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The Proximal Tubule and Albuminuria: Really!

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ABSTRACT

Recent data highlight the role of the proximal tubule (PT) in reabsorbing, processing, and transcytosing urinary albumin from the glomerular filtrate. Innovative techniques and approaches have provided exciting insights into these processes, and numerous investigators have shown that selective PT cell defects lead to significant albuminuria, even reaching nephrotic range in animal models. Thus, the mechanisms of albumin reabsorption and transcytosis are undergoing intense study. Working in concert with megalin and cubilin, a nonselective multireceptor complex that predominantly directs proteins for lysosomal degradation, the neonatal Fc receptor (FcRn) located at the brush border of the apical membrane has been implicated as the "receptor" mediating albumin transcytosis. The FcRn pathway facilitates reabsorption and mediates transcytosis by its pH-dependent binding affinity in endosomal compartments. This also allows for selective albumin sorting within the PT cell. This reclamation pathway minimizes urinary losses and catabolism of albumin, thus prolonging its serum half-life. It may also serve as a molecular sorter to preserve and reclaim normal albumin while allowing "altered" albumin to be catabolized *via* lysosomal pathways. Here, we critically review the data supporting this novel mechanism.

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Although the importance of urinary albumin in disease progression is known, the key mechanisms mediating the presence and toxic effects of albuminuria remain to be determined. Recently, the quantitative role of the glomerular filtration barrier (GFB) and the proximal tubule (PT) cell (PTC) in the development of albuminuria has been reexamined. Different lines of evidence, from multiple investigative teams, now suggest that the filtration of albumin, under physiologic conditions, is greater than previously determined. These data suggest an increased clinical role for the PT in minimizing albuminuria through the reabsorption of albumin. Emerging data also suggest that both glomerular permeability and PTCs play fundamental, physiologic, synergistic, interactive, and dynamic roles in the renal handling of albumin. Furthermore, it appears that PTCs, especially in the S1 segment, have specific mechanisms for efficiently and effectively reabsorbing and transcytosing albumin (reclamation). Therefore, the purpose of this review is to describe the emerging data regarding PTC albumin handling and provide a framework for considering future exciting, insightful, and novel studies with direct clinical relevance.

This review is not intended to debate the important role of the GFB but to emphasize that the PT should be considered important both under physiologic and pathologic conditions. We believe that glomerular or PTC defects can and do result in proteinuria. Specifically, we outline current data supporting PT uptake of albumin and mechanisms of reabsorption and transcytosis, and we propose a mechanism for intracellular sorting between degradation and transcytotic pathways based on pH-dependent binding.

DYSFUNCTIONAL PTCS LEAD TO ALBUMINURIA

For nearly 30 years albumin has been known to be reabsorbed by PTCs.[1] Albumin is a 66-kD, 585–amino acid, negatively charged globular protein found in plasma of mammals. It is produced and excreted by the liver and is the most abundant protein in plasma. Its half-life is approximately 35–39 hours in rodents and an impressive 19 days in humans.[2] Mammals have developed important cellular mechanisms for minimizing albumin turnover, thus enabling a long plasma half-life. Serum albumin is multifunctional as it buffers pH; provides oncotic pressure; and is a carrier protein for a wide range of molecules, including amino acids, fatty acids, inorganic ions, medications, and metabolites.[3,4] Preventing or reducing urinary albumin excretion thus makes the kidney a key player in "protecting" the organism from excessive loss of albumin

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and its ligands. Albumin loss in urine has long been used as a marker of kidney injury, whether it originates from glomerular dysfunction, defective PT reabsorption, or a combination.

Multiple investigative teams, using various preclinical model systems, have shown the PTCs, especially the S1 segment, have effective and efficient mechanisms of reabsorbing, transcytosing, and processing filtered albumin. Mechanisms for PTC uptake and metabolism of filtered albumin (Figure 1) include receptor-mediated clathrin-dependent endocytosis and fluid-phase endocytosis. Both processes can result in lysosomal targeting for degradation (degradation pathway),[5,6] and new data indicate that transcytosis moves albumin across the cell from apical to basolateral membranes into the extracellular fluid (reclamation pathway).[7–9] The overall quantitative importance of these processes is indicated by resulting albuminuria when selective defects occur in the involved tubular transport processes (Table 1). Individual disruption of numerous specific PTC processes, have been documented to cause proteinuria and albuminuria. In most cases the increase is mild to moderate, including loss of megalin and cubulin. Defects in the well studied multiligand endocytic receptor complex, megalin and cubilin, yield increased levels of albuminuria and proteinuria, suggesting a role in albumin reabsorption and metabolism.[5,6] Bardoxolone methyl, an anti-inflammatory mediator, is known to cause significant albuminuria by decreasing renal expression of megalin but not cubilin.[10] Rats receiving total-body irradiation lose the ability of albumin and megalin to bind to cubilin, resulting in albuminuria.[11] Knockout mice lacking the apical Na^+-H^+ exchanger isoform 3 (NHE-3)[12] develop albuminuria. Mutations in *ClC-5* in apical endosomes in three different mouse models, as seen in Dent disease, have demonstrated defective receptor-mediated endocytosis and fluid-phase endocytosis, deficient endosomal acidification, decreased internalization of the sodium-phosphate cotransporter 2 and NHE-3, and proteinuria.[13–15]

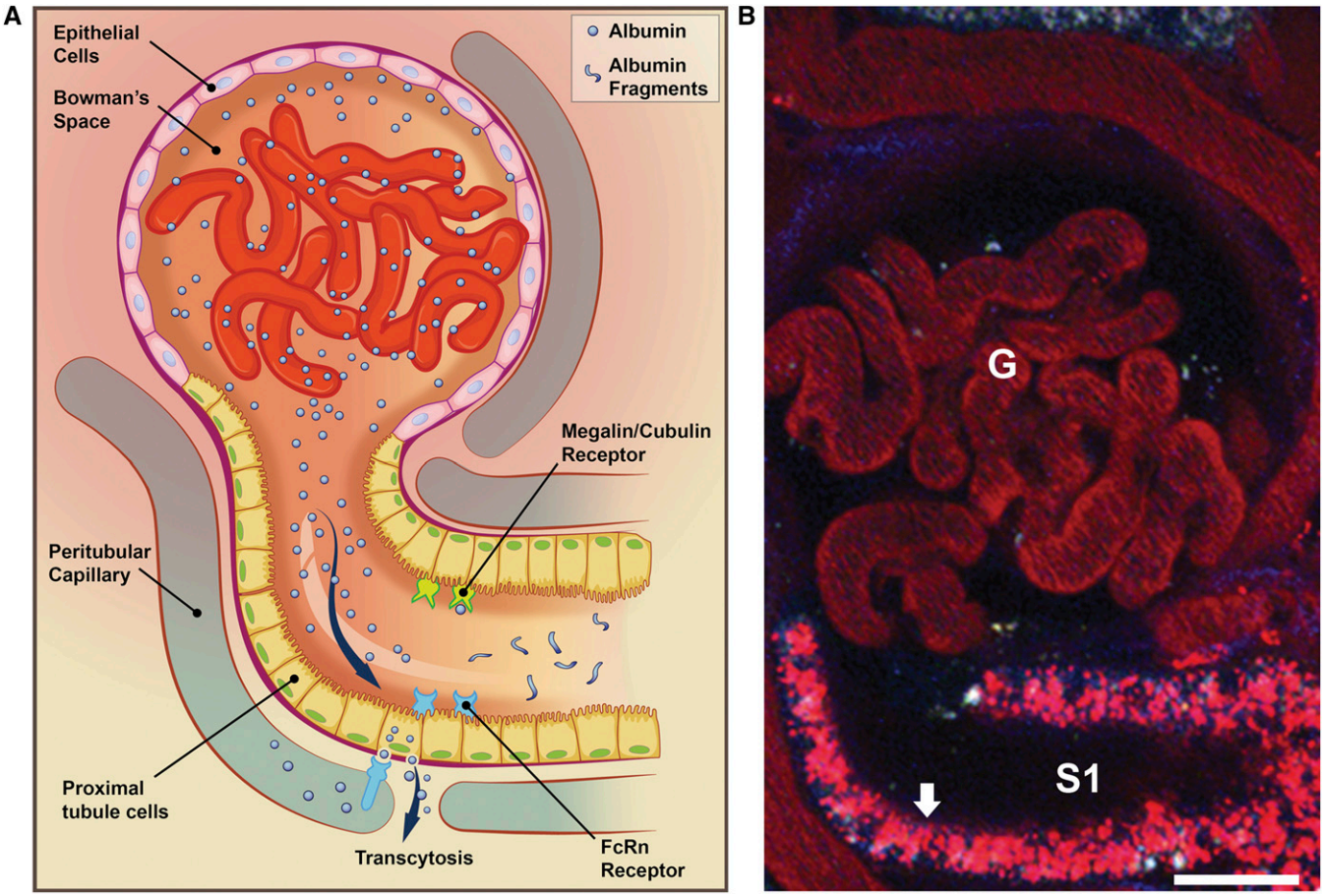

Figure 1. Albumin filtration across the glomerulus is greater than previously thought and reclaimed by the PTC, especially S1 cells. (A) Albumin filtered at the level of the glomerular capillaries into the Bowman space is taken up after binding by the megalin-cubilin receptor complex or perhaps by the FcRn lining the brush border of proximal tubular cells. Albumin is internalized to PTCs by receptor-mediated endocytosis *via* clathrin-coated vesicles and fluid-phase endocytosis. From there it can be catabolized *via* lysosomal degradation or can be transcytosed. Albumin fragments in the urinary lumen result from lysosomal exocytosis or peptide hydrolysis by apical membrane proteases. (B) *In vivo* image of 25-micron three-dimensional volume showing amounts of Texas red–labeled albumin uptake into PTCs (arrow), especially the S-1 segment (S1). G, glomerular capillaries. Bar=20 μm.

Table 1. Data implicating a role for the PT in albumin processing and/or albuminuria

Process Implicated or Defective	Reference
D-Serine–induced PTC injury	Carone and Ganote, 1975[23]
Megalin-cubilin complex	Birn, *et al.*, 2000[5]; Christensen and Birn, 2001[6]; Wang *et al.*, 2005[15]
ClC-5 knockout	Piwon *et al.*, 2000[13]; Christensen *et al.*, 2003[16]
Total-body irradiation	Yammani *et al.*, 2002[11]
NHE-3 knockout	Gekle *et al.*, 2004[12]
Statins	Sidaway *et al.*, 2004[20]; Verhulst *et al.*, 2004[21]; Atthobari *et al.*, 2006[22]
Rab 38	Rangel-Filho *et al.*, 2005[18]; Williams *et al.*, 2011[19]; Rangel-Filho *et al.*, 2013[17]
Increased GSCs	Russo *et al.*, 2007[7]
Transcytosis	Russo *et al.*, 2007,[7] Sandoval *et al.*, 2012[8]
FcRn	Sarav *et al.*, 2009[100]
Carbon nanotubes	Ruggiero *et al.*, 2010[126]
Bardoxolone	Reisman *et al.*, 2012[10]
Diphtheria toxin–induced PTC injury	Grgic *et al.*, 2012[25]; Sekine *et al.*, 2012[24]; Zhang *et al.*, 2012[26]

Multiple PTC defects have been shown to lead to significant albuminuria. Notably, selective PTC injury using dophtheria toxin induction, essentially eliminating any PT uptake of albumin, resulted in severe, but reversible, albuminuria without histologic or electron microscopic changes.

Defective endocytosis in *ClC-5* knockout mice is now known to be due to trafficking defects related to selective loss of brush-border cubilin and megalin, causing albuminuria.[16] Rab 38 dysfunction, or lack of function, is suggested to play a significant role in albuminuria in rats by decreasing endocytosis of colloidal gold-coupled albumin without modifying glomerular permeability.[17] The degree of proteinuria correlated best with the Rab 38 mutation, rather than the mutation in Fawn Hooded Hypertensive congenic rat strains associated with increased albumin permeability.[18,19] Statins have become of interest recently in albumin reabsorption. Studies have shown that statins may inhibit guanosine triphosphatase prenylation, which reduces PT endocytosis and enhances albuminuria and proteinuria.[20–22] Finally, in rats with selective PTC injury induced by using D-serine[23] or by expressing and activating the diphtheria toxin receptor on PTCs,[24–26] heavy albuminuria occurs without associated glomerular morphologic injury, neither histologic nor electron microscopic.[25,26]

These recent studies with diphtheria toxin, by three independent groups, have emphasized the magnitude of filtered albumin by selectively injuring the PTC, thus causing global PTC dysfunction and allowing all filtered albumin to end up in the urine. Thus, activating the receptor with diphtheria toxin[24–26] caused marked and sustained dose-dependent selective PTC injury that resulted in nephrotic range proteinuria.

IMPORTANCE OF PTC ALBUMIN REABSORPTION

There remains considerable controversy around glomerular albumin permeability. Numerous techniques and experimental approaches have been used to determine the quantitative role of glomerular albumin permeability in albuminuria. Values for the glomerular sieving coefficient of albumin have ranged from <0.001 to 0.07 under various physiologic and pathologic conditions using different techniques.[27] Of particular importance has been the use of Munich–Wistar (MW) rats that have surface glomeruli, allowing for direct dynamic visualization, instrumentation, and manipulation. MW Fromter (MWF) rats have many surface glomeruli, have been used in micropuncture studies, and spontaneously develop hypertension and progressive albuminuria beginning by week 8 and increasing to urinary albumin excretion >300 mg/24 hours by week 32. By week 40, 50% of glomeruli are sclerotic.[28–30] The Heymann nephritis rat model has also been used to study urinary protein loss and was significant in identifying megalin, found largely at the apical membrane of the PTC, as an autoantigen in membranous glomerulopathy.[31] MW Simonsen rats have fewer surface glomeruli and do not develop age-related spontaneous albuminuria under physiologic conditions. Mice unfortunately lack surface glomeruli, and therefore direct visualization methods cannot be performed, unless pathologic processes, such as ureteral obstruction for several days, are used.[32] This results in formation of atubular glomeruli and extensive alterations and remodeling of PT and glomerular cells.

Micropuncture studies in MWF rats with surface glomeruli measured low glomerular filtration of albumin in fasting states, with a glomerular sieving coefficient (GSC) of 0.00057–0.00062, consistent with low amounts of measured albumin observed in excreted urine (<30 mg/d).[33–38] This has been attributed to the charge barrier and size selectivity at the glomerular filtration barrier. Previous *in vivo* rat filtration studies and noninvasive studies using isolated perfused rat kidneys showed a much higher GSC of albumin using [^{3}H]albumin. Measuring total radioactivity in urine and inhibiting protein uptake in the PTC showed that the GSC of albumin may actually be approximately 0.074- to >120-fold greater than previously thought.[39] High GSCs for albumin were also observed by another group using glomerular volumetric analysis in rat glomeruli (0.02 ± 0.01).[40] This finding was strengthened by other groups showing that high-molecular-weight proteins

and dextrans, which have similar radius and molecular mass as albumin (3.6 nm and 66 kDa) and are not reabsorbed through receptor-mediated endocytosis, had similarly high GSCs in normal kidneys (pancreatic isoamylase: 3.4 nm, 45 kD, GSC of 0.03[41]; horseradish peroxidase: 3.0 nm, 40 kD, GSC of 0.06; Bence-Jones protein: 2.8 nm, 44 kD, GSC of 0.09[42]). Recent data using enhanced scanning electron microscopy have also shown that podocyte slit-diaphragm pore size is much larger than previously thought and is sufficiently large enough to allow for albumin filtration.[43]

Intravital *in vivo* two-photon microscopy studies, which allow four-dimensional analysis (volume and time) of physiologic processes, permit direct visualization and quantification of glomerular filtration and quantitation of PTC uptake.[44–46] This has allowed direct visualization and determination of GSCs for albumin (GSC_A), subcellular trafficking, transcytosis, catabolism, and reclamation of proteins from glomerular filtrate by PTCs.[45,46] Through use of this technique, MW Simonsen rats, which do not develop spontaneous albuminuria, have a GSC_A of 0.034 under physiologic fed states, while simultaneously measuring a GSC of 1.0 for inulin and approximately a 500-fold lower GSC for high-molecular-weight dextrans.[7,47] The GSC_A for MW Simonsen rats in fasting states is considerably lower at 0.016.[8] MWF rats, which develop albuminuria spontaneously with aging, have a lower fed GSC_A of 0.010 and also display a GSC_A reduction in fasting states to 0.007. Because micropuncture studies were always performed on fasting MWF rats, these studies indicate a much closer agreement between micropuncture studies and two-photon studies than previously reported when comparing two different rat strains.[8,48,49] They also indicate that feeding has a substantial effect on urinary albumin filtration.[8]

Controversy remains regarding the extent of glomerular filtration of albumin and similar-sized dextrans, used to model albumin filtration, as observed by two-photon microscopy. Detection of albumin in the glomerular filtrate requires maximizing the signal through use of the correct fluorescent probe, depth of study, site selection, detector sensitivity, and particular detail to background subtraction of existing autofluorescence.[8,50] Work by Peti-Peterdi and Tanner using two-photon microscopy reported values significantly lower than we have published,[51–53] which are closer to those from micropuncture studies. Peti-Peterdi and Tanner collectively pointed to multiple aspects of our studies as being causes for our elevated values. Addressing their points with data,[8] we refuted these assertions. Of particular importance, our most recent publication points to photo-multiplier tube detector offset settings as the factor probably explaining the observed differences.[50] These settings determine detector sensitivity and can cause variation in albumin permeability values spanning several orders of magnitude within the same glomerulus. Nakano *et al.*[51] state in their Methods section that the detector offset was adjusted for each individual glomeruli to reduce nonspecific fluorescence (autofluorescence) before and after dye injections. This is a departure from textbook approaches to correctly adjusting detector settings.[54] This approach was probably used to constrain tissue autofluorescence intensity to within the same low values derived from electronic detector noise; in fact, autofluorescence is a tangible phenomenon that when minimized by adjusting detector offset results in a progressively marked decrease in detector sensitivity. This lack of sensitivity is supported by a recent publication by Schießl and Castrop,[55] which also reports very low permeability values for albumin. In their work, the offset values used in their background image (Figure 1B, blue warning marker indicating values at zero) are an identical match to offset values in our study[50] showing decreased detector sensitivity to low-intensity fluorescence. A detailed method of our approach to determining GSC values can be found elsewhere.[56] Therefore, it appears the filtration of albumin across the GFB is dynamic with regard to feeding, varies between rat species, and is probably greater than previously determined by micropuncture studies.

ENDOCYTOSIS BY THE PT

Classically, cellular uptake of proteins and other molecules by endocytotic pathways has been attributed to receptor-mediated endocytosis by apical membrane-bound receptors, such as megalin and cubilin, clustering into clathrin-coated pits. Coated pits make up between 0.4% and 3.8% of the cell's surface, depending on the cell type.[57] These pathways have been studied extensively, and numerous reviews exist.[58,59] In addition, other mechanisms of protein internalization have also been described, including caveolin-dependent internalization and fluid-phase endocytosis. Molecules endocytosed in this manner are similarly routed to the sorting endosomal compartment and either are degraded through lysosomal pathways or undergo transcytosis back into circulation.[60] Albumin uptake by nonselective fluid-phase endocytosis is probably a quantitatively important process in PTCs, as shown by the rapid cellular uptake of molecules not having receptors on the apical membrane, such as neutral fluorescent dextrans (markers of fluid-phase endocytosis).[61,62]

The endocytic apparatus is found throughout the PT, although clathrin-coated pits and vesicles are notably fewer in the S3 segment.[63] Expectedly, protein reabsorption and degradation are greatest in the S1 segment of the PTCs and least in the S3 segment.[64–66] Kinetic studies of the rat PT have shown that internalization of cargo at the brush border is highly active. The membrane and trapped fluid (luminal fluid) contained in the apical membrane invaginations are internalized within 78 seconds.[67] This rapid rate of uptake means a great deal of luminal fluid is internalized *via* endocytic vesicles and probably indicates an important role for fluid phase endocytosis. However, quantifying the overall importance of fluid-phase

endocytosis has been difficult because all endocytic vesicles contain fluid and thus luminal contents.

Albumin degradation can occur in multiple sites. Degraded lysosomal albumin fragments were initially thought to be completely recycled back into circulation.[1] But newer studies using isolated perfused rat kidneys,[68] *in vitro* studies with HK-2 cells,[69] and *in vivo* models with Sprague-Dawley rats have shown that albumin can be rapidly degraded into small peptides and released back into the tubular fluid.[66] Use of the CD2AP knockout mouse showed that lack of lysosomal PTC albumin degradation resulted in high levels of intact albumin in urine.[68,70] Recent technologies using opossum kidney epithelial cells suggest that albumin uptake and degradation are significantly augmented by flow and fluid shear stress, not static conditions.[71] Urine proteases at the apical brush border can likewise hydrolyze and degrade tubular albumin into urinary fragments.[72] However, a note of caution is necessary. Modeling *in vivo* endocytosis to that occurring in cultured cells can be very revealing but also misleading. For example, *in vivo* PTCs have a rate of endocytosis that is far greater in magnitude than that of cultured PTCs.[67,73,74] In addition, the rate of apical endocytosis is many times that of basolateral endocytosis *in vivo,* but the two are equivalent in cell culture.[67,74,75] Therefore, one must not overinterpret cell culture data. Finally, intravenously injected albumin remains intact within the serum and is partially catabolized within PTCs.[76]

THE MEGALIN-CUBILIN COMPLEX

The megalin-cubilin receptor complex is well studied, and myriad reviews have described its function and role in protein absorption and metabolism.[6,59,77,78] The dissociation constant (K_d) of albumin to cubilin is estimated at 0.63 μM at a pH of 7.0,[5] resulting in a high-affinity, low-capacity pathway of endocytosis that primarily targets product to the lysosome for degradation. Subsequent lysosomal processing and trafficking of resulting amino acids back to the basolateral membrane for transport into plasma occur. Megalin and cubilin work in concert to reabsorb >40 filtered molecules.[6,59,79–81] Without this mechanism of retrieval and preservation, protein loss, malnutrition, vitamin deficiencies, and other consequences would ensue. Although both megalin[82] and cubilin[5] can bind albumin, megalin's principal role seems to be in catalyzing the retrieval and internalization of apical cubilin-albumin complexes from glomerular filtrate.

This multireceptor retrieval system is thought to have the capacity to process approximately 30–50 μg of albumin daily in mice.[83] Disruption of this mechanism results in proteinuria and albuminuria. In megalin knockout models, the internalization of endogenous ligands bound to apical cubilin, especially cubilin-albumin complexes, is markedly reduced. Urinary albumin excretion is increased 6-fold in cubilin knockout mouse models[83] and in humans,[84] although neither reaches nephrotic range, suggesting that an additional mechanism for albumin reabsorption exist. In fact, a missense mutation in one of the *CUBN* domains that binds megalin was found in microalbuminuric patients in the general population and in patients with diabetes using a genome-wide association study.[85] In two siblings with intermittent proteinuria reaching 2 g daily, exome sequencing was used to identify a homozygous frameshift mutation in cubulin, resulting in decreased albumin uptake in the PT.[86] Interestingly, mice deficient in megalin in addition to cubilin did not exhibit any more albuminuria than mice with cubilin deficiency alone,[83] suggesting that megalin's principal role is to facilitate cubilin-albumin internalization. In Dab2 knockout mice (Dab2 is a protein involved in coated pit formation), mild proteinuria was seen.[87] Patients with type 1 diabetes and albuminuria had significantly elevated urinary levels of megalin and cubilin, suggesting possible PT shedding of these proteins as a contributing factor to albuminuria in patients with diabetes.[88] Moreover, in early streptozotocin-induced diabetes in rats, the GSC_A was unchanged but PTC uptake of albumin was markedly reduced.[47]

NEONATAL FC RECEPTOR

The neonatal Fc receptor (FcRn), discovered by Jones and Waldmann in 1972,[89] is a heterodimer with class I MHC-like properties that contains a membrane-bound heavy chain and a β_2-microglobulin light chain. Its name originates the fact that it was purified and sequenced by Simister and Rees in 1985[90] from the intestine of an 11-day-old rat. Wild-type FcRn has two distinct and separate binding sites for albumin and IgG[91]; binding is low affinity and high capacity at a physiologic pH, with increasing affinity occurring at a lower pH. In humans, FcRn is derived from the *FCGRT* gene encoded on chromosome 19 located outside of the MHC class I locus on chromosome 6. Rat and mice FcRns are 91% identical, and both are encoded on chromosome 7. Human FcRn has one *N*-glycan moiety, and its molecular mass is approximately 42–44 kD, while rat FcRn's molecular mass is 48–52 kD (attributable to three additional *N*-glycan moieties).[92] It is known to reside on vascular endothelium; on epithelial cells of the proximal small intestine, liver, spleen, and lung; on placental syncytiotrophoblasts; on polymorphonuclear neutrophils, monocytes, and phagocytes; on dendritic cells; and in the kidney.[90,93–98] Within the kidney, FcRn is found in the vascular endothelia, podocytes, cortical collecting duct, and PT epithelial cells.[99] It has been labeled by immunofluorescence in human kidney sections at the brush border of PTCs and in endosomes.[99]

FcRn is known to transport albumin across membranes, preserving albumin's function and lifespan as a carrier protein, colloid, and buffer, and one that maintains oncotic hemostasis.[100] Its role in fetal immunity by transporting maternal IgG across neonatal placenta and by transcytosis across neonatal intestinal cells is well known.[90,101–103] In

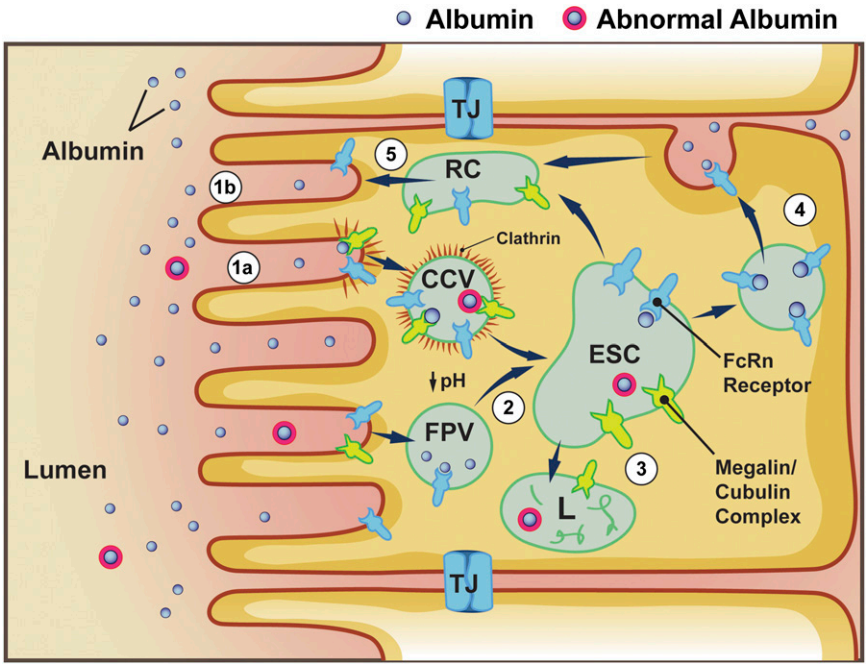

Figure 2. FcRn mediates pH-dependent transcytosis and intracellular sorting of reabsorbed albumin. Albumin is reabsorbed *via* both receptor-mediated clathrin-coated pits into vesicles (CCV) (1a) and by fluid-phase (clathrin-negative) endocytosis (1b). Following endocytosis, endosomal acidification occurs (2), causing dissociation of albumin from receptors, such as megalin-cubilin complexes. However, acidification enhances albumin binding to FcRn throughout endocytic compartments; thus, there is exchange of albumin from the megalin-cubulin complex to FcRn. Within the endosomal-sorting compartment (ESC), albumin is directed toward lysosomal degradation or the transcytotic pathway (3). Transcytosis occurs by both vascular and tubular structures mediating albumin delivery to the basolateral membrane (4). Upon fusion with the basolateral membrane, the increase in pH of the extracellular environment causes dissociation of albumin from FcRn; FcRn is then recycled back to the apical membrane *via* the recycling compartment. It is possible that albumin's binding to FcRn is reduced by alterations, such as glycosylation and carbamylation; thus, transcytosis of albumin would not occur and albumin would enter the lysosomal pathway. This would provide an intracellular molecular sorting mechanism to preserve physiologic albumin and facilitate catabolism of chemically altered albumin. FPV, fluid-phase vesicle; L, lysosome; RC, recycling compartment; TJ, tight junction.

fact, neonatal overexpression of FcRn in transgenic mice and rabbits increases serum albumin concentrations and further augments humoral immunity, resulting in a 3- to 10-fold increase in IgM and IgG concentrations in serum.[104,105] It is thought that elevated immunoglobulin levels would not interfere with albumin processing because of the distinct binding sites for albumin and immunoglobulins, but this concept has not been tested. FcRn mediates transcellular IgG transport in maternal milk during lactation to the newborn.[106,107] FcRn is also thought to preserve IgG and confers humoral immunity by podocyte clearance of IgG from the GBM.[108] Finally, FcRn is felt to mediate transcytosis and recycling of IgG by PTCs back into circulation.[109] The mechanism of FcRn-mediated transcytosis has been well studied in the small intestine, and its role in IgG endocytosis *via* clathrin-coated pits at low luminal pH is known.[110,111] In addition, data exist for other tissues for IgG transport, such as pneumocytes,[112] endothelia of small arterioles and capillaries of skeletal muscle and skin,[93,113] and vascular endothelia of the central nervous system and the choroid plexus.[114]

ROLE OF FcRn IN PTC ALBUMIN PROCESSING

Although not yet fully understood, the role of FcRn appears to be that of intracellular selection, sorting, and preservation of reabsorbed albumin and IgG. FcRn is concentrated into the apical area in the PT. Whether it participates in luminal albumin binding is not known, but this is not favored by the luminal pH. However, the megalin-cubilin–bound albumin within the clathrin-coated pits, and fluid-phase endocytosis vesicles undergo pH reduction to approximately 5.0. At the low pH found in endosomes, albumin dissociates from megalin-cubilin, while FcRn's affinity to bind both IgG and albumin increases dramatically.[90,91,100,115] Thus, albumin is capable of moving from a low-capacity lysosomal degradation pathway[39,116,117] to a high-capacity pathway of transcytosis and recycling mediated by FcRn based on inherent binding properties of the receptors.[39,118–120] Binding studies have shown that FcRn has a single binding site for albumin that is distinct from the IgG site and that both these interactions are pH dependent. The equilibrium dissociation constant, K_d, is much weaker at a pH of 7.0 (34–408 μM) versus a pH of 5.0 (0.2–0.7 μM).[91] Consequently, if albumin is internalized while bound to the megalin-cubulin complex and is trafficked to the late endosomes, it encounters acidic pH and a "handoff" of albumin to the FcRn receptor can occur, thus directing it down the transcytotic pathway. When the transcytotic vesicle fuses with the plasma membrane and encounter neutral physiologic pH, a rapid dissociation of albumin from FcRn will occur, thereby releasing it to the interstitium and ultimately back into the circulation *via* the FcRn-mediated pathway in the endothelium.[110,115,121] The FcRn receptor is recycled back to the apical membrane or apical compartment, ready for another cycle of albumin transcytosis. Of critical importance for albumin dynamics may be how modified albumins (*i.e.*, glycated, carbamylated, and various drugs bound to albumin) affect the albumin–FcRn pH-dependent

binding interaction. For instance, increased binding at a neutral pH or decreased binding at an acidic pH may both result in more targeting to lysosomes (Figure 2).

The first direct evidence for transcytosis of albumin came from PT microperfusion studies.[1] Subsequent studies using transmission electron microscopy immunogold studies revealed albumin uptake across the apical membrane and release across the basolateral membrane of PTCs.[7] Subsequent two-photon studies showed actual intracellular vesicles and tubules uniting with the basolateral membrane and releasing fluorescently labeled albumin into the interstitium.[8] Finally, Tenten *et al.*[9] showed that both negatively charged and neutral albumin released from transgenic podocytes was transcytosed from the filtrate into the blood. Furthermore, genetic deletion of the FcRn receptor in these mice abolished transcytosis of both types of albumin. These data prove FcRn is responsible for mediating albumin transcytosis in the PTC. However, the magnitude of this process remains to be determined.

FUNCTIONAL SIGNIFICANCE OF PTC RECLAMATION *VIA* FcRn

FcRn knockout mice lacking the neonatal Fc receptor and its ability to recycle filtered protein have been shown to result in plasma albumin with shorter half-lives (reduced to 75% of wild type) and plasma concentrations that are reduced by about 50%.[100,122,123] This was shown to result from greater catabolism and clearance of albumin.[122] Unfortunately, this study did not quantify urinary albumin excretion. In another study, FcRn knockout mice exhibited more albumin at the brush border and a modest increase in fractional excretion of albumin.[100] Mice that have β_2-microglobulin or FcRn mutations have reduced half-lives of both IgG and albumin,[123,124] and β_2-microglobulin knockout mice (which are therefore FcRn deficient) have increased urinary IgG excretion and albuminuria.[125]

PT-specific FcRn was further implicated when FcRn knockout kidneys were placed in wild-type mice and serum albumin declined to 40%–50% of baseline over 3 weeks. Conversely, serum albumin increased as wild-type kidneys were placed in FcRn knockout mice.[100] These data indicate the absence of FcRn results in increased urinary loss of albumin and enhanced catabolism. In PTCs, the lack of transcytotic pathway would shuttle all reabsorbed albumin into the degradation pathway. Further PT-specific studies are needed to more fully examine the exact role of FcRn within the kidney and its function in mediating transcytosis and preventing lysosomal degradation of albumin.

CONCLUSIONS

Currently the role of the PTC in albumin reabsorption and reclamation is being rewritten. Numerous single-site alterations cause proteinuria, and complete PTC dysfunction results in a high level of albuminuria, without histologic or electron microscopy structural alterations in the GFB, implying a GSC_A greater than previously believed. This has also been shown using intravital two-photon imaging. Reabsorption of filtered albumin involves a high-affinity, low-capacity megalin-cubulin receptor–mediated process and a low-affinity, high-capacity process that we believe is fluid-phase endocytosis. Data on the neonatal Fc receptor within the PT cell suggest that its principal function may be in pH-mediated binding, sorting and intracellular trafficking between transcytosis and degradation pathways. This latter function may be decided based on alterations in albumin binding at low pH to FcRn. Such a mechanism of selective processing and sorting would be evolutionarily critical in reclaiming normal albumin *via* transcytosis and in the lysosomal catabolism of chemically altered and potentially harmful albumin. Given the quantity of albumin reabsorbed daily, and the prolonged half-life of serum albumin, this is an absolutely essential process. Two-photon intravital studies to delineate the roles of altered GFB permeability, PTC endocytosis, and the intracellular trafficking of albumin are needed in the different animal models available to offer further insight into the renal handling of albumin. Additional reagents and approaches must be developed to allow assessment in human diseases.

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DISCLOSURES

None.

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