Fetal rhesus monkey model of obstructive renal dysplasia

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Background. Disorders of kidney development represent a major cause of renal failure and end-stage renal disease in the pediatric population. To understand further the prenatal pathogenesis of obstructive renal dysplasia, a fetal monkey model was developed using ultrasound-guided techniques.

Methods. Ureteropelvic obstruction (N = 13) was induced during the early or late second trimester by the injection of purified guluronic alginate spheres. All fetuses were monitored sonographically, and then fetal tissues were removed at varying time points during the second and third trimesters.

Results. There was no evidence of oligohydramnios during the course of gestation, and the obstructed kidneys were typically progressively smaller than the contralateral (nonobstructed) kidneys when monitored sonographically over time. Obstructed kidneys displayed most features of renal dysplasia, including numerous cortical cysts of various sizes derived predominantly from collecting ducts and glomeruli. Mesenchymal changes included expansion of both the cortical and medullary interstitium, as well as mesenchymal-myocyte transformation, expressed as pericystic and peritubular fibromuscular collar formation. An important feature of this model was the disruption of normal glomerular development and architecture, associated with significant podocyte apoptosis, evident as early as the prevascularized S-shaped nephron. As in other models, collecting duct apoptosis was apparent, particularly in areas of cyst formation and cellular atrophy.

Conclusions. These results demonstrate the importance of this nonhuman primate model for exploring the pathophysiology of congenital obstructive uropathy and highlight the potential role of podocyte injury in determining long-term renal function associated with this condition.

Developmental abnormalities of the kidney constitute a major cause of end-stage renal disease (ESRD) and chronic renal failure in childhood [1]. These congenital anomalies include obstruction of the fetal urinary tract at the level of the bladder outlet and ureters [2]. Although congenital obstructive nephropathy constitutes 10 to 15% of ESRD in children, little progress has been made toward understanding its pathophysiologic mechanism(s), developing methods for prevention and treatment, and predicting outcome.

To address these issues and to justify in utero intervention, models of congenital obstructive nephropathy have been developed in sheep [3–8], rabbits [9], and rodents [10–15]. These models have provided insight into the causal effect of congenital obstruction and developmental anomalies, although the mechanisms by which these kidney anomalies are induced are still unknown. Although single and multiple gene defects ultimately may be responsible for renal dysplasia, the final phenotypic expression of obstructive renal dysplasia is likely dependent on the dysregulation or altered expression of key developmental genes [16]. For example, angiotensin and transforming growth factor-β are overexpressed [17], and epidermal growth factor is underexpressed [18] in the obstructed developing mouse kidney, while obstruction of the fetal sheep kidney results in alterations of insulin-like growth factor (IGF) genes (abstract; Matsell et al, J Am Soc Nephrol 6:702, 1995). Furthermore, altered renal cell survival in obstructed fetal kidney models has been implicated in the pathogenesis of the disease, with an increase in collecting duct cell apoptosis and interstitial fibrosis, both features that improve with the administration of exogenous IGF-I [19].

Despite attempts at early in utero decompression, the outcome of severe human fetal bladder outlet obstruction remains poor [20, 21]. This has been attributable to a number of variables, but relate in part to lack of a clear indication of the optimal timing of intervention and how intervention may ultimately relate to postnatal renal outcome. At present, there are no reliable prenatal markers or predictors of postnatal renal function in the developing fetus, although the severity of renal dysplasia is associated with poor long-term outcome. In order to

Key words: end-stage renal disease, kidney failure, pediatric kidney failure, ultrasound techniques, congenital obstructive uropathy, podocyte injury.

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define further the prenatal pathogenesis of fetal obstructive nephropathy and to design strategies for improving outcome, a nonsurgical, ultrasound-guided method was used to induce ureteral/ureteropelvic obstruction in the fetal monkey during the early second trimester when active nephrogenesis is in progress. Nonobstructed, obstructed, and normal control fetal monkey kidneys were studied at various time points during the second and third trimesters to evaluate the histopathologic progression of obstructive nephropathy and to determine the role of cell-specific apoptosis.

METHODS

Animals

Normally cycling adult female rhesus monkeys (Macaca mulatta, N = 13) with a history of prior pregnancy were bred and identified as pregnant using established methods [22]. Pregnancy in the rhesus monkey is divided into trimesters by 55-day increments, with 0 to 55 days gestation representing the first trimester, 56 to 110 representing the second trimester, and 111 to 165 days gestation the third trimester (term 165 ± 10 days gestation) [23]. All pregnancies were sonographically assessed to confirm normal growth and development prior to obstruction [22]. The dams were administered ketamine hydrochloride (10 mg/kg) for these and subsequent ultrasound examinations.

Fetal obstruction and sample collection

Patented, custom-designed alginate spheres have been shown to be highly efficient in inducing a physiologic obstruction with no evidence of toxicity and were used for these studies. Alginate (high guluronic acid) was purified by charcoal adsorption, by filtration through cellulose nitrate membrane with filters of decreasing pore size (0.90, 0.45, and 0.20 μm) and a nylon filter (0.10 μm), by chemical extraction [Ca²⁺, Mg²⁺ precipitation, ethylenediaminetetraacetic acid (EDTA) dissolution, ethanolic precipitation], and by drying. The purified guluronic acid is dissolved in 0.9% sodium chloride solution with 10 mmol/L HEPES buffer and sterile filtered through a 0.1 μm nylon filter (UC patent No. 07/891564). Purified high guluronic alginate spheres (50 μm) were formed using an electrostatic droplet generator (UC patent no. 07/89982) and gelled in 120 mmol/L calcium chloride and 10 mmol/L HEPES.

Five hundred to 1000 spheres were injected into the fetal kidney at the exit of the ureter from the hilum in a 0.1 mL volume (0.9% sodium chloride solution with 6 mmol/L and 10 mmol/L HEPES buffer; Fig. 1A, B). Once the spheres were injected, they swelled to approximately twice their original size and conformed to the shape of the surrounding structures, which resulted in an immediate obstruction of urinary flow. Because the beads are echogenic, the obstruction was monitored by ultrasound (discussed later in this article). Obstruction was induced in fetuses on 70, 80, or 90 days gestation (early to mid-second trimester) or 100 or 120 days gestation (late second-early third trimester; Table 1). In three cases (fetuses 32, 131, and 133), the initial obstructions appeared incomplete sonographically, and the procedure was repeated on the contralateral side (Table 1). The remaining 10 fetuses were obstructed unilaterally. At the time of obstruction, the mean kidney length and width is 7.3 mm × 4.6 mm (75 ± 5 days gestation), 12.7 mm × 8.1 mm (100 days gestation), and 14.7 mm × 9.5 mm (120 days gestation), and the ureter is approximately 1 mm in diameter. The kidneys exhibit active nephrogenesis similar to humans during this range of gestational ages, with a well-demarcated nephrogenic zone with identifiable metanephric blastema cells, ureteric duct, S-shaped nephrons, and maturing glomeruli, demonstrating strong similarities to human nephrogenesis [24, 25]. Fetuses were monitored at 24 hours postobstruction and then every five to seven days until hysterotomy. Fetal blood samples (1 mL) were collected with ultrasound-guidance using standard techniques [26, 27] at 120 days gestation (second trimester) for complete blood counts (CBCs).

Fetal tissue harvest/necropsy

Fetuses were delivered by hysterotomy on 90, 120, or 150 days gestation (Table 1). Complete tissue harvests were performed using standard techniques [28]. Right and left kidneys were grossly evaluated, and lengths and widths were recorded. Total body weights and measures were assessed, and then all organs were removed and weighed, including the thymus, spleen, liver, lymph nodes, pancreas, adrenals, kidneys, gonads, small and large intestine, heart, lung, and brain. Representative sections of the right and left kidneys were fixed in 10% buffered formalin or quick frozen over liquid nitrogen or in OCT compound.

Immunocytochemistry

To define the specific histopathology and severity of renal dysplasia, immunocytochemistry (ICC) was performed utilizing smooth muscle α-actin (SMA), pan-cytokeratin, and vimentin immunoreactivity. We have demonstrated previously the expression of SMA immunoreactivity in kidneys in the fetal sheep model of ureteric obstruction, localizing it to the fibromuscular collars of primitive cysts, thus providing a marker of mesenchymal-myocyte transformation, and to the epithelium of dilated medullary collecting ducts [29]. Similarly, pan-cytokeratin immunoreactivity preferentially localizes to the collecting duct epithelium and identifies cysts of collecting duct origin. Vimentin has been previously shown to be a reliable marker of the undifferentiated mesenchyme in the
developing kidney and by some as a marker of tubular epithelial cell damage and dedifferentiation [19, 25].

Immunocytochemistry was performed as previously described [29]. Briefly, tissue sections were deparaffinized in xylene, rehydrated in alcohol, and then washed in phosphate-buffered saline (PBS). Endogenous peroxidase was inhibited with 1% hydrogen peroxide for 10 minutes. Sections were washed in PBS and then permeabilized with trypsin. Sections were then washed with PBS and incubated with primary antiserum for 24 hours. Antibodies used were a mouse monoclonal antibody to SMA (1:1000; Sigma, St. Louis, MO, USA), a mouse monoclonal antibody to vimentin (1:1000; Sigma), and a polyclonal antibody to pan-cytokeratin (1:1000; Dako, Carpinteria, CA, USA). Sections were then washed in PBS and incubated with a peroxidase-conjugated secondary anti-mouse IgG antibody (Dako). Immunoreactivity was identified with the avidin-biotin peroxidase complex (Vector Laboratories, Burlingame, CA, USA) and visualized using diaminobenzidine tetrahydrochloride (DAB) reaction. Sections were washed in PBS, counterstained with Carazzi’s hematoxylin, and then dehydrated in alcohol. Slides were mounted with Permount (Sigma).

Cellular proliferation was identified utilizing the same ICC technique with a rabbit polyclonal antibody to phospho-histone H3 (5 µg/mL; Upstate Biotechnology, Lake

Fig. 1. Fetal rhesus monkey kidney preobstruction and postobstruction. (A) Sonogram of a rhesus fetus at 100 days gestation during obstruction of the right kidney. Note the needle (arrow) and needle tip (curved arrow) located within the hilum of the kidney during injection of alginate spheres (small arrows; k = kidney). (B) Fetal kidney (k) postobstruction. Note the echogenic alginate spheres (small arrows) within the renal hilum. (C) Fetal kidney weights (obstructed and contralateral) at the time of necropsy compared to control specimens for each gestational age assessed (mean ± SEM). Symbols are: (■) control; (▲) obstructed; (□) contralateral.
Renal histology: Scoring system

Based on previously published descriptions of renal dysplasia and abnormal kidney development and for the purpose of analysis of fetal monkey kidneys, we devised a semiquantitative evaluation of renal dysplasia using the criteria of (1) glomerular cystic changes, (2) tubular cystic changes, (3) mesenchymal-myocyte transformation or the development of fibromuscular collars (peritubular, pericystic, and periglomerular), (4) mesenchymal expansion, and (5) gross architectural disruption. Each characteristic was scored from 0 to 3+ as follows: 0 = no change; 1+ = segmental changes; 2+ = diffuse changes involving the cortex; and 3+ = diffuse changes involving both cortex and medulla. Ultimately, the total score reflected the severity of dysplasia (Table 1).

RESULTS

Growth and development: Sonographic and gross findings

Sonographic assessments of fetal growth included standardized measures of the head, abdomen, and limbs. All fetuses showed normal growth and developmental patterns when compared with historical controls of comparable age, either sonographically or at necropsy (data not shown). Other parameters assessed (amniotic fluid volume, placental development) did not reveal any differences when compared with control fetuses. Assessments of kidney lengths and widths sonographically indicated that obstructed kidneys typically were smaller than the nonobstructed kidneys in each fetus assessed. These findings correlated with gross observations at necropsy (Fig. 1C). In some cases, there was limited growth of the obstructed kidneys over time, whereas the nonobstructed, contralateral kidneys appeared to exhibit compensatory growth when compared with controls of comparable gestational age, particularly at the later time points. Grossly, all of the kidneys were normal in shape with no evidence of hydronephrosis. All other organ systems were normal, including the lungs. Complete blood counts assessed at 120 days gestation and at hysterotomy were within the normal range when compared with fetuses of comparable gestational age (data not shown) [31].

Morphology

General gross morphology. As expected, there were variations among kidneys, but the specific histologic changes were consistent with dysplasia and included changes in glomerular, tubular, and interstitial development (Fig. 2 and Table 1). Obstructed kidneys were divided into early (70 to 90 days gestation) and late (100 to 120 days gestation) obstruction. With early obstruc-
**Table 1.** Overview of fetal rhesus monkeys obstructed on 70, 80, 90, 100, or 120 days gestation

<table>
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<th>Fetal number</th>
<th>Day of harvest</th>
<th>Duration of obstruction days</th>
<th>L × W-pre (mm)</th>
<th>L × W-post (mm)</th>
<th>Glomerular cysts</th>
<th>Tubular cysts</th>
<th>FM collars</th>
<th>Mesenchymal expansion</th>
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Abbreviations are: R, right kidney; L, left kidney; FM, fibromuscular. Contralateral kidneys (non-obstructed) are shown for fetuses with unilateral obstruction (identified in italics). L × W = kidney length and width (mm) measured sonographically immediately prior to obstruction (pre-) and immediately prior to necropsy (post-).

<sup>a</sup> Fetus #32, #131, and #133 were obstructed on 70 and 80 days gestation (see *Methods*).
Fig. 2. Histopathology of fetal monkey obstructive dysplasia. Ureteral obstruction in the fetal monkey resulted in renal dysplasia, including cystic transformation of glomeruli (g) and tubules (t), the formation of fibromuscular collars (fm), and interstitial or mesenchymal expansion (m). (A) Fetal kidney obstructed at 100 days gestation and removed by hysterotomy at 140 days gestation demonstrating smooth muscle actin (SMA) immunoreactivity (see Methods section for details) around glomerular cysts (arrows). (B) More pronounced changes in the fetal kidney obstructed on 70 days gestation and removed by hysterotomy at 140 days gestation. Glomerular and tubular cysts are apparent with significant expansion of the mesenchymal space and the formation of fibromuscular collars (arrows) as defined by smooth muscle actin (SMA) immunoreactivity. (C) One hundred fifty-days gestation normal control kidney demonstrating SMA immunoreactivity in arterial smooth muscle (arrowheads) and in the collecting duct epithelium (cd). Magnification ×100.

tion, kidneys were analyzed after a variable period of obstruction ranging from 10 to 70 days, while in the late group, the obstruction ranged from 30 to 50 days. In the early obstructions, renal dysplasia was generally more severe the longer the obstruction, as reflected by the higher score. Also, as anticipated, early ureteric obstruction resulted in more severe renal dysplasia than late ureteric obstruction. This was partly due to the fact that the length of obstruction was longer in the early group; however, even shorter obstructions in the early obstructed kidneys resulted in more severe dysplasia than in the late obstruction group (Fig. 2 A, B and Table 1). Interestingly, unilateral ureteric obstruction as late as 100 of 165 days gestation in the fetal monkey kidney (~60% gestation or the equivalent of 20 to 22 weeks gestation in the human) was still associated with significant histopathological changes of renal dysplasia, while obstruction after 120 days gestation was not associated with any dysplastic changes in the kidneys studied.

Specific histopathology: Glomerulus. In all kidneys studied, glomerular changes were apparent, but were most remarkable in the early obstructed kidneys. These changes included cystic dilation of Bowman’s space with variable degrees of glomerular tuft abnormalities, including almost complete lack of development (Fig. 3A). The changes noted also included cystic dilation of distal tubule, collecting duct, and ureteric duct structures. The dysplastic kidneys showed a paucity of glomeruli, as has been previously described [5, 8], in part from the cystic transformation of cortical glomeruli as well as from secondary defects in nephron and glomerular induction (Fig. 2B). Under low-power magnification (×10), at least 10 random subcapsular cortical regions were chosen, and the absolute number of identifiable glomeruli were counted in obstructed and control kidneys (120 days gestation, N = 2; 140 days gestation, N = 3; 150 days gestation, N = 2) and compared with age-matched controls (120 days gestation, N = 2; 140 days gestation, N = 3; 150 days gestation, N = 4). There were significantly less (P < 0.05) glomeruli in the dysplastic (obstructed) kidneys when compared with age-matched controls (Fig. 4).

Tubules. Tubular changes were diverse. The most striking abnormality of the proximal tubule was an absence of tubular development in the outer cortical region of the most severely affected kidneys (Fig. 2B). This was associated with more severe glomerulocystic changes and was most evident in the early obstructed kidneys. In the collecting ducts of obstructed kidneys, tubular atrophy was apparent, as previously described in other models (Fig. 3B). This was associated with both medullary and cortical collecting duct dilatation and, as expected, was most evident in the early obstructed kidneys. In fact, only subtle changes in the collecting duct epithelium were apparent in the early and late obstruction groups. Unlike reports in other models in which collecting duct epithelial dedifferentiation and mesenchymal-myocyte transformation have been suggested, we did not observe any significant vimentin-immunoreactivity in the collecting duct epithelium of the obstructed kidneys.

Interstitium. Renal interstitial changes consisted predominantly of two features: mesenchymal expansion with an increase in vimentin positive cells and mesenchymal-myocyte transformation with the formation of peritubular and periglomerular fibromuscular collars demonstrating SMA immunoreactivity. Although more severe in the early group, in all obstructed groups, both mesenchymal expansion and mesenchymal-myocyte transformation were observed. This was somewhat surprising
given the relatively short periods of obstruction in some kidneys (as short as 10 days). Nonetheless, the features were striking and consisted of a marked expansion of both the medullary and cortical interstitium with cells of mesenchymal phenotype, as evidenced from their vimentin staining (Fig. 3C). We found very little SMA immunoreactivity in the interstitium other than in the fibromuscular collars surrounding the cystic glomeruli and tubules, a finding at variance with the rodent model of postnatal obstructive hydronephrosis. The cortical mesenchymal expansion was predominant in the outer cortex correlating with the extent of cortical cystic changes and mesenchymal-myocyte transformation.

Peritubular and periglomerular fibromuscular collar formation or mesenchymal-myocyte transformation was a consistent finding in all obstructed kidneys studied. Periglomerular mesenchymal-myocyte transformation was invariably associated with glomerular cystic dilation (Fig. 2A, B). Peritubular mesenchymal-myocyte transformation was apparent in dilated collecting ducts, but was also evident around nonatrophic, nondilated tubules (Fig. 3D).

**Apoptosis**

Cellular apoptosis was studied in all kidneys at various stages of obstruction. The most significant degree of apoptosis was apparent in the kidneys that had been obstructed early and then allowed to develop to near term (Fig. 5A). An important unexpected finding that has not previously been described was abnormal apoptosis in less severely obstructed kidneys.

**Glomerulus.** Apoptosis in the obstructed kidneys was noted predominantly in the cystic glomeruli, specifically in the cystic parietal epithelium and in the visceral epithelium or podocyte cell layer of the developing glomerular tuft (Fig. 5B). In the early obstructed kidneys harvested on 140 to 150 days gestation, the changes were most pronounced in the outer cortical glomeruli; however, podocyte apoptosis was also evident in the nondilated, developed glomeruli of the corticomedullary region (Fig. 5C).

Kidneys obstructed early and harvested at 90 days gestation not only demonstrated features of early dysplasia, but also exhibited a unique temporal and spatial pattern of abnormal apoptosis. Apoptosis of both the
Fig. 5. Podocyte apoptosis in the obstructed fetal monkey kidney. (A and B) Apoptosis as determined by a DNA fragmentation assay (Methods section) was apparent in the early obstructed kidneys in the cystic epithelia as well as the podocyte cell layer (arrows) and parietal epithelium (arrowheads) of the developing glomeruli (g). Abbreviation bs is Bowman’s space. (C) Apoptosis in corticomedullary glomeruli of the early obstructed kidneys is predominant in the podocyte cell layer (arrows) in what appears to be otherwise normally developed glomeruli. (D) In the normal 150 days gestation fetal monkey kidney, glomerular cell and podocyte apoptosis is infrequent (arrow). Magnifications are: A, ×100; B, C, and D, ×400.

podocyte and visceral epithelial cell layers was evident in the obstructed kidneys as early as the induction and early determination of the S-shaped nephron with its epithelial commitment to the podocyte and parietal epithelial cell layers (Fig. 6A). Kidneys that were obstructed later in gestation and for variable periods of time also surprisingly demonstrated podocyte apoptosis in otherwise normal-appearing noncystic glomeruli (Fig. 6B). In control kidneys of comparable gestational age, an occasional apoptotic cell was noted in the developing glomerulus, as well as in the interposed metanephric blastema, and mesenchyme, as previously described by others (Fig. 6C) [32].

Tubules. Apart from a lack of development of proximal tubules in the dysplastic segments of the renal cortex of the obstructed kidneys, there was very little proximal tubule cell apoptosis evident in the otherwise normal-appearing proximal tubules. The pattern of apoptosis in the medullary collecting duct structures, on the other hand, was quite complex. As described in other mammalian systems [33], in the normal developing fetal monkey kidney, there was significant apoptosis in cells of the developing papillary collecting ducts at all developmental ages studied (90 to 150 days gestation; Fig. 7A, B). Also, as described by others, apoptosis in the collecting duct cells was more pronounced with ureteric obstruction and was particularly obvious in areas of tubular atrophy (Fig. 7C) [17].

Interstitium. In normal metanephric development and modeling, determination of the developing renal interstitium also appears to involve apoptosis of a subset of uninduced metanephric mesenchyme cells [32]. This was observed in the kidneys from earlier gestational time points with appreciable nephrogenic zones. In normal
medullary development, very little interstitial mesenchymal apoptosis is noted. With urinary obstruction, however, not only is there expansion of the mesenchymal space, but there also appears to be an increased number of apoptotic cells, particularly in the earlier obstructions (data not shown).

**Cellular proliferation**

Using an antiphospho-histone H3 antibody, we studied active mitosis in kidneys at all stages of obstruction. In the most severely affected kidneys (those obstructed early and harvested at 150 days gestation), proliferation was evident both in the cells of the glomerular mesangial stalk and in parietal cells of the cystic Bowman’s space (Fig. 8). Of note are podocyte cells shown to be undergoing apoptosis, which did not demonstrate evidence of active mitosis, as would be predicted. Mitosis in early short-duration obstructed kidneys appeared appropriate in the cells of the ureteric duct and induced metanephric blastema, while the collecting ducts of these kidneys, although dilated, also demonstrated active mitosis. Mitotic labeling was not apparent in the interstitium. Mitosis in the late obstructed kidneys was similar to that of control, near term kidneys with active cell proliferation in the mesangium, proximal tubule, and collecting ducts, and absence of mitosis in the podocyte cell layer.

**DISCUSSION**

These studies demonstrate the feasibility of reproducing human fetal obstructive nephropathy in the rhesus monkey. A major histopathologic consequence of early fetal urinary tract obstruction in humans is renal dysplasia, characterized by the architectural disorganization of the kidney and the development of immature glomeruli, primitive tubules surrounded by fibromuscular collars, nests of metaplastic cartilage, and cysts derived from formed tubular structures [34]. In this unique primate model, we have demonstrated that ureteric obstruction during the early or late second trimester, induced by a nonsurgical ultrasound-guided technique, results in renal pathology characteristic of fetal obstructive renal dysplasia with glomerular and tubular cystic transformation, mesenchymal-myocyte transformation, interstitial mesenchymal expansion, and architectural distortion.

Although other animal models have provided impor-
tant information on the pathogenesis of obstructive renal disease at different time points during gestation and in the postnatal period, this model is unique because of the methods used for inducing the obstruction, the similarities in histopathology when compared with humans, and the early in utero timing of intervention. The fetal monkey model of obstructive renal dysplasia solves the problem of interspecies variation of normal and abnormal nephron induction as well as the confounding effect of increased postnatal functional demand on normal and abnormal kidney development as seen in the rodent model. Given the close similarities in the length of gestation and the process of nephrogenesis between humans and nonhuman primates [24], this model is best suited to correlate early changes in histology and expression of molecular markers in the obstructed developing kidney with postnatal renal outcome. In addition, this model will be invaluable in identifying the effects of in utero decompression on renal development and functional outcome, and the identification of the critical time after which the elements of renal dysplasia are no longer reversible.

The morphologic features of renal dysplasia seen in this model and in human fetal kidneys suggest that altered proliferation, differentiation, and/or survival of mesenchymal and epithelial elements are involved in its pathogenesis. With obstruction of urinary flow at critical stages of nephrogenesis the inductive interaction between the metanephric blastema and the ureteric bud is altered [35, 36], resulting in an alteration of normal genetic programs and the expression of proteins necessary for appropriate cell-specific proliferation, differentiation, and survival [16–18]. An imbalance between cellular apoptosis and cellular proliferation, as described in multicystic renal dysplasia [37, 38], as well as acquired abnormalities of cell polarity, basement membrane formation, and cell–cell and cell–matrix attachment and interaction, are potentially the fundamental mechanisms responsible for such features as cystic transformation of tubules and glomeruli, mesenchymal expansion and fibrosis, and collecting duct cell atrophy and death.

Analysis of this primate model revealed a number of important and clinically relevant findings. As demonstrated by other animal models, in particular studies utilizing the fetal sheep model of unilateral ureteric obstruction [3, 8], the earlier the obstruction the more severe the extent of renal dysplasia. However, one of the more important findings was the temporal and spatial pattern of cellular apoptosis. Although it has been recognized that a number of factors contribute to compromised renal function in fetal obstructive nephropathy, including expansion of the interstitial mesenchymal space and fibrosis, tubular atrophy, and tubular apoptosis [39–43], this study is the first, to our knowledge, to implicate podocyte cell damage and apoptosis as a potential factor in determining long-term renal function. Although apoptosis is an important cellular event of normal development of many tissues and organs, including the kidney, inappropriate apoptosis precedes irreversible histopathologic changes in abnormally developing organs. We have identified significant podocyte cell death in the developing podocyte as early as the S-shaped nephron stage of development, with obstruction as short as 10 days, and in developing glomeruli that histologically otherwise appear normal. This has been previously noted in a sheep model after short-term unilateral ureteric obstruction at 90 days gestation [44]. It is of great interest that these changes are identified as early as the prevascularized S-shaped nephron. This implies that the potential mechanisms for altered glomerular development in this model are only indirectly a result of obstruction to urinary flow and may in fact be directly related to the effect of ureteric duct obstruction on vascularization of the developing glomerulus. This is further reinforced by the finding of decreased glomerular numbers in the obstructed kidneys (Fig. 4). In normal, control animals at similar gestational ages, podocyte apoptosis is virtually absent or undetected.

Collecting duct cellular atrophy and apoptosis have been previously demonstrated in the postnatal rat model of ureteric obstruction, which was shown to be rescued by exogenous administration of IGF-I, and implicated in the renal functional abnormalities of fetal obstructive nephropathy [17, 19]. Although these findings suggest the potential rescue of cellular apoptosis in obstructive nephropathy, this model does not appear to demonstrate significant glomerular abnormalities as we have shown in our fetal monkey model. We propose that these significant glomerular abnormalities, including a decrease in glomerular number, cystic glomerular changes, and podocyte damage and apoptosis, are also important contributing factors to the poor postnatal outcome of severe human obstructive nephropathy. In fact, given the spatial and temporal expression of various growth factors such as the IGFs in the developing human and primate fetal kidney [24, 25], these changes may lend themselves to prenatal intervention.

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REFERENCES


