Role of CFTR in Autosomal Recessive Polycystic Kidney Disease

KOICHI NAKANISHI, WILLIAM E. SWEENEY, JR., KATHERINE MACRAE DELL, CALVIN U. COTTON, and ELLIS D. AVNER

Rainbow Center for Childhood PKD, Department of Pediatrics, Rainbow Babies and Children's Hospital and Case Western Reserve University, Cleveland, Ohio.

Abstract. An extensive body of *in vitro* data implicates epithelial chloride secretion, mediated through cystic fibrosis transmembrane conductance regulator (CFTR) protein, in generating or maintaining fluid filled cysts in MDCK cells and in human autosomal dominant polycystic kidney disease (ADPKD). In contrast, few studies have addressed the pathophysiology of fluid secretion in cyst formation and enlargement in autosomal recessive polycystic kidney disease (ARPKD). Murine models of targeted disruptions or deletions of specific genes have created opportunities to examine the role of individual gene products in normal development and/or disease pathophysiology. The creation of a murine model of CF, which lacks functional CFTR protein, provides the opportunity to determine whether CFTR activity is required for renal cyst formation *in vivo*. Therefore, this study sought to determine whether renal cyst formation could be prevented by genetic complementation of the BPK murine model of ARPKD with the CFTR knockout mouse. The results of this study reveal that in animals that are homozygous for the cystic gene (*bpk*), the lack of functional CFTR protein on the apical surface of cystic epithelium does not provide protection against cyst growth and subsequent decline in renal function. Double mutant mice (*bpk* −/−; *cftr* −/−) developed massively enlarged kidneys and died, on average, 7 d earlier than cystic, non-CF mice (*bpk* −/−; *cftr* +/±). This suggests fundamental differences in the mechanisms of transtubular fluid secretion in animal models of ARPKD compared with ADPKD.

Polycystic kidney disease (PKD) is characterized by the massive enlargement of fluid-filled renal tubular and/or collecting duct cysts. Progressively enlarging cysts compromise normal renal parenchyma, eventually leading to renal failure. Autosomal dominant polycystic kidney disease (ADPKD) is caused by mutations in one of three genes (1,2), and autosomal recessive polycystic kidney disease (ARPKD) is caused by mutations in a single gene on chromosome 6 (3,4). Despite the impressive advances in genetic and molecular descriptions of PKD gene products, the normal cellular function of these proteins and their role in cyst formation and growth are unknown.

Studies using a variety of experimental models have identified three key pathophysiologic factors in cyst formation and progressive enlargement. A normal renal tubule can become cystic if (*1*) hyperplasia, localized to a distinct portion of a tubule, requires expansion to accommodate increasing cell numbers; (*2*) abnormal secretion leads to accumulation of intratubular fluid causing tubular dilatation; and (*3*) extracellular matrix abnormalities create an abnormal microenvironment for epithelial cell function (1,5,6).

Secretion is a critical pathogenic mechanism associated with cyst formation and growth in PKD. Fluid secretion, coupled with epithelial hyperplasia, is necessary and sufficient to account for the dynamics of cyst growth (7). In ADPKD, a large number of cystic lesions lack afferent and efferent tubule connections, suggesting that cysts, which arise from tubular segments, become disconnected from the influx of glomerular filtrate. The development and expansion of cystic lesions therefore requires net transtubular secretion (8). Transformation of normally absorptive epithelial cells into secretory epithelium is therefore fundamental in the development and expansion of renal cysts.

An extensive body of *in vitro* data implicates epithelial chloride secretion in generating or maintaining fluid-filled cysts in ADPKD. The cystic fibrosis transmembrane conductance regulator (CFTR) protein has been identified as a cyclic adenosine monophosphate (cAMP)-regulated chloride channel on the apical surface of many secretory epithelia. Chloride secretion through the CFTR has been implicated in the pathway of fluid secretion in ADPKD. *In vitro* experiments suggest that increased cAMP-mediated chloride secretion provides the electrochemical driving force, which mediates fluid secretion in cystic epithelia (9,10). Studies involving primary epithelial cultures from ADPKD cysts demonstrate that CFTR is expressed on the apical surface of cystic epithelia and that the electrochemical properties of forskolin-induced chloride secretion are identical to those of CFTR-mediated transport (11,12).

Analysis of intact isolated cysts, monolayer culture, and clonally derived microcysts from ADPKD kidneys demonstrate that these epithelial cells secrete fluid in response to adenylate cyclase agonists, cAMP analogues, and cyst fluid (9). It has been assumed that fluid secretion by cystic ARPKD epithelium is mediated by similar, if not identical, mechanisms.

Murine models of targeted disruptions or deletions of specific genes have created new opportunities to examine the role of individual gene products in normal development and/or disease pathophysiology. The creation of a murine model of CF, which lacks functional CFTR protein, provides the opportunity to determine whether CFTR activity is required for renal cyst formation *in vivo*. Therefore, we sought to determine whether renal cyst formation could be prevented by genetic complementation of the BPK murine model of ARPKD with the CFTR knockout mouse.

Materials and Methods
Animals

Balb/c-bpk/bpk Model.   The BPK model, a murine model of ARPKD, arose as a spontaneous mutation in an inbred colony of Balb/c mice. This model has been characterized extensively in our laboratory (13,14) and maintained for 8 yr without significant phenotypic drift. Homozygous *bpk* mice develop massively enlarged kidneys and die of renal failure at an average postnatal age of 24 d. Extrarenal manifestations include biliary proliferation and biliary ductal ectasia (BDE). Because of the recessive nature of this disease, wild-type +/+ and heterozygous *bpk* +/− mice are phenotypically normal.

CFTR Mouse Model.   CFTR knockout mice were generated from embryonic stem cells in which the CF gene was disrupted by gene targeting (15). The absence of functional CFTR in the animal model for CF (CFTR S489X or UNC) used in this study has been demonstrated extensively by Western analysis, ribonuclease protection assay, electrophysiologic analysis, and PCR analysis. The results demonstrate that when both alleles of CFTR are disrupted, CFTR −/− animals do not produce a normal CFTR mRNA and lack cAMP-regulated chloride channels in all tissues tested. Mice that are homozygous for the disrupted gene demonstrate absence of cAMP-activated chloride secretion in all epithelia tested (16) and display many features that are common to young CF patients, such as failure to thrive and meconium ileus, as well as pulmonary and pancreatic abnormalities. Although CFTR function was not measured directly in these mice, all mice used in this study were negative for *cftr* gene expression by PCR, the most sensitive method of identifying the presence of wild-type or mutant CFTR. Because of a propensity for these animals to develop intestinal obstruction when given a diet of solid food, these animals were fed a standard liquid diet (17). Previously published studies regarding survival of the CFTR −/− mice demonstrated dramatic improvement in survival when these mice were housed on corncob bedding and fed a low-residue liquid diet (Peptamin-Clintec Nutrition, Deerfield, IL). Therefore, once double heterozygous (bpk +/−; cftr +/−) breeding pairs were identified, they were maintained on Peptamin and housed on corn-cob bedding as were the offspring of successful pregnancies. All offspring, regardless of genotype, were housed on corncob bedding, and the mother was maintained on Peptamin while nursing the pups (17).

Generation of Double Homozygous Mice and Cystic Controls. Matings between female mice that were heterozygous for the *bpk* mutation and male mice that were homozygous for the CF mutation

(*cftr* −/−) generated compound heterozygous mice that were then used to generate double mutant mice. Mice that were homozygous for the *bpk* mutation were identified phenotypically, whereas CF mice (*cftr* −/−) were identified genotypically by PCR (16).

Histology, Immunohistology, and Determination of Segmental Nephron Cyst Localization

Kidney and liver tissues from five double homozygous mice (*bpk* −/− and *cftr* −/−), nine cystic, non-CF (*bpk* −/− and *cftr* +/±), and six control mice (*bpk* +/± and *cftr* +/±) were harvested for morphometric analysis at postnatal day 14. All kidney and liver tissues were weighed and fixed in 4.0% paraformaldehyde in phosphate buffer (pH 7.4) for 30 min at 4°C. Tissues were then washed, dehydrated through graded acetone, and embedded in Immunobed plastic embedding medium (Polysciences, Warrington, PA). Sections were cut at 4 μm on a Sorvall ultramicrotome (purchased from Energy Beam Science, Inc., Agawam, MA), mounted on glass slides, and stained with hematoxylin (all tissues) or segment-specific lectins (kidney only). Segmental nephron cystic localization and the cystic index were quantified by combining morphometric analysis with light microscopy and immunohistologic techniques (13,18–21). Cyst localization was determined by segment-specific lectin binding using Dolichos biflorus agglutinin (DBA) as a marker for collecting tubules (CT) and Lotus tetragonolobus (LTA) as a marker for proximal tubules (PT) (13,20,21). The immunohistology procedure used was our previously described postembedded staining technique developed specifically for localization of antigens and lectins in plastic sections (22). Sections 4 μm thick were etched in a solution of 95% ethanol and 1.5% NaOH for 8 min, rinsed 2× in 95% ethanol for 2 min, and rinsed briefly in H₂O. The sections were then trypsinized and incubated overnight at 4°C with biotinylated lectins (4 μg/ml for LTA and 6 μg/ml for DBA), followed by incubation with extraviden peroxidase (1:400) for 90 min at room temperature. Sections were then stained with 0.05% diaminobenzidine and 0.01% hydrogen peroxide for 10 min and were counterstained with hematoxylin.

Lectin Profile Analysis of Cyst Formation and Modified Renal Cystic Index

After immunohistologic preparation, LTA and DBA lectin profile analyses were performed on the kidney sections. The numbers of LTA-positive and DBA-positive cysts were counted in serial sections of bisected whole-mount kidneys from each animal, without knowledge of the animal genotype. PT cysts were identified by LTA binding, and CT cysts were identified by DBA binding. A minimum of 10 sets of serial sections evenly spaced through the kidney from the cortex to the inner medulla were used to determine the ratio of PT to CT cysts. The ratio of CT cysts to PT cysts in each section was calculated by dividing the number of DBA-positive cysts by the number of LTA-positive cysts. The degree of tubular cyst formation was quantified by use of a modified cystic index (21). The cystic index is derived from basic light microscopic morphometric methods and has been standardized to quantify cyst formation *in vivo* and *in vitro* (19). After lectin staining, serial bisected whole-mount kidney sections were graded for cyst formation in PT and CT segments (LTA positive and DBA positive) on the following scale: 0, no cysts observed; 1, single or multiple cysts >0.05 mm but ≤0.30 mm; 2, single or multiple cysts >0.30 mm but ≤0.60 mm; 3, single or multiple cysts >0.60 mm but ≤0.90 mm; 4, single or multiple cysts >0.90 mm but ≤1.20 mm; 5, single or multiple cysts >1.20 mm.

Hepatic BDE and Hyperplasia

After routine histologic preparation, eight evenly spaced (at least 80 μm apart), 4-μm-thick, hematoxylin-stained liver sections were graded (0 through 4) for BDE and biliary epithelial proliferation using the following scale (21): 0, all portal triads have normal biliary ductal profiles; 1, $\leq$15% of portal triads have ectatic biliary ducts and biliary epithelial hyperplasia; 2, >15% but $\leq$30% of portal triads have ectatic biliary ducts and biliary epithelial hyperplasia; 3, >30% but $\leq$50% of portal triads have ectatic biliary ducts and biliary epithelial hyperplasia; 4, >50% of portal triads have ectatic biliary ducts and biliary epithelial hyperplasia.

Analysis of Renal Function and Maximal Urinary Concentrating Ability

Animals were deprived of liquid for 12 h before the collection of urine samples for urine osmolarity measurements. Blood samples for serum blood urea nitrogen (BUN) and creatinine were obtained by orbital sinus collection. Serum BUN and creatinine were quantitatively determined using colorimetric-based assays from Sigma Diagnostics (Sigma Co., St. Louis, MO).

Survival Studies

The average life span of BPK cystic mice is 24 d (21). To determine the life span of double homozygotes, we closely monitored 12 animals (6 double homozygous mice (*bpk* $-/-$; *cftr* $-/-$) and 6 cystic controls (*bpk* $-/-$; *cftr* $+/\pm$) until animals demonstrated premorbid behavior including listlessness, especially when touched, and head drooping (20). The animals were then killed, and kidney and liver tissues were harvested for histologic analysis.

Results

Kidney Size and Survival

Double mutant mice (*bpk* $-/-$; *cftr* $-/-$) developed massively enlarged kidneys and died, on average, by postnatal day 17. Cystic control animals (cystic, non-CF, *bpk* $-/-$; *cftr* $+/\pm$) survived on average for an additional 7 d, to postnatal day 24, as described previously (21). Data for mortality rates were not recorded until it became apparent that the bpk-CF double mutants died prematurely compared with the bpk–non-CF animals. The premature death of the double mutants would have been unexpected if the lack of functional CFTR ameliorated the growth of the cystic lesions in these animals. It was obvious by the enlarged flanks of these double mutants that these animals were still developing large kidneys. Declining renal function, superimposed on the well-described fragility of CF mice, would increase the likelihood of premature death. Because one of the internal controls that we established for this study was to compare only bpk–non-CF and bpk-CF litter mates, we genotyped only litters that did have a loss of pups during first week. A total of 76 pups were genotyped as cftr $-/-$; 8 of these cftr $-/-$ pups (11%) died before day 14, the day of analysis. All analyses were performed on postnatal day 14 animals because of the decreased survival of the double mutants. Double mutant (*bpk* $-/-$; *cftr* $-/-$) kidneys were as large as or larger than cystic controls (*bpk* $-/-$; *cftr* $+/-$) and considerably larger than kidneys from control animals (*bpk* $+/\pm$; *cftr* $+/\pm$; Figure 1). As seen in Table 1, kidney weight of control (*bpk* $+/\pm$; *cftr* $+/\pm$) kidneys is 1.5% of the total
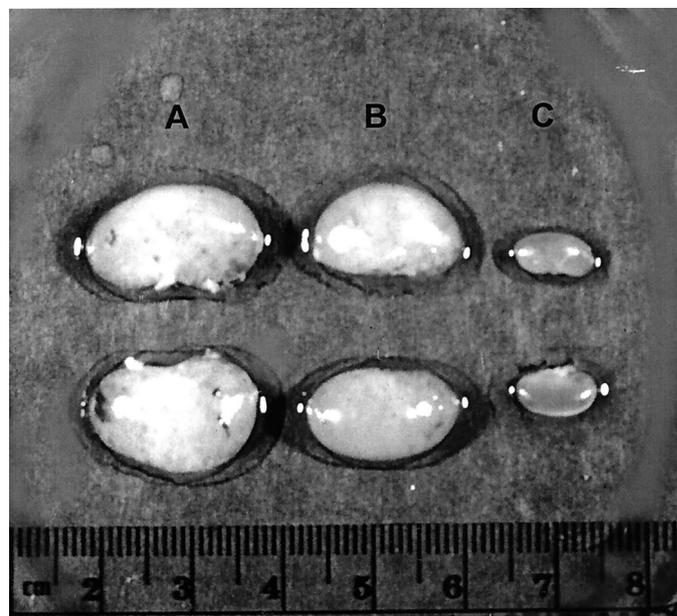

Figure 1. Whole kidney micrograph from day 14 double mutant animals (bpk $-/-$; cftr $-/-$) (A), cystic, non-CF animals (bpk $-/-$; cftr $+/-$) (B), and control animals (bpk $+/\pm$; cftr $+/-$) (C). The photograph demonstrates that both cystic specimens are considerably larger than the control kidneys.

body weight. This is significantly less than the kidney weight as a percentage of body weight of 15.3 $\pm$ 3.0% for the double homozygotes (*bpk* $-/-$; *cftr* $-/-$) and 14.3 $\pm$ 2.8% for the cystic, non-CF animals (*bpk* $-/-$; *cftr* $+/-$).

Morphometric Analysis: Lectin Profiling of Renal Cyst Formation and Modified Cystic Index

At postnatal day 14, the ratio of CT cysts to PT cysts for the double mutants (*bpk* $-/-$; *cftr* $-/-$) was 1.4 $\pm$ 0.1 (Table 1). This was identical to the CT/PT ratio of cystic control animals (*bpk* $-/-$; *cftr* $+/-$), indicating that the cyst localization profile of BPK mice was unchanged by the lack of CFTR.

The cystic index, a measure of the size of the cystic lesions, also revealed no statistical difference between the double mutants and the cystic, non-CF controls (Table 1). The PT cystic index for the double mutants (*bpk* $-/-$; *cftr* $-/-$) was 2.0 $\pm$ 0.7 with the cysts ranging in size from 0.05 to 0.63 mm. The PT cystic index for cystic, non-CF animals (*bpk* $-/-$; *cftr* $+/\pm$) was 2.1 $\pm$ 0.6 with a cysts ranging in size from 0.05 to 0.65 mm. The CT cystic index for the double mutants was 3.4 $\pm$ 0.9 with a maximum cyst size of 1.98 mm compared with a CT cystic index for cystic, non-CF animals (*bpk* $-/-$; *cftr* $+/\pm$) of 3.8 $\pm$ 1.0 with a maximum cyst size of 1.75 mm.

Figure 2 demonstrates the severity of the cystic lesions in both the double mutant kidneys and the cystic, non-CF animals. At postnatal day 14, normal renal parenchyma is scarce in both the double mutants (*bpk* $-/-$; *cftr* $-/-$; Figure 2A) and the cystic, non-CF controls (*bpk* $-/-$; *cftr* $+/-$; Figure 2B).

Table 1. Renal and biliary morphometrics

Genotype	KW/BW (%)	CT/PT Cyst Ratio (DBA (+) Cysts/ LTA (+) Cysts)	PT CI/CT CI	BDE
bpk −/−; cftr −/− (n = 5) BW(g) = 4.8 ± 0.9	15.3 ± 3.0	1.4 ± 0.1	2.0 ± 0.7/3.4 ± 0.9	2.6 ± 0.3
bpk −/−; cftr +/− (n = 9) BW(g) = 7.6 ± 2.1	14.3 ± 2.8	1.4 ± 0.2	2.1 ± 0.6/3.8 ± 1.0	2.5 ± 0.2
bpk +/+; cftr +/− (n = 30) BW(g) = 7.5 ± 2.1	1.5 ± 0.1	–	–	–
bpk +/±; cftr −/− (n = 26) BW(g) = 7.3 ± 1.5	1.5 ± 0.1	–	–	–

KW/BW, kidney weight as a percentage of body weight; CT, collecting tubule; PT, proximal tubule; DBA, Dolichos biflorus agglutinin; LTA, Lotus tetragonolubus; BDE, biliary ductal ectasia.

Biliary Ductal Ectasia

Morphologically, the livers from the double mutants and the cystic controls were indistinguishable. There was no statistically significant difference in the BDE index, a quantitative measure of the biliary ductal abnormality associated with the *bpk* gene (21) between the double mutants and the cystic, non-CF animals (Table 1).

Renal Function

The data in Table 2 demonstrate that both renal function and maximal urinary concentration were equally impaired in both double mutants and cystic, non-CF animals and significantly different from control mice (*bpk* +/±; *cftr* +/±). Serum BUN values of the double mutants and cystic, non-CF controls were 4 to 5 times higher than in control mice ($P < 0.02$). Maximal urinary osmolarity in double mutants and cystic, non-CF animals was reduced to approximately 40% of control mice ($P < 0.05$).

Discussion

The first systematic studies that investigated the role of fluid secretion in cyst formation began with the observations by McAteer *et al.* (23) that MDCK cells seeded within a three-dimensional matrix formed fluid-filled cysts. The only source of this cyst fluid was secretion by the cyst-lining cells. The use of these unique MDCK cells facilitated the development of techniques designed to elucidate mechanisms of fluid secretion by renal cystic epithelium. Studies of ADPKD cells in primary culture demonstrated fluid secretion stimulated by cAMP and by agonists of adenylyl cyclase (9). This, as well as direct measurement of ion concentrations, led to the conclusion that fluid secretion by ADPKD cystic epithelia was driven by chloride secretion (24) and that a likely mediator of this secretion was CFTR (25,26).

The CFTR chloride channel is a major component of transport systems involved in chloride and fluid secretion by secretory epithelia (27). CFTR is regulated by cAMP, which stimulates protein kinase A–mediated phosphorylation and membrane expression of CFTR (11). Although highly expressed in human fetal kidneys, CFTR is downregulated in adult kidneys. CFTR has been identified, at low levels, on the luminal membrane of normal proximal and distal tubules. However, it is highly expressed on the apical membrane of epithelial cells that line approximately half of the tubular cysts
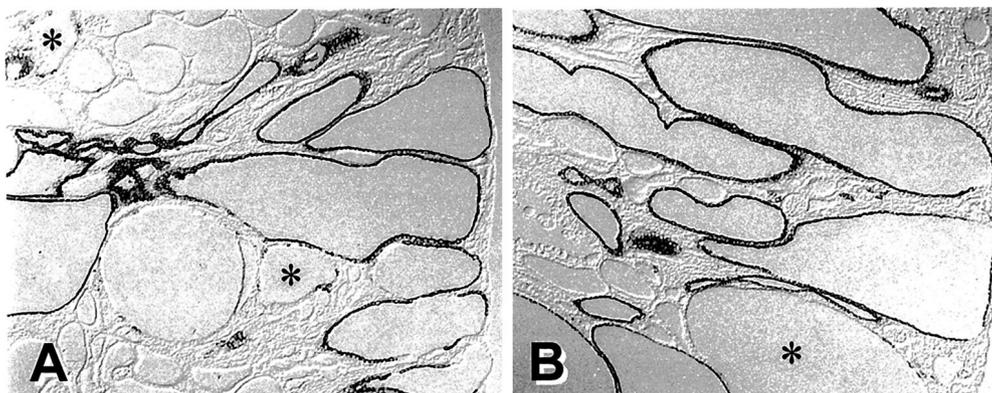

Figure 2. Lectin binding of day 14 kidney from double mutant (bpk −/−; cftr −/−) (A) and cystic non-CF (bpk −/−; cftr +/−) (B) animals. Segment-specific lectin binding with biotin-labeled Dolichos biflorus agglutinin (DBA; brown-stained cysts) demonstrates that both double mutants and cystic, non-CF animals developed large collecting tubule cysts, at equivalent rates, which compress normal renal parenchyma and ultimate lead to renal failure. Unstained cysts in this micrograph are proximal in origin (Lotus tetragonolobus [LTA]-positive in serial sections) with the exception of the cysts marked with an asterisk. These cysts are neither LTA nor DBA positive. Magnification, ×250.

Table 2. Renal function

Genotype	BUN (mg/dl)	Creatinine (mg/dl)	Maximal Urine Osmolarity (mOsm/L)
bpk −/−; cftr −/− (n = 5)	88 ± 29	0.5 ± 0.1	395 ± 70
bpk −/−; cftr +/− (n = 9)	71 ± 13	0.5 ± 0.1	419 ± 55
bpk +/±; cftr −/− (n = 4)	20 ± 4	0.2 ± 0.1	1115 ± 55
bpk +/±; cftr +/− (n = 6)	19 ± 4	0.2 ± 0.1	1104 ± 48

BUN, blood urea nitrogen.

in ADPKD kidneys (28,29). CFTR is also expressed in the apical membrane of ADPKD primary monolayer cultures and intact cysts dissected from ADPKD kidneys (25,30–32).

Treatment of the basolateral surface of ADPKD cystic epithelial cells with bumetanide inhibited short-circuit current and fluid secretion, suggesting that a transporter belonging to the bumetanide-sensitive Na-K-2Cl cotransporter family was responsible for basolateral chloride influx. The identification of the BSC2 isoform on the basolateral surface of CFTR-positive ADPKD cysts confirmed the presence of a basolateral Na-K-2Cl cotransporter in cysts from ADPKD kidneys (32). In ADPKD, it seems that transepithelial chloride secretion is mediated by basolateral entry into the cell via the BSC2 cotransporter and apical extrusion through the CFTR. In parallel with chloride movement, cations (particularly Na^+), driven through the paracellular pathway by the transepithelial voltage difference, accumulate within the cyst lumen.

An important role for CFTR in ADPKD secretion was also supported by the observation that interference with CFTR protein production (by treatment of ADPKD monolayers with antisense oligonucleotide against human CFTR) dramatically reduced fluid secretion by these cells (11). The tissue localization and functional data of these studies provide clear evidence for involvement of CFTR in chloride and fluid secretion by ADPKD cystic epithelium *in vitro*. Additional evidence supporting a role for CFTR in chloride secretion was obtained from immortalized CT cell lines isolated from CFTR mutant and CFTR wild-type mice. Wild-type cell lines formed numerous fluid-filled cysts in response to epidermal growth factor (EGF) and forskolin, when cultured in three-dimensional collagen gels, whereas CFTR mutant cell lines failed to form cysts under identical conditions (33). These results demonstrated that CFTR is required for cAMP-stimulated *in vitro* cyst formation.

Recent data concerning the role of CFTR in the progression of ADPKD *in vivo* suggest that CFTR may modulate cyst enlargement. In a single family affected with both ADPKD and CF, individual members with both ADPKD and CF had less severe renal disease than those family members with only ADPKD (34,35). These results suggest that *in vivo*, a mutant CFTR protein provides partial protection against renal cyst development and enlargement and suggests that modulation of epithelial chloride secretion may have therapeutic benefit in PKD.

Despite the extensive *in vitro* data regarding CFTR-medi-

ated chloride secretion in MDCK cells and human ADPKD epithelia, there are few data regarding the mechanisms of transtubular fluid secretion in ARPKD. It has been reasoned, by analogy, that the abnormal secretion of ARPKD epithelia is mediated by cAMP and CFTR-mediated chloride secretion, similar to that demonstrated in ADPKD epithelia.

In the current study, we were able to examine directly the effects of CFTR on cyst formation *in vivo* by genetic complementation of the BPK model of ARPKD with the CFTR knockout mouse. The results of this study reveal that in animals that are homozygous for a mutant cystic gene (*bpk*), the lack of functional CFTR protein on the apical surface of cystic epithelium does not provide protection against cyst growth and subsequent decline in renal function. Double mutant mice (*bpk* −/−; *cftr* −/−) developed massively enlarged kidneys and died, on average, 7 d earlier than cystic, non-CF mice (*bpk* −/−; *cftr* +/±). The kidney weight to body weight ratio of the double mutants was equivalent to the cystic, non-CF mice as was the ratio of CT to PT cysts. The maximum size of the cystic lesions in the double mutants (*bpk* −/−; *cftr* −/−) was slightly larger, though not statistically different, than the maximum size of the cystic lesions in the cystic, non-CF control animals (*bpk* −/−; *cftr* +/±). The most important measure of disease progression, renal function, showed no improvement as a result of the absence of CFTR. Serum BUN and creatinine levels of the double mutants (*bpk* −/−; *cftr* −/−) and cystic, non-CF animals (*bpk* −/−; *cftr* +/±) were equivalent and significantly higher than values of the control animals (*bpk* +/±; *cftr* +/±). Maximal urine osmolarity values in the double mutants and in the cystic, non-CF animals were significantly decreased from control mice ($P < 0.05$), indicating an inability to concentrate urine and a similar degree of CT disease. These results demonstrate that the absence of CFTR does not alter the course of cystic disease in *bpk* mice.

There are several possible explanations for why the loss of CFTR does not alter the course of cystic disease in the current study:

1. Alternative non-CFTR apical membrane chloride channels may compensate for the loss of CFTR and support fluid secretion by cystic epithelial cells (36). Because the renal deficit in CF is not as profound as in the pancreas or small intestine of CF patients (37), alternative chloride secretory mechanisms probably exist to compensate for loss of CFTR function (38). Ion transport studies in renal epithelial cells

demonstrate clearly the expression of non-CFTR chloride channels in the apical cell membrane (38). These channels include ClC chloride channels (39), pI_{cLn}, a chloride channel associated with cell swelling (40,41), and a calcium-activated chloride channel in murine renal inner medullary collecting duct cells (38,42). The expression and/or activity of such channels may be increased in renal tubules of CFTR knockout animals.

2. The immature state of cystic renal epithelia may unmask a constitutively activated embryonic chloride channel not normally active in mature renal epithelia (41).

3. Chloride secretion may play less of a role in ARPKD compared with ADPKD because of anatomic differences of cystic lesions in the two forms of PKD. Elongated ectatic nephron segments that are characteristic of murine models and human ARPKD may be fundamentally different from the detached spheroid, cystic structures found in human ADPKD (43). If the enlarged nephron remains in contact with the glomerulus, then the importance of fluid secretion to drive cyst expansion may be reduced.

4. Alterations in epithelial fluid absorption may be more crucial than chloride secretion in ARPKD. The dominant absorptive pathway for salt and water in the CT is amiloride-sensitive sodium absorption. We recently demonstrated that amiloride-sensitive sodium absorption is downregulated dramatically in an immortalized murine CT cell line during chronic exposure to EGF (44). Because the EGF receptor is overexpressed and mislocalized to the apical membrane of ARPKD (and ADPKD) cells (45), EGF that is present in urine and cyst fluid may promote chronic inhibition of sodium absorption and contribute to tubule enlargement independent of active chloride secretion.

In summary, renal cyst formation in a murine model of ARPKD does not require functional activity of CFTR. A number of mechanisms may explain the current findings that loss of CFTR did not affect the progression of cystic disease in the BPK model of ARPKD. Detailed studies of transport pathways in ARPKD cystic epithelial cells will be required to determine the relative importance of alterations in secretory and/or absorptive transport pathways in ARPKD cyst growth. Such studies may provide insight into the development of new therapeutic interventions to decrease cyst formation and enlargement in ARPKD.

Acknowledgments

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