Development of Autosomal Recessive Polycystic Kidney Disease in BALB/c-cpk/cpk Mice

JUSTIN L. RICKER,* VINCENT H. GATTONE II,* JAMES P. CALVET,† and CAROLYN A. RANKIN†

Departments of *Anatomy & Cell Biology and † Biochemistry & Molecular Biology, The University of Kansas Medical Center, Kansas City, Kansas.

Abstract. Autosomal recessive polycystic kidney disease (ARPKD) is a rare but devastating inherited disease in humans. Various strains of mice that are homozygous for the cpk gene display renal pathology similar to that seen in human ARPKD. The PKD progresses to renal insufficiency, azotemia, and ultimately a uremic death by approximately 3 wk of age. This study characterizes PKD in mice that are homozygous for the cpk gene on a BALB/c inbred mouse background. The BALB/c-cpk/cpk murine model displays renal as well as extrarenal pathology similar to that found in human ARPKD. The renal pathology includes the well-characterized early proximal tubule and, later, massive collecting duct cysts. The extrarenal defects in this murine model include common bile duct dilation, intrahepatic biliary duct cysts with periductal hyperplasia, and pancreatic dysplasia with cysts. Renal mRNA expression of c-myc, a proto-oncogene, and clusterin (SGP-2), a marker associated with immature collecting ducts, decreases during normal development but is upregulated in murine ARPKD. Expression of epidermal growth factor (EGF) mRNA is significantly diminished, whereas EGF receptor mRNA is upregulated in the BALB/c-cpk/cpk kidney compared with phenotypically normal littermates. To determine whether the altered EGF expression contributes to the development of PKD, neonatal mice were treated with exogenous EGF (1 μg/g body wt injected subcutaneously on postnatal days 3 through 9). EGF treatment reduced the relative kidney weight and common bile duct dilation and downregulated renal expression of clusterin and EGF receptor. However, exogenous EGF did not affect the degree of renal failure, the pancreatic pathology, or the misregulated renal expression of c-myc. In summary, the present study characterizes the renal and extrarenal pathology in the BALB/c-cpk/cpk murine model of ARPKD. Renal mRNA expression of EGF is diminished in this mouse model. EGF treatment did not prevent renal failure but ameliorated pathologic changes in the kidney and the biliary ducts of the BALB/c-cpk/cpk mouse.

Inherited polycystic kidney disease (PKD) is characterized by severe dilation of the renal tubules. Renal fibrosis and hepatic biliary duct changes accompany most forms of the disease. This multigenic disease can be inherited as autosomal dominant (AD) or autosomal recessive (AR) traits. In humans, ADPKD has a later onset and slower development than ARPKD, which usually affects newborns and young children (1). In the perinatal period, ARPKD can lead to pulmonary hypoplasia and death from respiratory insufficiency. Most individuals who survive the newborn period die from renal insufficiency associated with the PKD (1).

A number of murine models of ARPKD exist (2). Probably the most used animal model is the C57BL/6J mouse homozygous for the cpk gene, which exhibits renal pathology similar to that seen in human ARPKD. Although the cpk gene on the C57BL/6J background does not show evidence of extrarenal pathology, it does on other genetic backgrounds (2,3). In the present study, we sought to determine whether the cpk mutation on a BALB/c background would exhibit renal and extrarenal pathology similar to the human disease.

Renal expression of epidermal growth factor (EGF) seems to be significantly diminished in all forms of PKD, including C57BL/6J-cpk/cpk mice (4,5), DBA-2F1-pcy mice (6), and Han:SPRD cy/+ rats (7). Developmentally, EGF promotes epithelial cell maturation in multiple organs (8,9). EGF is initially localized in murine renal distal tubule cells at approximately 6 d of age (10,11). ProEGF remains on the extracellular surface of the apical membrane until it is enzymatically cleaved to EGF, displaced into the distal tubular fluid, and excreted into the urine (8,12,13). Therefore, the collecting duct is normally exposed to this growth factor, which may promote maturation of this tubule segment. The immature collecting duct cells have EGF receptors on the apical surface that could bind this protein and result in paracrine stimulation (14). Therefore, in cystic kidneys, the absence of this growth factor could delay epithelial maturation. Previously, exogenous EGF treatment of C57BL/6J-cpk/cpk mice led to an amelioration of collecting duct cysts and decreased renal expression of clusterin (SGP-2) (15), a marker of collecting duct immaturity (16). Exogenous EGF treatment also led to a reduction in tubular dilation and interstitial fibrosis as well as renal SGP-2 expression in neonatal rats with chronic unilateral ureteral ob-
struction (17). In this study, we evaluated the efficacy of exogenous EGF treatment on the amelioration of renal and extrarenal pathology in ARPKD using the BALB/c-cpk/cpk mouse model.

Materials and Methods
C57BL/6J-cpk/+ mice (colony established at KU Medical Center, stock originally purchased from Jackson Laboratory, Bar Harbor, ME) were crossed with BALB/c-+/ mice. BALB/c-cpk+ offspring were identified by PCR using D12Mit58 and D12Mit105 murine MapPairs (Research Genetics, Huntsville, AL) and DNA derived from the tail. Heterozygotes were backbred with BALB/c-+/ mice. These primers allowed the identification of +/+, +/cpk and cpk/cpk genotypes on the basis of a difference in the size of the PCR products. The size of the PCR product from C57 strain (which carries the mutated cpk gene) differs from the size of the BALB/c PCR product (with a normal cpk gene). Heterozygous mice are identified by selecting offspring that have both sizes of PCR products. The identification of heterozygotes was useful in the numerous backbreedings onto BALB/c mice. This procedure was followed for eight backcrossings, each time using PCR to identify cpk/+ mice. By the eighth backcross (or ninth generation), the cpk/+ mice possessed greater than 99.5% of the BALB/c background. The BALB/c-cpk/+ mice from the eighth backcross formed the colony that was used in the current characterization of this ARPKD model.

EGF Treatment
BALB/c-cpk litter received subcutaneous injections of EGF (1 μg/g body wt, Catalog #4001, mouse natural culture grade EGF, Collaborative Biomedical Products, Bedford, MA) on postnatal days 3 through 9. The EGF-treated litters were killed and evaluated at 15 d of age (see below). Eyelid opening was assessed to evaluate bioactivity of the EGF with this treatment (15).

Light and Electron Microscopy
Normal (+/+ or cpk/c+) and cystic (cpk/cpk) BALB/c mice were anesthetized (65 mg/kg sodium pentobarbital, intraperitoneally) and killed at 0, 5, 10, and 15 d of age. EGF-treated litters were similarly anesthetized and killed at 15 d of age. Total body weight, total kidney weight, and transverse bile duct diameter (using a micrometer) were determined. Blood was collected from the heart, and sera samples were used to assess kidney function. Serum urea nitrogen (SUN) was determined using a colorimetric assay (Sigma #640, Sigma Chemical Co., St. Louis, MO). The left kidney was removed and weighed, and the animals were then perfusion fixed with 4% paraformaldehyde in 0.1 M phosphate buffer for light microscopy or 2.5% glutaraldehyde, 2% paraformaldehyde in 0.1 M cacodylate buffer for electron microscopy (EM). The fixed kidneys were weighed, and the kidney, liver, and pancreas were processed for microscopy. Segments of the paraformaldehyde-fixed tissue were embedded in paraffin, and sections were stained with hematoxylin and eosin or periodic acid-Schiff reagent for histologic examination. Segments of glutaraldehyde-fixed kidney and liver were processed for transmission EM (TEM) and scanning EM (SEM). For TEM, samples were rinsed in phosphate-buffered saline and incubated in 1% osmium tetroxide for 1 h. The samples were then rinsed with distilled water and dehydrated in graded ethanol solutions and propylene oxide before being embedded in LX112 (Ladd Research, Burlington, VT). Thin sections were cut and viewed by JEOL 100S (JEOL USA, Inc., Peabody, MA) TEM. SEM samples were rinsed in phosphate-buffered saline and incubated in 1% osmium tetroxide for 30 min. The samples were then rinsed with distilled water, dehydrated in graded ethanol solutions, and dried in a critical point drier. The dried samples were mounted onto specimen stubs, sputter coated with gold-palladium alloy, and viewed with a Hitachi S2700 (Hitachi Corp., Tokyo, Japan) SEM.

Volume Density of Cystic Change
Transverse sections through the entire kidney from the 15-d-old control and EGF-treated cystic mice were analyzed using point-count stereology as described previously (15).

RNA Isolation and Northern Hybridization
RNA was extracted and purified from kidneys of 5-, 10-, and 15-d-old normal and cystic mice as well as 15-d-old EGF-treated normal and cystic mice using the acid guanidinium thiocyanate-phenol-chloroform extraction method as described previously (6). Briefly, the kidneys were snap-frozen in liquid nitrogen and stored at −80°C until processed for RNA extraction. The kidneys were homogenized in Tri-Reagent (Molecular Research Center, Inc., Cincinnati, OH), and RNA was isolated according to the manufacturer’s instructions. Five or 10 μg of total renal RNA was added to each lane of a 1% agarose/formaldehyde gel. After electrophoresis, the RNA was transferred to a Zetabind membrane (Cuno, Inc., Meriden, CT) and stained with methylene blue to ensure equal loading and transfer of RNA from each lane. The blots were baked for 2 h at 80°C and then hybridized overnight at 65°C in Church’s buffer (1% bovine serum albumin and 7% sodium dodecyl sulfate [SDS] in 0.5 M sodium phosphate with 1 mM ethylenediaminetetraacetate [EDTA]) (18) with 32P-labeled probes. DNA probes for c-myc (19), clusterin (SGP-2) (16), preproEGF (4), and EGF receptor (obtained from E. Adamson, LaJolla, CA), were labeled using a random prime labeling method (Rediprime II, Amersham Pharmacia Biotech, Arlington Heights, IL.). Hyridized blots were washed for 10 min each at 65°C; twice in Wash Buffer A (0.5% bovine serum albumin and 5% SDS in 40 mM sodium phosphate with 1 mM EDTA), and four times in Wash Buffer B (1% SDS in 40 mM sodium phosphate with 1 mM EDTA). The blots were exposed to x-ray film for autoradiography. The relative expression of specific mRNA was evaluated by densitometric quantitation using NIH Image software, including the subtraction of background and normalization to any RNA loading differences. All of the Northern blot hybridization studies were performed in duplicate with different sets of RNA.

Statistical Analyses
Statistical analysis of SUN, total body weight, kidney weight as a percentage of body weight, and transverse bile duct diameter were performed using ANOVA or a Mann-Whitney U analysis (P ≤ 0.05) with the aid of MINITAB software (State College, PA).

Results
The BALB/c-cpk/cpk Mouse Model
ARPKD in humans and BALB/c-cpk/cpk mice is a multorgan disease. Renal and extrarenal changes were evaluated in newborn through 15-d-old BALB/c-cpk/cpk mice. Newborn mice have cysts in the inner cortical proximal tubules (Figure 1, a and e). The cells that line the proximal tubular cysts have variable numbers of microvilli (Figure 1h). A multilaminated basement membrane was often associated with cells having less prominent microvilli (Figure 1h). In older mice, the kidneys rapidly enlarge (Table 1) and are associated with cyst development in the collecting ducts (Figure 1, b, c, and d). These collecting duct cysts are the predominant pathology seen...
Figure 1. Renal pathology in BALB/c-cp/cp mice. (a through d) Light micrographs of newborn and 5-, 10-, and 15-d-old BALB/c-cp/cp mouse kidneys. Proximal tubule cysts are evident early in the disease process with collecting duct cysts predominating as PKD progresses. (e, h) These proximal tubule cysts are lined by cells, most of which have a periodic acid-Schiff–positive staining brush border (e); however, some seem to lack this brush border (arrows). By transmission electron microscopy (TEM), many of these proximal tubule cyst lining cells have variable amounts of microvilli (h). The underlying basement membrane associated with cells that have less prominent microvilli can be multilaminated (large arrow). (f) The collecting duct cysts are lined by a single layer of epithelial cells (arrow). (g) By TEM, some cells exhibit stress actin fibers along their basal membrane (arrow). (i) By 10 d, much of the renal parenchyma is replaced by the collecting duct cysts as viewed in this scanning EM (SEM). (j) Cystic epithelia are composed mainly of principal cells; however, occasional intercalated cells (arrows) are seen with scanning electron microscopy. Magnifications: a through d, 60×; e, f, 240×; g, 3000×; h, 5000×; i, 50×; j, 2500×.
in the later, azotemic stages of PKD (Table 1, Figure 1, d and i). The collecting duct cysts are lined by a single layer of epithelial cells (Figure 1f). These cells have a polygonal principal-like cell appearance with a single cilium and short microvilli (Figure 1j). An occasional intercalated cell can be identified (Figure 1j). Some cells have basally located actin stress fibers (Figure 1g).

The extrarenal pathology includes pancreatic dysplasia (Figure 2a), common bile duct dilation (Figure 2b), and intrahepatic biliary duct cysts (Figure 3). The pancreatic pathology consists of dilated pancreatic ducts and periductal fibrosis with few acini and almost no observable islets of Langerhans (Figure 2a). The intrahepatic biliary duct cysts are associated with periductal hyperplasia and fibrosis (Figure 3, a and b) and are lined by epithelial cells with numerous microvilli and a single cilium (Figure 3, c, e, and f). The cystic intrahepatic bile duct

### Table 1. Balb/c-ckp mouse model of ARPKD

<table>
<thead>
<tr>
<th>Age</th>
<th>Phenotype</th>
<th>Body Weight (g)</th>
<th>KW/BW (%)</th>
<th>SUN (mg/dl)</th>
<th>Common Bile Duct (diameter in mm)</th>
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<tr>
<td>Newborn</td>
<td>Normal</td>
<td>1.42 ± 0.10</td>
<td>1.31 ± 0.05</td>
<td>22.19 ± 2.49</td>
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<tr>
<td></td>
<td>(N = 13)</td>
<td>(N = 13)</td>
<td>(N = 11)</td>
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<tr>
<td>Cystic</td>
<td></td>
<td>1.34 ± 0.08</td>
<td>1.41 ± 0.05</td>
<td>32.8 ± 4.20</td>
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<td>(N = 9)</td>
<td>(N = 9)</td>
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<tr>
<td>5 D</td>
<td>Normal</td>
<td>3.35 ± 0.08</td>
<td>1.22 ± 0.02</td>
<td>23.17 ± 2.36</td>
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<td>(N = 29)</td>
<td>(N = 29)</td>
<td>(N = 15)</td>
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<tr>
<td>Cystic</td>
<td></td>
<td>2.30 ± 0.09b</td>
<td>1.83 ± 0.06b</td>
<td>25.64 ± 0.88</td>
<td>1.08 ± 0.12</td>
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<td>(N = 12)</td>
<td>(N = 9)</td>
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<td>10 D</td>
<td>Normal</td>
<td>7.96 ± 0.29</td>
<td>1.44 ± 0.02</td>
<td>21.44 ± 1.00</td>
<td>0.30 ± 0.03</td>
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<tr>
<td>Cystic</td>
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<td>4.64 ± 0.18b</td>
<td>4.47 ± 0.27b</td>
<td>40.66 ± 3.10</td>
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<td>15 D</td>
<td>Normal</td>
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<td>1.55 ± 0.02</td>
<td>16.71 ± 0.86</td>
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<td>(N = 23)</td>
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<tr>
<td>Cystic</td>
<td></td>
<td>7.15 ± 0.42b</td>
<td>12.50 ± 0.63b</td>
<td>56.50 ± 9.86b</td>
<td>1.65 ± 0.17b</td>
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<td>(N = 12)</td>
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<tr>
<td>15 D EGF treatment</td>
<td>Normal</td>
<td>9.06 ± 0.29c</td>
<td>1.49 ± 0.03</td>
<td>15.49 ± 1.73</td>
<td>0.46 ± 0.02c</td>
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<td>(N = 22)</td>
<td>(N = 22)</td>
<td>(N = 17)</td>
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<tr>
<td>Cystic</td>
<td></td>
<td>5.78 ± 0.51b,c</td>
<td>9.74 ± 1.06b,c</td>
<td>48.15 ± 3.22b</td>
<td>1.16 ± 0.07b,c</td>
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<td>(N = 8)</td>
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<td>(N = 8)</td>
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- ARPKD, autosomal recessive polycystic kidney disease; KW/BW, kidney weight as a percentage of body weight; SUN, serum urea nitrogen.
- b Significant difference from normal, $p < 0.05$.
- c Significant difference with EGF treatment, $P < 0.05$.

**Figure 2.** Extrarenal pathology in 15-d-old BALB/c-ckp/cpk mice. Light micrographs of pancreas (a) and common bile duct (b). Notice the enormous pancreatic duct cysts (*) encompassed by the periductal fibrosis and a paucity of acini. The common bile duct is extremely large (see Table 1 for dimension). Magnification, 60×.
Figure 3. Hepatic pathology in BALB/c-cpk/epk mice. (a, b) Light micrographs reveal hepatic biliary duct cysts (*) with some periductal hyperplasia and fibrosis. Portal veins (v) and hepatic artery branches (a) are located near the cysts. (c, e, f) By SEM, the hepatic biliary duct cysts (*) are lined by epithelial cells with numerous microvilli and a single cilium. (d) This scanning micrograph displays a portal triad from the liver of a normal BALB/c mouse. The portal artery (a) and vein (v) are positioned on opposite sides of this normal bile duct (arrow). The bile duct is at the same magnification as the cystic bile duct in e and lined by cells with a similar appearance to those from cysts. Magnifications: a, 60×; b, 260×; c, 100×; d, e, 1000×; f, 4500×.
(Figure 3e) is much larger than a normal bile duct (Figure 3d, shown at the same magnification). The cystic pancreatic pathology is present in the newborn (Figure 4a) and may contribute to the overall smaller size of the cystic offspring at 5 to 15 d (Table 1) by limiting digestive capability. Common bile duct and intrahepatic biliary pathology are also evident early in the disease process (Figure 4b, Table 1).

**Altered Renal mRNA Expression**

The renal mRNA expression of SGP-2 and c-myc are increased in association with the development of ARPKD in BALB/c-cpk/cpk mice (Figure 5). The expression of both peaks at 10 to 15 d in cystic mice but remains very low in phenotypically normal mice. Renal EGF mRNA is virtually undetectable in BALB/c cystic kidney but is present at 10 d and to a much greater extent at 15 d in normal mice. The EGF-receptor (EGF-R) mRNA concentration is increased in the cystic kidneys at 10 and 15 d of age (Figure 5).

**Effects of EGF Treatment**

EGF caused precocious eyelid opening (day 8 versus day 12 in untreated controls) and was therefore biologically effective in vivo. Fifteen-d-old normal BALB/c mice that were treated with EGF exhibited reduced body weight compared with untreated controls (Table 1), which is consistent with the known effects of EGF given neonatally. Kidney size was therefore expressed as a percentage of total body weight. Kidneys of the BALB/c-cpk/cpk mice were smaller after treatment with EGF (Table 1). However, EGF did not significantly alter the relative kidney size of the phenotypically normal BALB/c mice (Table 1). EGF treatment did not improve renal function in BALB/c-cpk/cpk mice, as indicated by the elevated SUN levels comparable to those seen in untreated cystic mice (Table 1). Treatment with EGF reduced the common bile duct dilation in cystic mice (Table 1) as well as the histologic severity of the renal and hepatic cystic pathology (Figure 6). The volume density of renal cysts was reduced after EGF treatment in cystic mice (50.3 ± 2.55% versus 59.6 ± 2.56% in sham-treated mice, \( P < 0.05 \) by one-tailed analysis). EGF treatment of BALB/c-cpk/cpk mice reduced renal SGP-2 gene expression by 34%; however, renal expression of c-myc mRNA was not significantly altered (Figure 7). EGF treatment had no effect on EGF mRNA expression in cystic kidney but was increased 3.8-fold in normal kidney. Finally, renal EGF receptor mRNA expression was decreased 20% in the EGF-treated cystic mice but was increased 43% in normal mice after EGF treatment (Figure 7).

**The BALB/c-cpk/+ Phenotype**

The 1-yr-old BALB/c-cpk/+ heterozygous breeders all developed massive hepatic cysts (Figure 8). These hepatic biliary ductal cysts were associated with fibrosis (data not shown). In some of the older breeders, the entire hepatic parenchyma was almost completely replaced by cysts.

**Discussion**

**Renal Changes in BALB/c-cpk/cpk Mice**

In the present study, we characterized the renal and extra-renal pathology in the BALB/c-cpk/cpk murine model of ARPKD. In the kidney, proximal tubule cysts were found early in the disease process with collecting duct cysts predominating later. This pattern is similar to that described when the cpk gene was expressed in C57BL/6J (20–22), DBA (3), and CD1 (2) mouse strains. Furthermore, ARPKD in BALB/c-bpk/bpk mice (23) and PKD-1 knockout mice (24) also exhibit the same pattern of cyst progression from early proximal tubule to massive collecting duct cysts later in the disease. It is therefore possible that all of these genes may be involved in a common developmental cascade that is responsible for the development of PKD.

The BALB/c cystic mice misexpressed a number of renal mRNA. c-myc, a proto-oncogene associated with increased cellular proliferation (25,26), and SGP-2 were overexpressed in BALB/c-cpk/cpk mice. Increased expression of SGP-2 and c-myc
mRNA was previously localized to the epithelial cells of the cystic collecting duct in C57BL/6J-cpk/cpk mice (16,19). Furthermore, transgenic mice that overexpressed c-myc develop PKD (27,28), and the c-myc message is present in the cystic tubules (27). This suggests that c-myc may play an important role in the development of PKD, whereas epithelial cells that line cysts may remain in an immature proliferative state.

As in other PKD models, the kidneys of BALB/c-cpk/cpk mice had severely diminished expression of EGF mRNA (5–7,15) but an increased level of expression of the EGF-R (29).

Overexpression of EGF-R mRNA may result from the lack of downregulation by EGF (30,31).

**Extrarenal Changes in BALB/c-cpk/cpk Mice**

The extrarenal defects in the BALB/c-cpk/cpk mice included common bile duct dilation, pancreatic cystic dysplasia, and intrahepatic biliary duct cysts with periductal hyperplasia. Cystic dilation of the common bile duct associated with pancreatic cysts has also been found in cpk-induced PKD on other backgrounds (2,3). However, intrahepatic biliary duct dilation was limited (2,3) or absent (22) on these other backgrounds. Mouse strain background has previously been shown to play a role in the phenotype of PKD. Glucocorticoid-induced PKD is strain dependent (32), and pcy-induced PKD progresses differently on the DBA as compared with the C57BL/6 background (33). This is the first demonstration that the cpk gene induces hepatic pathology similar to human ARPKD. Therefore, the BALB/c background seems to be more permissive for the development of the PKD-associated intrahepatic pathology.

Like PKD-1 knockout mice (24), the BALB/c-cpk/cpk mice were significantly smaller than normal littermates and exhibited pancreatic cysts, which were present at birth. The enormous pancreatic cysts seemed to replace much of the normal pancreatic parenchyma and may have reduced the amount of functional pancreas. The digestive capability of the mice may have been limited, leading to the runted phenotype. However, other cystic mice with common bile duct dilation and pancreatic cystic dysplasia did not exhibit decreased body weight (2,3). The PKD-1 knockout mice are also runted at birth (24), further suggesting that digestive capability may not completely explain this body size difference. Therefore, other undetermined factors must be contributing to this runted phenotype as well.

**BALB/c-cpk/+ Mice**

The 1-yr-old BALB/c-cpk/+ breeders also developed extrarenal pathology, including massive intrahepatic biliary duct cysts. Similar hepatic pathology has been observed in other heterozygous PKD models (2,32,34). It is interesting to note that there is a spectrum of phenotypes in human ARPKD (35). Furthermore, the degree of extrarenal pathology generally is inversely related to the severity of the renal pathology (1). The BALB/c-cpk/+ mice require 1 yr to develop this significant hepatic pathology, whereas the BALB/c-cpk/cpk mice live only 2 to 3 wk and exhibit greater renal cystic pathology than hepatic pathology. In the absence of this morbid renal pathology, the BALB/c-cpk/cpk mice likely would progress to a phenotype expressing significant hepatic pathology, probably in a period of a few months rather than a year. However, because BALB/c-cpk/+ heterozygotes exhibit a cystic phenotype at 1 yr of age, there seems to be an apparent gene-dosage effect of the cpk gene. The development of the liver cysts may be due to either haploinsufficiency or a loss of heterozygosity of the normal allele in the biliary ducts as has been proposed for human ADPKD (36,37).
Role of EGF in PKD

The role of EGF in the development of PKD is unclear. EGF can act as a mitogen to enhance cyst formation and progression by activating EGF-R tyrosine kinase activity. Two in vivo studies support this role for EGF in PKD. orpk cystic mice homozygous for defective EGF-R develop a less severe form of PKD (38). pcy-induced PKD progresses more rapidly in mice that also express a transgene for transforming growth factor-α, a member of the EGF family (39). In addition, EGF-R tyrosine kinase inhibitors can inhibit the cystic phenotype in bpk mice with ARPKD both in vitro (40) and in vivo (41). However, these treatments are administered after the first postnatal week. All studies on the effects of EGF administration earlier in murine ARPKD have found that EGF inhibits the development of renal (4) and/or extrarenal cystic effects (42). Studies have shown that EGF is important in early development (43,44) and renal tubulogenesis (45). Transgenic CD1 mice with a targeted disruption of the EGF-R developed cystic dilation of the collecting ducts as well as hepatic abnormalities in the neonatal period (44). Maturation of collecting ducts (46,47) temporally correlates with renal EGF expression (10), whereas the lack of the growth factor is associated with renal cyst development in C57BL/6J-cpk/cpk mice (4). Cystic renal and biliary epithelia are also known to be responsive to EGF in vitro (48,49). Administration of exogenous EGF on days 3 through 9 led to an amelioration of the PKD in C57BL/6J-cpk/cpk mice in vivo (15). Therefore, EGF may serve a crucial role in the maturation of renal and possibly biliary epithelia. Furthermore, the lack of EGF could contribute to the persistence of epithelial immaturity in neonatal mice with ARPKD (50). After this neonatal period, EGF treatment seems to have distinctly different effects on the kidney and the cystic phenotype, which may explain the data from the orpk (38), transforming growth factor-α/cpy (39), and EGF-R tyrosine kinase inhibitor (40,41) studies.
EGF Treatment Effects in BALB/c Mice

In the present study, neonatal EGF treatment ameliorated the renal and extrarenal pathology in the BALB/c-cpk/cpk mice; however, the pancreatic pathology and the degree of azotemia were unaffected. It is unclear why the neonatal cystic kidney responds differently than the adult kidney to the affects of EGF. In another model, the BALB/c-bpk/bpk mouse, the SUN levels and common bile duct dilation were reduced, whereas relative kidney weight was unchanged after treatment with exogenous EGF (42). In general, the degree of cystic change has been shown to correlate with deterioration in renal function in human ADPKD (51). However, there may be exceptions to this rule as the progression to renal failure is highly variable in PKD and is affected by multiple factors (51). In studies on the effect of testosterone on ADPKD in rats (52), castration of cystic male rats led to a reduced kidney size but not to a change in the degree of azotemia. Conversely, when bpk mice were treated with EGF, renal function improved without any change in the amount of cystic pathology (42). Therefore, factors other than the amount of cystic change may influence the amount of residual renal function in PKD.

The role of EGF in promoting maturation of the distal tubule is supported by studies that show that EGF causes decreased proliferation of this segment (11). It has also been shown that exogenous EGF increases differentiation of the distal segment of the kidney (53). Therefore, a possible explanation for the upregulation of EGF mRNA after EGF treatment in normal distal tubule cells is that the exogenous EGF is advancing the development of the kidney to a later stage of maturation in which higher EGF synthesis would be expected. Although the expression level of the renal SGP-2 message remains higher in cystic than in normal mice, the expression is consistently lower in cystic kidneys after EGF treatment. This finding is similar to that seen when C57BL/6J-cpk/cpk mice (15) or neonatal rats...
with unilateral ureteral obstruction were treated with EGF (17). This suggests that neonatal EGF treatment stimulates matura-
tion of cystic epithelia.

BALB/c-cpklcpk mice, like many other models of PKD, had
dramatically reduced expression of EGF (5–7,15), whereas the
EGF-R mRNA expression was correspondingly upregulated.
After treatment with exogenous EGF, EGF mRNA expression
remained diminished, whereas EGF-R mRNA expression was
downregulated. Others have shown that EGF-R is decreased
after addition of EGF to renal epithelial cells (30) and endo
cytes (31) in vitro. Our data are consistent with an EGF-
induced downregulation of EGF-R in the EGF-treated mice
similar to other systems. In this study, EGF treatment of
neonatal BALB/c-cpklcpk mice also led to a reduction in renal
as well as common and hepatic biliary duct pathology. How-
ever, EGF treatment did not alter the pancreatic pathology or
the expression of c-myc mRNA in the kidney. Because the
pancreatic change is prominent at birth, it is not surprising that
EGF was ineffective in preventing this pathology. In general,
neonatal EGF treatment seems to partially ameliorate aspects
of ARPKD, whereas its effect later in the cystic process may be
different or even opposite what was seen neonatally. These
findings suggest that EGF may function differently in various
aspects and/or phases of PKD (54). Glucocorticoids given
neonatally cause an infantile form of PKD (32) but when given
later in the disease process actually ameliorates PKD (55).
Neonatal kidneys do not necessarily respond to agents in the
same way as mature kidneys do. Therefore, caution should be
exercised with any PKD intervention because the effect may be
dependent on the stage of kidney development.

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