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INVITED REVIEW

Role of albumin and its modifications in glomerular injury

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Abstract Albuminuria is both a characteristic hallmark and a known risk factor for progressive glomerular disease. Although the molecular basis for a potential causative role for albuminuria in progressive chronic kidney disease remains poorly understood, there have been several recent advances in our understanding of the role of albumin, and its molecular modifications, in the development and progression of glomerular disease. This review discusses recent findings related to the ability of albumin and its associated factors to directly induce podocyte and glomerular injury. Additional recent studies confirming the ability and mechanisms by which podocytes endocytose albumin are also discussed. Lastly, we present several known molecular modifications in the albumin molecule itself, as well as substances bound to it, which may be important and potentially clinically relevant mediators of albumin-induced glomerular injury. These recent findings may create entirely new opportunities to develop novel future therapies directed at albumin that could potentially help reduce podocyte and renal tubular injury and slow the progression of chronic glomerular disease.

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Introduction

Proteinuria, which reflects primarily urinary albumin, is well recognized not only as a marker but also a risk factor for progressive glomerular disease [14, 40]. In the normal state, only small amounts of albumin are able to cross the glomerular filtration barrier, and the filtered albumin is reabsorbed very efficiently by the renal tubular cells, resulting in negligible albuminuria [11, 34]. However, following injury to the glomerular filtration barrier, as occurs during glomerular disease, significantly greater amounts of albumin cross the barrier and enter the urinary space. Historically, this was recognized clinically simply as proteinuria. Over the last two decades, however, we have learned that such proteinuria was associated more proximally in the nephron with dramatically increased albumin uptake in renal tubular cells. Importantly, this albumin uptake was found to stimulate tubular cell toxicity, including stimulation of signaling pathways leading to the release of inflammatory, vasoactive, and fibrotic substances that cumulatively induced tubulointerstitial dysfunction and fibrosis, which if persistent, could lead to progressive renal disease [45, 46]. More recently, we have also begun to recognize that not only renal tubular cells, but also podocytes, are targets of injury resulting from leakage of albumin across a damaged glomerular filtration barrier [4]. This review focuses on our growing understanding of albumin-induced podocyte injury that also accompanies clinical proteinuria during glomerular disease (see Fig. 1). We also discuss our evolving appreciation that albumin can undergo multiple biomolecular modifications, and that albumin-induced podocyte toxicity results not only from the quantity of albumin delivered to podocytes, but also from the biomolecular characteristics of the albumin molecule itself.
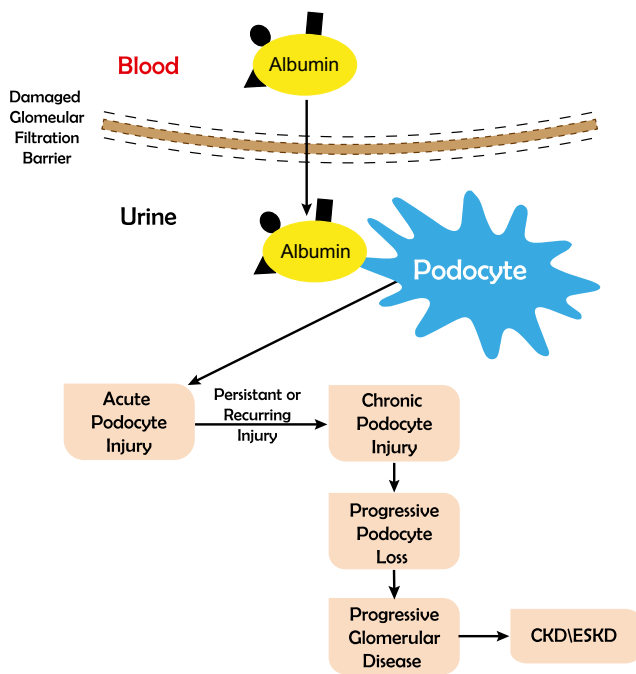
Fig. 1 Schematic of the causal role of albumin in progressive glomerular disease. Following injury to the glomerular filtration barrier, as occurs during glomerular disease, significantly greater amounts of albumin cross the filtration barrier and enter the urinary space. Prolonged exposure of podocytes to increased concentrations of albumin, including potentially toxic factors bound to it, leads to progressive podocyte loss, resulting in progressive glomerular disease, and ultimately in chronic kidney disease (CKD) and end-stage kidney disease (ESKD).

Finally, we discuss briefly how this knowledge might be able to be used to improve the treatment of glomerular disease in the future.

Albumin overload and glomerular injury

Albumin overload in animals has been an extensively studied model to elucidate the structural, pathological, and molecular changes in renal diseases [9, 25, 27, 47]. Tubulointerstitial injury has been an area of extensive focus in such animal models, while a few studies have also reported molecular changes in podocytes, in addition to structural and pathological changes [4, 9, 25, 47, 58, 71]. Albumin overload results in tubulointerstitial injury by eliciting a pro-inflammatory response, macrophage and lymphocyte infiltration, tubular atrophy, and interstitial fibrosis [7, 25–27]. While immune cell infiltration is not observed in the glomeruli, there are increases in IgG, IgM, C3, and C5 antigens, as well as glomerular enlargement and podocyte effacement and detachment from the GBM [9, 25, 47, 71]. Molecular changes in the glomeruli upon albumin overload include TNF-α and IL-1β induction in mice and increased osteopontin expression in rats [26, 58]. In our own work, we have reported the induction of COX-2,

the pro-inflammatory genes MCP-1 and CXCL1, and the small heat shock protein HSP25 in the glomeruli of rats subjected to albumin overload [4]. Thus, exposure to excessive amounts of albumin is clearly able to induce pro-inflammatory and pro-fibrotic changes in both the glomerular and tubulointerstitial compartments of the kidney.

Albumin deficiency and glomerular injury

On the other end of the spectrum, albumin deficiency resulting from a spontaneous mutation in Nagase analbuminemic rats (NAR) has been investigated in a few models of glomerular injury, with varying results [2, 32, 33, 54, 55, 59]. The existence of such analbuminemic rats was first recognized in the 1970s by Dr. Nagase in hypercholesterolemic Sprague Dawley rats (SDR) [54, 55]. While these rats lack albumin, they exhibit compensatory increases in other plasma proteins, as well as cholesterol and triglycerides. Following induction of puromycin aminonucleoside (PAN) nephrosis, while NAR did not exhibit proteinuria, the extent of podocyte foot process abnormality was found to be similar to SDR [2]. In a subtotal ¾ nephrectomy injury model, while proteinuria in NAR was significantly lower than that of SDR, NAR showed a higher frequency of glomerular lesions than SDR [48]. In an age-related glomerulosclerosis study, comparisons were made between NAR and SDR at 3 and 18 months of age. These studies revealed that NAR, unlike SDR, did not develop age-related glomerulosclerosis, which was attributed to be due to lower glomerular pressure and volume, and lower plasma triglyceride levels [32]. In an Adriamycin-induced nephropathy model, both NAR and SDR developed the same degree of focal glomerulosclerosis [59]. More recently, the role of albumin in injuring kidney cells has been underscored in Alport syndrome using an albumin knock-out model in mice [42]. Lack of albumin in $Col4a3^{-/-} Alb^{-/-}$ mice resulted in improved kidney outcome compared to $Col4a3^{-/-}$ mice. In addition, these albumin-deficient mice also were found to have improved survival, reduced transforming growth factor-β1signaling, as well as reduced tubulointerstitial, glomerular, and podocyte pathology. Together these studies provide additional, albeit indirect, support for a potentially important role for albumin during glomerular disease.

Albumin handling by podocytes

The appearance of albumin in the urine (i.e., albuminuria) is regulated by several factors. The first level of regulation is the amount of albumin filtered from the glomerular capillary lumen through the glomerular filtration barrier (which is composed of glomerular endothelial cells, GBM, podocytes, and podocyte slit diaphragms). Once albumin enters the urinary

space, a second level of regulation is the extent to which it is actively reabsorbed by renal tubular cells. In this regard, proximal tubule cells (PTCs) have been reported to very effectively reabsorb physiologic amounts of albumin in the urinary space [69], with albumin only reaching the urine (i.e., albuminuria) once the renal tubular capacity for albumin reabsorption has been exceeded. In this setting, however, it has been known for some time that exposure of PTCs to excessive amounts of albumin results in the activation of several molecular signaling pathways, and production of chemoattractants, profibrotic agents, and matrix proteins, which can ultimately result in interstitial inflammation and fibrosis [13].

Only more recently have we begun to appreciate that podocytes, like PTCs, can also reabsorb the albumin that enters the urinary space during glomerular disease. Podocytes have been found to have a variety of responses to albumin exposure, including endocytosis [23, 28], macropinocytosis [19], increased TGF-β and p38 MAPK signaling, and loss of synaptopodin [1, 76]. Podocyte exposure to albumin has also been reported to induce apoptosis in association with CD2AP downregulation and endoplasmic stress [37], TRPC6-mediated increases in intracellular Ca(2+) [18], increased MMP-2 and MMP-9 expression [29], modulation of the endothelin-1 gene with actin cytoskeletal reorganization [51], and induction of genes encoding COX-2 and proinflammatory and stress proteins [4, 58]. In our own work, we observed loss of cell viability, actin cytoskeleton rearrangement and significant inductions of COX-2, B7–1, MCP-1, CXCL1, HSP25, and HSP70i in podocytes exposed to normal serum concentrations of albumin [4]. We also observed albumin concentration-dependent COX-2 induction, suggesting that even subtle increases in albumin concentrations in the urinary space can result in increased COX-2 expression in podocytes. Thus, the ability of albumin to stimulate these various signaling and injury response pathways in podocytes suggests a variety of potential molecular mechanisms by which albumin may directly injure podocytes following injury to the glomerular filtration barrier.

Mechanism of albumin endocytosis by podocytes

As noted above, in the normal state, the components of the glomerular filtration barrier (glomerular endothelial cells, GBM, podocytes, and slit diaphragms) all act together to largely prevent the passage of large serum macromolecules into the urinary space. In addition, cellular endocytic mechanisms normally control the lipid and protein composition of cells, and act as gateways to extracellular components, thus playing an important role in many human disease processes [24]. In the setting of glomerular disease, endocytosis of plasma proteins leading to glomerular vacuolization in focal segmental glomerulosclerosis (FSGS) has long been reported as a

prognostic marker of kidney disease progression [77]. Additionally, rodent models of nephrotic syndrome (NS) injury have reported the presence of albumin-containing vesicles in podocytes [43]. In recent years many endocytic portals have been recognized in podocytes. These portals provide a mechanism for the clearance and trafficking of proteins in the normal state. However, they may also serve as a mechanism for the uptake of the excess albumin presented to podocytes during glomerular disease, which may induce direct podocyte injury (see Fig. 2).

Endocytosis is often reported to occur via both clathrin-dependent and independent pathways, and both of these pathways have been demonstrated to function in podocytes [39, 66]. Podocytes utilize clathrin-mediated pathways to internalize a variety of proteins, during both development and normal physiologic maintenance, as well as disease states [66]. Notably, the critical slit diaphragm protein, nephrin, has been reported to be internalized via clathrin- as well as raft-mediated endocytosis in podocytes [60]. Furthermore, a strong link has been demonstrated between clathrin-mediated endocytosis in podocytes and actin cytoskeleton arrangement [65, 66, 75], which is critical to the proper functioning of podocytes.

More recently, it has been demonstrated that podocytes endocytose albumin primarily via the caveolae-mediated pathway, rather than the clathrin-mediated pathway [23, 61]. Once internalized, a portion of the albumin gets degraded by lysosomes and a portion of it presumably gets transcytosed via the neonatal Fc receptor (FcRN). Moreover, most of the caveolae-mediated endocytosis takes place in the basal domain, rather than in the apical domain, as has been demonstrated by the use of confocal imaging and total internal reflection fluorescence microscopy [23].

FcRN, a known IgG and albumin transport receptor, is a well-studied receptor involved in PTC albumin uptake, and has also been shown to be expressed in podocytes [5, 36, 44, 63]. Its role was first identified in the transport of IgG antibodies from the mother to the fetus or neonate, and in protecting IgG from intracellular catabolism [63]. More recently, due to its well-recognized interaction with albumin, FcRN has emerged as a new target to design albumin-based therapeutics [63]. In the context of podocytes, it has been reported that mice deficient in FcRN demonstrated delayed IgG clearance from the GBM and potentiated the pathogenicity of nephrotoxic sera [5].

Even more recently, a new portal of endocytosis known as macropinocytosis has been recognized to function in podocytes [6, 19]. Macropinocytosis leads to the internalization of extracellular fluids and large molecules in a very efficient manner [24]. Podocytes uptake albumin by macropinocytosis, the rate of which is greatly increased by the free fatty acids (FFA) bound to albumin [19]. Moreover, this pathway is stimulated
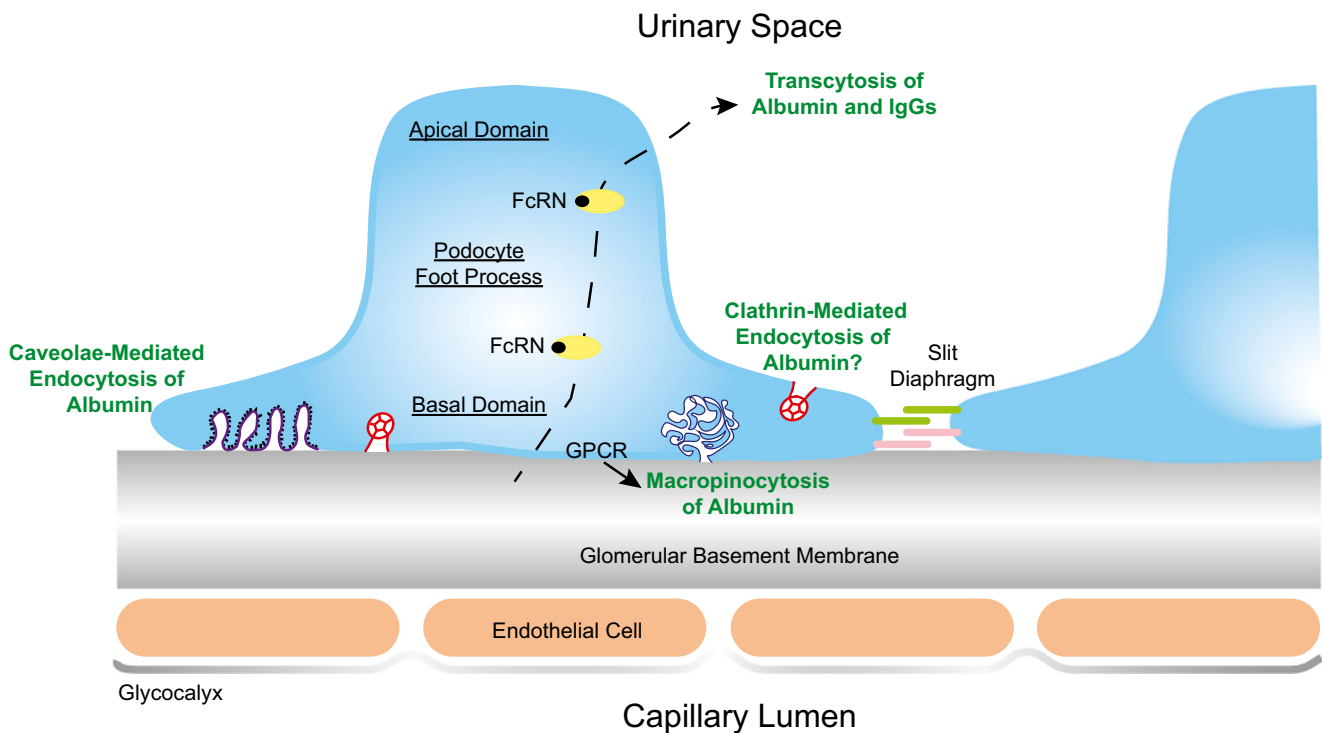
Urinary Space

Fig. 2 Schematic of recognized albumin endocytic portals in podocytes. The components of the glomerular filtration barrier (glomerular endothelial cells, GBM, podocytes, and slit diaphragms) all act together to prevent the passage of cells and larger serum macromolecules, including albumin, into the urinary space. The endocytic portals in podocytes internalize protein molecules in the normal state, and uptake increased amounts of albumin that leaks across the filtration barrier during glomerular disease. While the role of clathrin-mediated endocytosis in podocytes has been well described during development, maintenance and disease, its role in specific uptake of albumin in podocytes is unclear [39, 60, 65, 66, 75]. Albumin has been reported to be internalized in podocytes by caveolae-mediated endocytosis at the basal surface [23, 61]. Once internalized, part of the albumin molecule gets transcytosed into the urinary space via the neonatal Fc receptor (FcRN) [5]. More recently, macropinocytosis has been recognized as another major endocytic portal for albumin in podocytes [19]

by FFA-binding G-protein-coupled receptors (GPCRs) and Gβ/Gγ subunits, which in turn activate the Rho GTPases, RAC1, and CDC42, leading to cytoskeletal disruption and podocyte injury [19].

In summary, endocytosis of albumin by podocytes utilizing the different portals described above may serve as both a mechanism of clearance and trafficking of albumin in the healthy state, but also as a potentially important molecular mechanism of podocyte injury during glomerular disease.

Albumin modifications in glomerular and podocyte injury

The albumin protein is organized into three domains, I, II, and III, each divided into two subdomains, with 17 intramolecular disulfide bonds, and a free Cys^{34} residue [8]. The structure of albumin allows it to bind and transport diverse molecules in the plasma, including fatty acids, metal ions, hormones, drugs, etc. Due to the molecular characteristics noted above, albumin is also very susceptible to a wide variety of modifications induced by pH and other biophysical compounds.

Lipidated albumin

When albumin was first crystallized in 1941 by Kendall, the crystals were found to contain a small amount of FFA [67]. It was later shown that albumin is in fact a transport vehicle for FFA in blood [67]. Thus, it is this FFA-bound or lipidated form of albumin that is encountered by PTCs and podocytes once albumin crosses the glomerular filtration barrier. It has been long observed that the FFA carried on albumin may be the prime contributors of tubulointerstitial injury in the albumin overload model of renal injury, and several studies have also reported the lipotoxic roles of saturated fatty acids in proximal tubular injury [41, 49, 62, 70, 78]. More importantly, several different FFAs bound to albumin have also been shown to increase podocyte injury [4, 6, 19]. Macropinocytosis of albumin by podocytes has been shown to be enhanced with lipidated albumin via the stimulation of lipid-binding GPCRs [6, 19]. Moreover, mice fed a high-fat diet developed elevated levels of FFA, and were found to have increased susceptibility to Adriamycin-induced proteinuria compared to mice fed control chow [6, 19]. We have also recently reported that lipidated albumin induces significantly

greater glomerular injury and proteinuria in vivo in a rat albumin overload model, as well as greater podocyte injury in vitro in cultured podocytes, compared to de-lipidated albumin [4]. Furthermore, the FFA content of the albumin retained in the plasma in NS has been reported to be greater than the albumin lost in the urine, which in combination with hypoalbuminemia results in an increased plasma FFA/albumin ratio [20, 35]. These changes have been further reported to culminate in increased angiopoietin like-4 (Angptl-4) expression and secretion from peripheral organs, leading to hypertriglyceridemia [20, 35].

Oxidized albumin

Increased reactive oxygen species (ROS) have been reported to play an important role in the pathophysiology of many diseases that result in inflammatory organ damage or neoplasia [15]. Since albumin is the most prevalent protein in the plasma, it thus plays a key role in the antioxidant function present in plasma. The oxido-redox potential of albumin changes upon oxidation, and this modification thus represents a chemical marker of oxidative stress. The ROS react primarily with the free cysteine (Cys^{34}) residue of albumin, which drives the formation of sulfenic acid (SOH-Alb) → sulfinic acid (SO_2-H-Alb) → sulfonic acid (SO_3-H-Alb). Upon stable oxidation to the sulfonic form, the properties of albumin are altered and it becomes more prone to faster degradation. A few reports have suggested an indirect association of ROS and free radicals with FSGS [52, 53]. Importantly, massive oxidation of plasma albumin has also been demonstrated in FSGS patients, leading to its chemical modification. The finding of in vivo oxidation of plasma albumin in patients with FSGS, given its greatly increased risk for disease progression, adds further evidence suggesting a potential role for modification of the albumin molecule as an additional risk factor for progressive chronic kidney disease (CKD) in FSGS, and potentially other glomerular diseases.

Cationic albumin

The cationic form of albumin (cBSA; cationic bovine serum albumin) has been long used to induce immune complex glomerulonephritis (ICGN) in animal models to replicate human membranous nephropathy [73, 74]. Membranous nephropathy is characterized by in situ immune complex deposition in the glomerular subepithelial space, GBM thickening, nephrotic syndrome, and a predominantly type-2 immune response [38]. Animal models of ICGN using cBSA injections have also been widely reported in rats, mice, dogs, cats, and rabbits [10, 12, 17, 56, 57, 72–74]. In addition, multiple children with membranous nephropathy have also been found to have circulating cBSA, which has been suggested to have imparted its pathogenic role through binding to the anionic GBM, and

subsequent formation of immune complexes [22]. Moreover, less anionic forms of albumin have also been reported to be present in the urine of patients with NS [35].

Glycated albumin

Albumin is highly sensitive to glycation, as it is highly abundant in plasma and it undergoes structural and functional changes upon binding to reduced sugars [8]. In fact, due to stable structural changes associated with glycation, it is emerging as a new marker for diabetes, superseding HbA1C [8, 21]. Interestingly, new findings are also implicating a causal role of glycated albumin in promoting proteinuria and glomerulosclerosis, in addition to its potential role as a biomarker [21]. In this context, it is notable that glycated albumin has been found to penetrate the glomerular filtration barrier deeper than non-glycated albumin, thus modifying the glomerular filtration properties [50]. This effect, however, was not as a direct result of changes in the anionic charge distribution and density of the GBM by glycated albumin [50]. Thus, glycation of albumin represents yet another molecular modification able to alter the physical and chemical characteristics of albumin, which could also potentially alter its glomerular permeability and toxicity.

Role of the glomerular filtration barrier in albumin permeability

The glomerular filtration barrier (described above) functions as a single entity that prevents leakage of large proteins from the plasma. The various layers and cells comprising it each play important unique roles and exhibit crosstalk in maintaining the overall filtration barrier function (see Fig. 2). The glomerular endothelium is characterized by fenestrations and a surface glycocalyx, and is the most proximal barrier to the filtration of albumin from the plasma [64]. Dysfunction in the endothelium results in increased glomerular permeability in disease, and crosstalk between the endothelial cells and podocytes also regulates the ability of podocytes to handle albumin. The GBM provides the second barrier to the filtration of albumin, and is a non-cellular layer between the endothelium and the interdigitating foot processes of podocytes [68]. It is composed of four types of extracellular matrix macromolecules: laminin, collagen, agrin, and nidogen, which together provide an interwoven meshwork imparting size and charge-selective properties to the GBM. Human mutations reported in Alport and Pierson syndromes, as well as animal knock-out studies of specific GBM components, have underscored the essential role of the GBM in regulating glomerular filtration [68]. The podocyte layer, complete with their interdigitating distal foot processes and intercellular connecting slit diaphragms (see Fig. 2), comprise the third

and final barrier to the filtration of albumin from the plasma. Finally, while not a recognized component of the glomerular filtration barrier, the parietal epithelial cells lining Bowman's capsule are positioned between the filtration barrier and the beginning of the proximal tubule. Interestingly, similar to podocytes, these cells have also been reported to undergo increased apoptosis via Erk1/2 signaling upon exposure to albumin [16].

Albuminuria as a therapeutic target in glomerular disease

There is controversy within the nephrology community about whether albuminuria is or is not an appropriate therapeutic target in patients with CKD [30, 31, 45, 46]. Additional studies are clearly needed to better define the future clinical utility of albuminuria as a therapeutic target in glomerular disease. However, our improving understanding of the molecular and cellular handling, and related toxicity, of albumin within both the glomerulus and renal tubules during glomerular disease will likely have an important future role in shaping these determinations. There is growing evidence for a potentially clinically important role for albumin, and its molecular modifications, in glomerular and podocyte injury. In addition, we now recognize that the most widely utilized clinical therapy for glomerular disease, glucocorticoids, as well as the PPARγ agonist, pioglitazone (a potential future therapy for glomerular disease) both inhibit albumin-induced podocyte signaling pathways such as COX-2 [3, 4] (see Fig. 3). Finally, our evolving appreciation that the toxicity of albumin itself is variable, and could potentially be modified for future therapeutic interventions, may also create novel opportunities for potential new approaches to slow the progression of CKD in chronic glomerular disease.

Summary

There have been several recent advances in our understanding of the role of albumin, and its molecular modifications, in the development and progression of glomerular disease. It has become increasingly clear that albumin and its associated factors are able to directly induce both podocyte and glomerular injury. Moreover, it appears that albumin-bound lipids have a notable role in mediating this injury. In contrast, studies using rodent models of albumin deficiency have reported reduced proteinuria in albumin-deficient rats and mice following glomerular injury induction, compared to normal animals, further suggesting a role for albumin in glomerular injury and proteinuria. Additional recent studies have now also confirmed that podocytes endocytose albumin, and do so utilizing mainly the caveolae-mediated pathway and macropinocytosis.
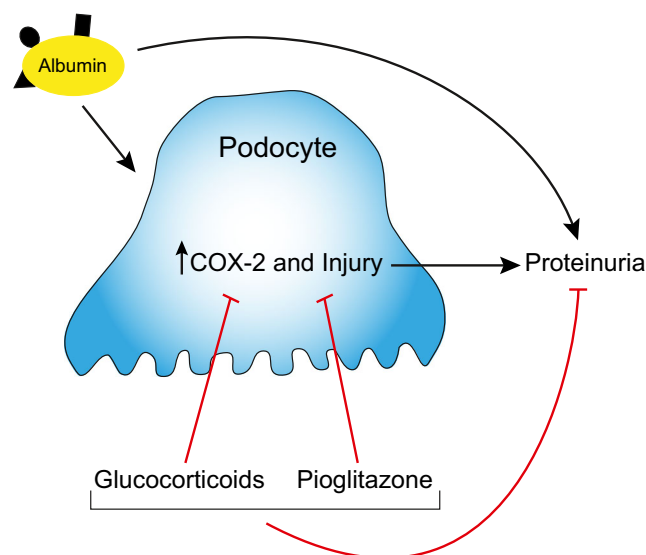

Fig. 3 Schematic of inhibitory effects of glomerular disease therapy on albumin-induced podocyte injury. The most widely utilized clinical therapy to reduce proteinuria in glomerular disease, glucocorticoids, as well as the PPARγ agonist, pioglitazone (a potential future therapy for glomerular disease), both inhibit albumin-induced podocyte COX-2 signaling and pro-inflammatory injury pathways, suggesting these molecular effects may mediate, at least in part, their beneficial effects on proteinuria and podocyte injury [3, 4]

Finally, it is also becoming clearer that modifications in the albumin molecule itself, as well as substances bound to it, may be important regulators of albumin-induced glomerular injury. These recent findings may create entirely new opportunities to develop novel future therapies directed at albumin that could potentially help reduce podocyte and renal tubular injury and slow the progression of chronic glomerular disease.

References

1. Abbate M, Zoja C, Morigi M, Rottoli D, Angioletti S, Tomasoni S, Zanchi C, Longaretti L, Donadelli R, Remuzzi G (2002) Transforming growth factor-beta1 is up-regulated by podocytes in response to excess intraglomerular passage of proteins: a central pathway in progressive glomerulosclerosis. Am J Pathol 161:2179–2193
2. Abe H, Shibuya T, Odashima S, Arichi S, Nagase S (1988) Alterations in the glomerulus in aminonucleoside nephrosis in analbuminemic rats. Nephron 50:351–355
3. Agrawal S, Chanley MA, Westbrook D, Nie X, Kitao T, Guess AJ, Benndorf R, Hidalgo G, Smoyer WE (2016) Pioglitazone enhances the beneficial effects of glucocorticoids in experimental nephrotic syndrome. Sci Rep 6:24392
4. Agrawal S, Guess AJ, Chanley MA, Smoyer WE (2014) Albumin-induced podocyte injury and protection are associated with regulation of COX-2. Kidney Int 86:1150–1160
5. Akilesh S, Huber TB, Wu H, Wang G, Hartleben B, Kopp JB, Miner JH, Roopenian DC, Unanue ER, Shaw AS (2008)

Podocytes use FcRn to clear IgG from the glomerular basement membrane. Proc Natl Acad Sci U S A 105:967–972

6. Allison SJ (2015) Free fatty acid-induced macropinocytosis in podocytes. Nat Rev Nephrol 11

7. Anderson MS, Recant L (1962) Fine structural alterations in the rat kidney following intraperitoneal bovine albumin. Am J Pathol 40: 555–569

8. Arasteh A, Farahi S, Habibi-Rezaei M, Moosavi-Movahedi AA (2014) Glycated albumin: an overview of the in vitro models of an in vivo potential disease marker. J Diabetes Metab Disord 13:49

9. Ashworth CT, James JA (1961) Glomerular excretion of macromolecular substances. Electron microscopic study of rat kidney after administration of human serum albumin. Am J Pathol 39:307–316

10. Bass PS, Wang Y, al Nawab M, Evans B, Thomas H, Davies DR (1992) The effect of cyclosporin A on cationized bovine serum albumin-induced nephropathy in NZW rabbits. J Pathol 167:41–47

11. Birn H, Christensen EI (2006) Renal albumin absorption in physiology and pathology. Kidney Int 69:440–449

12. Border WA, Kamil ES, Ward HJ, Cohen AH (1981) Antigenic changes as a determinant of immune complex localization in the rat glomerulus. Lab Invest 45:442–449

13. Brunskill NJ (2000) Albumin handling by proximal tubular cells: mechanisms and mediators. Nephrol Dial Transplant 15(Suppl 6): 39–40

14. Brunskill NJ (2004) Albumin signals the coming of age of proteinuric nephropathy. J Am Soc Nephrol 15:504–505

15. Candiano G, Petretto A, Bruschi M, Santucci L, Dimuccio V, Prunotto M, Gusmano R, Urbani A, Ghiggeri GM (2009) The oxido-redox potential of albumin methodological approach and relevance to human diseases. J Proteome 73:188–195

16. Chang AM, Ohse T, Krofft RD, Wu JS, Eddy AA, Pippin JW, Shankland SJ (2012) Albumin-induced apoptosis of glomerular parietal epithelial cells is modulated by extracellular signal-regulated kinase 1/2. Nephrol Dial Transplant 27:1330–1343

17. Chen JS, Chen A, Chang LC, Chang WS, Lee HS, Lin SH, Lin YF (2004) Mouse model of membranous nephropathy induced by cationic bovine serum albumin: antigen dose-response relations and strain differences. Nephrol Dial Transplant 19:2721–2728

18. Chen S, He FF, Wang H, Fang Z, Shao N, Tian XJ, Liu JS, Zhu ZH, Wang YM, Wang S, Huang K, Zhang C (2011) Calcium entry via TRPC6 mediates albumin overload-induced endoplasmic reticulum stress and apoptosis in podocytes. Cell Calcium 50:523–529

19. Chung JJ, Huber TB, Godel M, Jarad G, Hartleben B, Kwoh C, Keil A, Karpitskiy A, Hu J, Huh CJ, Cella M, Gross RW, Miner JH, Shaw AS (2015) Albumin-associated free fatty acids induce macropinocytosis in podocytes. J Clin Invest 125:2307–2316

20. Clement LC, Mace C, Del Nogal AM, Marshall CB, Chugh SS (2015) The proteinuria-hypertriglyceridemia connection as a basis for novel therapeutics for nephrotic syndrome. Transl Res 165:499–504

21. Cohen MP, Chen S, Ziyadeh FN, Shea E, Hud EA, Lautenslager GT, Shearman CW (2005) Evidence linking glycated albumin to altered glomerular nephrin and VEGF expression, proteinuria, and diabetic nephropathy. Kidney Int 68:1554–1561

22. Debiec H, Lefeu F, Kemper MJ, Niaudet P, Deschenes G, Remuzzi G, Ulinski T, Ronco P (2011) Early-childhood membranous nephropathy due to cationic bovine serum albumin. N Engl J Med 364:2101–2110

23. Dobrinskikh E, Okamura K, Kopp JB, Doctor RB, Blaine J (2014) Human podocytes perform polarized, caveolae-dependent albumin endocytosis. Am J Physiol Renal Physiol 306:F941–F951

24. Doherty GJ, McMahon HT (2009) Mechanisms of endocytosis. Annu Rev Biochem 78:857–902

25. Eddy AA (1989) Interstitial nephritis induced by protein-overload proteinuria. Am J Pathol 135:719–733

26. Eddy AA, Giachelli CM (1995) Renal expression of genes that promote interstitial inflammation and fibrosis in rats with protein-overload proteinuria. Kidney Int 47:1546–1557

27. Eddy AA, Kim H, Lopez-Guisa J, Oda T, Soloway PD (2000) Interstitial fibrosis in mice with overload proteinuria: deficiency of TIMP-1 is not protective. Kidney Int 58:618–628

28. Eyre J, Ioannou K, Grubb BD, Saleem MA, Mathieson PW, Brunskill NJ, Christensen EI, Topham PS (2007) Statin-sensitive endocytosis of albumin by glomerular podocytes. Am J Physiol Renal Physiol 292:F674–F681

29. Fang Z, He F, Chen S, Sun X, Zhu Z, Zhang C (2009) Albumin modulates the production of matrix metalloproteinases-2 and -9 in podocytes. Journal of Huazhong University of Science and Technology Medical sciences = Hua zhong ke ji da xue xue bao Yi xue Ying De wen ban = Huazhong keji daxue xuebao Yixue Yingdewen ban 29:710–714

30. Fried LF, Lewis J (2015) Albuminuria is not an appropriate therapeutic target in patients with CKD: the con view. Clin J Am Soc Nephrol: CJASN 10:1089–1093

31. Fried LF, Lewis J (2015) Rebuttal of the pro view: albuminuria is an appropriate therapeutic target in patients with CKD. Clin J Am Soc Nephrol: CJASN 10:1095–1098

32. Fujihara CK, Limongi DM, De Oliveira HC, Zatz R (1992) Absence of focal glomerulosclerosis in aging analbuminemic rats. Am J Phys 262:R947–R954

33. Fujihara CK, Limongi DM, Falzone R, Graudenz MS, Zatz R (1991) Pathogenesis of glomerular sclerosis in subtotally nephrectomized analbuminemic rats. Am J Phys 261:F256–F264

34. Gekle M (2005) Renal tubule albumin transport. Annu Rev Physiol 67:573–594

35. Ghiggeri GM, Ginevri F, Candiano G, Oleggini R, Perfumo F, Queirolo C, Gusmano R (1987) Characterization of cationic albumin in minimal change nephropathy. Kidney Int 32:547–553

36. Haymann JP, Levraud JP, Bouet S, Kappes V, Hagege J, Nguyen G, Xu Y, Rondeau E, Sraer JD (2000) Characterization and localization of the neonatal Fc receptor in adult human kidney. J Am Soc Nephrol 11:632–639

37. He F, Chen S, Wang H, Shao N, Tian X, Jiang H, Liu J, Zhu Z, Meng X, Zhang C (2011) Regulation of CD2-associated protein influences podocyte endoplasmic reticulum stress-mediated apoptosis induced by albumin overload. Gene 484:18–25

38. Hirayama K, Ebihara I, Yamamoto S, Kai H, Muro K, Yamagata K, Kobayashi M, Koyama A (2002) Predominance of type-2 immune response in idiopathic membranous nephropathy. Cytoplasmic Cytokine Analysis. Nephron 91:255–261

39. Inoue K, Ishibe S (2015) Podocyte endocytosis in the regulation of the glomerular filtration barrier. Am J Physiol Renal Physiol 309: F398–F405

40. Iseki K, Ikemiya Y, Iseki C, Takishita S (2003) Proteinuria and the risk of developing end-stage renal disease. Kidney Int 63:1468–1474

41. Iwai T, Kume S, Chin-Kanasaki M, Kuwagata S, Araki H, Takeda N, Sugaya T, Uzu T, Maegawa H, Araki SI (2016) Stearoyl-CoA desaturase-1 protects cells against lipotoxicity-mediated apoptosis in proximal tubular cells. Int J Mol Sci 17

42. Jarad G, Knutsen RH, Mecham RP, Miner JH (2016) Albumin contributes to kidney disease progression in Alport syndrome. Am J Physiol Renal Physiol 311:F120–F130

43. Kinugasa S, Tojo A, Sakai T, Tsumura H, Takahashi M, Hirata Y, Fujita T (2011) Selective albuminuria via podocyte albumin transport in puromycin nephrotic rats is attenuated by an inhibitor of NADPH oxidase. Kidney Int 80:1328–1338

44. Kobayashi N, Suzuki Y, Tsuge T, Okumura K, Ra C, Tomino Y (2002) FcRn-mediated transcytosis of immunoglobulin G in human renal proximal tubular epithelial cells. Am J Physiol Renal Physiol 282:F358–F365

45. Lambers Heerspink HJ, Gansevoort RT (2015) Albuminuria is an appropriate therapeutic target in patients with CKD: the pro view. Clin J Am Soc Nephrol: CJASN 10:1079–1088

46. Lambers Heerspink HJ, Gansevoort RT (2015) Rebuttal of the con view: albuminuria is an appropriate therapeutic target in patients with CKD. Clin J Am Soc Nephrol: CJASN 10:1099

47. Lawrence GM, Brewer DB (1981) Effect of strain and sex on the induction of hyperalbuminaemic proteinuria in the rat. Clin Sci 61: 751–756

48. Lemley KV, Kriz W (1991) Glomerular injury in analbuminemic rats after subtotal nephrectomy. Nephron 59:104–109

49. Li C, Lin Y, Luo R, Chen S, Wang F, Zheng P, Levi M, Yang T, Wang W (2016) Intrarenal renin-angiotensin system mediates fatty acid-induced ER stress in the kidney. Am J Physiol Renal Physiol 310:F351–F363

50. Londono I, Gingras D, Bendayan M (2003) Circulating glycated albumin and glomerular anionic charges. Exp Diabesity Res 4:83–92

51. Morigi M, Buelli S, Angioletti S, Zanchi C, Longaretti L, Zoja C, Galbusera M, Gastoldi S, Mundel P, Remuzzi G, Benigni A (2005) In response to protein load podocytes reorganize cytoskeleton and modulate endothelin-1 gene: implication for permselective dysfunction of chronic nephropathies. Am J Pathol 166:1309–1320

52. Musante L, Bruschi M, Candiano G, Petretto A, Dimasi N, Del Boccio P, Urbani A, Rialdi G, Ghiggeri GM (2006) Characterization of oxidation end product of plasma albumin 'in vivo'. Biochem Biophys Res Commun 349:668–673

53. Musante L, Candiano G, Petretto A, Bruschi M, Dimasi N, Caridi G, Pavone B, Del Boccio P, Galliano M, Urbani A, Scolari F, Vincenti F, Ghiggeri GM (2007) Active focal segmental glomerulosclerosis is associated with massive oxidation of plasma albumin. J Am Soc Nephrol 18:799–810

54. Nagase S, Shimamune K, Shumiya S (1979) Albumin-deficient rat mutant. Science 205:590–591

55. Nagase S, Shimamune K, Shumiya S (1980) Albumin-deficient rat mutant: an animal model for analbuminemia. Jikken dobutsu Exp Anim 29:33–38

56. Nash AS, Mohammed NA, Wright NG (1990) Experimental immune complex glomerulonephritis and the nephrotic syndrome in cats immunised with cationized bovine serum albumin. Res Vet Sci 49:370–372

57. Oite T, Batsford SR, Mihatsch MJ, Takamiya H, Vogt A (1982) Quantitative studies of in situ immune complex glomerulonephritis in the rat induced by planted, cationized antigen. J Exp Med 155: 460–474

58. Okamura K, Dummer P, Kopp J, Qiu L, Levi M, Faubel S, Blaine J (2013) Endocytosis of albumin by podocytes elicits an inflammatory response and induces apoptotic cell death. PLoS One 8:e54817

59. Okuda S, Oochi N, Wakisaka M, Kanai H, Tamaki K, Nagase S, Onoyama K, Fujishima M (1992) Albuminuria is not an aggravating factor in experimental focal glomerulosclerosis and hyalinosis. J Lab Clin Med 119:245–253

60. Qin XS, Tsukaguchi H, Shono A, Yamamoto A, Kurihara H, Doi T (2009) Phosphorylation of nephrin triggers its internalization by raft-mediated endocytosis. J Am Soc Nephrol 20:2534–2545

61. Razzak M (2014) Glomerular disease: albumin endocytosis is caveolin-mediated. Nat Rev Nephrol 10:242

62. Ruggiero C, Elks CM, Kruger C, Cleland E, Addison K, Noland RC, Stadler K (2014) Albumin-bound fatty acids but not albumin itself alter redox balance in tubular epithelial cells and induce a peroxide-mediated redox-sensitive apoptosis. Am J Physiol Renal Physiol 306:F896–F906

63. Sand KM, Bern M, Nilsen J, Noordzij HT, Sandlie I, Andersen JT (2014) Unraveling the interaction between FcRn and albumin: opportunities for Design of Albumin-Based Therapeutics. Front Immunol 5:682

64. Satchell S (2013) The role of the glomerular endothelium in albumin handling. Nat Rev Nephrol 9:717–725

65. Soda K, Balkin DM, Ferguson SM, Paradise S, Milosevic I, Giovedi S, Volpicelli-Daley L, Tian X, Wu Y, Ma H, Son SH, Zheng R, Moeckel G, Cremona O, Holzman LB, De Camilli P, Ishibe S (2012) Role of dynamin, synaptojanin, and endophilin in podocyte foot processes. J Clin Invest 122:4401–4411

66. Soda K, Ishibe S (2013) The function of endocytosis in podocytes. Curr Opin Nephrol Hypertens 22:432–438

67. Spector AA (1975) Fatty acid binding to plasma albumin. J Lipid Res 16:165–179

68. Suh JH, Miner JH (2013) The glomerular basement membrane as a barrier to albumin. Nat Rev Nephrol 9:470–477

69. Tenten V, Menzel S, Kunter U, Sicking EM, van Roeyen CR, Sanden SK, Kaldenbach M, Boor P, Fuss A, Uhlig S, Lanzmich R, Willemsen B, Dijkman H, Grepl M, Wild K, Kriz W, Smeets B, Floege J, Moeller MJ (2013) Albumin is recycled from the primary urine by tubular transcytosis. J Am Soc Nephrol

70. Thomas ME, Harris KP, Walls J, Furness PN, Brunskill NJ (2002) Fatty acids exacerbate tubulointerstitial injury in protein-overload proteinuria. Am J Physiol Renal Physiol 283:F640–F647

71. Weening JJ, Van Guldener C, Daha MR, Klar N, van der Wal A, Prins FA (1987) The pathophysiology of protein-overload proteinuria. Am J Pathol 129:64–73

72. Wright NG, Mohammed NA, Eckersall PD, Nash AS (1985) Experimental immune complex glomerulonephritis in dogs receiving cationized bovine serum albumin. Res Vet Sci 38:322–328

73. Yamamoto T, Kihara I, Morita T, Oite T (1978) Bovine serum albumin (BSA) nephritis in rats. I. Experimental model. Acta Pathol Jpn 28:859–866

74. Yamamoto T, Miyazaki S, Kawasaki K, Yaoita E, Kihara I (1986) Rat bovine serum albumin (BSA) nephritis. VI. The influence of chemically altered antigen. Clin Exp Immunol 65:51–56

75. Yarar D, Waterman-Storer CM, Schmid SL (2005) A dynamic actin cytoskeleton functions at multiple stages of clathrin-mediated endocytosis. Mol Biol Cell 16:964–975

76. Yoshida S, Nagase M, Shibata S, Fujita T (2008) Podocyte injury induced by albumin overload in vivo and in vitro: involvement of TGF-beta and p38 MAPK. Nephron Exp Nephrol 108:e57–e68

77. Yoshikawa N, Ito H, Akamatsu R, Hazikano H, Okada S, Matsuo T (1986) Glomerular podocyte vacuolation in focal segmental glomerulosclerosis. Arch Pathol Lab Med 110:394–398

78. Zuo N, Zheng X, Liu H, Ma X (2015) Fenofibrate, a PPARalpha agonist, protect proximal tubular cells from albumin-bound fatty acids induced apoptosis via the activation of NF-kB. Int J Clin Exp Pathol 8:10653–10661