THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 290, NO. 25, pp. 15679–15686, June 19, 2015

Exploitation of the Polymeric Immunoglobulin Receptor for Antibody Targeting to Renal Cyst Lumens in Polycystic Kidney Disease*

Received for publication, August 27, 2014, and in revised form, April 21, 2015 Published, JBC Papers in Press, April 28, 2015, DOI 10.1074/jbc.M114.607929

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Background: Mitogenic cyst fluid in polycystic kidney disease is not accessible to therapeutic IgG antibodies.
Results: STAT6 drives expression of the polymeric immunoglobulin receptor, which can transcytose dimeric IgA from the circulation into cyst fluid.
Conclusion: Dimeric IgA antibodies target to renal cyst lumens.
Significance: Therapeutic antibodies, reformatted to dIgA, could be evaluated for the treatment of polycystic kidney disease.

Autosomal-dominant polycystic kidney disease (ADPKD) is a common life-threatening genetic disease that leads to renal failure. No treatment is available yet to effectively slow disease progression. Renal cyst growth is, at least in part, driven by the presence of growth factors in the lumens of renal cysts, which are enclosed spaces lacking connections to the tubular system. We have shown previously shown that IL13 in cyst fluid leads to aberrant activation of STAT6 via the IL4/13 receptor. Although antagonistic antibodies against many of the growth factors implicated in ADPKD are already available, they are IgG isotype antibodies that are not expected to gain access to renal cyst lumens. Here we demonstrate that targeting antibodies to renal cyst lumens is possible with the use of dimeric IgA (dIgA) antibodies. Using human ADPKD tissues and polycystic kidney disease mouse models, we show that the polymeric immunoglobulin receptor (pIgR) is highly expressed by renal cyst-lining cells. pIgR expression is, in part, driven by aberrant STAT6 pathway activation. pIgR actively transports dIgA from the circulation across the cyst epithelium and releases it into the cyst lumen as secretory IgA. dIgA administered by intraperitoneal injection is preferentially targeted to polycystic kidneys whereas injected IgG is not. Our results suggest that pIgR-mediated transcytosis of antagonistic antibodies in dIgA format can be exploited for targeted therapy in ADPKD.

Autosomal-dominant polycystic kidney disease (ADPKD)[2] is a common genetic disease, affecting over 12 million people worldwide, caused by mutations in the genes coding for polycystin-1 (PC1) or polycystin-2 (PC2) (1–3). Excessive proliferation of renal tubule epithelial cells leads to the growth of renal cysts, which eventually destroys the normal renal parenchyma, resulting in renal failure in at least 50% of patients. No approved treatment is available to slow disease progression, although this is an active area of research.

Numerous signaling molecules and pathways have been shown to be aberrantly activated in cyst-lining epithelial cells. This may be caused, at least in part, by the presence of several growth factors that are detectable in cyst fluid including EGF, hepatocyte growth factor, ouabain, TGF-α, TGF-β, TNF-α, and IL-1β (4–10). Renal cysts in ADPKD are enclosed spaces that have typically lost their connection to the tubular system (3). Therefore, any growth factors secreted into the luminal space will be trapped and can potentially lead to persistent auto/paracrine activation of the cyst-lining cells that may express the corresponding receptors on their apical surfaces.

Previous work from our laboratory has indicated that PC1 can regulate the activity of the transcription factor STAT6 (11) and that aberrant activation of the STAT6 pathway contributes to renal cyst growth (12). STAT6 is typically activated by the cytokines IL4 or IL13, which bind to the heterodimeric IL4/13 receptor (13). Our results indicated that activation of STAT6 in cyst-lining cells causes a positive feedback loop involving the secretion of IL13 into cyst fluid and IL4/IL13 receptor activation at apical membranes of cyst-lining epithelial cells. Gene ablation of STAT6 or use of the pharmacological inhibitor leflunomide lead to reduced renal cyst growth in a PKD mouse model (12).

Because of its non-specificity toward STAT6 and its side effect profile, leflunomide is unlikely to be useful as a clinical therapy for ADPKD. To our knowledge, no small-molecule specific inhibitors of STAT6 are presently available. Highly specific inhibition of signaling pathways can often be achieved by the use of antagonistic antibodies against growth factors or their receptors. For example, antibodies against IL13 or the IL4/13 receptor are currently being tested for asthma therapy. Similarly, antibodies against other growth factors implicated in the

* This work was supported, in whole or in part, by National Institutes of Health Grant R01 DK62338 and a gift from the Lillian Goldman Charitable Trust (to T. W.). This work was also supported by a Swiss National Science Foundation early postdoctoral mobility grant (to M. R). The authors declare that they have no conflicts of interest with the contents of this article.
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[2] The abbreviations used are: ADPKD, autosomal-dominant polycystic kidney disease; PKD, polycystic kidney disease; pIgR, polymeric Ig receptor; pIg, polymeric Ig; SC, secretory component; dIgA, dimeric IgA; mIgA, monomeric IgA; qPCR, quantitative PCR.

pathogenesis of ADPKD, such as EGF and TNF-α, are used for cancer or autoimmune disease therapy (14, 15). Such antagonistic antibodies may potentially be effective for the treatment of ADPKD. Given that highly effective antibodies against numerous promising targets are already available or under development as therapeutics for other indications, the time seems ripe to evaluate and repurpose them for ADPKD therapy.

However, if aberrant activation of a targeted signaling pathway in ADPKD indeed involves growth factor/receptor interaction between the cyst fluid and the apical plasma membrane of cyst-lining cells, then an antagonistic antibody would need to be present in cyst fluid to be effective. Of the five different immunoglobulin isotypes, the biopharmaceutical industry has almost exclusively focused on developing IgG antibodies as therapeutics because of their long half-life in serum and established production technology. Almost all of the approved antibody therapeutics are human IgG1 antibodies, with relatively few being IgG2 or IgG4 (16). Although IgG antibodies are ideal for targets that are accessible via the circulatory system and interstitial fluids, it appears unlikely that IgG antibodies would effectively gain access to the lumens of renal cysts in ADPKD. Therefore, the use of IgG antibodies for ADPKD therapy is not promising.

Here, we report an alternative strategy and exploit the polymeric immunoglobulin receptor (pIgR) to overcome this obstacle. The pIgR is a transmembrane protein that is expressed in many mucosal epithelial cell types. pIgR present at the basolateral plasma membrane can bind to polymeric immunoglobulins (pIgs) of the IgA and IgM isotype. Upon binding, the pIgR-pIg complex undergoes transcytosis across the cell to the apical membrane, where the extracellular region of pIgR is proteolytically cleaved. This releases the pIg in a complex with the extracellular portion of pIgR termed secretory component (SC) (17). Secretory IgA, the complex of dimeric IgA (dIgA) and the SC, is the major antibody isotype in external secretions, such as the intestinal lumen, saliva, milk, and bile, protecting the mucosal environment from infectious agents such as bacteria, viruses, fungi, and parasites (18).

dIgA is composed of two monomeric IgA (mIgA) subunits linked together by disulfide bonds with the so-called J chain. Because pIgR recognizes the J chain of dIgA, only dIgA, but not mIgA, can be transcytosed. dIgA is typically produced by plasma cells located in the lamina propria, near the basolateral surface of mucosal epithelia. pIgR has been found in mouse and rat kidney tubule epithelial cells (19), and pIgR expression can be regulated by water deprivation, vasopressin administration, or renal ischemia-reperfusion in rats (20, 21). dIgA can be found in urine, suggesting that it can reach the urinary space by pIgR-mediated transcytosis (21). pIgR expression has been shown to be regulated by IL-4, TNF-α, and IFN-γ in airway, intestinal, and mammary gland epithelial cells (22), and a STAT6 binding domain has been identified in intron 1 of the pIgR gene (23, 24). Altogether, these data suggest that the kidney can use the dIgA/pIgR system and that it can be up-regulated to protect the urinary space against pathogens.

Because we found previously that STAT6 is activated in cyst-lining cells in PKD, we hypothesized that this may lead to increased pIgR expression and that pIgR could be exploited to transport dIgA across the epithelium into the cyst lumens. Here we show that pIgR is indeed highly expressed in renal cysts and is processed into the SC, indicative of active transcytosis. Consequently, murine and human cyst fluids contain high levels of dIgA. When dIgA is injected into mice, it accumulates preferentially in polycystic kidneys compared with normal kidneys. In contrast, very little injected IgG accumulates in polycystic kidneys. These results indicate that therapeutic antibodies can be targeted by pIgR-mediated transcytosis to the lumens of renal cysts if they are in the dIgA format. Because renal cysts are enclosed spaces, dIgA antibodies are expected to accumulate in their lumens over time, whereas they are eliminated rapidly from other secretions and the circulation. Therefore, pIgR-mediated targeting of dIgA antibodies may be a highly promising approach for ADPKD therapy and is expected to lead to high specificity toward the target organ.

Experimental Procedures

Animals—The Institutional Animal Care and Use Committee of the University of California at Santa Barbara approved all animal experiments. Pkd1$^{cond/cond}$, Pkd1$^{cond/cond}$:Nestin^{cre}, and WT/bpk colonies were maintained under standard vivarium conditions. STAT6$^{-/-}$ animals on a BALB/c background were obtained from The Jackson Laboratory (Bar Harbor, ME) and crossed with the WT/bpk animals as described previously (12).

Antibodies—Goat anti-mouse pIgR antibody was obtained from R&D Systems, Inc. (Minneapolis, MN). Rabbit anti-human IgA and mouse anti-β-actin antibody were from Sigma-Aldrich (Saint Louis, MO). Mouse anti-pIgR (C terminus, catalog no. SC166) and guinea pig anti-SC were provided by Keith Mostov (University of California San Francisco) (25). HRP- and fluorescence-conjugated secondary antibodies were obtained from Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA) and Santa Cruz Biotechnology, Inc. (Dallas, TX). Rhodamine-conjugated *Dolichos biflorus* agglutinin was from Vector Laboratories, Inc. (Burlingame, CA).

Human Samples—Normal and ADPKD kidney samples were obtained through the National Disease Research Interchange.

In Vivo Immunoglobulin Injection—10 μg of biotinylated mouse IgA or biotinylated mouse IgG (BD Biosciences) was injected intraperitoneally into wild-type or bpk/bpk mice on postnatal day 21. Animals were euthanized 24 h post-injection. 5-μm sections from formalin-fixed paraffin-embedded kidney tissue were deparaffinized in xylene and rehydrated through a series of alcohol, followed by antigen retrieval using 4×5 min microwave sessions in 10 mM trisodium citrate (pH 6.0). Sections were blocked with 1% BSA in Tris-buffered saline with 0.1% Tween 20, followed by blocking of endogenous peroxidase activity using 3% H_2O_2 in Tris-buffered saline. Sections were incubated with ABC reagent from Elite Kit (Vector Laboratories), followed by application of 3,3′-diaminobenzidine.

100 μg of purified human IgA (provided by Keith Mostov, UCSF) or human IgG (Sigma-Aldrich) was injected intraperitoneally into wild-type or bpk/bpk mice on postnatal days 18–19 or into Pkd1$^{cond/cond}$:Nestin^{cre} mice at 6 months of age. Animals were euthanized 12 or 24 h post-injection, and kidney tissues were either flash-frozen in liquid nitrogen or cyst fluids

were aspirated with a fine needle. Flash-frozen kidneys and cyst fluids were lysed in SDS buffer. Samples were separated by non-reducing 6% SDS-PAGE or 4–15% gradient SDS-PAGE gels, transferred to nitrocellulose, and subjected to immunoblot analysis using anti-human IgA, anti-human IgG, or an anti-human κ light chain HRP conjugate (Life Technologies, Thermo Fisher Scientific). Western blots were quantified by film densitometry. The amount of human IgA retained in both kidneys was calculated and represented as the percentage of original injected material.

qPCR—Total RNA was isolated from mouse inner medullary collecting duct (IMCD3) cells treated for 18 h with Dulbecco's PBS (Mediatech, Inc., Manassas, VA), 100 ng/ml mouse IL4 (R&D Systems, Inc., Minneapolis, MN), or 100 ng/ml mouse IL13 (Cell Signaling Technology, Inc., Danvers, MA) using the RNeasy Plus mini kit (Qiagen, Inc., Valencia, CA). RNA (2 μg) was converted to cDNA using Moloney murine leukemia virus reverse transcriptase reverse transcriptase (Promega Corp., Madison, WI). The following primers were used for qPCR amplification: ms pIgR, 5′-gccaaaccttgaggtgacg-3′ (forward) and 5′-taccagccttcatcttccttactgaccggg-3′ (reverse) (19); cross-species β-actin, 5′-gaagtgtgacgttgacatcc-3′ (forward) and 5′-acagagtacttgcgctcagg-3′ (reverse) (26). The amplification program included an annealing temperature of 55 °C and used GoTaq qPCR Master Mix (Promega) and the Stratagene Mx3000P qPCR system (Agilent Technologies, Inc., Santa Clara, CA). Data analysis and statistics used GraphPad Prism (GraphPad Software, Inc., La Jolla, CA) (27).

Results

pIgR Is Expressed in Renal Cyst-lining Cells and Processed into the SC—We found previously that STAT6 is aberrantly activated in renal cyst-lining epithelial cells in two mouse models of PKD, the Bpk model and the human-orthologous $Pkd1^{cond/cond}$:$Nestin^{cre}$ model (12). In addition, significant amounts of the STAT6 activating cytokine IL13 are present in cyst fluid in these models (12). We tested whether STAT6 activation may lead to increased pIgR expression in these models. Immunoblotting revealed that pIgR expression is increased in Bpk polycystic kidneys compared with kidneys from age-matched control animals (Fig. 1*A*). In addition, a significant fraction of pIgR is processed, leading to the SC cleavage product (Fig. 1*A*). This indicates that a significant fraction of pIgR must have transcytosed polymeric immunoglobulins across renal epithelial cells and that the resulting SC fragment is unable to be excreted into the urinary space, suggesting that it is trapped in cyst lumens. To test whether increased pIgR expression may be due to aberrant STAT6 activation, we investigated crossed mice lacking STAT6 (12). As shown in Fig. 1*A*, pIgR expression is reduced strongly, but not eliminated completely, in kidneys of bpk/bpk:$STAT6^{-/-}$ mice compared with bpk/bpk mice. This indicates that STAT6 is not necessary for a basal level of pIgR expression but is responsible for the observed increased expression in bpk/bpk mice. Similarly, pIgR is expressed and processed into the SC in the human orthologous $Pkd1^{cond/cond}$:$Nestin^{cre}$ mouse model compared with control $Pkd1^{cond/cond}$ mice (Fig. 1*B*).

Immunofluorescence microscopy revealed that pIgR expression in polycystic kidneys of bpk/bpk mice is confined to epithelial cells. Virtually all cysts exhibit at least a basal expression level with particularly intense staining in numerous smaller cysts (Fig. 1*C*). pIgR is expressed both in cysts that stain positively or negatively for the collecting duct marker *D. biflorus* agglutinin. In control kidneys, pIgR expression is low or absent in most tubules, except for occasional cells in *D. biflorus* agglutinin-negative tubules (Fig. 1*C*), consistent with a previous report (19).

Treatment of mouse inner medullary collecting duct cells *in vitro* with IL4 or IL13 to activate STAT6 significantly increases the mRNA expression of pIgR (Fig. 2). Although the results of IL4 treatment are consistent with previous findings in intestinal epithelial cells (23, 24, 28), importantly, IL13 treatment has a similar or greater effect on pIgR expression, which has not been demonstrated previously. Together, these results indicate that STAT6 regulates the expression of pIgR in renal epithelial cells and that pIgR expression is increased in cyst-lining cells. Furthermore, the results suggest that pIgR actively undergoes transcytosis in polycystic kidneys, leading to the accumulation of the SC fragment.

pIgR and the SC Are Highly Expressed in Human ADPKD Kidneys—Next we determined whether the increased pIgR expression and accumulation of SC observed in mouse models of PKD also occurs in human ADPKD kidneys. As shown in Fig. 4*A*, pIgR expression is strongly increased in ADPKD kidneys compared with normal human kidneys. Additionally, strong signals for the SC fragment of pIgR are detected in aspirated cyst fluids from ADPKD kidneys (Fig. 3*B*). Because pIgR undergoes transcytosis and cleavage at the apical plasma membrane when it is bound to polymeric IgA or IgM, this result indicates that pIgR must actively transport these secretory immunoglobulins across cyst-lining epithelial cells into cyst fluid. Immunofluorescence microscopy shows little to no detectable pIgR expression in normal human kidneys (Fig. 3*C*). This is consistent with previous findings from normal human kidneys (29). In contrast, there is strong pIgR immunostaining in epithelial cells lining most cysts in ADPKD kidneys.

Endogenous dIgA Accumulates in Renal Cyst Fluids—IgA in normal serum is primarily in the monomeric form, but a fraction is dimeric and, in mice, is largely cleared by transport into bile (30). To directly determine whether the observed high expression level of pIgR and its processing into SC leads to transport of dIgA into renal cyst fluids, we examined total kidney lysates from wild-type and cystic mice by immunoblot analysis. Under non-reducing conditions, dIgA is partially preserved during electrophoresis and visible as an ~250-kDa band in contrast to mIgA at ~130 kDa. As shown in Fig. 4*A*, the amount of dIgA is strongly increased in kidneys from cystic $Pkd1^{cond/cond}$:$Nestin^{cre}$ mice in comparison with normal $Pkd1^{cond/cond}$ mice. The relative amount of dIgA to mIgA in cystic kidneys is also increased in comparison with serum, consistent with the view that dIgA actively accumulates in cystic kidneys by pIgR-mediated transport. When examining aspirated cyst fluid from $Pkd1^{cond/cond}$:$Nestin^{cre}$ mice, we observed strong bands for dIgA (Fig. 4*B*) and SC (Fig. 4*C*), suggesting that

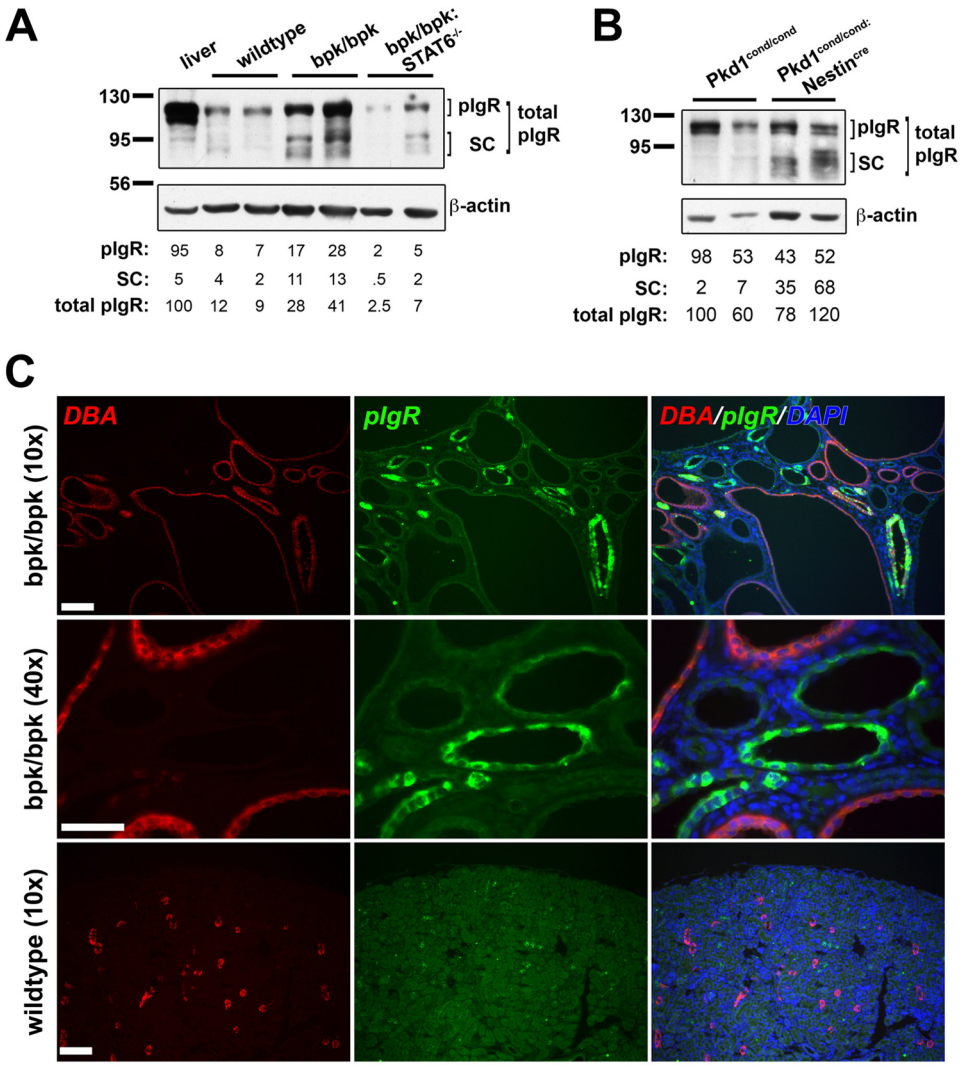

FIGURE 1. **Increased amounts of pIgR and SC in renal cysts in PKD mouse models.** *A* and *B*, total kidney lysates were analyzed by immunoblotting for β-actin and for pIgR using an antibody against the pIgR ectodomain that recognizes both uncleaved pIgR and SC. Positions of full-length pIgR and SC are indicated with *brackets*. Quantified pIgR/SC levels in arbitrary units, normalized to the actin loading control, are displayed below each lane. *A*, 21-day-old wild-type, bpk/bpk, or bpk/bpk:STAT6$^{-/-}$ animals. Total liver lysate from a wild-type animal was used as a positive control. *B*, 11-month-old Pkd1$^{cond/cond}$ or Pkd1$^{cond/cond}$:Nestin^{cre} animals. *C*, immunofluorescence for pIgR. Cyst-lining renal epithelial cells exhibit pIgR staining (*green*), with some smaller cysts staining more intensely. *D. biflorus* agglutinin staining (*red*) of collecting ducts shows some overlapping signals. *Blue*, DAPI nuclear stain. *Scale bars* = 100 μm (×10) and 50 μm (×40).

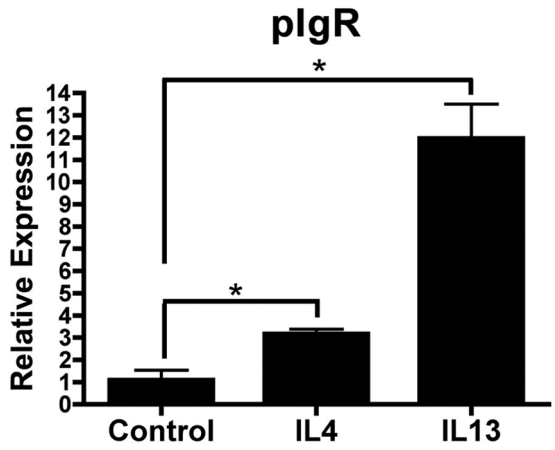

FIGURE 2. **IL4 and IL13 induce expression of pIgR in kidney epithelial cells.** Treatment of inner medullary collecting duct cells with 100 ng/ml mouse IL4 or mouse IL13 increases the expression of pIgR mRNA when analyzed by qPCR. *, $p < 0.05$, two-tailed Student's *t* test. Shown is one representative graph from three independent experiments.

dIgA indeed undergoes pIgR-mediated transport across the cyst-lining epithelium and accumulates in cyst fluids.

Parenterally Administered dIgA Is Targeted to Renal Cysts More Effectively Than IgG—To determine whether exogenous dIgA can be effectively delivered to renal cysts, biotinylated mouse IgA (a mixture of mIgA and dIgA) or biotinylated mouse IgG was administered by intraperitoneal injection into bpk/bpk mice. 24-hours post-injection, the localization of biotinylated immunoglobulins was analyzed by immunohistochemistry. No biotin signals were detected in the kidneys of uninjected mice or mice injected with biotinylated IgG. In contrast, cyst-lining epithelial cells stained positively in mice injected with biotinylated IgA, suggesting that the exogenous IgA has been endocytosed in these cells and may be undergoing transcytosis (Fig. 5*A*).

To directly observe the *in vivo* transport and renal accumulation of exogenous unmodified immunoglobulins, we administered human dIgA (or human IgG) into bpk/bpk mice or age-

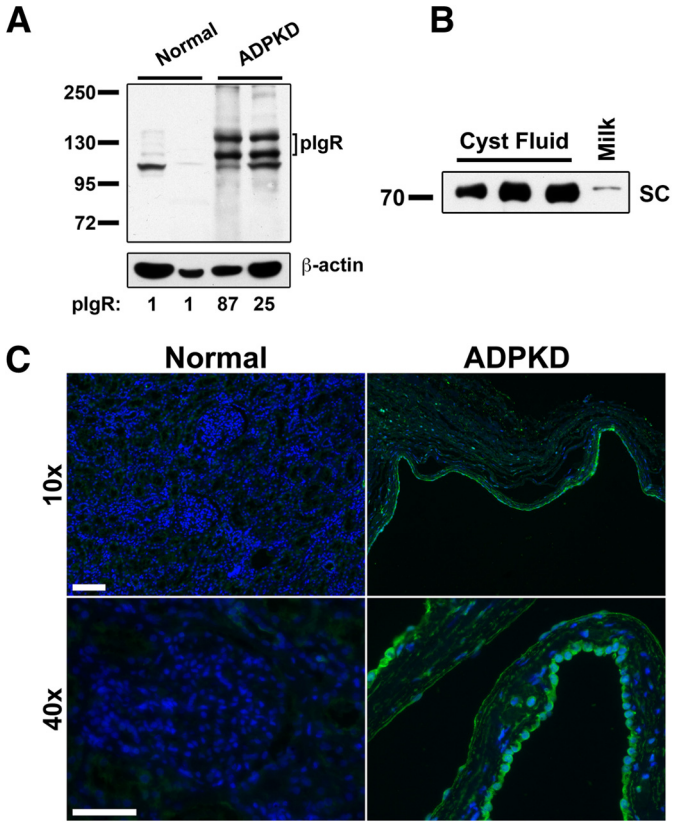

FIGURE 3. **pIgR and SC in ADPKD human kidney samples.** *A*, total kidney lysates from ADPKD patients or normal controls were analyzed by immunoblotting using an antibody against the cytoplasmic domain of pIgR that recognizes only uncleaved full-length pIgR. pIgR migrating as a doublet of the expected molecular weight is indicated by a *bracket*. The nature of the band below the bracket is unknown and may reflect nonspecific binding. Quantified pIgR levels in arbitrary units, normalized to the actin loading control, are displayed below each lane. ADPKD kidneys show increased pIgR expression compared with normal tissue. *B*, immunoblotting of ADPKD patient aspirated cyst fluids using an antibody against the ectodomain of pIgR that recognizes SC. Human breast milk was used as a positive control. *C*, immunofluorescence microscopy of normal or ADPKD kidney tissue. pIgR, *green*; DAPI nuclear stain, *blue*. Strong staining for pIgR is visible in cyst-lining epithelial cells. *Scale bars* = 100 μm (×10) and 50 μm (×40).

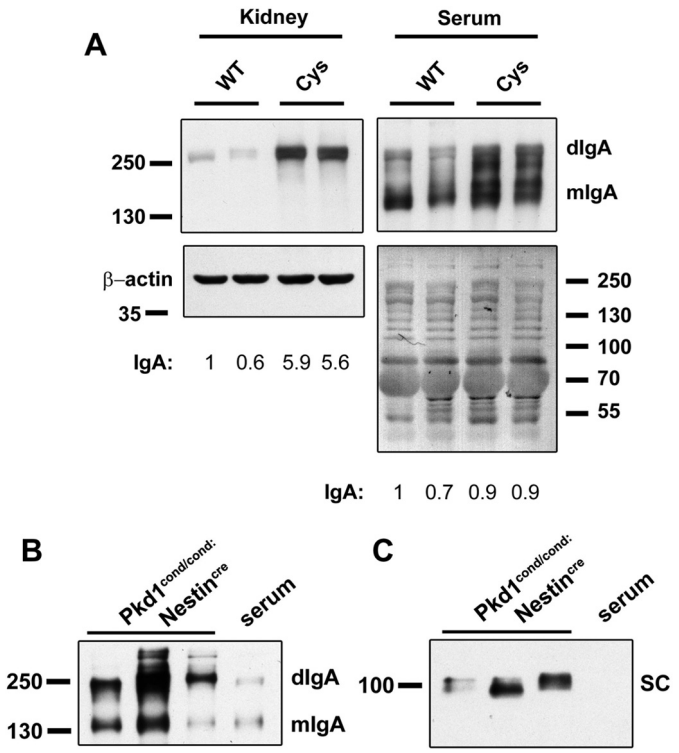

FIGURE 4. **Dimeric IgA accumulates in polycystic kidneys in PKD mouse models.** *A*, total kidney lysates or serum from 11-month-old Pkd1$^{cond/cond}$ or Pkd1$^{cond/cond}$:Nestin^{cre} animals were analyzed by immunoblotting for mouse IgA under non-reducing conditions to visualize dIgA *versus* mIgA. Actin immunoblot (*left*) or total protein stain (Ponceau S, *right*) were used as loading controls. Quantified IgA levels in arbitrary units, normalized to loading control, are displayed below each lane. *B* and *C*, cyst fluids from 11-month-old Pkd1$^{cond/cond}$:Nestin^{cre} animals were analyzed by immunoblotting for mouse IgA under non-reducing conditions (*B*) and for mouse SC under reducing conditions (*C*).

matched wild-type mice. The dIgA/pIgR interaction is highly conserved among mammalian species. Consequently, human dIgA, when injected into rodents, is recognized and transcytosed normally by rodent pIgR *in vivo* (31). The use of human-specific antibodies against IgA (or IgG) then enables the detection of injected human immunoglobulins over the large background of endogenous murine immunoglobulins. 12 h post-intraperitoneal injection of human IgA (a mixture of mIgA and dIgA, Fig. 5*B*) or human IgG, kidney lysates were analyzed by immunoblotting. As shown in Fig. 5*B*, dIgA accumulates preferentially in kidneys of bpk/bpk cystic mice compared with wild-type controls. In contrast, very little, if any, injected human IgG is detectable in kidneys of either cystic or control mice (Fig. 5*C*). Together, these results suggest that IgG antibodies do not effectively target to polycystic kidneys but that dIgA antibodies undergo pIgR-mediated transport into the lumen of renal cysts, where they accumulate.

We hypothesized that the human dIgA that was found retained in kidneys of wild-type mice 12 h after intraperitoneal injection (Fig. 5*B*) will eventually be cleared by urinary excretion, whereas human dIgA retained in polycystic kidneys should be retained long-term because it will be trapped inside cysts. To test this hypothesis, we repeated the above experiment but analyzed kidneys 24 h post-injection of human dIgA. As shown in Fig. 5, *D* and *E*, at this time point very little injected dIgA is still retained in wild-type kidneys. In contrast, ~7% of the injected human dIgA can be recovered from polycystic kidneys. On the basis of kidney weight and the fact that kidneys in this model consist of ~50% cyst fluid (12), we can estimate that the concentration of parenterally administered dIgA reached 7 μg/ml in cyst fluid after 24 h. This assumes that the retained dIgA in polycystic kidneys of Bpk mice is primarily present in cyst fluid and may be an over-estimate. However, given that the 50% effective concentration of currently used therapeutic IgG antibodies is typically in the range of 1–200 ng/ml or less, it appears likely that therapeutically effective concentrations of dIgA antibodies should be achievable in renal cyst fluid.

To directly test whether injected dIgA is transported into cyst lumens, human dIgA was injected into either 16-day-old bpk/bpk mice or into 6-month-old Pkd1$^{cond/cond}$:Nestin^{cre} mice. Cyst fluids were aspirated and analyzed by immunoblotting. As shown in Fig. 6, injected human dIgA can be recovered from cyst fluids from both mouse models after 24 h, indicating that parenterally administered dIgA is taken up by cyst-lining cells and transcytosed into the lumen. Together, these results

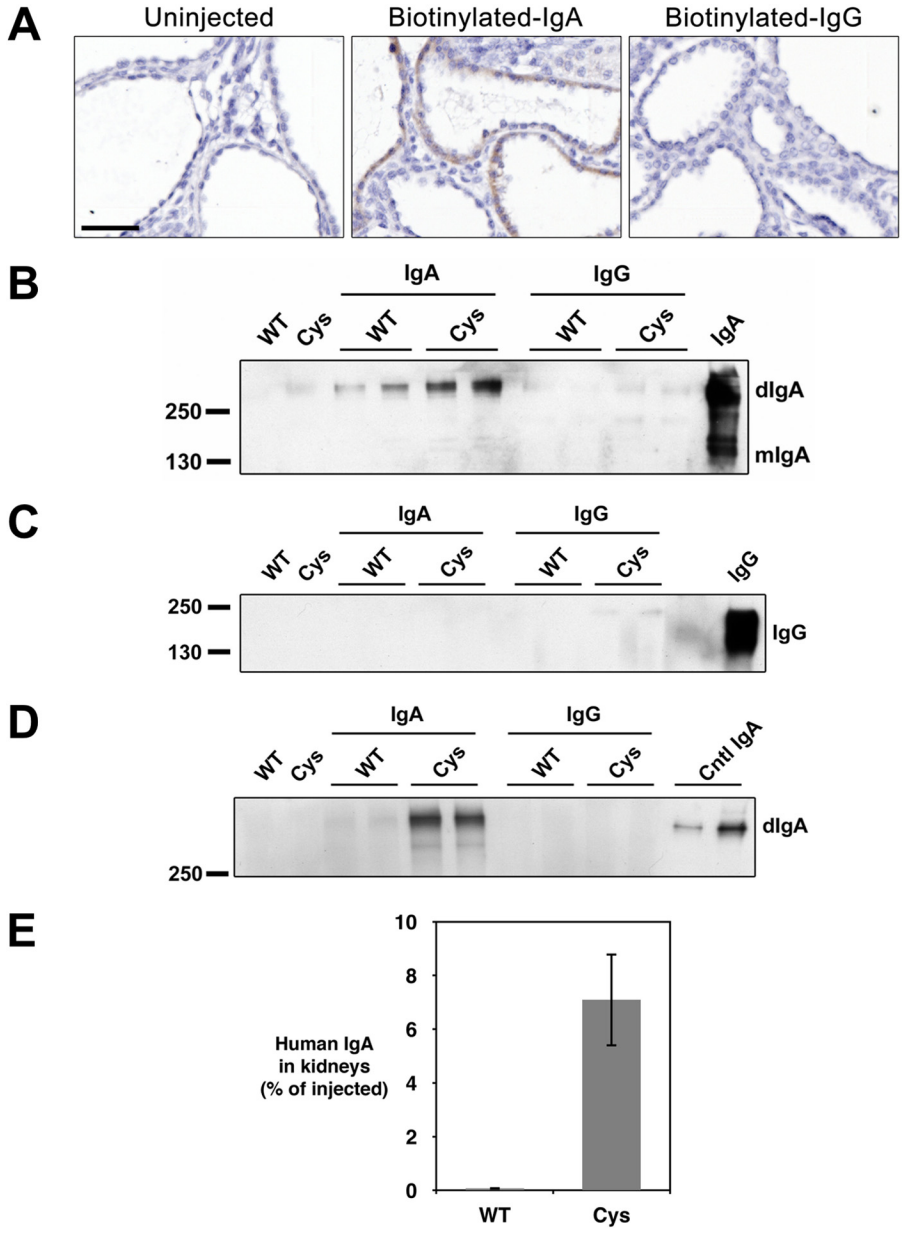

FIGURE 5. **Dimeric IgA is transported and accumulates in polycystic kidneys *in vivo*.** *A*, streptavidin-HRP staining of kidneys from bpk/bpk (day 21) mice either uninjected or injected with biotinylated IgA or biotinylated IgG, respectively. 24-hours post-injection shows biotinylated-IgA present in cyst-lining epithelial cells, indicating that it is undergoing transcytosis. Counterstaining was done with hematoxylin. *Scale bar* = 50 μm. *B* and *C*, 100 μg of human IgA or human IgG were injected intraperitoneally into wild-type or bpk/bpk animals, and total kidney lysates were analyzed by immunoblotting 12 h post-injection (60 μg of total protein loaded per lane) under non-reducing conditions using antibodies specific to human IgA or human IgG, respectively. *B*, dIgA accumulates preferentially in polycystic kidneys (*Cys*) of bpk/bpk animals compared with kidneys of age-matched wild-type animals. The injected starting material (purified human IgA) was used as a positive control (*right lane*). *C*, human IgG is undetectable in kidney lysates using an antibody specific to human IgG. The injected starting material (purified human IgG) was used as a positive control (*right lane*). *D*, accumulation of human dIgA in polycystic kidneys compared with wild-type kidneys when analyzed 24-hours post-injection. Detection used an antibody specific to human κ light chain. Purified human IgA (1 and 10 ng, respectively) was used as a positive control and as the standard for quantification (*right lanes*). *E*, quantification of the experiment shown in *D*, represented as the percentage of injected dIgA present in the kidneys 24 h post-injection (n = 2, mean ± range).

indicate that dIgA efficiently accumulates in polycystic kidneys, where it persists for extended periods of time.

Discussion

We report here a novel strategy to target therapeutic or diagnostic antibodies to polycystic kidneys and, in particular, to the lumens of renal cysts. Although virtually all immunoglobulins designed for clinical use are in IgG format, these antibodies are not expected to gain access to the luminal space in polycystic kidneys. Therefore, such antibodies will likely be ineffective if their targets are present on the apical surface of cyst-lining cells or within the cyst fluid. Several growth factors have been identified previously in renal cyst fluid and implicated in driving cyst growth, such as EGF, hepatocyte growth factor, and TNF-α (7–9). Similarly, our previous results indicated that STAT6 is aberrantly activated in cyst-lining cells because of auto/paracrine stimulation of the IL4/13 receptor by IL13 present in cyst fluid (12). Inhibition of these pathways using antagonistic anti-

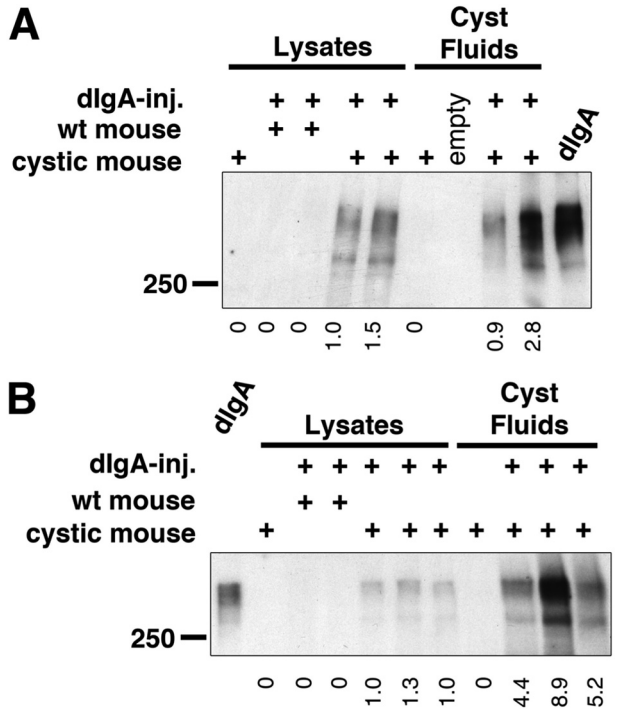

FIGURE 6. **Parenterally administered dIgA is transcytosed into cyst fluids.** *A* and *B*, 100 μg of human-IgA was administered by intraperitoneal injection into either 16-day-old bpk/bpk mice (*A*) or 6 month old Pkd1$^{cond/cond}$:Nestin^{cre} mice (*B*) or age-matched WT control mice. After 24 h, kidneys were removed, and cyst fluids were aspirated from one kidney per animal. The remaining intact kidneys were used to prepare total kidney lysates. Lysates and cyst fluids, standardized by an equal mass of tissue/fluid, were analyzed by immunoblotting using an antibody specific to human κ light chain. Purified human dIgA (5 ng) was run as a positive control as indicated. Each lane represents an individual animal. Band quantifications are indicated in arbitrary units underneath each blot.

bodies could be therapeutically promising but requires that the antibodies gain access to the cystic space.

Using a combination of PKD mouse models and human ADPKD tissues, we show that renal cyst-lining epithelial cells express the pIgR and that this results in active transport of dIgA from the circulation across the epithelium, where secretory IgA accumulates in the cyst fluids. Our results suggest that aberrant STAT6 activation contributes to the observed high level of pIgR expression. IFN-γ and TNF-α induce expression of pIgR in other epithelial cell types (24), and these cytokines have also been found in ADPKD cyst fluid (9). Therefore, IL13-induced STAT6 signaling may not be the only mechanism leading to increased pIgR expression in cystic kidneys.

Together, our results suggest that the pIgR-mediated transport of dIgA into renal cyst lumens can be exploited to target therapeutic antibodies to this compartment. Besides the ability to initially target dIgA antibodies to renal cysts, an additional benefit would be that the dIgA antibodies will remain and accumulate in cyst fluids because renal cysts lack a connection to the tubular system. This is in contrast to virtually all other epithelial tissues that express the pIgR and transport dIgA to external secretions, which are lost over time. For example, in mice, the bulk of dIgA present in plasma is cleared via transcytosis into bile (30). Similarly, in humans, dIgA is excreted via the intestinal epithelium, salivary glands, and lungs (18). Therefore, parenteral administration of dIgA is not expected to lead to accumulation in tissues that normally express the pIgR, which should limit off-target side effects. Interestingly, IgA and SC have been found in hepatic cyst fluid from ADPKD patients (32), which suggests that therapeutic dIgA antibodies may also effectively target to liver cysts. Given that secretory IgA is highly stable and can withstand extreme environments (33), it is likely that it exhibits a long half-life in renal cyst fluid, which may allow low-frequency dosing similar to established therapies with IgG antibodies.

Although there are currently no approved antibodies using isotypes other than IgG, the idea of using IgA antibodies for cancer treatment has been investigated recently (34–36). Beyond treatment for ADPKD, we propose that dIgA antibodies may be useful for the treatment of other disorders in which pIgR-mediated antibody targeting to epithelial luminal spaces would be desirable. This could include other renal disorders such as chronic kidney disease, lung diseases such as asthma or cystic fibrosis, and so forth. An alternative to the use of polymeric immunoglobulins are pIgR-binding peptides that can be linked to payloads, an area of investigation for targeting other mucosal epithelia (37, 38).

Acknowledgments—We thank Kevin Kipp and Brian Su for help with experiments.

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