A New Mechanism for Albuminuria-Induced Podocyte Injury

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A large body of evidence describes the relationship between albuminuria and progressive renal damage.1–3 Podocyte injury contributes to progressive albuminuria in diabetic and nondiabetic glomerular diseases, with antiproteinuric regimens providing a renoprotective effect.4–6 However, it remains unclear whether progressive albuminuria is merely a consequence of podocyte injury or a potential aggravating factor to podocytopathy. Recent in vitro studies have suggested that high doses of albumin may contribute to podocyte loss7,8 as a result of cellular uptake of albumin, leading to activation of proapoptotic and proinflammatory pathways.9 Podocytes are terminally differentiated cells with limited capacity for self-renewal, but recent evidence suggests that regeneration of podocytes by renal progenitor cells along the Bowman capsule occurs in the setting of podocyte loss.10,11

Retinoids are derivatives of vitamin A and have multiple cellular functions, such as inhibition of proliferation, regulation of apoptosis, and induction of cell differentiation.12 In the canonical retinoic acid (RA) synthesis pathway, retinol is taken up by retinol-binding protein in circulation and transferred intracellularly, where it is transformed into retinaldehyde and eventually oxidized to RA. Subsequently, RA exerts its effect by binding to retinoic acid receptors, which forms heterodimers with retinoid X receptors, leading to activation of RA response elements (RARE) in the nucleus.12

Specifically, in kidney development, RA plays a crucial role in regulating tubulogenesis and nephron number.12 In addition to the well known therapeutic benefits in skin disorders and malignancies,12 treatment with retinoids attenuates kidney disease in multiple murine models.13 In fact, several studies have revealed that RA induces the expression of podocyte differentiation markers, thereby abrogating stress-induced podocyte injury.13,14

In this issue of JASN, Peired and colleagues provide evidence for albuminuria attenuating podocyte regeneration via the sequestration of RA.15 Initial in vitro studies revealed that albumin overload impairs podocyte differentiation markers without affecting podocyte survival. This is in stark contrast to findings observed by Okamura et al. and others, in which albumin exposure activated inflammatory cytokines, resulting in cell death in a dose-dependent manner.7–9 Furthermore, RA-induced podocyte differentiation was attenuated in the setting of increasing albumin concentration, suggesting this process is mediated via the sequestration of RA by albumin, leading to downregulation of RARE. To confirm that albumin overload attenuates RA-induced podocyte differentiation, the authors used the in vivo murine model of Adriamycin (ADR)-induced nephropathy in three distinct mouse backgrounds. Using the Cre-lox system, podocytes were irreversibly tagged with green fluorescent protein, thereby making expression of this protein a direct marker of podocyte survival. In vivo, albuminuria induced by ADR treatment was associated with both podocyte loss and dedifferentiation. The authors also observed that administration of disulfiram (aldehyde dehydrogenase 1A inhibitor) in ADR-treated mice reduced survival with an aggravation in glomerular injury. Thus, they concluded that a loss in endogenous RA may accelerate glomerular disease.

Although the precursors and enzymes involved in RA synthesis were unchanged with ADR treatment, the authors identified that with increasing albuminuria, urinary concentration of retinol increased while RA decreased, thereby suggesting that RA is sequestered by albumin. In contrast, key observations from previous studies reveal alteration of key RA synthesis enzymes in other kidney diseases.16,17

In the final set of experiments, the authors validated a recent study by Zhang et al.18 by showing that RA-induced podocyte differentiation results from restoration of novel podocytes from the transdifferentiation of renal progenitor cells into podocytes. ADR treatment of RARE-lacZ transgenic mice showed an increase in endogenous RARE activity exclusively in the renal progenitor cells, and not in podocytes. With subsequent RA treatment, RARE activity increased in the renal progenitor cells, with a loss of RARE activity in mice not treated with RA. Interestingly, the RARE activity was not observed in renal progenitor cells in cellular lesions, but only in renal progenitor cells along the Bowman capsule. Combined with the evidence provided by the authors from previous studies, a subset of renal progenitor cells are likely responsive to RA treatment.
Using established techniques, the authors provide a novel explanation of albumin sequestration of RA for the progression of podocyte injury in nephrotic diseases. One limitation of this thought-provoking study is the failure to determine the amount of cellular uptake of albumin in the setting of ADR-induced nephropathy. Because RA synthesis occurs intracellularly with subsequent translocation to the nucleus and activation of RARE, it is important to determine whether inhibiting cellular uptake of albumin attenuates RA sequestration. Another striking observation in this study is that RARE activity was exclusively present in the parietal epithelial cells in the setting of glomerular injury. Because many groups have shown that podocytes express receptors for RA, some endogenous RARE activity in podocytes would have been expected in the RARE-lacz mice. Moreover, Vaughn et al. showed that RA induces podocyte differentiation and increases nephrin and podocin expression in the setting of podocyte injury. In addition to activation of transcription factors involved in podocyte differentiation, KLF15, by RA, we have previously shown that RA can attenuate podocyte dedifferentiation by activation of a cAMP-dependent pathway via retinoic acid receptor-α in the podocytes. However, expression of receptors for RA has not previously been confirmed in the renal parietal epithelial cells or renal progenitor cells. Nonetheless, with these new findings, RA appears to exert a similar effect on parietal epithelial cells as in podocytes.

The current evidence provided by the authors suggests that albumin can sequester RA. However, a similar pattern of sequestration would have been expected with retinol because retinol is typically transported by retinol-binding protein. In addition, the authors failed to reconcile one conclusion between their in vitro and in vivo studies. In the initial set of experiments, albumin overload attenuated podocyte differentiation markers independent of podocyte survival. However, a loss of podocyte differentiation markers in ADR-treated mice was associated with podocyte loss. Finally, previous groups have shown that ADR-induced podocyte injury can occur in resistant mouse strains at high doses of ADR. However, it is noteworthy that a similar magnitude of proteinuria and FSGS lesions was observed in all three strains.

Despite a few limitations, the findings of this study are undoubtedly provocative with regard to identifying the mechanism by which proteinuria exacerbates glomerular disease. The current study also further validates the critical role of RA in the treatment of glomerular disease. Additional studies using previously published renal progenitor cell lineage tracing techniques are necessary to confirm that RA-induced regeneration of podocytes occurs via the transdifferentiation of renal progenitor cells. Although all-trans retinoic acid has been shown to attenuate podocyte injury, clinical studies are limited because of significant adverse effects. Novel derivatives of RA receptor agonists, such as Am580 and BD4, induce the expression of podocyte differentiation markers in the setting of podocyte injury. Future studies will need to be conducted to determine whether these new derivatives provide similar efficacy of podocyte regeneration with lower toxicity.

DISCLOSURES
None.

REFERENCES
Proximal Tubules Forget “Self-Eating” When They Meet Western Meals

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Autophagy is an evolutionarily conserved cellular catabolic process in which cytoplasmic components are isolated from the rest of the cell within double-membrane structures (autophagosomes) and degraded through lysosomal degradation. Autophagy is activated under conditions of metabolic stress in which there is a shortage of energy for cells to maintain cellular homeostasis.1 A number of recent studies demonstrated that the induction of autophagy serves a crucial role in protecting renal proximal tubular cells from many stresses, including ischemia/reperfusion and nephrotoxic agents.2–4 However, few studies have examined the role of proteinuria in the regulation of autophagy and the conditions that impair the activity of autophagy in the course of renal dysfunction.

By characterizing an albumin-overload mice model and tissues from obese patients with proteinuria, Yamahara and coworkers, as described in this issue of JASN,5 present evidence that autophagy plays an important role in protecting renal proximal tubular cells from harmful proteinuria. They demonstrated that proteinuria is a strong cue for autophagy induction in the proximal tubular cells of mice with albumin overload. Furthermore, high-fat diet and obesity blunt proteinuria-induced autophagy induction in the proximal tubules and thereby exacerbate tubular cell damage by proteinuria.

The term “autophagy” is derived from the Greek words “phagy,” meaning eat, and “auto,” meaning self. Interestingly, this “self-eating” behavior was first discovered in renal proximal tubular cells of newborn mice in 1957.6 Dr. Clark described the observations of autophagy in his paper:

The large round bodies in the proximal tubules consist of an amorphous material and contain concentrically lamellar structures and mitochondria. They resemble the cytoplasmic droplets produced in the proximal tubules of adult rats and mice by the administration of proteins. The large round bodies disappear from the proximal tubules of infant mice during the first week after birth, but the concentric lamellar structure may be found in adult mice.

From these observations, Clark hypothesized that the formation of the large round bodies (autophagosome) in the proximal tubules was likely due to the absorption of proteins that passed through immature newborn glomeruli. Now, Yamahara and coworkers have re-evaluated the process of renal tubular autophagy first described more than a half-century ago using their modern technologies under clinically relevant conditions.

Compared with control mice, mice lacking autophagy activity in the renal proximal tubular cells displayed more severe damage of their tubular cells in response to free fatty acid (FFA)-albumin–induced proteinuria. Furthermore, the renoprotective activity of proteinuria-induced autophagy in proximal tubular cells was suppressed in obese mice and humans. Yamahara and coworkers found that the activation of 5’AMP-activating protein kinase (AMPK) but not the enhanced endoplasmic reticulum stress played a critical role in the induction of autophagy by proteinuria. Moreover, the activation of mammalian target of rapamycin complex 1 (mTORC1) in the proximal tubules of obese mice or patients was responsible for

See related article, “Proteinuria Impairs Podocyte Regeneration by Sequestering Retinoic Acid,” on pages 1756–1768.