetic kidney disease is glomerular basement membrane thickening, inflammation and apoptosis markers in urine (58) and serum (41) early in diabetes can predict declines in renal function in longitudinal studies. Tubulointerstitial inflammation is found in human diabetic nephropathy specimens (4).

We focused on ischemia-reperfusion renal injury, because it drastically depresses capillary renal blood flow in the postischemic period in obese-diabetic mice and, subsequently, reduces renal blood flow (44). Renal hemodynamic and inflammatory changes may interact and worsen renal injury. For example, angiotensin II, which is critical in diabetic nephropathy and arterial hypertension, stimulates the expression of cytokines and growth factors (38) and results in renal infiltration of inflammatory cells in experimental CKD (49). Inflammation can result in synthesis of angiotensin II (57). Systolic and diastolic blood pressure were decreased in patients treated with immunosuppression for rheumatoid arthritis or psoriasis, and systolic blood pressure was correlated with urine levels of inflammatory mediators (19). Furthermore, AKI has synergistic morbid effects on diabetic nephropathy, and, conversely, diabetic nephropathy enhances the risk for AKI (20). Accordingly, we tested whether the dynamic interaction of “acute-on-chronic” renal injury was fueled by renal inflammation. We reasoned that the proinflammatory role of renal hypoxia has been undeniably demonstrated in the ischemia-reperfusion model of renal injury (22, 27, 28). Moreover, renal hypoperfusion also complicates diabetic nephropathy (36), likely resulting in a proinflammatory state (16, 37). However, this earlier work does not fully address the self-sustained chronic nature of the postischemic proinflammatory state reported here. In fact, in otherwise normal rats, an acute episode of ischemic injury results in long-lasting vascular effects, including ran-
efaction of the peritubular capillary network many weeks after the ischemic episode (3). Our results, consistent with these data, clearly demonstrate that one early episode of ischemia-reperfusion has powerful and long-lasting effects in obese-diabetic rats. Indeed, the postischemic obese-diabetic rats demonstrated an enhanced and sustained renal proinflammatory state, which most likely accelerated apoptosis, a critical turning point after reperfusion injury (8, 25, 26), as well as the delay of renal function and structure.

Our novel model of acute-on-chronic renal failure reveals that accelerated persistent renal inflammation is a critical morbid element of progressive renal failure complicated by acute injury. We have termed this condition the “postischemic inflammatory syndrome of diabetic nephropathy.” The damaging inflammatory process goes on for months after the acute ischemic insult, and it seems to critically modify the renal outcome. The remarkable features of the long-lasting postischemic inflammatory syndrome have received little attention, although its existence was described in uninephrectomized rats subjected to ischemia-reperfusion consequential to renal autotransplantation (18). The autotransplanted rat model also had a sustained inflammatory response, and the main driving stimulus appeared to be ischemia and the loss of kidney mass (18). In contrast, our rat model has the metabolic syndrome, and this morbid entity was likely the driving self-sustaining force, although a significant role of progressive loss of renal mass could not be discounted (13). In either case, our data show that AKI exacerbates the sustained renal proinflammatory state, accelerates apoptosis and renal decay, and causes far more severe renal fibrosis and failure. This critical discovery validates, at least in obese-diabetic rats, the view that acute ischemia can result in chronic renal failure (2). In addition, from our findings, we conclude that anti-inflammatory renal rescue therapy, used successfully by us (11) and others (52), can be employed to limit the long-term damage inflicted by ischemia-reperfusion injury in diabetes (unpublished observations).

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