Chronic kidney disease induced in mice by reversible unilateral ureteral obstruction is dependent on genetic background

Tipu S. Puri,1 Mohammed I. Shakaib,1 Anthony Chang,2 Liby Mathew,1 Oladunni Olayinka,1 Andrew W. M. Minto,1 Menaka Sarav,1 Bradley K. Hack,1 and Richard J. Quigg1

1Section of Nephrology, Department of Medicine and 2Department of Pathology, University of Chicago, Chicago, Illinois

Submitted 7 July 2009; accepted in final form 19 January 2010


Chronic kidney disease (CKD) begins with renal injury; the progression thereafter depends upon a number of factors, including genetic background. Unilateral ureteral obstruction (UUO) is a well-described model of renal fibrosis and as such is considered a model of CKD. We used an improved reversible unilateral ureteral obstruction (rUUO) model in mice to study the strain dependence of development of CKD after obstruction-mediated injury.

C57BL/6 mice developed CKD after reversal of three or more days of ureteral obstruction as assessed by blood urea nitrogen (BUN) measurements (>40 mg/dl). In contrast, BALB/c mice were resistant to CKD with up to 10 days ureteral obstruction. During rUUO, C57BL/6 mice exhibited pronounced inflammatory and intrinsic proliferative cellular responses, disruption of renal architecture, and ultimately fibrosis. By comparison, BALB/c mice had more controlled and measured extrinsic and intrinsic responses to injury with a return to normal within several weeks after release of ureteral obstruction. Our findings provide a model that allows investigation of the genetic basis of events during recovery from injury that contribute to the development of CKD.

THE EPIDEMIOLOGY OF KIDNEY disease reveals striking differences in incidence and prevalence rates of chronic kidney disease (CKD) and end-stage kidney disease (ESKD) between racial groups, with highest rates seen in the African-American population. The fundamental pathophysiology underlying this variability from group to group is a question of great clinical importance that remains largely unexplained.

In human kidney diseases, there are often periods of injury/inflammation followed by repair processes, including those induced by therapeutic maneuvers. Repair after injury may result in regeneration of renal structures and recovery of function, or may result in replacement of renal structures by nonfunctional matrix. Predominance by the latter process of destructive fibrosis is widely considered to be responsible for the development and progression of CKD. It seems likely that both the injury phase and the repair phase would have an underlying genetic basis, given the considerable diversity in progression of renal diseases to ESKD (1, 23).

Unilateral ureteral obstruction (UUO) is a well-described model of renal fibrosis and as such is considered a model of CKD (13). Advantages of this model include that kidney injury and fibrosis occur over a time course of days to weeks and that it can be used in mice of any strain. Our primary goal in these studies was to determine whether there were differences in the development of CKD between strains of mice. We have used an improved method of reversible unilateral ureteral obstruction (rUUO) to study strain-dependent differences in both injury and repair phases and their functional consequences.

Our findings provide a model that allows investigation of the genetic basis of events during injury and recovery that contribute to the development of CKD.

MATERIALS AND METHODS

rUUO protocol. All aspects of the use of vertebrate animals in these studies were approved by the University of Chicago Institutional Animal Care and Use Committee (IACUC). Chevalier and colleagues (5, 6) have demonstrated that transient obstruction in neonatal rats leads to chronic renal insufficiency in adulthood as a consequence of impaired growth, glomerular sclerosis, tubular atrophy, chronic inflammation, and interstitial fibrosis. With older mice (>12 wk), increased periureteral fat raised concerns about the reliability of obstruction by the microvascular clips and problems with adhesions due to damage to the fat. Given these considerations, 6- to 8-wk-old adult mice were used for these studies. Mice underwent anesthesia with continuous inhaled isoflurane, and all techniques were performed under strict aseptic conditions. After a standard laparotomy, the bowel was gently displaced from the abdomen to one side and covered with sterile saline-soaked sterile gauze. The right ureter was isolated by blunt dissection and clamped (right ureteral obstruction; RUO) with a nontraumatic microvascular clip (5–15 g/mm², 7 mm S&T Vascular Clamp, Fine Science Tools, Foster City, CA). The bowel was then laid back in place. The muscle and fascia were closed with 4-0 nylon sutures, and the skin was then closed with sterile surgical wound clips. Prophylactic chlorhexidine ointment (150 µl) was applied to the abdominal wound.

In studies using increasing times of obstruction (Fig. 1), we noted a nearly linear inverse relationship between the time that the microvascular clip was left in one position on the ureter and the reversal rate (Supplementary Fig. 1, dotted line; all supplementary material for this article is available on the journal web site). This suggested that failure of obstruction to reverse might reflect progressive injury to the clamped segment of the ureter. We determined that changing the position of the clip by moving it distally every 2 days during the obstruction period greatly improved the rate of successful reversal of obstruction to >70% after a total of 6 days of obstruction compared with <20% if the clip was left in one position for 6 days (Supplementary Fig. 1). Based on this, the surgical procedure was repeated every 2 days with a microvascular clip placed immediately distal to the clipped site on the right ureter and then the proximal clip was removed. To maintain uninterrupted obstruction, the distal clip was placed on the ureter before the proximal clip was removed. Supplementary Fig. 2 graphically shows the procedure for a 6-day obstruction. After the desired total time of obstruction, the microvascular clip was removed (right ureteral obstruction release; RUOR). Placement or removal of the microvascular clip required <5 min, and total surgery time per animal from induc-
tion of anesthesia to close of laparotomy was 25–30 min. Surgeries could be comfortably performed on 10–12 animals during a typical 6- to 7-h operative session. Mice tolerated the multiple surgeries without difficulty as evidenced by a rapid return to normal activity (grooming, feeding, drinking, etc.) after recovery from anesthesia. There was no evidence for wound or systemic infection as a consequence of the protocol.

After 7 days of recovery, reversal of obstruction was confirmed by resolution of hydrenephrosis (Supplementary Fig. 3). As has also been noted by others (7, 26), the function of the normal contralateral kidney is sufficient to keep serum markers of kidney function at normal to near normal levels. Thus, after allowing a sufficient period of recovery for the previously obstructed kidney, the function of the normal contralateral kidney needed to be removed to allow evaluation of the functional consequences of obstruction-mediated injury. If contralateral kidney function was removed <1 wk after the release of the obstruction, the previously obstructed kidney had not recovered sufficient function and the majority of animals died (data not shown). Preterminal blood urea nitrogen (BUN) and serum potassium levels (S_k) were >200 mg/dl and >12 meq/l, respectively, suggesting that animals died of renal failure. After 1 wk, the previously obstructed kidney had sufficiently recovered to allow removal of contralateral kidney function without consequent death from renal failure. This was accomplished by a left nephrectomy (LNX) or more typically left ureteral obstruction (Luo) by ligation of the left ureter with a silk suture. No difference in function was observed with either Luo or LNX (Supplementary Fig. 4). Luo was typically used as it is a faster procedure (<5 min for Luo vs. 20–25 min for LNX), and it avoided a small but measurable increase in animal loss due to bleeding (5%).

With the exception of the study in Supplementary Fig. 4, Luo at 7 days after relief of obstruction was used in all studies where contralateral kidney function was removed. Serum for BUN and potassium measurements was obtained by retroorbital bleeding. Urine was collected by aspiration from the bladder under anesthesia before tissue harvest.

Tissue harvest. Kidneys were surgically removed under anesthesia and sectioned after removal of the renal capsule and extrarenal structures. Each kidney was sectioned along the transverse axis into three pieces: upper (3- to 4-mm length), middle (2- to 3-mm length), lower (3- to 4-mm length). The upper and lower pieces contained the upper and lower poles of the kidney. The middle piece was cut in half again along the transverse axis, and the resulting pieces (1- to 1.5-mm length/thickness each) were processed for light microscopy (as described below) or frozen in a block using Histoprep (Fisher Scientific, Fair Lawn, NJ) and stored at −80°C. The upper and lower pieces were cut in half along the coronal axis, and the resulting four equal-size pieces were flash frozen in liquid nitrogen and then stored at −80°C for RNA and protein studies. All pieces contained both cortex and medulla.

Light microscopy. Pieces of renal tissue no more than 1–1.5 mm thick were fixed in 4% paraformaldehyde in 1× PBS (pH 7.4) for 24 h at 4°C. Following fixation, tissue samples were routinely processed and embedded in paraffin wax (TissuePrep II, Fisher Scientific). Tissue sections, of 5-μm thickness, were cut onto SuperfrostPlus slides (Fisher Scientific), dewaxed, and hydrated through a descending series of alcohols. Tissue sections were routinely stained by Mayer’s hematoxylin and Putt’s eosin (H&E), Masson’s trichrome, and Sirius red.

Immunohistochemistry. Immunohistochemical staining was conducted using a Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA) with some modifications. Briefly, 5-μm sections were deparaffinized, and endogenous peroxidase activity was quenched by incubation in hydrogen peroxide. Epitope retrieval was carried out on tissue sections by microwave treatment (10 min on high setting) followed by blocking for endogenous biotin and nonspecific background staining. Blocking was followed by incubation with the affinity-purified antibody [rabbit anti-mouse F4/80 antibody (sc-25830; Santa Cruz, CA) or rabbit anti-mouse S100A4 (ab27957; Abcam, Cambridge, MA)] or control IgG. Sections were then washed in Tween 20-buffered saline (TBS) and incubated with biotinylated secondary antibody. After further washes with TBS, the sections were incubated with an avidin-biotinylated horseradish peroxidase complex. Finally, the sections were rewashed and developed by diaminobenzidine tetrahydrochloride (Sigma, St. Louis, MO). Staining of kidney tissue from untreated kidney or staining with nonimmune serum or rabbit IgG was performed for controls. Staining with control reagents was negative in all cases. All procedures were carried out at room temperature unless otherwise noted.

Immunofluorescence staining. For immunofluorescence staining, 5-μm frozen sections were air dried at room temperature for 30 min. Sections were fixed with ice-cold acetone for 10 min, then washed with 1× PBS. Fixed sections were incubated for 1 h at room temperature in a humidified chamber with the cocktail of primary antibodies [rabbit anti-mouse S100A4 (ab27957; Abcam, Cambridge, MA) and rabbit anti-mouse laminin (T40269R; Meridian Life Science, Cincinnati, OH)] preconjugated to different fluorescent labels and diluted 1:100 in 1× PBS. Sections were washed three times (5 min wash) with 1× PBS. Stained sections were stored at 4°C in the dark.

Flow cytometry. Single-cell suspensions of isolated renal cells were prepared from whole kidneys at the indicated time using modification of previously described methods (29). Whole kidneys were minced into small pieces (<1 mm) in ice-cold 1× HBSS media. Tissue fragments were centrifuged at 250 g × 5 min. Pelleted fragments were resuspended in HBSS with 1 mg/ml collagenase (type IA, catalog no. C-9891, Sigma) and 0.1 mg/ml deoxyribonuclease (DNase, type I, catalog no. DN25, Sigma) and then incubated at 37°C for 25 min with gentle shaking every 5 min to disaggregate renal cells. The suspension was again centrifuged at 250 g × 5 min, and the supernatant was discarded. The pellet was resuspended in 2 ml of RBC lysing reagent (150 mM NH4Cl, 10 mM KHCO3, 0.5 M EDTA, pH 8) and incubated for 5 min at room temperature. The suspension was again centrifuged at 250 g × 5 min, and the supernatant was discarded. The pellet was resuspended in 1–2 ml of ice-cold 1× PBS. The suspension was passed through a nylon mesh (BD-Falcon 40-μm self-strainer) to remove undigested fragments. Cells suspensions were stained with 0.4% trypan blue and counted using a hemocytometer. In our protocol,
Table 1. Primers for quantitative real-time PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Accession No.</th>
<th>Sense Primer (5’-3’)</th>
<th>Antisense Primer (5’-3’)</th>
<th>Product, bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen IIIa</td>
<td>NM_009930</td>
<td>aagccacagtgttctcctg</td>
<td>gacctctgtgetcagttacg</td>
<td>149</td>
</tr>
<tr>
<td>S100A4</td>
<td>NM_011311</td>
<td>cagccasaggtgtgcaacag</td>
<td>cttttccccaggaagtctgg</td>
<td>88</td>
</tr>
</tbody>
</table>

Typically $1 \times 10^7$ live cells/ml were isolated from one whole kidney. One hundred microliters of single-cell suspensions were incubated with 1–1.5 μL (based on the manufacturer’s recommendations) of the indicated fluorescent labeled antibody, on ice, for 25 min in the dark (F4/80 antibody, catalog no. MCA497APC, Serotec, Raleigh, NC). Labeled cell suspensions were washed a total of two times with ice-cold 1× PBS (4 ml/wash) followed by centrifugation at 250 g × 5 min to pellet cells between washes. As a final step, cells were resuspended in ice-cold PBS and then analyzed on a flow cytometer (FACScanto, Becton-Dickinson, Franklin Lakes, NJ). Data were analyzed using FlowJo v 8.7 software.

Quantitative real-time PCR. Total RNA was extracted from frozen kidneys using TRIZol Reagent (Invitrogen, Carlsbad, CA). Five micrograms of RNA from each sample were reverse transcribed according to the manufacturer’s protocol (Superscript III, Invitrogen). Quantitative PCR was performed utilizing the ABI 7900 HT Fast Real Time PCR System and SDS 2.3 software (Applied Biosystems, Foster City, CA). Gene-specific primers are provided in Table 1. The reaction product was quantified by monitoring the fluorescence levels of the intercalating SYBR green dye. A sample of 5 μL of 1:10 diluted cDNA was analyzed along with the control cDNA standard. Tubes contained 10 μL of SYBR Green dye mix (QuantiTect SYBR Green PCR kit, catalog no. 204145, Qiagen, Valencia, CA), 250 nM each forward and reverse primers, 5 μL of template cDNA, and water to bring all final reaction volumes to 20 μL. Reactions were monitored by confirming single reaction products by agarose gel electrophoresis, and single peaks of melting temperature curves were determined at the end of the reaction.

Statistical analyses. All data were analyzed with Minitab 15 (College Park, MD) and initially evaluated by Anderson-Darling normality testing. For numeric data collected over time, values from C57BL/6 and BALB/c mice subjected to sham RUO at each time period ($n = 4 – 8$) were used to normalize experimental data. Univariate ANOVA was then used to compare experimental data between the two strains; when significantly different, follow-up comparisons at individual time points post-RUOR were made using Tukey’s tests. When two groups of parametric data were compared, a two-sample $t$-test was used.

RESULTS

Irreversible loss of kidney function with increasing duration of obstruction. We investigated the development of irreversible loss of kidney function after increasing time of obstruction followed by the release of the obstruction. In normal C57BL/6 mice, renal function was dependent upon the time of obstruction, such that with 1–2 days of obstruction, there was full function of the obstructed kidney following removal of contralateral kidney function (Fig. 1). With ureteral obstructions of 3 or more days, there was obstruction-time dependent renal failure (Fig. 1). Immediately following the removal of contralateral (left) kidney function, there was a considerable but transient rise in BUN, which then fell and stabilized at a level elevated from baseline. The transient rise and then fall in BUN likely reflects the expected shift in blood flow to the previously obstructed (right) kidney followed by hyperfiltration (28). Notably, compared with the contralateral (left) kidney during the initial reversible obstruction, the previously obstructed (right) kidney is not able to bring BUN levels down to previous baseline levels, reflecting loss of function in this kidney. BUN levels remained stable at this ”new baseline” for times up to 6 mo (Fig. 1). Therefore, this model successfully and reliably produced CKD in mice.

Strain differences in susceptibility to rUUO. In our initial work to develop the rUUO model, we noted that outbred CD-1 mice had considerable variability in development of CKD (as assessed by BUN values reflecting the function of the previously obstructed kidney; data not shown), while inbred C57BL/6 mice were much more consistent in their responses. This suggested there was a genetic component to the injury and repair responses of the rUUO model. Therefore, in the next prospective experiments, we compared development of CKD in C57BL/6 mice to mice of 129 and BALB/c strains following reversal of a 6-day obstruction. In these studies, 129 mice exhibited irreversible loss of function comparable to C57BL/6 mice (data not shown), while the BALB/c strain was completely resistant to loss of function (Fig. 2). To further examine the BALB/c response to injury, we increased the durations of ureteral obstruction. Yet, even after 10 days of obstruction, the BALB/c strain remained nearly completely resistant to renal functional impairment, with final BUN levels of 32.8 ± 2.0 compared with baseline levels of 27.3 ± 0.9 mg/dl ($n = 5$).

In subsequent studies, it is important to note that in the rUUO protocol the function of the contralateral unmanipulated kidney was removed 7 days after RUOR. Therefore, for time points up to and including day 7 post-RUOR, the contralateral unmanipulated kidney was in place and functional.
Histological features in rUUO. In the next set of studies, we examined histological features of the 6-day rUUO model and compared those in C57BL/6 and BALB/c mice at various times up to 28 days following RUOR. Representative histopathology demonstrated by H&E staining is shown in Fig. 3A. The day 0 post-RUOR time point reflects events occurring after 6 days of UUO before RUOR. Scores reflecting global interstitial inflammation based on H&E staining were accumulated by a renal pathologist blinded to origin of slides (strain and rUUO time point). Inflammation scores after 6 days of obstruction and following RUOR for each strain are shown in Fig. 3B.

As is known to occur, there was considerable tubular injury after 6 days of UUO, as manifested by tubular dilatation and epithelial cell flattening (and loss of brush border in the proximal segments) (Fig. 3A, day 0). In addition, interstitial inflammation was readily apparent around the tubules at day 0. At this time point before RUOR, inflammation was somewhat more prominent in kidneys from the C57BL/6 strain compared with those from the BALB/c strain. This observation was supported by inflammation scores at day 0 (Fig. 3B). Further strain-dependent differences in the progression of inflammation and alterations in tubular architecture were clearly observed post-RUOR. From 0 to 7 days post-RUOR, the C57BL/6 strain developed further inflammation and tubular atrophy and interstitial fibrosis (as evidenced by decreased tubular lumen sizes and loss of back-to-back tubular organization) (Fig. 3A, left column). At 7 days post-RUOR, the C57BL/6 strain had remarkable cellular infiltration and proliferation, particularly in the outer cortex. In kidneys from BALB/c mice, a modest increase in inflammation was also observed from 0 to 2.5 days post-RUOR (Fig. 3A, right column, and B). By contrast, at 7 days post-RUOR, resolving inflammation, retention of back-to-back tubular organization, and a gradual return toward normal histology was apparent in the BALB/c strain. Slight histological differences remained between strains at 28 days post-RUOR. C57BL/6 kidneys had a persistence of mild interstitial inflammation associated with patchy areas of interstitial fibrosis around atrophic tubules, whereas BALB/c kidneys had a normal histological appearance. Assessment of interstitial fibrosis by histochemical staining supported these observations (see Fig. 5).

Macrophage/monocyte infiltration in rUUO. The pathophysiology of renal injury in the UUO model itself remains enigmatic, despite a concentrated effort by many investigators (3, 13, 16). Given the significant cellular infiltration/proliferation, we examined markers of extrinsic cellular infiltration. Flow cytometry was utilized, as others have during irreversible UUO (29). To more specifically characterize the significant monocyte/macrophage infiltration, F4/80 (29) was elevated numbers of F4/80⁺ cells in C57BL/6 mice. By contrast, F4/80⁺ cells in BALB/c mice declined to percentages similar to those seen in sham control mice of either strain at 7 days post-RUOR. In addition to their increased proportions in cellular suspensions from C57BL/6 kidneys, there were increased numbers of F4/80⁺ cells by immunohistochemistry (Fig. 4B shows anti-F4/80 staining 7 days post-RUOR).

Assessment of fibrosis after rUUO. To quantify chronic tubulointerstitial pathological changes, scores reflecting global fibrosis were accumulated by a renal pathologist blinded to the origin of slides (strain and rUUO time point). Renal fibrosis scores reflected tubular atrophy and increased interstitial matrix, as visualized with Sirius red (Fig. 5A) and Masson’s trichrome-stained (not shown) sections with a range from 0 to 6. As shown in Fig. 5A, the mean fibrosis score at 28 days post-RUOR in C57BL/6 mice was nearly threefold higher than in BALB/c mice.

Collagen III is a major component of deposited interstitial matrix that accumulates with development and progression of CKD. Collagen III α1 mRNA levels assessed by qPCR showed dramatic changes during obstruction (not shown) and after release of obstruction in both C57BL/6 and BALB/c mice (Fig. 5B). Consistent with the greater degree of fibrosis, C57BL/6 mice had greater collagen III (α1) mRNA levels following release of UUO.

Markers of epithelial-to-mesenchymal transition in rUUO. Epithelial-to-mesenchymal transition (EMT) with the resulting generation of tubulointerstitial myofibroblasts is believed relevant to the development of renal fibrosis after injury. We examined the mRNA levels of S100A4, a potential marker of epithelial cell transformation to myofibroblasts, to investigate the underlying repair processes in the rUUO model. Figure 6A shows the change in mRNA levels during recovery from obstruction compared with mRNA levels from sham-operated control mice. After 6 days of obstruction (0d RUOR time point), elevated levels of S100A4 mRNA compared with sham were observed in both strains, with levels in C57BL/6 mice double those in BALB/c. S100A4 mRNA levels continued to rise through 2.5 days post-RUOR in C57BL/6 mice and thereafter showed a modest decline. A similar time course was observed post-RUOR in BALB/c mice however, S100A4 levels were consistently lower throughout and approached sham control levels by 14 days post-RUOR.

Figure 6B shows immunohistochemical staining of kidney sections at 2.5 days post-RUOR with an anti-S100A4 antibody. While S100A4 was expressed in the kidneys of both strains, it was interesting that in C57BL/6 mice it was identified between (beneath) tubules (Fig. 6B, arrows), while in BALB/c mice, it was restricted to expression in tubular cells that remained in place on the tubular basement membrane (Fig. 6B, asterisk). At later time points, these cells were shed, rather than appearing in interstitial spaces as apparent in C57BL/6 mice. To further investigate this, we performed dual immunofluorescent-labeled antibody staining of kidney sections at 2.5 days post-RUOR with an anti-laminin antibody to outline the tubular basement membrane (TBM) along with anti-S100A4. Immunofluorescence staining for S100A4 and laminin in Fig. 6C clearly demonstrated morphology consistent with staining of tubular epithelial cells in both strains. In sections from C57BL/6 mice, anti-laminin staining was consistent with a loss of integrity of the TBM, and S100A4⁺ cells were observed to be traversing the TBM into the tubulointerstitial spaces (Fig. 6C, top). In
Fig. 3. Hematoxylin and eosin (H&E) staining at various times following release of ureteral obstruction. A: representative H&E-stained sections from C57BL/6 and BALB/c at various times following release of 6 days of RUO (post-RUOR; ×20 magnification). In C57BL/6 mice, inflammatory cells persisted through 7 days post-RUOR and were accompanied by significant and lasting disruption of tubular architecture. In BALB/c mice, inflammation developed rapidly and then resolved. Tubular architecture was better preserved throughout with a return to near-normal structure by day 28 post-RUOR. B: average inflammation score at 0, 2.5, 7, and 28 days post-RUOR. H&E-stained sections from C57BL/6 and BALB/c mice were scored (0–3) for severity of global interstitial inflammation by a renal pathologist blinded to their origin (strain and rUUO time point). A score of 0 was used for absence of interstitial inflammation. Scores of 1, 2, or 3 reflected the presence of inflammation involving 1–25, 26–50, or >50% of the interstitium, respectively. *P < 0.05 vs. BALB/c (Kruskal-Wallis test).
contrast, anti-laminin staining in sections from BALB/c mice demonstrated preservation of TBM integrity, and S100A4+ cells were confined to the intratubular space (Fig. 6C, bottom).

DISCUSSION

The ureteral obstruction model has become widely used to study renal interstitial fibrosis, a hallmark of development and progression of CKD (13, 22). Chevalier and colleagues (4) recently reviewed the model, including an important discussion of technical aspects. Specific considerations included 1) the importance of rodent species or strain; 2) the age of the animal; 3) the use of sham-operated animals for comparison; and 4) the advantage provided by surgical models in allowing variations in obstruction parameters and further provided by reversible...
models allowing studies of events during recovery. These important considerations have been addressed in the present study. In particular, the importance of rodent strain is clearly demonstrated by our results. With regard to the surgical procedure, typically in the UUO model obstruction is achieved by irreversible ligation of the ureter. Kidney injury and fibrosis are assessed by histology or other ex vivo methods with the background of ongoing injury from obstruction. Reversible ureteral obstruction offers several key advantages for studying development and progression of CKD, including 1) the ability to reliably assess functional consequences of kidney injury and repair using serum or urine markers; 2) the ability to study renal pathophysiology during repair after injury; and 3) the potential for translational and long-term studies in living animals with CKD.

Reversible approaches have had more limited use in rodent models, where injury to the ureter appears to be an important limiting factor. The approach has been most used in the rat, where complicated and delicate surgical methods have been used to either protect the ureter from injury or reestablish patency despite injury, thereby allowing reversal of obstruction (2, 5, 6, 10, 17, 19, 21). In the mouse, there are just a few published reports of rUUO. In one study, transient ureteral obstruction of only 24-h duration by an microaneurysm clip was used to demonstrate subsequent protection from ischemiareperfusion-induced injury (20). In another study of induction and reversal of partial ureteral obstruction in neonatal mice, stainless steel wires of varying diameters were used to protect the ureter from injury, but functional data were not reported (27). Two more recent reports describe reversal of 7–14 days of complete ureteral obstruction in adult mice (7, 26). Cochrane and colleagues (7) used microvascular clips to facilitate reversal of obstruction followed by bilateral cannulation of the ureters. This approach allowed separate functional assessment of postobstructed and contralateral control kidneys; however, it is less amenable to longer term serial assessment of renal function, and a significant number of animals were reported lost due to shredding of the ureter during the delicate cannulation procedure. Tapmeier and colleagues (26) reestablished ureteral patency by reimplantation of the ureter into the bladder and excision of the suture-ligated segment of ureter followed by contralateral nephrectomy and assessment of the postobstructed kidney function using BUN measurements. This approach allowed a longitudinal study of renal function, but reimplantation of the ureter requires an experienced animal surgeon and delicate microsurgical procedures performed while operating with a binocular microscope.

Our approach to reversible ureteral obstruction in adult mice focused on minimizing injury to the ureter, thereby allowing an independent return of patency after relief of complete obstruction rather than reestablishing patency after injury. The multiple surgeries required by our protocol are extremely well tolerated by the mice with no evidence of systemic or wound infections and a rapid return to normal feeding and grooming behaviors after each surgery. Our method combines attractive features of existing approaches, including 1) the use of microvascular clips to facilitate obstruction and removal of obstruction; and 2) longitudinal assessment of renal function in the postobstructed kidney. Furthermore, it offers the advantages of utilizing simple surgical techniques that can be performed by laboratory personnel with basic technical skills and obviates the need for an experienced animal surgeon to perform delicate microsurgical procedures. Therefore, we believe this is an improved and relatively simple approach to achieve rUUO in adult mice.
Using our rUUO model, we have identified C57BL/6 mice to be susceptible and BALB/c mice to be resistant to development of CKD after obstruction-mediated injury. Here, it is important to emphasize that all mice were subjected to the exact same protocol with the only variables being 1) the omission of actual clip placement in sham animals and 2) the genetic strain of mice used. The degree of hydronephrosis was assessed by visual inspection on days 2, 4, and 6 of the surgical protocol during obstruction (Supplementary Fig. 1).

No differences in the degree of hydronephrosis were observed between strains based on these repeated assessments of all mice reported in this study. Published reports of differences in susceptibility or resistance between inbred mouse strains in models of kidney disease are limited. Strain dependence of susceptibility or resistance to development of glomerulosclerosis has been reported in mouse models using genetic mutations to reduce nephron number and the nephrectomy model (9, 15, 31). Strain dependence has also been reported in an experimental model of human focal glomerular sclerosis using adriamycin-induced nephropathy. In this model, BALB/c mice were susceptible to development of chronic proteinuric renal disease after adriamycin injection while C57BL/6 mice were found to be resistant (12, 30).

As expected, major histological findings in our studies were predominantly in the tubulointerstitial compartment with no significant findings in the glomeruli in mice of either strain. Consistent with this, we found no significant change in proteinuria throughout the rUUO protocol between experimental and sham animals or between strains (Supplementary Fig. 5). To our knowledge, ours is the first report of a difference in proteinuria in mice of either strain.

Histopathologically, there was a strong correlation between the degree of hydronephrosis and renal functional loss at the latest time points post-RUOR. Consistent with this was the considerably upregulated expression of collagen III (α1) mRNA in C57BL/6 mice. Epithelial cell injury, as a consequence of obstruction or otherwise, results in induction of EMT and local fibroblast generation (11, 18). Many studies have highlighted the potential role of EMT in the development of fibrosis after kidney injury (8, 11, 25). We have demonstrated intriguing differences between strains in mRNA expression and immunohistochemistry of S100A4, a potential marker of EMT, during recovery from obstruction. These findings suggest that differential induction of EMT and/or associated downstream events might have an important role in susceptibility or resistance to development of fibrosis and CKD and warrant further investigation. Based on our data, the mechanism by which myofibroblasts gain access to the tubulointerstitial space, including the identity and activity of specific matrix-degrading enzymes, would be a particular area of focus.

In summary, we have identified inbred strains of mice that are either susceptible (C57BL/6) or resistant (BALB/c) to development of CKD after rUUO. Our impression is that the extrinsic and intrinsic responses to injury are more controlled and measured in the BALB/c mice with rUUO. Either contributing to this or as a consequence, renal architecture is better maintained throughout injury and recovery from injury. In contrast, the response to injury in C57BL/6 mice is more pronounced and prolonged with rUUO. Confirmation and further investigation of these observations are necessary and will be the focus of future work with this model. Strain-dependent differences in inflammatory and tubular cell responses during the injury and recovery/repair phases provide intriguing potential targets for further studies of susceptibility or resistance to the development of CKD.

ACKNOWLEDGMENTS

We thank Ryan Duggan for his expert technical assistance with the flow cytometry studies. We thank Brian Mack for work during the early development of the rUUO surgical protocol.

GRANTS

This work was supported by National Institutes of Health Grants R01DK041873, R01DK055357, T32DK007510, and T32HL07381, and a Young Investigator Award from the National Kidney Foundation of Illinois and an Early Career Award from the Howard Hughes Medical Institute to T. S. Puri.

DISCLOSURES

No conflicts of interest are declared by the authors.

REFERENCES


