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

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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) 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
pathogenesis of ADPKD, such as EGF and TNF-α, are used for cancer or autoimmune disease therapy (14, 15). Such antago-
nistic antibodies may potentially be effective for the treatment of
ADPKD. Given that highly effective antibodies against nu-
merous promising targets are already available or under develop-
ment 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 path-
way in ADPKD indeed involves growth factor/receptor interac-
tion 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 es-

tablished production technology. Almost all of the approved anti-
body 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 poly-
meric immunoglobulin receptor (pIgR) to overcome this obsta-
cle. The pIgR is a transmembrane protein that is expressed in
many mucosal epithelial cell types. pIgR present at the baso-
lateral plasma membrane can bind to polymeric immunoglobu-
lins (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 protein-
tically cleaved. This releases the pIg in a complex with the extra-
cellular 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
trat 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 kid-
ney can use the dIgA/pIgR system and that it can be up-regu-
lated 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. Con-
sequently, murine and human cyst fluids contain high levels of
dIgA. When dIgA is injected into mice, it accumulates prefer-
entially in polycystic kidneys compared with normal kidneys. In
contrast, very little injected IgG accumulates in polycystic kid-
neys. 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-me-
diated 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 Committ-
ee of the University of California at Santa Barbara approved all
animal experiments. Pkd1cond/cond, Pkd1cond/cond:Nestincre,
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-hu-
man IgA and mouse anti-β-actin antibody were from Sigma-
Aldrich (Saint Louis, MO). Mouse anti-pIgR (C terminus, cat-
alog 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). Sec-
tions were blocked with 1% BSA in Tris-buffered saline with
0.1% Tween 20, followed by blocking of endogenous peroxidase
activity using 3% H2O2 in Tris-buffered saline. Sections were
incubated with ABC reagent from Elite Kit (Vector Laborato-
ries), 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 intraperi-
toneally into wild-type or bpk/bpk mice on postnatal days
18–19 or into Pkd1cond/cond:Nestincre 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’-gcaaagcttggaggtcagc-3’ (forward) and 5’-tacaggctcttctctctcagcggg-3’ (reverse) (19); cross-species β-actin, 5’-gaagttgatggcatcacct-3’ (forward) and 5’-acagatgctctctcagcggg-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

plgR 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 Pkd1cond/cond:Nestincre 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 plgR expression in these models. Immunoblotting revealed that plgR expression is increased in Bpk polycystic kidneys compared with kidneys from age-matched control animals (Fig. 1A). In addition, a significant fraction of plgR is processed, leading to the SC cleavage product (Fig. 1A). This indicates that a significant fraction of plgR 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 plgR expression may be due to aberrant STAT6 activation, we investigated crossed mice lacking STAT6 (12). As shown in Fig. 1A, plgR 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 plgR expression but is responsible for the observed increased expression in bpk/bpk mice. Similarly, plgR is expressed and processed into the SC in the human orthologous Pkd1cond/cond; Nestincre mouse model with control Pkd1cond/cond mice (Fig. 1B).

Immunofluorescence microscopy revealed that plgR 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. 1C). plgR is expressed both in cysts that stain positively or negatively for the collecting duct marker D. biflorus agglutinin. In control kidneys, plgR expression is low or absent in most tubules, except for occasional cells in D. biflorus agglutinin-negative tubules (Fig. 1C), 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 plgR (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 plgR expression, which has not been demonstrated previously. Together, these results indicate that STAT6 regulates the expression of plgR in renal epithelial cells and that plgR expression is increased in cyst-lining cells. Furthermore, the results suggest that plgR actively undergoes transcytosis in polycystic kidneys, leading to the accumulation of the SC fragment.

plgR and the SC Are Highly Expressed in Human ADPKD Kidneys—Next we determined whether the increased plgR expression and accumulation of SC observed in mouse models of PKD also occurs in human ADPKD kidneys. As shown in Fig. 4A, plgR expression is strongly increased in ADPKD kidneys compared with normal human kidneys. Additionally, strong signals for the SC fragment of plgR are detected in aspirated cyst fluids from ADPKD kidneys (Fig. 3B). Because plgR undergoes transcytosis and cleavage at the apical plasma membrane when it is bound to polymeric IgA or IgM, this result indicates that plgR must actively transport these secretory immunoglobulins across cyst-lining epithelial cells into cyst fluid. Immunofluorescence microscopy shows little to no detectable plgR expression in normal human kidneys (Fig. 3C). This is consistent with previous findings from normal human kidneys (29). In contrast, there is strong plgR 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 plgR 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. 4A, the amount of dIgA is strongly increased in kidneys from cystic Pkd1cond/cond;Nestincre mice in comparison with normal Pkd1cond/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 plgR-mediated transport. When examining aspirated cyst fluid from Pkd1cond/cond;Nestincre mice, we observed strong bands for dIgA (Fig. 4B) and SC (Fig. 4C), suggesting that
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. 5A).

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-
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. 5B) or human IgG, kidney lysates were analyzed by immunoblotting. As shown in Fig. 5B, 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. 5C). 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. 5B) 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 ~70 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 Pkd1cond/cond:Nestincre 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
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-
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 plgR and that this results in active transport of dlgA 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 plgR expression. IFN-γ and TNF-α induce expression of plgR 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 plgR expression in cystic kidneys.

Together, our results suggest that the plgR-mediated transport of dlgA into renal cyst lumens can be exploited to target therapeutic antibodies to this compartment. Besides the ability to initially target dlgA antibodies to renal cysts, an additional benefit would be that the dlgA 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 plgR and transport dlgA to external secretions, which are lost over time. For example, in mice, the bulk of dlgA present in plasma is cleared via transcytosis into bile (30). Similarly, in humans, dlgA is excreted via the intestinal epithelium, salivary glands, and lungs (18). Therefore, parenteral administration of dlgA is not expected to lead to accumulation in tissues that normally express the plgR, 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 dlgA 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 dlgA antibodies may be useful for the treatment of other disorders in which plgR-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 plgR-binding peptides that can be linked to payloads, an area of investigation for targeting other mucosal epithelia (37, 38).

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