

More on Renal Disease Progression: Is Interstitial Inflammation Truly Protective?

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Patients with a chronic glomerulopathy are at risk for progressively declining renal function that results in end-stage renal failure irrespective of etiology. The underlying pathophysiology is at least partly dependent on maladaptive functional changes that occur in the surviving nephrons upon destruction of a critical number of glomeruli. In proteinuric nephropathies, the dysfunction of the glomerular filtration barrier causes the abnormal passage of large amounts of potentially toxic plasma proteins and protein-bound molecules from the bloodstream into the tubular lumen. One widely accepted mechanism underlying the acceleration of progressive injury to the kidney is that the ensuing tubulointerstitial damage is induced by direct toxicity of filtered protein, because proteinuria has long been known to correlate with both the severity of glomerular barrier impairment and the rate of decline of renal function. An important question that has not yet been adequately addressed in this area is whether albumin in proteinuric ultrafiltrate may promote proximal tubule cell injury through enhanced endocytosis.

The proximal convoluted tubule has a critical role in handling albumin and other filtered proteins in the mammalian kidney *via* its well-developed endocytic function. This field is rapidly expanding as a result of the identification of molecules that cooperate in receptor-mediated endocytosis at the brush border region. Megalin, a 600-kD glycoprotein that belongs to the LDL superfamily, is a multifunctional molecule that acts as a low-affinity receptor that mediates normal uptake of albumin and other filtered plasma proteins (1). Lack of megalin causes low molecular weight proteinuria in mice.

In this issue of *JASN*, Theilig *et al.* (2) used mice with partial deficiency for megalin (megalin *lox/lox*;apoE Cre mice [Cre(+)mice]) to assess whether megalin-dependent enhanced protein endocytosis by tubular epithelial cells would lead to upregulation of mediators that promote inflammation, tubular degeneration, and fibrosis. Megalin immunostaining shows a mosaic in these kidneys—that is, both megalin-positive and megalin-negative cells reside in tubules—with 60 to 80% of tubular profiles showing megalin deficiency in the mice that

were used in this study. To assess effects of proteinuria on tubulointerstitial injury, anti-glomerular basement membrane glomerulonephritis (GN) was induced in megalin-deficient mice [Cre(+)GN] and in control mice [Cre(-)GN], and animals were killed on day 18 after injection of nephritogenic antiserum. Preliminary to discussion of the data, it should be acknowledged that this is an acute and immunologically mediated disease, making it difficult to infer to what extent the observations at this relatively early time point may apply to the pathophysiology of chronic proteinuria.

A more prolonged exposure to proteinuria is usually needed for significant tubulointerstitial damage to ensue. For instance, in Adriamycin nephrosis, one of the most severe animal models of nephrotic proteinuria that invariably progresses to glomerulosclerosis and tubulointerstitial injury, mild tubulointerstitial changes develop only after 1 mo. Moreover, in this model, the deficiency of megalin was incomplete within individual nephrons. In proteinuric mice, only megalin-positive cells were engaged in protein endocytosis, as revealed by immunostaining for mouse IgG, which permitted differences in the localization of marker molecules of interest to be compared in megalin-deficient and -sufficient cells. However, it can be difficult to draw inferences from the topographic relation with interstitial changes, particularly when they involve mediators that can diffuse from their site of origin along the nephron toward the contiguous interstitial compartment.

Nephritic mice that lacked megalin developed both proteinuria and glomerular crescentic lesions, resulting in tubular obstruction and degeneration. The phenotype of the animal model of partial megalin deficiency included worse glomerular injury in response to anti-glomerular basement membrane serum. Therefore, it is possible that more glomerular damage could have overwhelmed any potential protective effect of tubular megalin deficiency. Nevertheless, one major finding was that the expression of the proinflammatory and fibrogenic molecules endothelin-1, TGF- β 1, and TGF- β 3 was increased exclusively in megalin-positive cells. This is in agreement with available *in vitro* data showing upregulation of such molecules by proximal tubular cells in response to protein load (3–8) and suggests that the stimulatory effect could be mediated by megalin. Intercellular adhesion molecule-1 and vascular cellular adhesion molecule-1 showed a similar pattern, whereas the upregulation of monocyte chemoattractant protein-1 was variable.

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An association was not found, however, between the expression of proinflammatory mediators and the evidence of renal inflammation, which instead was more associated with megalin-deficient tubular profiles. Fibrotic changes were present but did not show preferential association with either cell phenotype in Cre(+)/GN kidneys. Notably, more apoptosis was found in megalin-deficient cells than in megalin-positive cells of Cre(+)/GN mice. Theilig *et al.* suggest that, in contrast to the conclusions that have been drawn to date in a large majority of studies, the expression of inflammatory mediators by proximal tubular cells that are exposed to excess ultrafiltered protein is in some way protective rather than harmful. The evidence to support this interpretation, however, is problematic, being largely based on the negative finding of lack of a tight topographic association with the inflammatory infiltrate at a single time point in a short-term study. In addition, in this study, both proteinuria of glomerular origin and renal damage were greater in nephritic megalin-deficient mice than in nephritic control mice. This hypothesis being tested therefore merits investigation in a suitable noncrescentic model in which the full time course of changes in protein uptake and parameters of injury and inflammation can be studied.

A potentially important finding of the study by Theilig *et al.* is the observation that megalin-positive cells displayed more pronounced proliferation. This important finding has been taken as evidence for an antistress response promoted by enhanced protein uptake. However, the opposite has also been documented in an elegant recent study (9). High concentrations of albumin induced apoptosis of proximal tubular cells by decreasing the transcription and membrane expression of megalin and causing a dissociation of the serine/threonine kinase protein kinase B from megalin; the resulting inhibition of protein kinase B activity was associated with dephosphorylation of Bad at Ser-136, thus activating an apoptotic pathway. Another recent article further suggested that cultured proximal tubular cells respond to ligand stimulation of megalin by regulated intramembrane proteolysis (10). This process, which links receptor-mediated endocytosis with intracellular signaling events, has been proposed to underlie the transcriptional regulation of specific genes in the proximal tubule (11). Of note, with the use of laser capture microdissection of renal proximal tubular epithelial cells that were isolated from kidney biopsies of patients with proteinuric nephropathies, a significant up-regulation of genes that were involved in cell proliferation and cell-cycle control (*e.g.*, IGF-1) and cell differentiation (*e.g.*, bone morphogenetic protein 7) was documented (12). As our knowledge of these events improves, further studies in the megalin-deficient kidney models could be of major help to clarify how megalin-dependent processes may be relevant to the pathophysiology of proteinuric nephropathy.

As a consequence of proteinuria, the intrarenal activation of the complement cascade may also promote injury through the formation of membrane attack complex (13–15) and possibly other biologically active products that interact with specific receptors. Complement activation is a powerful mechanism underlying tubular and interstitial injury *via* cytotoxic, proinflammatory, and fibrogenic effects. Abnormal C3 and C5b-9

staining in proximal tubular cells and along the brush border is a long recognized feature of both experimental models and human chronic proteinuric diseases. Therefore, both excess ultrafiltration and proximal tubular cell synthesis of complement (6) could underlie complement-mediated injury independent of megalin-related mechanisms in chronic proteinuric renal diseases.

In conclusion, the findings of Theilig *et al.* are very interesting and provocative. However, the fact that megalin deficiency is heterogeneous in the proximal tubule in Cre(+) mouse kidney, together with the assessment of changes that occur at an early time point of crescentic glomerulonephritis, precludes the definitive dissection of the role of megalin-dependent renal injury induced by chronic proteinuria in this model and leaves open the door for additional investigation of the many proposed mechanisms by which filtered proteins may lead to chronic interstitial disease and progression.

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Disclosures

None.

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See the related article, “Abrogation of Protein Uptake through Megalin-Deficient Proximal Tubules Does Not Safeguard against Tubulointerstitial Injury,” on pages 1824–1834.