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Editorial Comments

Proteinuria and interstitial injury

Allison A. Eddy

Children's Hospital and Regional Medical Center, Department of Pediatrics, University of Washington, Seattle, WA, USA

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Introduction: proteinuria as a risk factor for progressive renal disease

It has long been recognized that patients with high-grade proteinuria due to chronic glomerular disease are more likely to develop chronic renal failure than a matched group of patients with low-grade or no proteinuria [1,2]. Although this association may seem intuitively obvious, suggesting correlation with the severity of glomerular damage, two important observations promote an alternative compelling hypothesis. First is the fact that renal functional outcome for patients with chronic glomerulopathy is best predicted histologically by the severity of chronic extraglomerular damage—peritubular capillary loss, tubular atrophy and interstitial fibrosis. Second is research evidence that urinary proteins themselves may elicit pro-inflammatory and pro-fibrotic effects that directly contribute to chronic tubulointerstitial damage. For example, rodents injected daily with large doses of albumin develop 'overload proteinuria' that consists of both the exogenous albumin as well as several endogenous plasma proteins [3,4]. Although there does not appear to be an immunological response to the albumin, shortly after the onset of proteinuria, interstitial inflammation develops and fibrosis ensues. While this exact scenario would be unusual in humans, analogous cases have been reported including two children with hemolytic uremic syndrome, severe hyperproteineamia and proteinuria associated with aggressive plasma-infusion therapy [5]. The existence of a direct proteinuria–interstitial inflammation–fibrosis connection has important clinical implications including avoidance of unnecessary albumin and plasma infusions and the use of therapies that reduce protein excretion rates, both immunosuppressive therapy for the primary disease and angiotensin II blockade, especially for hypertensive patients.

The proteinuria–interstitial inflammation–fibrosis connection

Effects of proteinuria on renal tubules

Interstitial inflammatory cells are recruited from the circulation via an integrated series of steps involving interactions between leukocyte adhesion molecules (selectins and integrins) and their counter-receptors/ligands on peritubular capillaries (mucins and IgG-like receptors). Leukocyte migration is directionally regulated by chemokines and chemoattractants. Several *in vitro* studies have demonstrated that certain urinary proteins (albumin and transferrin are commonly used) can stimulate proximal tubular cells to synthesize chemokines: monocyte chemoattractant protein-1 (MCP-1), RANTES and fractalkine that recruit monocytes and T-cells and interleukin-8 that attracts neutrophils [6–10]. Such findings suggest that a similar sequence of events may occur *in vivo*, providing a plausible mechanistic link between proteinuria and interstitial inflammation (Figure 1). Indeed, *de novo* tubular production of MCP-1 has been observed in several proteinuric renal diseases including overload proteinuria [4]. Anti-MCP-1 gene therapy was shown to significantly reduce the severity of interstitial inflammation and fibrosis in animals with overload proteinuria [11]. Studies in knockout mice provide further evidence that MCP-1 is involved in the interstitial inflammatory and fibrogenic response in mice with anti-GBM and lupus nephritis [12,13]. Although a role for chemokines such as MCP-1 is most convincing, other tubular-derived chemoattractants have been implicated in this process including the adhesive glycoprotein osteopontin [14] and activated complement C3 fragments [15].

Correspondence and offprint requests to: Allison Eddy, Children's Hospital and Regional Medical Center, Division of Nephrology, Mail Stop M1-5, 4800 Sand Point Way NE, Seattle, WA 98105, USA. Email: allison.eddy@seattlechildrens.org

Given the apparent effects of tubular-derived proteins, several questions need further consideration. First, which urinary protein(s) activates tubular cells? Despite the fact that albumin elicits several tubular responses *in vitro*, it is still unclear whether albumin alone is an important activator of tubular cells *in vivo*. Progressive renal disease is less common in patients with steroid-responsive nephrotic syndrome and familial nephropathy associated with highly selective proteinuria [16], composed predominantly of albumin and lower molecular weight proteins. Although animals with albumin-induced overload proteinuria develop interstitial disease, the urine of these animals does contain non-albumin endogenous plasma proteins. Analbuminemic rats [17] treated with adriamycin develop proteinura and interstitial damage, although the latter could be a response to the drug rather than proteinuria. Albumin might act as a carrier for other inflammatory mediators such as lipids [18,19]. Several other urinary proteins have already been shown to activate tubular cells including complement proteins, transferrin and certain growth factors [20–22]. Many other candidates remain to be investigated.

Second, how do urinary proteins activate tubular cells? Megalin and cubilin are multi-ligand and multifunctional receptors expressed by proximal tubular cells and involved in the endocytosis of luminal proteins [23]. While logical candidates, it is not yet clear if megalin and/or cubulin are the primary receptors that initiate tubular cell pro-inflammatory responses to proteinuria. Activation of nuclear factor kappa B (NF-κB) within proximal tubular cells exposed to urinary proteins appears to be one intracellular signalling event that leads to transcriptional activation of chemokine genes. *In vivo* treatment with the NF-κB inhibitor (IκBa) significantly reduced interstitial inflammation and fibrosis in rats with overload proteinuria although specific effects on chemokine expression were not evaluated [24].

Third, how do tubular-derived mediators of inflammation reach their target cells, which are circulating within peritubular capillaries? Some *in vitro* studies using confluent monolayers of polarized tubular cells have nicely demonstrated that apical presentation of proteins can lead to synthesis and release of potential inflammatory mediators across the basolateral membrane. However, *in vivo* tubular basement membranes (TBM) still present a significant barrier between tubules and capillaries (Figure 1). In more advanced disease, the TBM is often damaged, permitting direct communication between tubular cells and the interstitial space. For example, TBM disruption appears to be an important prerequisite for the migration of transdifferentiated tubular cells into the interstitium where they contribute to matrix protein synthesis [25]. However interstitial inflammation typically precedes overt TBM damage and the channels of communication between the tubular epithelium and the capillary endothelium remain to be determined. Along the distal nephron, tubules may be damaged by obstructing protein casts, with subsequent damage to epithelial cells and their basement membranes. Little is currently known about specific cellular effects of proteinuria on more distal tubular cells.

While the pro-inflammatory response of renal tubules to proteinuria has been implicated as the important early event leading to interstitial fibrosis, other effects may also contribute to progressive renal

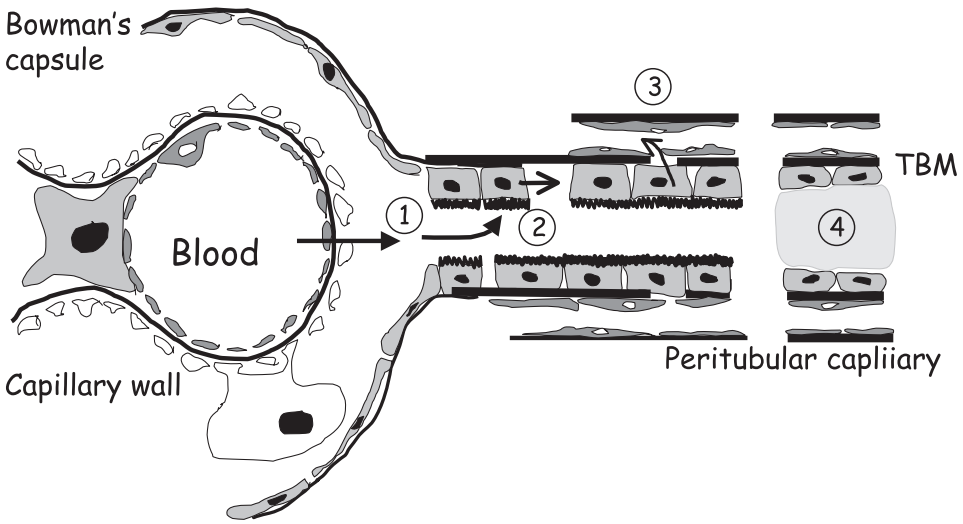

Fig. 1. Effects of urinary proteins on tubular cells. (1) In glomerular diseases associated with proteinuria, proximal tubular cells may be activated by elevated levels of normal and novel urinary proteins. (2) Activated tubular cells may synthesize pro-inflammatory mediators, especially monocyte chemoattractant molecules (e.g. MCP-1, RANTES, fractalkine, complement component 3) and fibrosis-promoting molecules (e.g. endothelin, angiotensin II, TGF-β). (3) Damage to tubular basement membrane facilitates the passage of tubular-derived products into the interstitium and peritubular capillaries spaces. (4) Along the distal nephron, protein casts may obstruct urinary flow and aggravate tubulointerstitial damage. (This figure was adapted from Segerer S, Nelson, PJ, Schlondorff D, Chemokines, chemokine receptors, and renal disease: from basic science to pathophysiologic and therapeutic studies. *J Am Soc Nephrol* 2000; 11: 152–176 [34] with permission of Lippincott, Williams and Wilkins.)

destruction including increased tubular cell synthesis of extracellular matrix proteins as a direct response to urinary proteins, especially pro-fibrotic growth factors such as transforming growth factor β [22] and the activation of pro-apoptotic pathways that lead to tubular cell death [26,27].

Other pathways from the glomerulus to the interstitium

While the response of proximal tubules to excessive and/or novel urinary proteins has been strongly implicated in the proteinuria–interstitial inflammation connection, additional pro-inflammatory mediators may originate within glomeruli, either produced systemically or locally by inflammatory and resident glomerular cells as part of the primary nephritic process. Under this scenario, the glomerular-derived products may elicit additional tubular responses, but these proteins may also gain access to the tubulointerstitium via alternative pathways (Figure 2). Analogous to pulmonary venous blood enriched with oxygen, blood in the glomerular efferent arterioles may be enriched with glomerular inflammatory mediators before perfusing the peritubular capillaries. Although it is easy to imagine how activation of peritubular capillary endothelium could facilitate leukocyte recruitment and migration into the interstitium, this pathway has not been investigated in any detail.

In crescentic glomerular diseases, breaks in Bowman's capsule are not uncommon and allow leakage of proteins in the glomerular ultrafiltrate directly from Bowman's space into the interstitium. Evidence that such a pathway may be important comes from careful observation of human biopsies, demonstrating that the periglomerular interstitial space is often most severely inflamed in crescentic forms of nephritis. In animal models of anti-GBM nephritis, interleukin-1 has been identified as one glomerular mediator of interstitial inflammation [28].

As glomerular diseases progress to a chronic phase, regions of sclerosis of the glomerular tuft develop. Often these sclerotic regions become adherent to and disrupt Bowman's capsule. Such lesions provide an additional site for direct leakage of the glomerular ultrafiltrate into the peritubular interstitial space—misdirected ultrafiltatrion as described by Kriz *et al*. [29].

Why is the inflamed interstitium a concern?

The recruitment of macrophages to sites of acute injury represents a fundamental step in wound healing at any site within the body. However, if these cells persist after the initial damage is repaired, the fibrogenic responses are often sustained with damaging consequences to renal architecture. As the renal interstitium becomes progressively expanded by extracellular matrix proteins, there are damaging and ultimately irreversible effects on peritubular capillaries and tubular epithelium. Macrophages are multifunctional cells that are capable of synthesizing a number of secretory products that contribute to ongoing tissue injury (Figure 3). They may also regulate matrix accumulation, primarily by producing fibrosis-promoting growth factors (e.g. TGF-β) and vasoactive products (e.g. endothelin-1, angiotensin II) and products that impair matrix degradation (e.g. plasminogen activator inhibitor-1, tissue inhibitors of metalloproteinases). Macrophages are not thought to be a significant source of the actual matrix proteins that accumulate in the interstitium but they may participate in the recruitment of the matrix-producing interstitial myofibrobasts [30].

Many therapeutic interventions tested in experimental models and used in humans with chronic proteinuric renal diseases decrease interstitial inflammation and fibrosis. For example, following surgical 5/6 nephrectomy of rats, the initially normal remnant kidney undergoes progressive destruction by a process that involves interstitial inflammation and fibrosis. In this non-immune model of injury, immunosuppression with mycophenolate mofetil was surprisingly protective [31]. The treated animals developed less interstitial inflammation and fibrosis and had better renal function than control animals. In several studies designed to identify the molecular pathways involved in interstitial monocyte recruitment, specific inhibition and/or deletion of a

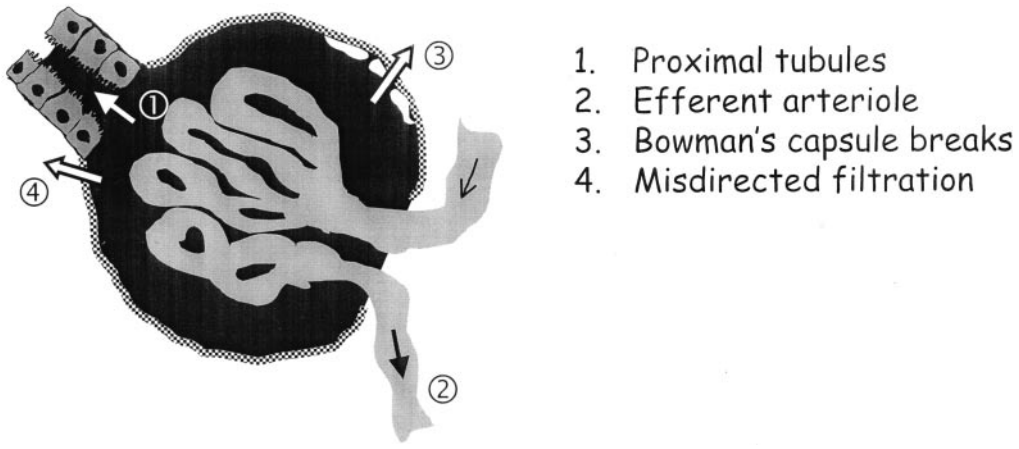

Fig. 2. Points of direct communication between proteins in the glomerular ultrafiltrate and the tubulointerstitium.

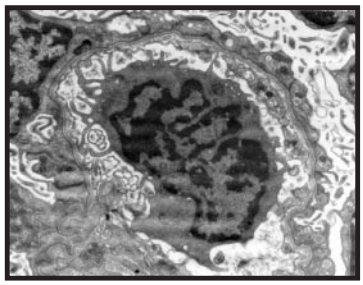

Fig. 3. Macrophage secretory products that have been implicated in the pathogenesis of tubulointerstitial damage and progressive renal disease. While most products promote injury, scavenging functions of macrophages may help to dampen the severity of the damage.

critical mediator has not only reduced the severity of the interstitial inflammation but has been associated with less fibrosis and chronic kidney damage. For example, such beneficial outcomes have been reported in animals with overload proteinuria that were treated with inhibitors of MCP-1 and NF-κB [11,24].

However, there is one important caveat that needs to be considered. Macrophages are multi-functional cells that may also impart important beneficial functions, especially pertaining to their ability to serve as phagocytic scavengers. There is an increasing number of examples where blockade of specific macrophage receptors has caused worse chronic kidney damage despite fewer numbers of interstitial macrophages, including macrophages lacking the urokinase receptor (uPAR) and the angiotensin II type 1 receptor [32,33]. Both of these receptors were shown to modify specific macrophage phagocytic functions that may serve to dampen the intensity of the fibrogenic response. Thus future studies will need to delineate not only the number but also the phenotype of interstitial macrophages in an effort to differentiate protective from harmful responses.

Conclusion

There is now a substantial body of evidence that sustained high-grade proteinuria is an independent mediator of progressive kidney damage. Effects on renal tubules appear to provide a critical link between proteinuria and tubulointerstitial injury although several other mechanisms have also been implicated (Figure 4). These studies offer rationale for the development and testing of therapies to modulate the

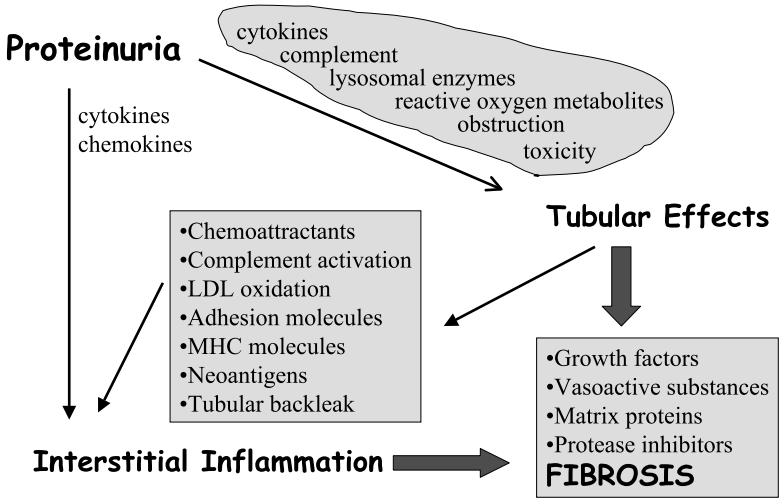

Fig. 4. Schematic summary of some of the pathways and mediators that may explain how proteinuria causes interstitial inflammation and fibrosis. (This figure was modified from Eddy and Schnaper [35] with permission from Elsevier.)

severity of proteinuria and/or mediators of proteinuria-induced tubulointerstitial disease.

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Conflict of interest statement. None declared.

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