Disease-dependent mechanisms of albuminuria

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Department of Biochemistry and Molecular Biology, Monash University, Clayton, Victoria, Australia; CVK Research, New York, New York; Department of Nephrology and Monash University Department of Medicine, Monash Medical Centre, Clayton, Victoria, Australia; and Center for Systems Biology, Program in Membrane Biology, and Division of Nephrology, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts

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Comper WD, Hilliard LM, Nikolic-Paterson DJ, Russo LM. Disease-dependent mechanisms of albuminuria. Am J Physiol Renal Physiol 295: F1589–F1600, 2008. First published June 25, 2008; doi:10.1152/ajprenal.00142.2008.—The mechanism of albuminuria is perhaps one of the most complex yet important questions in renal physiology today. Recent studies have directly demonstrated that the normal glomerulus filters substantial amounts of albumin and that charge selectivity plays little or no role in preventing this process. This filtered albumin is then processed by proximal tubular cells by two distinct pathways; dysfunction in either one of these pathways gives rise to discrete forms of albuminuria. Most of the filtered albumin is returned to the peritubular blood supply by a retrieval pathway. Albuminuria in the nephrotic range would arise from retrieval pathway dysfunction. The small quantities of filtered albumin that are not retrieved undergo obligatory lysosomal degradation before urinary excretion as small peptide fragments. This degradation pathway is sensitive to metabolic factors responsible for hypertrophy and fibrosis, particularly molecules such as angiotensin II and transforming growth factor-β1, whose production is stimulated by hyperglycemic and hypertensive environments. Dysfunction in this degradation pathway leads to albuminuria below the nephrotic range. These new insights into albumin filtration and processing argue for a reassessment of the role of podocytes and the slit diaphragm as major direct determinants governing albuminuria, provide information on how glomerular morphology and “tubular” albuminuria may be interrelated, and offer a new rationale for drug development.

proximal tubular cell; albumin retrieval; albumin degradation

It was well over 200 years ago that the first identification of proteinuria was made. In an extensive article associated with a symposium on the history of nephrology, J. S. Cameron (17) identifies Domenico Cotugno as writing the first scientific paper on proteinuria in 1770. There were a number of others in the late eighteenth century who detected albumin in diabetic urine—identified by heating the urine in a spoon so the protein would coagulate, like heating the white of an egg. Maher (65) describes William Wells, who in 1812 identified “serum” in the urine of some persons with dropsy. Similar findings were made by Cochrane in 1813 (65). It appears that the perception at the time was that the cause of the “serum” in urine was nonrenal pathology. However, it was Richard Bright in 1827 who first related kidney disease [granular degeneration of the kidney—known as Bright disease (14)] with albuminous urine.

Classic View of Glomerular Permeability and Albuminuria

The study of albumin filtration by the glomerulus has been strongly influenced by the fact that only trace amounts of intact, immunoreactive albumin are excreted in normal urine (~2–20 mg/day). A series of studies in the late 1960s and 1970s suggested that albumin permeability across the glomerular capillary wall (GCW) was markedly impeded, and that this was controlled by both size and charge selectivity (electrostatic repulsion of negatively charged albumin by the fixed negative charges of the GCW). Analysis of samples of glomerular filtrate collected by micropuncture (73, 116) indicated that the albumin content was very low, whereas in nephrotic states the albumin content increased accordingly (73). Glomerular sieving coefficient (GSC) studies of dextran sulfate demonstrated that it had a much lower sieving than its uncharged counterpart (9, 20, 21), and this was interpreted as being due to the electrostatic repulsion by the fixed negative charges of the GCW at a concentration of 170 meq/l (28). At the same time, heparan sulfate was being recognized as an important component in the GCW controlling charge-dependent transglomerular passage (52). The putative repulsive force associated with charge selectivity must be very large to account for the ~100-fold difference in albumin GSC of 0.0006 by micropuncture compared with the GSCs in the range of 0.02–0.1 of albumin-sized transport probes based on size selectivity alone (7, 11, 13, 21, 38, 43, 59, 75). Many studies subsequently interpreted albuminuria as a result of either changes in glomerular charge selectivity or changes in size selectivity, or both, leading to leakage of albumin across the GCW (4, 5, 9, 11, 13, 33, 40, 41, 49, 68, 75–77, 92, 101, 102).

More recently, the marked albuminuria identified with genetic mutations of components of the slit pore (such as nephrin,
podocin, actinin-4) (118), or of the glomerular basement membrane (GBM) (such as laminin) (1) has reemphasized the important role these components have in governing the level of albuminuria in glomerular disease (128).

A schematic diagram representing the essential elements of the classical view of albuminuria and glomerular permeability is shown in Fig. 1. However, over the last 17 years there has been substantial criticism of this classical model of albuminuria, and this is described in the next sections.

Charge Selectivity Cannot Account for the Low Glomerular Permeability Suggested by Micropuncture Studies

The first study to address whether negatively charged polysaccharides or glycosaminoglycans like heparan sulfate at concentrations up to 170 meq/l could restrict dextran sulfate transport was a physicochemical study utilizing frontal gel chromatography, in which it was demonstrated that the restriction was based on size but not on charge (131). Subsequently it was demonstrated that dextran sulfate is desulfated during filtration in vivo (27) and that this cell-mediated desulfation process is responsible for the apparent differences in glomerular sieving of dextran sulfate compared with uncharged dextran (16, 124). Dextran sulfate itself does not bind to albumin (16) under physiological conditions, and therefore this does not contribute to apparent differences in transglomerular transport.

A follow-up physicochemical study demonstrated that albumin transport through heparan sulfate regions was based only on size and not charge (108). This was consistent with many other physicochemical studies that had shown that albumin interaction with negatively charged glycosaminoglycans is based on size, not charge (58, 71, 107, 109).

The first demonstration that no charge selectivity is exerted on globular charged molecules in vivo came from studies comparing the GSC of negatively charged Ficoll with a radius of 36 Å (with a negatively charged valence 3 times that of albumin) compared with uncharged Ficoll of the same radius (38). This has been followed now by many permeability studies with stable charged inert probes, both globular and random coil, demonstrating that charge selectivity does not exist (6, 43, 72, 104, 124) (summarized in Table 1).

![Fig. 1. Models of renal albumin processing and mechanism of albuminuria. There are essentially 2 models involved. In the traditional model (top), increases in glomerular permeability lead to increasing amounts of albumin filtered per day as represented by the increasing thickness and length of the blue arrows. Small amounts of this filtered albumin may be endocytosed by 1 receptor, the megalin/cubilin receptor, and directed to the lysosome, where it is degraded to amino acids that are returned to the blood supply (purple dashed arrow, although this pathway has not been demonstrated in vivo). The resulting levels of albuminuria extending from microalbuminuria to macroalbuminuria to nephrotic levels are thought to be directly related to the level of glomerular permeability dysfunction. One aspect of this model not commonly appreciated is that it implies that there can be increases in glomerular permeability without albuminuria provided that the excess filtered albumin is endocytosed by the proximal tubular cell (PTC). At higher levels of filtered albumin it is assumed that the endocytotic pathway will become saturated. The proposed new tubular model (bottom) states that the level of glomerular albumin filtration is relatively high and essentially unchanged for acquired and chemically induced albuminuria. It is also a 2-receptor model, consistent with the Park and Maack (87) concept; there is a high-capacity, low-affinity receptor associated with the retrieval pathway of filtered albumin that returns filtered albumin to the peritubular blood supply through transcytosis in the PTC. The second receptor is a high-affinity, low-capacity receptor that lysosomally processes unretrieved filtered albumin (degradation pathway) and returns the resultant peptides (green arrow) back to the tubular lumen for ultimate excretion. Relatively low levels of albuminuria (micro and macro) will be associated with dysfunction in the degradation pathway and minor dysfunction of the retrieval pathway, where unretrieved albumin may saturate the degradation pathway. Nephrotic levels of albuminuria are primarily associated with dysfunction of the retrieval pathway. Actual amounts of filtered albumin will be heavily influenced by plasma albumin concentration and glomerular filtration rate (GFR) (not shown). The model predicts that a high GFR may generate albuminuria because of overload saturation of PTC albumin receptors. Conversely, albuminuria occurring at low GFR or with hypoalbuminemia suggests an intrinsic uptake dysfunction of the PTC.]

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Table 1. Charge selectivity is not observed with these negatively charged transport probes

<table>
<thead>
<tr>
<th>Negative Polymer</th>
<th>Method</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Dextran sulfate 0.3 sulfates/glucose</td>
<td>IPK</td>
<td>124</td>
</tr>
<tr>
<td>Dextran sulfate 1.0 sulfates/glucose</td>
<td>IPK</td>
<td>124</td>
</tr>
<tr>
<td>Dextran sulfate 1.2 sulfates/glucose</td>
<td>IPK</td>
<td>124</td>
</tr>
<tr>
<td>Dextran sulfate ~1.7 sulfates/glucose (used at high concentration)</td>
<td>IPK</td>
<td>124</td>
</tr>
<tr>
<td>Carboxymethyl Ficoll</td>
<td>In vivo</td>
<td>38</td>
</tr>
<tr>
<td>Carboxymethyl dextran</td>
<td>In vivo</td>
<td>104</td>
</tr>
<tr>
<td>Hydroxyethyl starch</td>
<td>In vivo</td>
<td>104</td>
</tr>
<tr>
<td>Bikunin</td>
<td>IPK</td>
<td>72</td>
</tr>
<tr>
<td>Hyaluronan</td>
<td>IPK</td>
<td>72</td>
</tr>
<tr>
<td>Denatured albumin</td>
<td>IPK</td>
<td>23</td>
</tr>
</tbody>
</table>

IPK, isolated, perfused kidney.

The first direct measurement of albumin GSC was made by two-photon microscopy, a technique that enables the noninvasive, real-time observation of fluorescence-labeled albumin being filtered and processed by renal cells in the intact live animal, and gave a GSC value of 0.034 (99). To corroborate these data, we took a low polydisperse 69-kDa FITC-dextran (which has a radius of 5.3–6.5 nm) and determined its GSC to be 0.025 ± 0.0041 (n = 4) (7). This result is in agreement with the GSC measurement obtained for similar dextrans in an independent study using a glomerular uptake technique in nondiabetic rats, which reported a GSC of 0.022 (Russo LM, Sandoval RM, Campos S, Molitoris BA, Comper WD, Brown D, unpublished observation). This agreement between two different methods further substantiates the use of two-photon technology to quantitate albumin GSC. The GSC for albumin is in the range that would be expected for albumin filtration based on size selectivity alone (7, 11, 13, 21, 38, 43, 59, 75) and suggests that albumin filtration is at much greater levels than previously thought. This GSC in rats is also similar to the value obtained in lower vertebrates by Tanner et al. (112) in the salamander Necturus maculosus, also utilizing two-photon microscopy. All these results demonstrate that the GSC is primarily determined by size selectivity alone. They also confirm earlier studies that identified a similar GSC for albumin by employing proximal tubular cell (PTC) poisons without altering glomerular permselectivity (84), or the use of slightly denatured albumin that is not that is not taken up by PTCs (23).

Apparent charge selectivity with anionic and neutral proteins has been shown to be due to their differential tubular uptake and degradation (79, 84). This has been confirmed by two-photon microscopy, where the GSC for neutral albumin was 0.038 (unpublished work), just slightly higher than for native albumin.

An exception to these findings of the lack of charge selectivity are the studies by Haraldsson’s group (5, 72), who claim to measure charge selectivity due to the difference in the clearance of albumin from that of uncharged Ficoll (Fig. 2) in a cold perfused kidney. However, this measurement of apparent charge selectivity is extremely glomerular filtration rate (GFR) dependent, and at normal GFR charge selectivity may not occur (Fig. 2). The marked GFR dependence is probably an artifact of the very low albumin filtration and postfiltration binding to PTC albumin receptors because of the hypoalbuminic and hypofiltration conditions used in these studies (25, 98). The change in apparent charge selectivity is compounded by the marked drop in the fractional clearance of Ficoll with increasing GFR, which has been noted previously and has been described as a decreasing role of diffusion over convective transport (90). A comparison of the clearance of negatively charged versus uncharged Ficoll in this unique system would help to resolve this issue.

A number of recent biochemical and genetic studies have also demonstrated little influence of the major glomerular charge component, heparan sulfate, on albuminuria (22, 45, 91, 121, 123). Glomerular heparan sulfate appears to function, in part, to allow protein filtration and minimize clogging (2). There have been some reports implicating heparinase activity in proteinuric states (57, 60, 67, 122), but the conclusion, in light of the studies cited above, is that it is acting on something other than glomerular heparan sulfate or affecting the release of bioactive compounds bound to heparan sulfate (122).

The relatively high GSC for albumin is also consistent with the occasional observations that fluorescent albumin when injected into the blood supply is rapidly seen in the postfiltration space and associated with PTC brush border (3, 66). This would not be the case if the albumin GSC is as low as 0.0006.
Micropuncture studies have yielded very low GSCs of albumin, which would indicate that under normal circumstances the primary filtrate is essentially albumin free. However, there is an underlying confounding issue in this type of measurement of how to deal with the active retrieval of filtered albumin by the PTCs (see below). This will give rise to time-dependent and therefore collection method-dependent results, and this is exactly what has been observed. Tojo and Endou (116) reported a nearly 50-fold difference in tubular lumen concentrations of serially collected fractions and had to eliminate 75% of their data in order to obtain a GSC of 0.00062 where they thought that the early collected fractions were contaminated by plasma albumin. Oken and Flamenbaum (73) reported that over a 500-fold variation in tubular lumen albumin concentrations could be observed, particularly when relatively large samples of tubular fluid were collected. They too considered this to be the result of the contamination of tubule fluid with extraneous plasma albumin, and they modified their collection technique. The contamination issue certainly seems specific for albumin; the GSC of polysaccharides from micropuncture analysis was the same as the fractional clearance determined by urine analysis, indicating that little contamination occurred in the micropuncture measurement (9, 13, 21).

Overall, charge selectivity cannot account for the GSC measured by micropuncture. The repulsive force required to explain charge selectivity certainly has not been observed in other fenestrated capillary beds (93, 113) or in any other biological system (93). It is most likely that the micropuncture result is due to other factors such as the rapid removal of filtered albumin by the retrieval pathway during the course of micropuncture sampling of the tubular lumen. It is this dynamic cellular uptake of albumin that may confound the results depending on the method of collection. The retrieval pathway will also confound other purported measurements of albumin GSC if they are not made directly.

If There is No Charge Selectivity, Do Changes in Glomerular Permeability Account for Albuminuria in Glomerular Disease?

Size selectivity studies have been performed with inert probes, particularly dextran (a slightly branched random coil polymer) and Ficoll (a cross-linked polysaccharide that takes on a globular conformation more closely akin to albumin). There is general agreement in the literature about this concept. The results are fairly consistent among laboratories that the GCW exhibits size selectivity, with GSCs in the range of 0.02–0.1 in rats and humans for a molecule of equivalent GCW radius (36 Å) (7, 11, 13, 21, 38, 43, 59, 75).

In studies of a broad range of acquired or chemically induced kidney diseases (Table 2) there is also universal agreement in that there is essentially no change in Ficoll or dextran clearance for molecules up to 40-Å radii (Fig. 3). In stark contrast, the fractional clearance of albumin may increase by 100- to 10,000-fold in these diseases. Many of these glomerular diseases come under the spectrum of podocytopathies where there are characteristic glomerular podocyte morphological changes (128). The loss of podocyte foot processes is a common, though not invariant, finding in albuminuric disease (120). This tight association has been assumed to be a cause and effect relationship, although experimental studies have shown little or no change in the GSC for albumin-size molecules in nephrotic renal diseases (Table 2). No study published to date has used an inert transport probe to mimic these changes seen for albumin. It is interesting that in nephrotic syndrome the GSCs of albumin are in the range of 0.03–0.06 (38, 80, 114) for rats and in the range of 0.008–0.022 (11, 41, 101) for humans, similar to the values that would be obtained if there were inhibition of albumin PTC uptake. These GSC values approach those obtained for inert probes like dextran and Ficoll of hydrodynamic size similar to albumin. Furthermore, it has been shown in nephrotic states that increased elimination of plasma albumin can be fully accounted for by its increased excretion in urine (55, 78). This was not accompanied by any changes in the plasma clearance of dextran or Ficoll of radii up to 85 Å (55) and strongly argues that GCW porosity is not the major factor governing plasma albumin loss by the kidney in nephrotic states or for albumin excretion.

Previous size selectivity studies demonstrated that the fractional clearance of large molecules with hydrodynamic radii >50 Å may be increased in nephrotic states, thus suggesting the formation of large pores. Despite these postulated large pores, there were no changes in the clearance for molecules of <40-Å radii (see also Fig. 3). This means that albuminuria in acquired and chemically induced kidney disease is not the result of changes in glomerular permeability. In fact, recent studies by two-photon microscopy have demonstrated that for diabetic rats the GSC for albumin remains high, ~0.03, and that there is no change in size selectivity for a 70-kDa dextran with GSC = 0.025 (Russo LM, Sandovol RM, Campos S, Molitoris BA, Comper WD, Brown D, unpublished observation). This would mean that changes in albumin excretion seen in diabetic rats are very unlikely to be associated with changes in the permeability of the GCW as previously proposed (125).

All the studies described above point to the fact that it is the albumin processing by the PTC whose dysfunction is responsible for marked increases in albumin excretion rate in disease states.

### Table 2. Kidney diseases in which albumin excretion increases 100- to 10,000-fold without changes in glomerular permeability of albumin-size dextran and Ficoll transport probes

<table>
<thead>
<tr>
<th>Disease</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Diabetic nephropathy (humans)</td>
<td>33, 59, 68, 92, 102</td>
</tr>
<tr>
<td>Diabetic nephropathy (rats)</td>
<td>94</td>
</tr>
<tr>
<td>Hypertension</td>
<td>94, 96</td>
</tr>
<tr>
<td>Nondiabetic nephrotic syndrome (humans)</td>
<td>11</td>
</tr>
<tr>
<td>Anti-GBM disease (rats)</td>
<td>38</td>
</tr>
<tr>
<td>Puromycin amino nucleoside nephrosis (rats)</td>
<td>38, 79</td>
</tr>
<tr>
<td>Adriamycin nephrosis (rats)</td>
<td>126</td>
</tr>
<tr>
<td>Sclerosis after renal ablation (rats)</td>
<td>76</td>
</tr>
<tr>
<td>Minimal change disease (humans)</td>
<td>41</td>
</tr>
<tr>
<td>Membranous glomerulopathy (rats, humans)</td>
<td>40, 101</td>
</tr>
<tr>
<td>Nephrotic serum nephritis (humans)</td>
<td>4</td>
</tr>
<tr>
<td>Diffuse proliferative lupus nephritis (humans)</td>
<td>101</td>
</tr>
<tr>
<td>Focal segment glomerulosclerosis (humans)</td>
<td>101</td>
</tr>
<tr>
<td>Overload proteinuria (rats)</td>
<td>54</td>
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</tbody>
</table>

GBM, glomerular basement membrane.
Major Albumin Processing Pathways in the Proximal Tubular Cell

While the GCW barrier offers major resistance to albumin filtration, significant amounts of albumin are still normally filtered. The filtered albumin that does not appear in the urine must be processed by a post-GCW process. There appear to be two major cellular pathways involved in this process, namely, the retrieval pathway and the degradation pathway (Fig. 1). Most (>95%) of the filtered albumin is taken up by the retrieval pathway and returned to the blood supply. The small amount (<5%) of albumin not taken up by the retrieval pathway is destined for processing by the degradation pathway and subsequent urinary excretion. The capacities of the different pathways are in accord with the original observations of Park and Maack (87), who analyzed albumin binding and uptake in perfused rabbit proximal tubules (Fig. 4). They identified two binding sites: one with a Michaelis-Menten constant ($K_m$) value of 0.031 mg/ml and a maximum binding range of 0.1–0.2 mg/ml and a second with a $K_m$ value of 1.2 mg/ml and a maximum of ~10 mg/ml. A number of reports from cell culture studies found similar binding parameters (15, 19, 35, 106). The receptor associated with the high-capacity/low-affinity uptake of albumin is consistent with the retrieval pathway, whereas the high-affinity/low-capacity receptor is consistent with uptake by the degradation pathway. It is interesting that the nature of the high-capacity receptor has not attracted a great deal of investigation. Its capacity is entirely consistent with an albumin GSC of 0.03, which would yield an albumin flux of 22.5 ng·min$^{-1}$·nephron$^{-1}$ (assuming 40,000 nephrons per rat kidney and a GFR of 1 ml·min$^{-1}$·kidney$^{-1}$). The length of the rat proximal tubule is 8 mm (64), which would require a receptor to take up albumin at a rate of 2.8 ng·min$^{-1}$·mm$^{-1}$, which is within the range of the high-capacity receptor examined by Park and Maack (Fig. 4).

The substantial levels of albumin that are normally filtered would suggest that albumin itself is not toxic to PTCs. This is consistent with the demonstration that proteinuria and tubular damage derived from anti-GBM disease and puromycin amonucleoside nephrosis can be induced in albumin-deficient analbuminemic rats in exactly the same manner as in normoalbuminemic rats (85).

Retrieval pathway. The initial demonstration of glomerular filtered albumin returning to the renal vein was made through the introduction of a small pulse of radioactive $[^3]$H]albumin into the renal artery in vivo or in the isolated perfused kidney, followed by examination of the radioactive profile from the renal vein effluent (29). It is viewed that the filtered portion of $[^3]$H]albumin in the pulse will be taken up by the tubular pathway, which will return the albumin to the blood supply undegraded. This recycled albumin appeared as a second peak(s) in renal vein effluent after the initial bolus had passed. Quantification of the glomerular flux of albumin from the size of the second peak relative to the control (controls included $[^3]$H]horseradish peroxidase, $[^14]$C]inulin, or $[^3]$H]albumin in a nonfiltering kidney) accurately estimated the predicted glomerular flux of albumin governed by size selectivity alone, i.e., it gave a GSC = 0.06 ± 0.006 (29). Overall, the albumin
retrieval pathway in humans is predicted to process ~250 g albumin/day (99).

Further evidence for the existence of the retrieval pathway in vivo has recently been provided by two-photon microscopy studies in live rats (99). With the use of fluorescence-labeled albumin, which has a long plasma half-life, it was demonstrated that albumin filtration and uptake by PTCs was very rapid and of high capacity in accord with the relatively high GSC. Large vesicles (diameter 500 nm) laden with intact endogenous albumin, as determined by immunogold labeling, appear to track through the PTC from the apical to the basolateral side (Fig. 5A). This correlates with the observation of regular cytoplasmic channels seen in the three-dimensional reconstruction of the two-photon image containing fluorescent albumin (Fig. 5B). While the capacity of the retrieval pathway concurs with the capacity of the low-affinity/high-capacity receptor of Park and Maack (87), the exact nature of the receptor(s) is not known.

Retrieval pathway disruption and nephrotic albuminuria. It is apparent from the discussion in the previous sections that changes in glomerular permeability cannot account for the nephrotic levels of albuminuria seen in kidney disease. Evidence linking retrieval pathway disruption and nephrotic levels of albuminuria have come from studies using agents that disrupt intracellular trafficking such as colchicine or cytochalasin B (84). In addition, high concentrations of ANG II are known to affect cytoskeletal organization (24), and albumin retrieval can be restored through ANG II blockade (117). Furthermore, in experimental models of anti-GBM disease (38) and puromycin aminonucleoside nephrosis (38, 99), it was estimated that PTC albumin uptake was inhibited by 80%. Other investigators have used this mechanism to explain albuminuria in acute tubular necrosis (127) and sirolimus-induced albuminuria (119). Massive albuminuria is not observed in acute tubular necrosis because of the markedly reduced GFR and therefore lower albumin filtration together with the fact that damage is most severe in the S3 part of the proximal tubule rather than S1, where retrieval is postulated.

In a study of reversible overload proteinuria induced by intraperitoneal injection of exogenous albumin (Fig. 6) there is a 20% increase in plasma protein but an ~400% increase in urinary protein excretion. This disproportionate increase in albuminuria is consistent when the albumin receptors on the PTC become saturated and unretrieved albumin is excreted at nephrotic levels (54). This study points out the possibility of the lack of a PTC defect but albuminuria associated with exceeding the capacity of the PTC retrieval pathway and therefore the indirect involvement of glomerular function. Conditions that may allow excess filtration of albumin across the GCW, such as an increased GFR in
pregnancy or in genetic alteration of the size selectivity of the GCW, may cause a similar effect.

The marked albuminuria associated with genetic mutations in the components of the podocyte slit diaphragm (such as nephrin, podocin, actinin-4, etc.) (118) or of the GBM (such as laminin) (1) has been associated with many of the morphological changes associated with glomerular podocytes seen in acquired and chemically induced kidney disease. However, there is a dilemma here. It is very unlikely that the structural changes that are responsible for the proteinuria lead directly to increases of 100- to 10,000-fold for the glomerular permeability of albumin (classical model). The question to address is, How does genetically altering components of the GCW generate proteinuria when there are already nephrotic levels of albumin being normally filtered? This has yet to be investigated. The issues discussed above strongly argue that any increase in the GSC for albumin in such genetic causes of nephrotic syndrome is likely to be minor or entirely absent, with the major cause of albuminuria being dysfunction or saturation of the retrieval pathway. Since the retrieval pathway is likely to be operating at close to maximum capacity in the normal kidney, minor increases in the amount of albumin filtered in these genetically induced diseases through changes in glomerular permeability may induce albuminuria due to saturation of the PTC albumin receptors [similar to what occurs in models of protein overload (54)].

Degradation pathway. The detailed evidence for the existence of the degradation pathway has been reviewed elsewhere (26, 48, 93). Briefly, the degradation pathway is proposed to be a high-affinity/low-capacity pathway, associated with the megalin/cubilin receptor, transporting the relatively small quantities of unretrieved filtered albumin to lysosomes. The kidney has been shown to produce large quantities of albumin peptides (37, 42, 47, 79, 110) that are sensitive to proteolytic digestion (47) (Fig. 7). Fragmentation can be inhibited by specific lysosomal inhibitors (84) and inhibitors of v-ATPase and dynamin GTPase (47). The relatively large quantities of lysosomal degradation products found in the urine are in accord with the significant lysosomal armory of the kidney relative to all other organs (Refs. 8, 56; Hilliard, Russo, Comper, unpublished observation). For example, the rat kidney has ~300 times more cathepsin B activity than the left ventricle of the heart (Hilliard, Russo, Comper, unpublished observation). Also, this degradation pathway is not a specific albumin pathway but appears to process all filtered proteins (26, 93). For normal rats the capacity of the degradation pathway is ~0.05–0.1 g albumin-derived material per day (38). This would correspond to an albumin uptake of 0.1–0.2 ng·min⁻¹·mm⁻¹ in the proximal tubule, which is of the same order as the capacity of the high-affinity site identified by Park and Maack (Fig. 4). The size of the fragments determined in rat urine are in the range 200–10,000 Da (79). For humans the capacity of the degradation pathway is 1–3 g albumin-derived material per day (37), with the size of the fragments in the range of 300–500 Da (110). Partial reversible inhibition of this degradation process can be achieved in vivo by a bolus injection of albumin peptides, resulting in urinary excretion of larger fragments (30). Peptides excreted in the isolated, perfused kidney are also of an intermediate size, 10,000–60,000 Da, presumably because of the ischemic effects on the degradation process (Fig. 7) (47). The degradation pathway may serve a number of functions: (1) to dispose of denatured proteins (23), (2) to dispose of proteins with toxic ligands, or (3) to liberate important ligands bound to proteins.

Purified radiolabeled peptides from human urine account for the major biuret-reacting material in urine as well as 214-nm absorbing material (110). The low-molecular-mass nature of the fragments may explain why analysis of peptides >750 Da failed to detect substantial albumin peptides in human urine (70). Criticism by Hortin and Meilinger (50) that the peptides are present only at low concentration on the basis that the biuret assay only detects interfering compounds in urine is unsubstantiated given that they failed to account for the major biuret-reacting material in urine samples. Other criticisms involving the suggestion that there is a 0.1% contamination from free tritium (25) in the radiolabeled albumin preparations are unfounded because the preparations used are highly purified by chromatography and contain <0.003% contamination, if any (38, 79, 110).

It is widely thought that the products of this lysosomal degradation are transported to the basolateral side of the PTCs based on in vitro perfusion studies (87). However, in vivo studies have failed to identify any labeled albumin fragments in
Degradation pathway disruption. Changes in the degradation pathway generally do not change the net albumin that is excreted, only the form in which the albumin is excreted (Fig. 1). It is apparent that inhibition of the degradation pathway could yield the nonnephrotic levels of albuminuria when measured by assays that detect intact protein only. That is, albuminuria is promoted by metabolic events or hormonal signals to affect lysosomal function and/or internalization and/or intracellular trafficking (Fig. 8), rather than changes in glomerular permeability. For the same amount of albumin or albumin-derived material being excreted, the relative balance between intact albumin and degraded albumin will be metabolically controlled. This will determine the relative amount of albuminuria. This mechanism is likely to account for albuminuria at levels $\geq 30$ mg/day to 1–3 g/day and includes the microalbuminuria to macroalbuminuria that can be observed in situations such as exercise, hyperlipidemia, diabetic nephropathy, cardiovascular disease, orthostasis, and tubular causes. Other disease states such as Fanconi syndrome and Fabry disease are likely candidates for degradation pathway dysfunction.

The degradation pathway mechanism clearly has an important role in many of the studies associated with albumin clearance in diseased states: it is apparent that much of the observed increased clearance of albumin in diseased states is associated with the limited detection by the immunochemical-based assays used for quantification of urine albumin. The relatively low fractional clearance of albumin in normal humans and animals with standard assays (range $10^{-5}$–$10^{-6}$) is in part the result of an underestimation of albumin excretion because albumin fragments in urine are not detected. The relatively high clearances of albumin in diabetes (39, 82, 94, 97), hypertension (Refs. 94, 96, 97; Hilliard, Russo, Comper, unpublished observation), and anti-Thy1 disease (31) are due to the high content of intact immunoreactive albumin in the urine. This occurs because of the lack of degradation of intact albumin, which is independent of GCW permeability. One report has suggested that the Rf-2 gene may modulate albuminuria through the lysosomal degradation pathway (89).

Fig. 8. Schematic diagram linking the renin-angiotensin system (RAS) and transforming growth factor (TGF)-β production to lysosomal dysfunction and albuminuria. Production of renal ANG II and TGF-β may be increased in hypertension and diabetes by increased glucose, glycated albumin, and increased stretch forces induced by hypertension. In addition to the development of glomerulosclerosis, increased TGF-β and ANG II in hypertension and diabetes can lead to decreased lysosomal activity promoting hypertrophy in the kidney and heart and albuminuria in the kidney. Changes in glomerular morphology, while not directly responsible for albuminuria, will be correlated with albuminuria because both events have the same underlying cause. We also acknowledge that there may be other biochemical events and cytokine/growth factor/mediators that contribute directly or indirectly to lysosomal dysfunction in both hypertension and diabetes.

Fig. 7. Large quantities of albumin peptides are excreted in the urine of an isolated, perfused kidney (IPK) that are protease sensitive (47). Comparison of representative size-exclusion chromatography profiles of where peptides are present from fractions 45–80 in control IPK urine ($n = 6$, A and B) and trypsin-digested IPK urine ($n = 6$, C and D), obtained by radioactivity analysis and absorbance at 214 nm, is shown.
(TGF-β) production (132). Although many studies have implicated ANG II and TGF-β in the development of renal hypertrophy and progressive glomerulosclerosis, these factors may also play an important role in the induction of albuminuria through promotion of lysosomal dysfunction as outlined in Fig. 8. The discussion of these pathways serves an important example of the central role of lysosomal processing in nonnephrotic albuminuria. Other factors in the pathogenesis of these disorders, including hyperglycemia, reactive oxygen species, macrophage infiltration and activation, and other cytokines and growth factors may also affect PTC function to directly or indirectly suppress the albumin degradation pathway.

The association of renal hypertrophy and inhibition of lysosomal enzymes is well established (74, 88, 103, 115). Furthermore, in addition to its prosclerotic function, TGF-β has an inhibitory effect on lysosomal function. Schenk et al. (105) demonstrated that TGF-β suppresses the activity of lysosomal enzymes, particularly lysosomal cysteine proteinases (cathepsins), which are primarily responsible for tissue turnover in the kidney (105).

There is increasing evidence that TGF-β decreases lysosomal function and by doing so it decreases the activity of the degradation pathway on filtered albumin. Studies in spontaneously hypertensive rats with and without streptozotocin-induced diabetes demonstrated that albuminuria was accompanied by increased TGF-β expression and reduced lysosomal activity (94, 96). Studies by Gekle et al. (34) and Russo et al. (95) have further demonstrated in PTC cultures that addition of TGF-β can disrupt both albumin uptake and degradation. Studies have demonstrated that quantitative effects of angiotensin-converting enzyme inhibitors (ACEi) in reducing albuminuria can be explained by their ability to activate lysosomal processing (Refs. 83, 86; Hilliard, Russo, Comper, unpublished observation). In other words, ACEi treatment activates lysosomal activity by inhibiting ANG II production. With lysosome activation filtered albumin will be degraded before excretion, thus explaining the relatively rapid ability of ACEi treatment to reduce albuminuria in patients with diabetic nephropathy (10). Recent studies have shown that a genetic deficiency of decorin, which inhibits active TGF-β, exacerbates the development of albuminuria and diabetic nephropathy in mice (130). However, it is clearly difficult to separate the direct potential role of TGF-β and ANG II in causing albuminuria through lysosomal dysfunction from other factors that may mediate these activities.

Relationship of glomerular morphology to proximal tubular cell lysosome-mediated albuminuria. Glomerular morphological changes such as expansion of the extracellular matrix, foot process effacement, loss of the slit diaphragm, an abnormal endothelial cell layer, and thickening of the GBM have often been correlated with albuminuria. These changes have been thought to be directly responsible for increases in glomerular permeability to albumin, although, as discussed above, the evidence would suggest that glomerular permeability to albumin does not change. We suggest that the factors that drive these changes to glomerular morphology are the same as those that affect PTC processing. Therefore, targeting the factors that affect PTC albumin lysosomal processing will be beneficial not only in reducing albuminuria but also in improving glomerular morphology in terms of fibrosis.

Cardiovascular disease and albuminuria. It is notable that increased RAS and TGF-β, as shown in Fig. 8, are also implicated in the disruption of cardiovascular function and manifestation of cardiac hypertrophy and fibrosis resulting in structural changes in the heart associated with significant cardiovascular risk. ANG II indirectly stimulates cardiac hypertrophy, the proliferation of cardiac fibroblasts, the phenotypic conversion of fibroblasts to myofibroblasts, and extracellular matrix deposition via the induction of TGF-β1, the most common human isoform of TGF-β. Increased TGF-β expression has been observed during cardiac hypertrophy and fibrosis in humans and animal models (18, 46, 61, 62). Similar to the kidney, associations between decreased lysosomal enzyme activity and cardiac hypertrophy have been established (111, 129), while ACEi and ANG type 1 receptor (AT1) antagonists have also been demonstrated to restore lysosomal enzyme activity (83, 86, 111, 129) and successfully prevent or reverse cardiac hypertrophy and fibrosis (12, 32, 36, 51, 53, 63, 69, 129). Hilliard et al. (unpublished observation) recently demonstrated that ANG II-stimulated TGF-β in hypertension causes cardiac hypertrophy and albuminuria that is associated with significant decrease in lysosomal activity in both the kidney and the heart. These changes can be prevented by treatment with the ACEi ramipril.

Concluding Remarks

Albuminuria is the most widely recognized molecular marker of kidney disease. More recently, it has been directly associated with cardiovascular disease. Understanding the mechanism of albuminuria has the potential to give us direct insight into the molecular events underlying these diseases.

We conclude that albuminuria is predominantly a tubular dysfunction. Subnephrotic-range albuminuria (<3 g/day) is often equated with metabolic conditions that result in systemic hypertension and fibrosis related to vascular diseases like hypertension, cardiovascular disease, and diabetes. In such diseases, these metabolic events act on the PTC to inhibit the lysosomal albumin degradation pathway that directly influences albuminuria. Therefore, nonnephrotic albuminuria in such diseases is more a marker of vascular disease and organ damage than a specific vehicle for damage itself. In the situation of a nephrotic range of albuminuria (>3 g/day), this is likely to involve inhibition of the retrieval pathway of the kidney.

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REFERENCES


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Review


