CD36 mediates proximal tubular binding and uptake of albumin and is upregulated in proteinuric nephropathies

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Submitted 17 January 2012; accepted in final form 2 July 2012

Baines RJ, Chana RS, Hall M, Febbraio M, Kennedy D, Brunskill NJ. CD36 mediates proximal tubular binding and uptake of albumin and is upregulated in proteinuric nephropathies. Am J Physiol Renal Physiol 303: F1006–F1014, 2012. First published July 11, 2012; doi:10.1152/ajprenal.00021.2012.—Dysregulation of renal tubular protein handling in proteinuria contributes to the development of chronic kidney disease. We investigated the role of CD36 as a novel candidate mediator of albumin binding and endocytosis in the kidney proximal tubule using both in vitro and in vivo approaches, and in nephrotic patient renal biopsy samples. In CD36-transfected opossum kidney proximal tubular cells, both binding and uptake of albumin were substantially enhanced. A specific CD36 inhibitor abrogated this effect, but receptor-associated protein, which blocks megalin-mediated endocytosis of albumin, did not. Mouse proximal tubular cells expressed CD36 and this was absent in CD36 null animals, whereas expression of megalin was equal in these animals. Compared with wild-type mice, CD36 null mice demonstrated a significantly increased urinary protein-to-creatinine ratio and albumin-to-creatinine ratio. Proximal tubular cells expressed increased CD36 when exposed to elevated albumin concentrations in culture medium. Expression of CD36 was studied in renal biopsy tissue obtained from adult patients with heavy proteinuria due to minimal change disease, membranous nephropathy, or focal segmental glomerulosclerosis. Proximal tubular CD36 expression was markedly increased in proteinuric individuals. We conclude that CD36 is a novel mediator influencing binding and uptake of albumin in the proximal tubule that is upregulated in proteinuric renal diseases. CD36 may represent a potential therapeutic target in proteinuric nephropathy.

albumin; CD36; proteinuria; proximal tubule

PROTEINURIA IS AN INDEPENDENT risk factor for progressive renal disease (32). Considerable evidence has established a causal relationship between glomerular filtered proteins and the tubulointerstitial injury observed in proteinuric nephropathies (1, 5). Consequently, the mechanisms of renal glomerular and tubular handling of proteins have attracted considerable interest but remain intensely controversial.

Recently, it has been suggested that the healthy glomerulus is freely permeable to proteins and that a tubular retrieval pathway may be responsible for the reabsorption of very large quantities of intact filtered proteins (14, 15). However, there is little direct evidence in support of such a pathway. The favored traditional view remains that the glomerulus is relatively impermeable to macromolecules (8, 33) and that those molecules escaping the filtration barrier and entering the glomerular filtrate are reabsorbed by receptor-mediated endocytosis in the proximal tubule before lysosomal degradation.

Extrapolating from rodent micropuncture studies, it is calculated that in humans 3–5 g of albumin may be filtered per 24 h (18, 23, 31), but human urine contains negligible amounts of albumin. A tandem complex of the giant receptors megalin and cubilin mediates the endocytosis of albumin and other proteins by proximal tubular cells (PTC) (10, 11). However, several lines of evidence suggest that additional PTC receptors for proteins may exist. Megalin knockout animals display only modest low-molecular weight proteinuria (24), as do dogs and humans deficient in cubilin and/or its partner amnionless (12). Furthermore, in some mice with a renal-specific megalin knockout displaying a mosaic PTC pattern of megalin expression, up to 15% of non-megalin-expressing PTC continue to exhibit appreciable intracellular albumin accumulation (27). In cell culture, inhibition of the megalin/cubilin complex with receptor-associated protein and/or intrinsic factor B12 complex only partially inhibits albumin endocytosis (12).

CD36 is a class B scavenger receptor with widespread tissue distribution, including PTC, where its role is uncertain (13, 29). In macrophages, CD36 is a major endocytic receptor for oxidized lipoproteins, and a central role for CD36 in atherosclerosis has been proposed (13). In human diabetic nephropathy, PTC CD36 expression and apoptosis are positively associated (39). Expression of CD36 in PTC is increased by albumin (43) and following exposure to advanced oxidation protein products (AOPPs). Endocytosis of AOPP-modified human serum albumin by PTC is CD36 dependent (21). Downstream, CD36-stimulated signaling events may mediate proteinuria-induced PTC injury (21, 37). In hypercholesterolemic mice, CD36 deficiency attenuates transforming growth factor (TGF)-β signaling, NF-κB activity, and renal fibrosis (29).

Clearly, PTC CD36 is well placed to bind filtered lipoproteins and other negatively charged proteins such as albumin in proteinuria, but binding of unmodified albumin to CD36 has not been studied in detail. We performed a number of studies to determine the ability of CD36 to mediate albumin endocytosis in PTC and to establish whether expression of CD36 was altered in proteinuric human renal diseases.

METHODS

Reagents and materials. The irreversible CD36 inhibitor sulfosuccinimidyl-oleate (SSO) and human full-length CD36 cDNA in pcDNA3.0-Flag vector (CD36-Flag) were obtained from Dr. N. A. Abumrad (Washington University, St. Louis, MO). FuGENE 6 transfection reagent was purchased from Roche Diagnostics (Lewes, UK). Rabbit polyclonal anti-CD36 antibodies were obtained from Santa Cruz...
Biotechnology (sc9154, Autogen Bioclear UK, Calne, UK) or Novus Biologicals (NB400–144, Littleton, CO). Mouse anti-human CD36 monoclonal antibodies and rabbit polyclonal anti-megalin antibodies were from Abcam (ab-17044 and ab-101011, respectively, Cambridge, UK). Alexa Fluor 594-conjugated rabbit anti-mouse immunoglobulin was purchased from Invitrogen (Paisley, UK). Rabbit anti-human CD36 monoclonal antibody was purchased from Cayman Chemical. Polyclonal swine anti-rabbit IgG/biotinylated swine F(ab)2 was purchased from Vector Laboratories (Burlingame, CA). Anti-β-actin antibody (A5441), anti-mouse and anti-rabbit horseradish peroxidase-conjugated secondary antibodies (A4416 and A6154, respectively), and FITC-albumin were purchased from Sigma-Aldrich (Dorset, UK). ECL plus kits were from Amersham Pharmacia (Little Chalfont, UK).

**Cell culture.** Wild-type (WT) opossum kidney (OK) cells were maintained in DMEM F-12 supplemented with 10% FCS, 2 mmol/l l-glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin. For an uptake/binding assay, cells were plated in 24-well plates, grown until confluent, then growth arrested in serum-free medium for 24 h.

**Preparation of proximal tubule fractions.** Lysate was prepared from WT and CD36 null kidneys using the method of Tertyn et al. (40) with minor modifications. Briefly, renal cortices were dissected visually in ice-cold dissection solution (DS; HBSS with 10 mM glucose, 5 mM glycine, 1 mM alanine, 15 mM HEPES, pH 7.4) and minced into 1-mm pieces. These were transferred to DS containing 0.1% (wt/vol) type II collagenase and digested in a shaking (120 rpm) water bath for 30 min at 37°C. The supernatant was sieved through two nylon mesh sieves (pore size 235 and 70 µm). Collection in the 70-µm sieve yielded a large number of long PT fragments, while other nephron segments and glomeruli were visualized in the flow-through. Both fractions (i.e., <235 to >70 µm, and 70-µm sieve flow-through) as well as a fraction of total kidney lysate (positive control) were resuspended and homogenized in a buffer containing 20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM sodium orthovanadate, 1 mM β-glycerophosphate, and protease inhibitor cocktail. Samples were rotated at 4°C for 30 min then centrifuged at 13,000 rpm for 15 min. The supernatant was collected, and lysate total protein was determined in duplicate by a detergent-compatible modified Lowry assay.

Equal amounts of protein were subjected to SDS-PAGE, transferred to Immobilon-P membranes (Millipore, Billerica, MA), and membranes were incubated with anti-CD36 antibodies (1:1,000; Novus Biologicals) and developed using the SNAP-i.d. protein detection system (Millipore).

**Generation of CD36 stably transfected cells and Western blotting.** OK cells were stably transfected with CD36-Flag (OK-CD36) or empty pcDNA3.0-Flag vector (OK-empty) at ~50% confluence using Fugene 6 transfection reagent according to the manufacturer’s instructions. Plasmid DNA/Fugene 6 mixtures were added to cells in six-well plates in fully supplemented media. Media were removed after 48 h and replaced with selection medium containing G418 (500 µg/ml). After 4 days in this selection medium, WT OK cells were all dead (data not shown). After 8-wk culture in selection media, surviving transfected colonies were isolated, expanded, and screened for CD36 expression.

To detect CD36 in OK cell transfsects, cells were washed with cold PBS and lysed in Laemmli buffer (60 mM Tris, pH 6.8, 10% glycerol, 2% sodium deoxycholate, 100 mM dithiothreitol, and 0.01% bromophenol blue). Cell lysates were heated at 100°C for 5 min and subjected to 10% SDS-PAGE. Proteins were transferred to nitrocellulose membranes, and CD36 and Flag proteins were detected using rabbit polyclonal anti-human CD36 antisera at 1:400 dilution (1:400, Santa Cruz Biotechnology) or anti-Flag monoclonal antiserum at 1:1,000 dilution (F7425, Sigma), respectively. A secondary anti-rabbit HRP-conjugated antibody was used at 1:5,000 dilution (A6154, Sigma), and bound antibodies were visualized by the ECL system. To assess loading, blots were stripped and reprobed for β-actin using a mouse anti-β-actin antibody at 1:2,000 dilution and a goat anti-mouse HRP-conjugated antibody at 1:2,000 dilution.

**Immunofluorescence.** CD36- and empty vector-transfected OK cells were grown in four-well chamber slides and fixed with 4% paraformaldehyde for 15 min at room temperature. Cell nonspecific binding was blocked using 10% goat serum in PBS for 4 h, followed by incubation overnight at 4°C with a mouse monoclonal anti-human CD36 antibody diluted 1:100 in PBS. After several washes in PBS, cells were exposed to goat anti-mouse Alexa Fluor 594-conjugated secondary antibody diluted 1:400 in PBS for 1 h. To assess nonspecific binding, cells were treated with secondary antibody alone. After washing, cells were then stained with 4,6-diamidino-phenylindole (DAPI), mounted in Citifluor Aquamount, and examined by confocal laser-scanning microscopy.

To assess colocalization of FITC-albumin and CD36, cells grown in chamber slides were growth arrested for 24 h and then incubated with FITC-albumin (5–200 µg/ml) in Ringer solution pH 7.4 at 37 or 4°C for 1 h. After washing in Ringer solution, cells were fixed in 4% paraformaldehyde, blocked for nonspecific binding, exposed to the CD36 antibody overnight, the secondary antibody, for 1 h, and stained with DAPI before Citifluor Aquamount mounting. Slides were examined by confocal microscopy.

**Uptake and binding of FITC-albumin.** Albumin uptake and binding were measured using FITC-albumin as described previously (4, 36) using OK cells as a model (4, 35, 36). Briefly, confluent cells in 24-well plates were growth arrested in serum-free medium for 24 h. For uptake studies, cells were incubated with FITC-albumin for 30 min at 37°C, whereas to assess binding cells were incubated with FITC-albumin at 4°C for 30 min, or overnight to allow binding to reach equilibrium (4). Unbound FITC-albumin was removed by rinsing five times with Ringer solution, pH 7.4, and cells were lysed by addition of 0.1% Triton X-100 in 20 mM MOPS. Fluorescence was measured in a 100-µl aliquot of cell lysate, and lysate protein content was determined. To determine nonspecific binding, cells were incubated with various concentrations of FITC-albumin at 4°C in the presence of a 100-fold excess of unlabeled albumin, and these values were subtracted from total binding to give specific binding. In some experiments, cells were coinubated with FITC-albumin and the specific CD36 inhibitor sulfoconimidyl-oleate (SSO) or with 1 µM receptor-associated protein (RAP). FITC-albumin uptake and binding results were then expressed as micrograms albumin per milligram cell protein. Where different cell clones were compared, each experiment was performed at least three times in each clone.

**Urine protein excretion by CD36 null mice.** Spot urine was collected from male WT and CD36 null mice. Urine albumin concentration was determined colorimetrically using the bromocresol green method (Quantichrom BCG Albumin assay, BioAssay Systems, Hayward, CA). Urine protein and albumin concentrations were normalized to urine creatinine concentration, which was measured colorimetrically using the modified Jaffe method (QuantiChrom creatinine assay, BioAssay Systems). All assays were performed according to the manufacturer’s protocols.

All animal procedures were carried out at the Cleveland Clinic and were approved by the Institutional Animal Care and Use Committee, carried out in an AAALAC-accredited facility, and the investigations conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996).

**Immunostaining of paraffin sections.** Immunostaining of mouse kidneys was performed using deparaffinized 5-µm serial sections. Heat-induced epitope retrieval was performed in citrate buffer (10 mM sodium citrate, 0.05% Tween 20, pH 6.0). Endogenous peroxidases were inactivated using 3% H2O2, followed by blocking with 5% milk. Serial sections were incubated overnight (4°C) with anti-CD36 (1:300, Novus Biologicals) or anti-megalin (1:200). This rabbit poly-
CD36 AND PROTEINURIA

Fig. 1. Expression of CD36 in transfected opossum kidney (OK) cells. OK cells were stably transfected with human CD36 in vector pcDNA3.0-Flag. After selection, transfected cell lysates were subject to Western blotting using anti-CD36 antisera, and then after stripping were reprobed with anti-Flag antisera. Expression of CD36 in wild-type (WT) cells and 3 different OK-CD36 cell clones, designated clones 1, 2, and 3, is shown. Blots were again stripped and reprobed for β-actin as a loading control. The blot depicted is representative of 3 identical experiments.

clonal anti-CD36 antibody yielded optimum results compared with several other monoclonal antibodies, as previously described by our group (25). Nonimmune rabbit serum was used as a negative control. Histochemical reactions were performed using a peroxidase- and alkaline phosphatase-based detection kit, and sections were counterstained with hematoxylin.

Immunostaining of human renal biopsies was performed using 3-μm paraffin sections of formalin-fixed, embedded biopsy samples from patients. All biopsies were obtained from patients newly presenting with minimal change disease, focal segmental glomerulosclerosis (FSGS), or membranous nephropathy. At the time of biopsy, all nephrotic patients were normotensive, manifested ≥3.5 g proteinuria/24 h, and had a Modification of Diet in Renal Disease Estimated Glomerular Filtration Rate >60 ml/min. Normal control kidney tissue was taken from the unaffected pole of kidneys removed due to renal cell carcinoma, from an area well clear of the tumor margin. All studies using human kidneys were approved by the Leicestershire Research Ethics Committee.

The primary antibody was rabbit anti-human CD36 polyclonal antibody (1:200, Cayman). Sections were heated in citrate buffer and blocked in 0.5% BSA, 3% milk in 10% goat serum. Immunoreactive signals were visualized using a peroxidase- and alkaline phosphatase-based detection kit, and sections were counterstained with hematoxylin.

To semiquantify the expression of CD36 in stained sections, slides were scored by three blinded examiners using a grading scale where a minimum value of 0 represented no brown staining for CD36, and a value of 4 represented maximal brown staining for CD36. Each examiner studied five overlapping cortical fields sequentially along the length of a stained biopsy section from three different patients with each condition, and controls. The mean score of the five fields for one section from one examiner was regarded as n = 1. Each of three examiners studied three sections, from different patients or controls, thus giving n = 9 for each condition.

Data and statistical analysis. Data were analyzed using GraphPad Prism (La Jolla, CA), and FITC-albumin binding data were analyzed by nonlinear regression assuming specific one-site binding. FITC-albumin uptake kinetics were analyzed using a Michaelis-Menten model to determine maximal (Vmax) velocity of uptake, and the albumin concentration required to achieve half-maximal velocity (Km) of uptake. Statistical analyses used either an unpaired or paired Student’s t-test, or for multiple comparisons ANOVA followed by Bonferroni’s multiple comparisons test. Results are expressed as means ± SE unless otherwise stated, and P < 0.05 was considered significant.

RESULTS

A number of stably transfected OK cell clones displaying a range of CD36 overexpression levels were generated (Fig. 1), as revealed by immunoblotting using anti-CD36 or anti-Flag antisera. We were unable to detect expression of native CD36 in WT OK cells by Western blotting using this anti-human CD36 antibody. Similarly, immunocytochemistry revealed little or no evidence of CD36 expression in OK-empty cells, but in

Fig. 2. Immunocytochemistry depicting FITC-albumin (alb) binding and uptake and CD36 expression in OK-empty and OK-CD36 cells. OK cells were prepared for immunofluorescence as described in METHODS. In some experiments, nuclei were counterstained blue with 4,6-diamidino-phenylindole. A: comparison, in different cells, of CD36 expression, FITC-alb binding at 4°C, and FITC-alb uptake at 37°C in OK-empty cells (left) and OK-CD36 cells (right). B: colocalization, in the same cells, of bound-FITC alb and CD36 on the surface of OK-CD36-transfected cells. Each image represents a single focal plane from the Z-axis (XT) and 3-dimensional reconstructions along the YZ axis and XZ axis for CD36 (red) and bound FITC-alb (green). A merge of both channels (bottom) reveals superimposition of FITC-alb and CD36 by a yellow signal. The images were prepared using Imaris 7.4 software (Bitplane, Zurich, Switzerland) and are representative of 3 separate experiments. Original magnification ×400.
OK-CD36-transfected cells large amounts of CD36 protein was clearly observed in a plasma membrane distribution (Fig. 2A). When incubated with FITC-albumin and observed by fluorescence microscopy, OK-CD36 cells exhibited greater binding of FITC-albumin to the plasma membrane at 4°C and enhanced levels of FITC-albumin uptake at 37°C compared with OK-empty cells (Fig. 2A). Cells overexpressing CD36 were incubated with FITC-albumin at 4°C, then stained for CD36, and examined by fluorescence microscopy. When the images were merged, CD36 and FITC-albumin were seen to colocalize where the superimposition of green and red fluorescence gave a yellow signal (Fig. 2B).

OK-CD36 cells demonstrated increased specific binding and uptake of FITC-albumin over time compared with OK-empty cells (Fig. 3A). There were no differences in nonspecific binding of FITC-albumin between OK-empty and OK-CD36 cell clones (Fig. 3B). In OK-empty cells, nonspecific binding represented ~50% of total binding. In OK-CD36 cells, specific albumin binding was seen to increase but nonspecific binding was unaffected, and thus the proportion of nonspecific binding fell. Both OK-empty- and OK-CD36-transfected OK cells demonstrated specific, saturable binding of FITC-albumin to their cell surfaces, typical of a receptor-mediated process (Fig. 3C). The observed increases in both FITC-albumin binding and uptake were related to the degree of CD36 overexpression in OK-CD36-transfected cells. The binding affinity ($K_d$) and $B_{max}$ values for the OK-empty cells and the various CD36-overexpressing clones are given in Table 1. Expression of CD36 resulted in significantly increased capacity for albumin binding ($B_{max}$) by cells at 4°C, the increase in binding paralleling the increased level of CD36 expression. The $K_d$ for albumin of OK-CD36 cells was indistinguishable from that of OK-empty cells. Cells overexpressing CD36 also displayed significantly greater $V_{max}$ of FITC-albumin at 37°C (Table 1), in proportion to the amount of CD36 present (Fig. 3D). Notably, the shape of the albumin uptake curve in OK-CD36 transfects at 37°C was distinct from that in OK-empty cells, with the $K_m$ for uptake being significantly lower in OK-CD36 cells (Table 1).

Excess specific albumin binding in OK-CD36 cells was completely inhibited by the specific CD36 antagonist SSO to levels equivalent to those observed in OK-empty cells (Fig. 4A). In the presence of RAP, specific albumin binding was reduced by ~30% in OK-empty cells and by ~16% in OK-CD36 cells (Fig. 4B).

To determine whether CD36 may influence renal tubular protein handling in vivo, we analyzed the urine of CD36 null mice and compared urine protein excretion to that in WT mice (Fig. 5). The CD36 null animals demonstrated increased urinary albumin excretion ratio of $1.8 \pm 0.12$ mg/mg creatinine, compared with $1.2 \pm 0.03$ mg/mg creat-
inine in WT mice. Similarly, CD36 null animals also had greater total urinary protein excretion measured as urine protein-to-creatinine ratio (14.2 ± 0.9 mg/mg creatinine) compared with WT mice (8.8 ± 0.3 mg/mg creatinine).

Immunostaining of kidney tissue and Western blotting of tubular fragments was performed to determine expression patterns of CD36 in the kidney. CD36 was clearly present in PTC of WT animals but absent in CD36 null mice. In contrast, there was no difference in PTC megalin expression between these animals where it was present in an apical distribution. Similarly by immunoblotting, CD36 protein was detected in whole kidney and tubular fragments of WT animals but not CD36 null mice (Fig. 6).

Tubular CD36 expression may be dynamically regulated by proteinuria. When human HK-2 cells were incubated in albumin-containing medium for 48 h, expression of CD36 was modestly increased (Fig. 7, A and B). Renal biopsy tissue was obtained from newly presenting nephrotic patients, stained for CD36 expression, and analyzed semiquantitatively (Fig. 7C). When control sections derived from normal kidneys, or from the kidneys of nephrotic patients, were incubated only with secondary antibody, no specific staining was seen. In kidney sections from patients with normal kidney function and no proteinuria, modest background staining for CD36 was observed in PTC and podocytes. However, in kidney sections from patients with membranous nephropathy, minimal change disease, and FSGS, a diffuse increase in CD36 expression was

Table 1. FITC-albumin binding and uptake parameters for opossum kidney (OK)-empty (WT)- and different OK-CD36-transfected clones

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<td>Binding</td>
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<td>$K_d$, mg/l</td>
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Values are means ± SE. Clone numbers refer to those CD36-transfected cell lines depicted in Fig. 1 and 2. *P < 0.05 compared with relevant value for WT cells (n = 4 for each clone and WT cells). Immunostaining of kidney tissue and Western blotting of tubular fragments was performed to determine expression patterns of CD36 in the kidney. CD36 was clearly present in PTC of WT animals but absent in CD36 null mice. In contrast, there was no difference in PTC megalin expression between these animals where it was present in an apical distribution. Similarly by immunoblotting, CD36 protein was detected in whole kidney and tubular fragments of WT animals but not CD36 null mice (Fig. 6).

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seen in PTC and in some distal tubules. In some PTC, a generalized increase in cytoplasmic staining was observed, whereas in others apical accentuation was seen.

**DISCUSSION**

Mechanisms of proteinuria and the tubular handling of filtered proteins remain of intense interest. Although recent suggestions that the glomerulus filters proteins freely (15) with a high-capacity PTC system responsible for their subsequent retrieval seem incorrect (8, 33), there is evidence that other receptors for proteins may complement the well-described role of megalin/cubilin in PTC protein handling. We have used a variety of in vitro and in vivo techniques in addition to studying clinical samples to identify CD36 as a mediator of protein binding and reabsorption by PTC.

The overexpression of recombinant human CD36 led to both increased albumin-specific binding and uptake in OK-CD36 cells that closely paralleled the level of CD36 protein expression, suggesting a direct relationship between these observations. Albumin $K_d$ values were similar in OK-empty- and OK-CD36-transfected cells, suggesting that CD36 shares a similar affinity for this ligand with the native megalin/cubilin receptor complex previously described in this cell type (44). In contrast, albumin uptake experiments showed that the $K_m$ for albumin uptake by OK-CD36 cells was significantly lower than that seen in OK-empty cells. This implies mechanistic differences in the processes underlying uptake/internalization mediated by CD36 and those mediated by megalin/cubilin.

Binding of ligands to megalin is substantially inhibited by RAP (9, 16, 26), and therefore to help exclude the possibility that CD36 transfection resulted in increased megalin expression, consequently increasing albumin uptake, we used RAP as a competitive inhibitor. This concentration of RAP has been shown to completely inhibit the megalin-mediated component of albumin uptake in OK cells, equivalent to $\sim$40% of total specific albumin uptake (44). We found that RAP inhibited a similar proportion of specific albumin uptake in OK-empty cells but a significantly lower proportion of total specific albumin uptake in OK-CD36 transfects, indicating that megalin is not responsible for increased albumin reabsorption by CD36-transfected cells. To further confirm that CD36 itself was directly contributing to increased albumin uptake, we used the irreversible CD36 inhibitor SSO at previously published concentrations (28). The effect of SSO on albumin uptake by OK-empty cells was modest, but suggestive of low levels of native CD36 expression by OK-empty cells that were undetectable by Western blotting or immunofluorescence, most likely due to lack of specificity of antisera for the native opossum protein. The complete blockade by SSO of enhanced FITC-albumin binding to CD36-transfected cells further supports the contention that CD36 itself is a mediator of albumin binding.

CD36 null mice are viable but display lipid abnormalities with reduced uptake of oxidized LDL by macrophages and long-chain fatty acids by adipocytes (17), and hepatic insulin resistance but peripheral insulin sensitivity (19, 20). There is no documented renal phenotype in CD36 null mice, and their urinary parameters have not been previously reported. Taken together with the current in vitro studies, the finding of albuminuria and generalized proteinuria in these animals, with unaltered tubular megalin expression provides further support for CD36 as a mediator of protein binding to PTC. In comparison, whole-animal megalin-deficient mice exhibit low-molecular weight proteinuria but no excess urinary albumin excretion.
(24), and renal-specific megalin knockout mice with a mosaic pattern of megalin expression in 40% of PTC similarly display low-molecular weight proteinuria but no measurable increase in urinary protein-to-creatinine ratio (27). An effect of CD36 deficiency on glomerular protein filtration cannot be completely excluded; however, electron microscopy revealed no differences in glomerular ultrastructure between WT and CD36 null animals (data not shown).

A previous study failed to demonstrate CD36 expression in mouse kidney proximal tubules (39), but microarray data obtained using the Affymetrix MOE430 mouse developing kidney gene set, available from the GUDMAP consortium database (2), clearly shows increasing CD36 expression in the developing mouse PT. In the current study, using kidneys from WT mice, CD36 was clearly seen in PTC, with CD36 null animals acting as a valuable negative control. Therefore, the evidence suggests that CD36 is indeed expressed in PTC but that detection is technique dependent.

In the current studies, expression of CD36 by human PTC is upregulated under proteinuric conditions in vitro and in vivo. Similar increases in CD36 expression have been observed in LLC-PK1 porcine PTCs exposed to albumin (43) and in whole kidney homogenates of nephrotic rats (22). Susztak et al. (39) reported increased CD36 expression in the kidneys of patients with diabetic nephropathy with a diffuse cellular distribution, very similar to that observed in the nephrotic kidneys in the present study, but no increased expression in the kidneys of patients with FSGS. In contrast, we found increased CD36 expression in PTC of patients with three different glomerular pathologies resulting in nephrotic syndrome, including FSGS. The reasons for this discrepancy are unclear, although the biopsies examined in the current study were from patients with better preserved renal function.

In nephrosis, PTC architecture is often profoundly distorted due to the presence of vacuoles containing proteins reabsorbed from the tubular lumen. The effects of proteinuria on PTC megalin expression are poorly studied, but in cultured cells exposed to albumin overnight, megalin levels are suppressed (6). Similarly, in proteinuric animal models of diabetic nephropathy megalin expression is downregulated (34, 41, 42).
Therefore, megalin may not be responsible for increased intra-PTC protein accumulation in nephrosis. Overall, the current data support the concept that CD36 positively regulates binding and uptake of albumin, and potentially other proteins, in the proximal tubule. The data do not allow the firm conclusion that CD36 is itself an albumin receptor, although the data do provide support for this contention. It also remains possible that CD36 binds moieties such as fatty acids carried by albumin and/or other proteins, rather than the protein itself. This issue is currently under study in our laboratory.

CD36 expression is upregulated in proteinuric glomerular disease, possibly as a compensatory mechanism to retrieve excess filtered proteins that overwhelm the capacity of the megalin/cubulin complex. CD36 is a peroxisome proliferator-activated receptor (PPAR)-γ-regulated gene (7), and we have previously demonstrated activation of PPAR-γ in PTC by albumin-bound fatty acids (37). Thus PTC CD36 transcription may be driven in vivo by this mechanism. In addition to acting as a scavenger receptor, CD36 is able to signal (37). Therefore, the consequences of CD36 upregulation may be deleterious by either mediating apotOPSIS (39) or promoting sterile inflammation (38), events prominently observed in the proteinuric kidney (4). Thus renal tubular CD36 may be a logical therapeutic target to prevent progressive excretory functional loss in proteinuric nephropathies.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: R.J.B., R.S.C., M.F., and N.J.B. provided conception and design of research; R.J.B., R.S.C., M.F., and D.J.K. performed experiments; R.J.B., R.S.C., M.F., D.J.K., and N.J.B. analyzed data; R.J.B., R.S.C., M.F., M.F., D.J.K., and N.J.B. interpreted results of experiments; R.J.B., R.S.C., M.F., D.J.K., and N.J.B. approved final version of manuscript; R.S.C., D.J.K., and N.J.B. drafted manuscript; D.J.K. and N.J.B. edited and revised manuscript.

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