The Fate of Nephrons in Congenital Obstructive Nephropathy: Adult Recovery is Limited by Nephron Number Despite Early Release of Obstruction

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**Purpose:** Urinary tract obstruction and reduced nephron number often occur together as a result of maldevelopment of the kidneys and the urinary tract. We determined the role of nephron number on adaptation of the remaining nephrons of mice subjected to neonatal partial unilateral ureteral obstruction followed through adulthood.

**Materials and Methods:** Wild-type and Os/+ mice (the latter with 50% fewer nephrons) underwent sham operation or partial unilateral ureteral obstruction in the first 2 days of life. Additional mice underwent release of unilateral ureteral obstruction at 7 days. All kidneys were harvested at 3 weeks (weaning) or 6 weeks (adulthood). Glomerular number and area, glomerulotubular junction integrity, proximal tubular volume fraction and interstitial fibrosis were measured by histomorphometry.

**Results:** In the obstructed kidney unilateral ureteral obstruction caused additional nephron loss in Os/+ but not in wild-type mice. Glomerular growth from 3 to 6 weeks was impaired by ipsilateral obstruction and not preserved by release in wild-type or Os/+ mice. Proximal tubular growth was impaired and interstitial collagen was increased by ipsilateral obstruction in all mice. These conditions were attenuated by release of unilateral ureteral obstruction in wild-type mice but were not restored in Os/+ mice. Unilateral ureteral obstruction increased interstitial collagen in the contralateral kidney while release of obstruction enhanced tubular growth and reduced interstitial collagen.

**Conclusions:** Unilateral ureteral obstruction in early postnatal development impairs adaptation to reduced nephron number and induces additional nephron loss despite release of obstruction. Premature and low birth weight infants with congenital obstructive nephropathy are likely at increased risk for progression of chronic kidney disease.

**Key Words:** ureteral obstruction; renal insufficiency, chronic; growth and development; disease progression; nephrons

Most pediatric CKD results from congenital anomalies of the kidneys and urinary tract. It is now recognized that the majority of all patients in whom renal failure develops as a result of congenital obstructive nephropathy are older than 18 years. It was demonstrated that a reduction in nephron number accelerates the progression of renal disease by provoking a maladaptive response in the remaining nephrons.
as characterized by glomerular hypertrophy, hyperfiltration and eventual sclerosis. Since then, the cellular and molecular basis for progression of CKD has undergone intense investigation with emphasis on renal interstitial cellular infiltration and collagen matrix deposition. Complete UUO, the animal model most widely used to elucidate the underlying mechanisms, is characterized by widespread death of proximal tubular cells and formation of atubular glomeruli within 1 to 2 weeks.

We developed a technique to create variable partial, reversible ureteral obstruction in the mouse within the first 2 days of life, at which time nephrogenesis is incomplete. In this model renal injury evolves from birth through to adulthood and release of obstruction allows for recovery and remodeling of the renal parenchyma.

Frequently associated with congenital renal anomalies, reduced nephron number at birth is an independent risk factor for adult CKD. We wished to determine the role of nephron number on the adaptation of remaining nephrons in mice subjected to neonatal partial UUO that were followed through adulthood. In additional mice obstruction was released to examine the process of recovery and remodeling. Histomorphometry and immunohistochemical techniques were used to measure the effects of these variables on glomerular, tubular and interstitial development, and response to injury. Results revealed that a congenital reduction in nephron number accelerates obstructive renal injury and impairs recovery when assessed in adults, despite early release of obstruction.

**MATERIALS AND METHODS**

**Experimental Animals and Surgical Procedures**

A total of 132 Os+/+ mutant mice with reduced nephron number and their WT litter mate controls were derived from B6.ROP/Le +/- and C57BL/6 breeding pairs. Sample sizes were based on prior studies using this model. Pups were weaned at age 21 days. The protocol was approved by the University of Virginia institutional animal care and use committee. All surgical procedures were performed using sterile technique and anesthesia with isoflurane and oxygen. Neonates were subjected to 0.2 mm diameter partial UUO or sham operation within the first 2 days of life and underwent sham operation or release of obstruction allows for recovery and remodeling of the renal parenchyma.

A total of 15 WT and 20 Os+/+ male and female mice were studied at age 21 days. However, only 40 WT and 33 Os+/+ males were studied at age 42 days because the fraction of glomeruli with tall parietal epithelial cells (Lotus lectin positive glomeruli) is greater in males than females after age 21 days. In all mice that underwent partial UUO patency of the ureter was documented by passage in the bladder of India ink injected in the renal pelvis at kidney harvest. Additional nonoperated mice were studied at birth (4 WT and 4 Os+/+) and at age 9 days (12 WT and 4 Os+/+).

**Tissue Fixation and Embedment**

Animals were anesthetized with pentobarbital sodium phenytoin sodium solution (Euthasol®). Kidneys were perfusion fixed or immersed in phosphate buffered formalin solution, embedded in paraffin and sectioned on a RM2155 microtome (Leica, Nussloch, Germany). For plastic embedment kidneys were perfused with Hank’s Balanced Salt Solution followed by 2.5% glutaraldehyde in Hank’s Balanced Salt Solution. They were cut into 50 μm sections with a vibrating microtome, postfixed in osmium tetroxide, infiltrated with Poly/Bed 812 resin and embedded on microscope slides. Semithin sections (0.1 to 0.2 μm) were cut on a Sorvall® MT-2B ultramicrotome. All sections were examined with DMLS light microscopes (Leica) equipped with a QColor3™ digital camera.

**Staining**

Morphological features were examined in paraffin sections stained with PAS/hematoxylin. Collagen was stained with picrosirius red. Lotus tetragonolobus agglutinin staining was used to identify proximal tubules and

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**Figure 1.** Experimental design. Three groups of mice were studied. In each group C57BL/6 WT mice were compared to Os/+ mice, latter with 50% congenital reduction in nephron number. Early and late recovery was determined at ages 21 and 42 days (d), respectively. A, normal renal development. B, obstructive injury. C, recovery from obstructive injury.
tall epithelial cells in the Bowman capsule, which is characteristic of mature murine glomerulotubular junctions. Plastic sections were stained with alkaline toluidine blue solution.

**Morphometry**

Nephron number was determined by counting glomeruli in PAS stained median sagittal sections, a technique that was validated by the dissector technique. ImagePro Plus 5.1 or 7.0 image analysis software (MediaCybernetics®) was used to determine glomerular area by sampling the entire cortical thickness as well as the volume fraction of collagen and proximal tubules, termed Vc/PT. Proximal tubular maturation in the mouse is characterized by the acquisition of avid binding by Lotus tetragonolobus lectin in the first 3 weeks of life. Conversely UUO leads to the progressive loss of Lotus lectin binding, which is a reflection of epithelial injury and cell death that begins at the glomerulotubular junction and extends down the proximal tubule with continued obstruction. Therefore, the fraction of total glomeruli in a sagittal section that contains any Lotus lectin staining along the Bowman capsule or the glomerulotubular junction index represents the fraction of mature nephrons with functional integrity (a combined measure of maturation and injury). The proximal tubular volume fraction is calculated as the ratio of Lotus lectin staining tubules to total cortical parenchyma, which represents a measure of proximal tubular mass.

Tissue collagen was measured in 20 microscopic fields of picrosirius red stained sections, alternating between cortical and medullary zones. Parenchymal area was determined using digital images of median sagittal sections of kidneys scanned with a Super Coolscan 4000 slide scanner (Nikon, Tokyo, Japan). All morphometric measurements were made using tissue labeled with unique identifier numbers.

**Statistical Analysis**

Comparisons between WT and Os/+ strains for individual parameters were made using the Student t-test for normally distributed data and the Mann-Whitney rank sum test for data not normally distributed. Comparisons among the 3 treatment groups (sham operated, UUO and UUO release) for each strain (WT or Os/+), age (21 or 42 days) and kidney (obstructed or contralateral) were made using 1-way ANOVA. For data that passed the equal variance test ANOVA was followed by Holm-Sidak pairwise multiple comparison. For data that did not pass the equal variance test Kruskal-Wallis ANOVA was performed, followed by Dunn pairwise multiple comparison. Comparisons of the overall effects of age, strain and UUO on each parameter were made using 2-way ANOVA. All statistical analyses were performed with SigmaStat®. Statistical significance was considered at p < 0.05.

**RESULTS**

Os/+ and Wild-Type Kidney Glomeruli

Os/+ mouse kidneys had fewer, larger glomeruli than WT mouse kidneys. Compared to the C57BL/6 WT kidney glomeruli appeared larger and more widely dispersed among larger tubules in the Os/+ kidney at age 42 days (fig. 2, A to F). Detectable by 9 days of age, mean glomerular area was significantly greater in Os/+ than WT kidneys (fig. 2, G).

**Growth and Protein Excretion in Os/+ Mice**

Normal body growth and urine protein excretion were maintained by Os/+ mice from birth to adulthood and were not affected by obstruction. Somatic growth from birth through adulthood was not affected in Os/+ compared to WT mice (fig. 2, G). At 21 days the body weight of Os/+ mice with persistent UUO lagged behind that of sham operated mice but by 42 days body weight was not affected by UUO regardless of strain (fig. 3, A). The urine protein-to-creatinine ratio did not differ between groups. The mean ± SE ratio was 39.8 ± 2.0 in sham operated WT mice, 34.4 ± 1.8 in sham operated Os/+ mice, 39.5 ± 26.1 in UUO WT mice and 49.2 ± 11.8 in UUO Os/+ mice (2-way ANOVA not significant).

**Effects of Obstruction**

**Kidney Growth.** Kidney growth was irreversibly impaired by obstruction. Kidney weight remained significantly lower in Os/+ than in WT mice from birth through age 42 days (fig. 2, I). Kidney weight was not affected by 21 days of ipsilateral UUO but continued growth to 42 days was impaired by persistent UUO in Os/+ mice or following release of UUO regardless of strain (fig. 3, B). Compared to sham operated mice the weight of the contralateral kidney was increased 42 days after UUO regardless of release of obstruction (2-way ANOVA p = 0.001, fig. 3, C). Renal parenchymal area after release of ipsilateral UUO was reduced by 29% compared to that of sham operated kidneys of WT mice and by 76% compared to sham operated kidneys of Os/+ mice (figs. 3, D and 4, A). Parenchymal area of the contralateral kidney was not affected by UUO or release (fig. 3, E).

**Glomerular Growth and Nephron Number Reduction.**

Glomerular growth was irreversibly impaired by obstruction and nephron number was further reduced in Os/+ mice. Glomerular area increased at a greater rate in Os/+ than in WT mice with significant differences between strains detectable even at 9 days (fig. 2, D). This revealed that compensatory glomerular growth in Os/+ mice begins prior to the completion of nephron maturation. The maturational increase in glomerular area from 21 to 42 days was suppressed in the post-obstructed kidney of WT mice and compensatory glomerular growth in Os/+ mice was also impaired despite the release of UUO (fig. 5, A). There was no effect of UUO on glomerular area of the contralateral kidney in either strain (fig. 5, B).
Figure 2. Growth and development. A, C and D, WT mice. B, E and F, Os/+ mice. A and B, survey micrographs show sections of 42-day-old sham operated mice comparing glomerular size and distribution between WT and Os/+ strains. Average area of 14 WT and 8 Os/+ kidney glomeruli was 3,437 and 4,978 μm², respectively. PAS staining, scale bar indicates 250 μm (also applies to A). C to F, semithin plastic sections of glutaraldehyde perfused kidneys from 42-day-old sham operated mice. C and D, WT mouse. E, glomerulus with proximal tubule, showing typical extension of tall epithelial cells on Bowman capsule. D, transversely sectioned proximal tubules in subcapsular cortex. E and F, Os/+ mouse. F, proximal tubules in cross-section. They were noticeably larger in diameter than WT proximal tubules. Scale bar indicates 25 μm (also applies to C to E). G, area of glomeruli, which underwent compensatory growth detectable at 9 days that continued through adulthood. H, body weight. There was no difference in somatic growth between Os/+ and WT mice. I, from birth through adulthood kidney weight in Os/+ mice remained approximately 50% lower than in WT mice. Bars represent mean ± SE. Blue bars represent C57BL/6 WT. Red bars represent Os/+ mice. Asterisk indicates vs WT t-test p < 0.05.
There was no effect of UUO on the number of glomeruli in the obstructed kidney of WT mice. However, the number of glomeruli decreased further in the obstructed kidney of Os/+ mice with no benefit from release of obstruction (fig. 5, C). There was no effect of UUO on the number of glomeruli in the contralateral kidney of mice of either strain (fig. 5, D).

**Effects of Obstruction and Release**

**Glomerulotubular Junction.** Obstruction impaired maturation of glomerulotubular junction in all mice but release preserved maturation in WT mice only. Persistent partial UUO resulted in numerous glomeruli lacking capsular Lotus lectin staining, consistent with immature glomerulotubular junctions (fig. 4, B). There was a significant increase in maturation of the glomerulotubular junction from 21 to 42 days in kidneys of sham operated animals (fig. 5, E), which did not differ in the contralateral kidneys of either strain (fig. 5, F). The fraction of mature glomerulotubular junctions was decreased in the obstructed kidney of WT mice at 21 and 42 days but normalized with release.
of obstruction (figs. 4, B and 5, E). A decreased fraction of mature glomerulotubular junctions observed in mice with persistent UUO is the result of a process of morphological alteration and selective death of cells in this nephron segment, culminating in the formation of atubular glomeruli.7 Although the integrity of the glomerulotubular junction was similarly impaired by ipsilateral UUO in Os/þ mice, release of obstruction resulted in only partial recovery (figs. 4, B and 5, E). There was no effect of UUO or release on glomerular number, area or glomerulotubular junction index of the contralateral kidney (figs. 5, B, D and F).

**Proximal Tubular Volume Fraction and Interstitial Collagen.** Proximal tubular volume fraction and interstitial collagen accumulation were inversely affected by obstruction and release in obstructed and contralateral kidneys. The proximal tubular volume fraction increased with age in sham operated mice, was suppressed by UUO and resumed following release of obstruction in WT but not Os/þ mice (fig. 6, A). Notably interstitial collagen accumulated after 42 days of ipsilateral UUO and decreased following release of obstruction in WT but not Os/þ mice (fig. 6, C). Unexpectedly the proximal tubular volume fraction was significantly greater in the contralateral kidney of WT and Os/þ mice that underwent release of obstruction compared to that in mice with persistent UUO (fig. 6, B). This was accompanied by significant increases in interstitial collagen content in the contralateral kidney of both strains of mice with persistent UUO with normalization of interstitial collagen in mice that underwent release of obstruction (fig. 6, D). There was a significant negative correlation between the volume fraction of interstitial collagen and proximal tubules in obstructed kidneys and a similar trend in contralateral kidneys (figs. 6, E and F).

**DISCUSSION**

**Development of Congenital Obstructive Nephropathy Model**

In infants with congenital obstructive nephropathy there is an increased incidence of preterm birth and renal hypoplasia, which are each associated with a decreased nephron number.19,20 Since nephrogenesis in the mouse continues through day 3 of life (followed by continuation of nephron maturation during the next 3 weeks), surgical partial UUO within 12 to 36 hours of birth is analogous to human ureteropelvic junction obstruction, the most common cause of obstructive nephropathy.18,21,22 This model allows for control of the severity, timing and duration of obstruction, of which all are significant determinants of clinical obstructive injury.7 To our knowledge the current study is the first to investigate in an animal model from birth to adulthood the consequences of reduced nephron number on the effects of persistent partial UUO as well as on recovery after release of obstruction. Study intervals of ages 21 and 42 days were selected because the former is the age of weaning and the latter is the age of sexual maturity in the mouse. This is relevant to human disease since progression of chronic renal insufficiency in children is accelerated at the end of infancy and in early adolescence.23
Within 7 to 14 days of complete UUO in the adult mouse proximal tubules undergo massive cell death, resulting in the formation of tubular fragments and atubular glomeruli. In contrast to the adult, proximal tubular cell death following complete UUO in the neonatal mouse is delayed past 14 days with atubular glomeruli appearing only after 21 to 28 days. These observations led to the development of histomorphometric techniques that quantitate the integrity of the glomerulotubular junction and the proximal tubular volume fraction, as applied in the current study.

Additive Maturational and Adaptive Nephron Growth

The current study demonstrates that adaptive accelerated growth by glomeruli in Os/+ mice begins before the completion of nephron maturation. Despite the lower kidney weight in Os/+ compared to WT mice the rate of kidney growth is similar in the 2 strains. Glomerular and proximal tubular growth in sham operated Os/+ mice continued to exceed that in WT mice throughout maturation (figs. 5, B and 6, B). These observations imply short-term preservation...
of adaptation to reduced nephron number in the adult.

While nephron growth is normally accelerated in Os/+ mice, chronic UUO impairs the adaptive growth of glomeruli and tubules in the ipsilateral kidney (figs. 5, A and 6, A). Although nephron number was not decreased by ipsilateral partial UUO in WT mice, the obstructed kidney of Os/+ mice experienced a further reduction in nephron number (fig. 5, C). This may have resulted from impaired completion of nephrogenesis or subsequent loss of nephrons. Because nephron immaturity appears to be protective against proximal tubular damage due to UUO, accelerated maturation/growth of nephrons in both kidneys of Os/+ mice may increase their susceptibility to obstructive injury.

**Figure 6.** Proximal tubular and interstitial response to partial UUO and its release in WT and Os/+ mice. A, C and E, obstructed kidney. B, D and F, contralateral kidney. A and B, proximal tubular volume fraction of renal parenchyma. C and D, fractional interstitial collagen. Light blue bars indicate 21-day WT. Dark blue bars indicate 42-day WT. Light red bars indicate 21-day OS/+. Dark red bars indicate 42-day OS/+.

§UUO, within strain 2-way ANOVA p < 0.05. §/NN, within strain nephron number 2-way ANOVA p < 0.05. E and F, fractional interstitial collagen decreased as function of proximal tubular volume fraction in obstructed and contralateral kidneys (r = 0.95, p < 0.001 and r = 0.75, p = 0.09, respectively). Blue symbols indicate WT. Red symbols indicate Os/+.

Squares indicate sham operated. Filled triangles indicate UUO. Open triangles indicate release.
Reduced Nephron Number Impairs Recovery from Neonatal Obstruction

After release of obstruction glomerular growth did not normalize regardless of nephron number (fig. 5, A). These results are consistent with a limited regenerative capacity for glomeruli. Although parietal epithelial cells of the Bowman capsule can replace damaged podocytes, the current study indicates that accelerated glomerular growth in Os/+ mice in response to reduced nephron number could not be maintained after release of partial UUO.

There was complete proximal tubular recovery in WT mice but impaired recovery in Os/+ mice (fig. 6, A). The close negative correlation between interstitial collagen and the proximal tubular volume fraction suggests that extracellular matrix deposition depends on the severity of tubular injury (fig. 6, E). Thus, in early adulthood renal interstitial fibrosis is reversible if tubular mass is preserved. However, a decrease in nephron number blunts this response, as underscored by the persistence of significant interstitial collagen accumulation in Os/+ mice.

The increased weight of the contralateral kidney following 42 days of UUO (fig. 3, C) was consistent with compensatory growth regardless of strain or release of obstruction. This was likely due to increased interstitial collagen during UUO and persistent tubular hypertrophy after release of obstruction (fig. 6, B and D). Regardless of nephron number, compared to mice with persistent UUO there was an increase in proximal tubular volume fraction in the contralateral kidney following release of UUO, which was paralleled by a concomitant decrease in interstitial collagen (fig. 6, B, D and F). These responses are consistent with reversal of oxidative proximal tubular stress in the kidney contralateral to a chronically obstructed neonatal kidney.

Mouse Model Clinical Implications

There is a great need for new predictors of progression of obstructive nephropathy. Correlation of outcome with renal pelvic dilatation or with renal scintigraphy is poor and reliable biomarkers are not readily available. The most widely used current index of progression of CKD, that is urinary protein excretion, has poor predictive value in early stages of the disease. The lack of sensitivity of the urine protein-to-creatinine ratio in the current study contrasts with the reduction in renal mass in Os/+ mice with persistent or post-obstructed UUO. However, these data suggest predictive value in the measurement of renal parenchymal area in patients by ultrasound or magnetic resonance imaging. This has been borne out in male infants younger than 6 months with lower urinary tract obstruction in whom lower renal parenchymal area is associated with an increased risk of renal failure later in life. To complement the measurement of renal parenchymal area a promising new technique is being developed to measure the number and size of glomeruli using magnetic resonance imaging in whole human kidneys labeled with cationic ferritin.

CONCLUSIONS

The current study reveals that a congenital reduction in nephron number impairs recovery from obstructive injury and contributes to additional nephron loss. These findings suggest that infants born with a nephron number below the median (approximately 900,000 nephrons per kidney) are at increased risk for progressive renal injury due to obstructive nephropathy, which is the leading cause of chronic kidney disease in children. Key questions require investigation. 1) What are the signals responsible for adaptive glomerular and tubular growth before and after the completion of nephrogenesis? 2) What are the determinants of the fate of individual cells responding to the injurious stimuli, leading to remodeling/regeneration or cell death?

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