Tubular injury in glomerular disease

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CASE PRESENTATION

A 40-year-old man was admitted to the hospital for a renal biopsy; three years earlier his first renal biopsy had revealed focal segmental glomerulosclerosis. Proteinuria first had been noted on a routine physical examination at approximately age 20. The proteinuria, initially in the subnephrotic range, was not accompanied by notable activity of the urinary sediment. Around the age of 30, the patient developed hypertension and hypercholesterolemia, which were treated with an angiotensin-converting-enzyme (ACE) inhibitor and a statin, respectively. Serial urinalyses by his personal physician revealed persistent subnephrotic proteinuria. By age 37, however, the urinary protein excretion had increased to 4 g/day and the serum albumin had fallen to 3.4 g/dL. The serum creatinine level remained normal at 1.0 mg/dL.

Further workup to determine the cause of proteinuric renal disease was initiated. The patient had no history of childhood renal disease or other urinary tract disease. He had no history of drug use, and serologic studies for autoimmune and infectious diseases associated with nephrotic-range proteinuria were negative.

The initial renal biopsy disclosed 16 glomeruli on light microscopy, five of which had global sclerosis and one segmental sclerosis. Another glomerulus showed periglomerular fibrosis and wrinkling, consistent with ischemia. Several glomeruli dem-

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DISCUSSION

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This patient’s second renal biopsy showed changes typical of proteinuric glomerular disease advancing to renal insufficiency. The diagnosis of focal segmental glomerulosclerosis was based on the finding of segmental sclerotic lesions in 2 of 13 glomerular profiles. In addition, 2 glomeruli were globally sclerosed, and 2 appeared shrunken and collapsed with wrinkling of the basement membranes. Glomerular injury was accompanied by tubulo-interstitial injury. Some tubular segments were hypertrophic, but many were atrophic. Some were filled with protein casts. The interstitium was expanded, especially...
around the most damaged tubules, and contained a patchy mononuclear cell infiltrate.

**Intact nephron hypothesis**

How are these changes in glomerular, tubular, and interstitial structure related? The organization of glomeruli and tubules into individual nephrons cannot be appreciated on examination of routinely prepared tissue sections. But physiologic studies have suggested that even when disease causes structural changes like those seen in this biopsy specimen, glomerular and tubular function remain closely integrated. Evidence that glomerular and tubular function remain linked in diseased kidneys can be usefully summarized by the term “intact nephron hypothesis,” as originally proposed by Neal Bricker in 1960 [1]. This hypothesis does not imply that each nephron is either untouched by disease or obliterated. Rather, the initial population of homogeneous nephrons is replaced by a lesser population in which neighboring structures exhibit heterogeneous patterns of form and function.

Important support for the intact nephron hypothesis was provided by micropuncture studies by Carl Gottschalk and colleagues in the 1970s [2–4]. These studies showed that in rats with chronic glomerulonephritis, glomerular filtration rate (GFR) for single nephrons varied from one-third to three times normal. Yet the fraction of sodium and water reabsorbed was the same in proximal tubules connected to hypofiltering and hyperfiltering glomeruli. Similar results were obtained in rats with tubular disease caused by heavy metals. Filtration was markedly reduced in glomeruli attached to the most severely damaged tubules, but the balance between glomerular filtration and proximal fluid reabsorption was maintained.

The mechanisms that keep glomerular and tubular function linked as renal disease progresses are not fully understood. Tubuloglomerular feedback might reduce single-nephron GFR when damaged tubules fail to reabsorb a normal amount of filtrate, and changes in peritubular Starling forces might limit proximal reabsorption when filtration is reduced in damaged glomeruli. Ultimately, these functional changes are associated with structural changes. If the flow of filtrate is stopped, tubules atrophy [5]. If tubules are injured, upstream glomeruli shrink and can become sclerotic [3, 6–8]. More than 60 years ago, Jean Oliver used serial histologic sections to reconstruct individual nephrons from patients dying with renal failure. He demonstrated that the kidneys of these patients contained large glomeruli connected to hypertrophic tubules and shrunken glomeruli connected to atrophic tubules (Fig. 1) [9]. Oliver also microdissected individual nephrons from diseased kidneys. Of note, he was not convinced that tubular atrophy and glomerular atrophy were invariably associated. He identified some aglomerular tubules in his microdissec-

**Contribution of tubular injury to loss of renal function**

When we see a damaged glomerulus attached to an atrophic tubule, has glomerular injury caused tubular injury, has tubular injury caused glomerular injury, or both? The answer might be different for individual nephrons as well as for individual patients. In today’s patient, a biopsy was first performed more than 10 years after renal disease was detected. We cannot exclude the possibility that focal glomerulosclerosis developed in response to an antecedent tubular or interstitial injury, but we do have strong evidence that once established, proteinuric glomerular injury can cause tubular injury [10–16]. The question then becomes, to what extent does tubular injury contribute to loss of nephron function? In some nephrons, the tubule might atrophy only when glomerular injury lowers the single-nephron GFR. In others, proteinuric glomerular injury could cause tubular injury by means other than reduction in filtration, and this secondary tubular injury then might cause loss of function while the glomerulus is still perfused and part of its surface remains available for filtration. To the extent that this is the case, treatment to limit tubular injury could slow the loss of renal function even when glomerular injury cannot be prevented.

Early studies that analyzed renal biopsy specimens are often said to have established that tubular and interstitial injury are the major contributors to loss of renal function, even in primary glomerular diseases [17–19]. These studies found that renal function is better correlated with scores for tubular atrophy and interstitial expansion than with scores for glomerular injury. Their results have stimulated a great deal of valuable work. But the correlations of function and structure originally reported did not in fact provide conclusive evidence that tubular injury is more often the proximate cause of nephron loss than is glomerular injury. One problem has been unequal sampling of glomeruli and tubules. Glomerular injury has been scored by examination of as few as 10 profiles, while tubular injury has usually been assessed by examination of a larger sample. A potentially greater problem has been posed by the semiquantitative methods used to assess glomerular injury. The structural features on which injury scores have been based might not accurately reflect filtration capacity. A particular problem is that glomerular size and capillary surface area usually have not been measured. When more detailed morphometric measurements have been made, GFR has been correlated with changes in glomerular structure [20, 21]. Overall, it
Fig. 1. Individual nephron units in the kidney of a patient dying with “hemorrhagic Bright’s disease.” The patient was a physician who provided Addis and Oliver (case xv [10]) with a detailed account of his 11-year course of nephritis attributed to streptococcal infection. Progressive decline of renal function to ~10% of normal over the last 6 years was documented with Addis’ then new urea ratio test. The upper panel provides a general view of the renal cortex showing the situation of a hypertrophic nephron (A) and an atrophic nephron (B) that were selected for further analysis (×28). Serial sections were made through these selected areas at 5 μ intervals and wax reconstructions of the individual nephrons were prepared from the serial sections as illustrated by the figures in the lower panel. The micrograph labeled (C) is from the section passing through the center of the atrophic nephron (×100) and (D) is a picture of the wax reconstruction of this nephron. (E) and (F) provide similar illustrations of the hypertrophic nephron. (Reproduced from [9] with permission.)
seems likely that tubular and glomerular structure change together, as originally suggested by Bricker [1] and Gottschalk [2]. Analysis of biopsies has emphasized that tubules can be injured and renal function lost when glomeruli are not globally sclerosed. But further study is required to determine the extent to which tubular injury per se contributes to loss of function.

We could better estimate the extent to which tubular injury causes loss of function if we could identify glomerular structural changes that are caused by tubular injury. The use of serial sectioning to examine glomeruli and tubules of individual nephrons provides one means of doing this. The serial sectioning technique was first applied by Marcussen to analyze structural changes responsible for loss of renal function in rats with nephrotic injury [6]. Remarkably, Marcussen and colleagues found large numbers of shrunken “atubular” glomeruli with open capillary loops but no attached tubules in rats with renal insufficiency caused by cis platinum and by lithium [6–8]. Other glomeruli with similar appearance were attached only to atrophic structures representing the vestiges of normal tubular segments. The reduction in GFR closely correlated with the fraction of glomeruli that were either atubular or attached to vestigial tubules.

We applied the serial section technique to examine disease progression in experimental glomerular disease. An initial study examined renal structure at 10 and 25 weeks after five-sixths renal ablation [22]. As expected, renal ablation induced hypertension and progressive glomerular injury manifested by heavy proteinuria. By 25 weeks, remnant kidney GFR had decreased to approximately 25% of the value seen at 10 weeks. Examination of serial sections revealed that this decrease in renal function was associated with an increase in the percentage of glomeruli no longer connected to normal tubules. At 10 weeks, an average of 20% of glomeruli were atubular or connected only to vestigial tubules, while at 25 weeks this portion had increased to approximately 75%. Of note, the prevalence of glomeruli no longer connected to normal tubules greatly exceeded the prevalence of glomeruli that were globally sclerosed. In a subsequent study that examined glomerular and tubular injury in rats with adriamycin nephrosis, we found that GFR fell to approximately 20% of normal after 16 weeks of nephrosis [23]. The reduction in GFR again closely correlated with the appearance of glomeruli that were atubular or connected only to vestigial tubules. Most recently, we have found that some glomeruli become atubular during even a brief episode of puromycin nephrosis [24].

Limitations in the interpretation of serial sections must be acknowledged. In the models of toxic injury studied by Marcussen and colleagues [6–8], atubular glomeruli were reduced in size but otherwise appeared normal. It could reasonably be presumed that loss of nephron function was due entirely to tubular injury. But this was not the case in our studies of proteinuric glomerular disease. In these studies, many atubular glomeruli also exhibited segmental sclerotic injury [22, 23]. Thus, the extent to which prevention of tubular injury would have preserved function was not clear. Moreover, the finding of atubular glomeruli does not identify the cause of tubular atrophy. Marcussen and coworkers also found atubular glomeruli in ischemic renal injury [7]. It is possibly relevant to the current case that we have observed a subpopulation of glomeruli with reduced volume in rats with renal insufficiency caused by chronic cyclosporine administration [25]. Serial sections were not made, but these glomeruli looked like those found to be atubular in other studies. Thus, overall, the serial section technique has confirmed that glomerular structure does not remain normal as tubules atrophy. It also has provided improved quantitation of the extent of tubular loss. But morphologic criteria for determining the cause of tubular loss have not yet been developed, and we cannot currently tell whether tubular injury is due to ischemia, obstruction, protein leakage from the glomerulus, or other causes.

Finally, I should note that serial sectioning has been carried out mostly in rat models, and we do not know how the appearance of atubular glomeruli might change over the long course of human renal disease. Despite these caveats, serial sectioning might provide an index of the contribution of tubular injury to loss of renal function. In the patient described today, a separate biopsy was embedded in Epon and serially sectioned at 3 micron intervals. A total of 16 glomeruli were examined, of which 4 were globally sclerotic, 2 were atubular, and 4 were connected to vestigial tubules (Fig. 2). Only 6 glomeruli remained connected to normal-appearing tubules. The volume of these 6 glomeruli averaged 8.8 ± 1.7 (SD) \times 10^6 \mu m^3, a value much greater than the average value of 2.6 ± 0.8 \times 10^6 \mu m^3 obtained using the same method in normal subjects [21]. In contrast, the volume of glomeruli that were atubular or connected only to vestigial tubules averaged 2.0 ± 1.0 \times 10^6 \mu m^3, similar to the value of 1.7 ± 0.5 \times 10^6 \mu m^3 obtained in globally sclerotic glomeruli. These results are consistent with the hypothesis that tubular injury contributed significantly to nephron loss in this case while hypertrophy of remnant functioning nephrons limited the reduction in GFR. Clearly, further studies are required to assess the contribution of tubular injury to the progression of human glomerular disease.

**Potential mechanisms of tubular cell injury**

Acknowledging that the extent to which tubular injury contributes to the progression of glomerular disease has not been fully established, we pass on to the question of how tubules are injured. The processes responsible for
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Fig. 2. Glomerular profiles from the patient described in this forum. The glomerulus in (A) retains a connection to a proximal tubule segment that appears normal (arrow). In contrast, the glomerulus in (B) is connected to a markedly atrophic tubular segment (arrow). Tubular atrophy in this case did not appear to be associated with a tuft adhesion. Serial sections revealed that the glomerulus in (C) did not have any tubular connection. This glomerulus has a cystic appearance that is only occasionally seen in atubular glomeruli in experimental studies (toluidine blue, ×180).

Fig. 3. Effect of single-nephron obstruction for five weeks. The distal tubule of a superficial glomerulus in a Munich-Wistar rat was occluded by microinjection of silicon rubber. A remnant of the obstructing “cast,” which shrunk during tissue processing, is visible within a dilated tubule profile (wide arrow). The glomerulus belonging to the obstructed tubule appears collapsed and retains only a vestigial connection to an atrophic proximal tubule (arrowhead). Several profiles of the proximal tubule appear as nests of epithelial cells without visible lumina (thin arrows). A normal glomerulus and proximal tubules are seen to the left of the picture. These findings largely replicate the work of Tanner and Evan [5], except that the obstruction here was in the distal tubule rather than the proximal tubule. (From Pagtalunan, Olson, Meyer, unpublished observations.)

Table 1. Potential mechanisms of tubular injury

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<th>Mechanism</th>
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<td>Obstruction of tubular lumen by casts</td>
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<td>Obliteration of tubular neck by glomerular tuft adhesions</td>
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<td>Effects of filtered macromolecules on tubular cells</td>
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<td>- Actions of proteins with specific tubular receptors (growth factors, etc.)</td>
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<td>- Results of “excessive” endocytosis of filtered plasma proteins</td>
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<td>- Toxic effects of molecules bound to plasma proteins (lipids, etc.)</td>
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tubular injury of course might differ in different cases. At present, we can only compile a list of potential mechanisms of injury (Table 1). One simple but often-neglected mechanism is luminal obstruction. Dilated tubular segments with flattened epithelial cells and lumina filled with protein casts are commonly seen in diseases characterized by heavy proteinuria, especially in animal models. The potential contribution of cast formation to tubulointerstitial disease was pointed out by Bertani et al. in their original description of chronic adriamycin nephrosis [26, 27]. That luminal obstruction causes atubular atrophy was elegantly demonstrated by Tanner and Evan [5, 28], who showed that chronic obstruction of single proximal tubules led to atrophy of both upstream and downstream segments. The latter result suggests that tubules atrophy when delivery of filtrate is reduced for any reason. Glomeruli of obstructed tubules eventually shrink and appear collapsed. Indeed, as Figure 3 illustrates, such glomeruli have the same appearance as the atubular glomeruli seen by Marcussen and coworkers in models of toxic tubular injury [6–8]. The extent to which cast formation causes tubular loss in glomerular disease will become clear only when cast formation can be prevented. So far, this has not been accomplished. We recently tried to limit cast formation in rats with adriamycin nephrosis by decreasing urine concentration with a vasopressin V2 receptor blocker [23]. This maneuver had no effect on cast formation or any other aspect of renal injury, however.

Kriz and colleagues described another mechanism by which glomerular injury can cause tubular loss before glomeruli are completely sclerosed [29, 30]. They examined the relation of tubular and glomerular structure
in experimental models of heavy proteinuria in which podocyte injury induced adhesion of the tuft to Bowman's capsule. Adhesion formation was followed by accumulation of amorphous material in a paraglomerular space that sometimes extended along the capsule to surround the neck of the tuft. Tracer studies indicated that the amorphous material included plasma proteins delivered to the paraglomerular space by “misdirected” filtration from glomerular capillary loops adherent to the capsule [31]. Tubular degeneration was observed where the material extended down the tubule, separating the tubular cells from the underlying basement membrane. Presumably, infiltration of the amorphous material disrupted cell-membrane connections essential for maintenance of normal cell structure [32].

Kriz et al initially described the extension of adhesions as causing tubular loss in Fawn hooded and Milan normotensive rats, but also identified this process in other rodent models [29–31]. We recently observed this form of injury in adriamycin nephrosis [23]. Small tuft-to-capsule adhesions formed early in the course of nephrosis. Subsequently, the adhesions enlarged and amorphous material spread circumferentially along the capsule beneath a layer of fibroblast-like cells. Tubular atrophy leading to obliteration of the glomerular-tubular connection was present where the amorphous material reached the tubular neck. One difference between our results and those of Kriz et al [29, 30] was that Bowman’s space was usually not enlarged around glomeruli that had their connections to tubules severed by spreading adhesions. Enlargement of Bowman’s space also has not been observed in studies of tubular injury induced by nephrotoxins or luminal obstruction [5, 7, 8]. Thus, the appearance of cystic glomeruli in routine histologic sections probably does not provide a reliable index of the extent of tubular loss. Adhesions between the tuft and capsule also can be hard to detect on routine sections of renal tissue that has not been perfusion fixed. When carefully looked for, however, adhesions are observed with remarkable frequency in animal models of nephrosis. Using the serial section technique, Rasch et al identified adhesions in more than 90% of glomeruli following recovery from a single episode of acute puromycin nephrosis [24]. Whether such adhesions expand to cause nephron loss in the absence of ongoing podocyte injury remains to be determined. The contribution of adhesions to nephron loss in human glomerular disease also requires further study. Lesions similar to those observed in Fawn hooded and Milan rats have been described in a few humans [30]. However, serial sections did not show that tuft-to-capsule adhesion and subcapsular accumulation of paraglomerular material caused tubular loss in the patient we are discussing today. Of course we need to examine other cases, but this apparent discrepancy brings up a general problem. The mechanisms of tubular injury that predominate in commonly studied rodent models of glomerular disease might not be the same as those operating in more slowly progressive human disease.

Currently, the most widely proposed cause of tubular injury in glomerular disease is excessive tubular uptake of filtered proteins or protein-bound substances [10–16]. According to this hypothesis, injury begins in the proximal tubule, where filtered proteins are endocytosed. Injury then extends downstream and is multiplied by secondary processes of inflammation and fibrosis. Studies showing that the rate of disease progression correlates with the amount of proteinuria and that maneuvers that reduce proteinuria also retard progression are often cited in support of the hypothesis that filtered proteins damage tubules. But these findings could be equally well accounted for by other proposed mechanisms of injury, such as tubular obstruction by protein casts or the tendency of glomeruli that leak protein to become sclerotic or form adhesions. The hypothesis that filtered proteins injure tubular cells has received more direct support from experiments showing that plasma proteins induce tubular cell changes in vitro [15, 16, 33–36]. Protein-induced changes have included activation of common stress response systems, elaboration of chemokines, and apoptosis. Cell culture experiments are necessarily of short duration, and the protein concentrations used to induce tubular cell changes have been high in some cases.

However, many of the same changes seen in proximal tubule cells exposed to plasma proteins in vitro also have been observed in proximal tubules of nephrotic animals and humans [37–40]. For example, increased expression of monocyte chemoattractant protein-1 (MCP-1) has repeatedly been demonstrated in both settings [34, 37, 39, 40]. An increased rate of apoptosis also has been observed in proximal tubules of nephrotic animals and in tubular cells exposed to plasma proteins in vitro [35, 38]. Studies are accumulating rapidly in this area, and their results are difficult to summarize in a brief review. In general, however, the in vitro results are consistent with, although they cannot prove, the hypothesis that filtered proteins cause tubular injury in glomerular disease.

One advantage of the in vitro studies is that they might distinguish which filtered macromolecules are most injurious to tubular cells. Although this question remains unsettled, some studies have indicated that albumin, while quantitatively the most important filtered protein, is not the most injurious. In vivo and in vitro evidence suggests that filtered complement is particularly injurious [41]. A further intriguing possibility is that a protein-bound substance, rather than a filtered protein itself, causes injury. Schreiner’s group originally suggested that an inflammatory lipid is produced when proximal tubule cells metabolize fatty acids carried by filtered albumin [13]. Harris et al initially suggested that uptake of iron carried by filtered transferrin causes oxidative injury to
tubular cells [42]. However, the subsequent finding by the same group that iron depletion does not limit tubular injury associated with proteinuria in the remnant kidney model stands against their original hypothesis [43]. Other substances, however, are actively under investigation. Interest has focused largely on proteins and protein-bound substances, but tubular injury also might be caused by glomerular leakage of red cells and the resultant exposure of tubular cells to hemoglobin [44]. Hemoglobin, like other heme-containing proteins, is a potent cause of tubular cell injury [45]. Nath et al showed that sustained exposure of tubules to hemoglobin causes tubulointerstitial injury similar to that seen in nephrotic models [46], and similar injury is seen in some patients with hemolytic disease [46]. Addis and Oliver routinely quantified excretion of red cells and casts as well as protein in patients with chronic glomerular disease. In the patient illustrated in Figure 1, for example, they documented excretion of more than 7 million red cells/day and 2 million casts/day as well as 7 g of protein/day [10]. It is possible that tubular obstruction and hematuria would be more often considered as contributors to progression if such measurements still were made.

The mechanism by which uptake of heme proteins causes cell injury has been extensively investigated [45]. It is not so clear how other substances that pass from diseased glomeruli into the tubular lumen can injure tubular cells. Signaling receptors for specific bioactive proteins on the apical surface of some tubular segments might be activated when increased amounts of proteins such as IGF-1, HGF, or TGF-β are filtered from the circulation or produced by damaged glomeruli [47]. In vitro experiments have shown that activation of receptors for HGF and TGF-β stimulates tubular cell production of inflammatory chemokines. However, signaling receptors do not exist on tubular cells for most filtered proteins. Normally, the tubule takes up only small amounts of these proteins. But in vitro studies have shown that tubular cells exposed to large amounts of these proteins exhibit potentially injurious changes. One logical explanation of these findings is that “excessive” uptake of proteins somehow causes cell injury.

Tubular uptake of most proteins and protein-bound substances is accomplished by endocytosis [48]. The endocytic apparatus is most prominent in the proximal tubule, and studies of the hypothesis that excessive protein uptake causes injury have therefore focused on this segment. Endocytic function in normal proximal tubules has been beautifully described. Tracer studies have revealed that filtered proteins bind to the membrane of the coated invaginations, which then pinch off to form endocytic vesicles [48]. The endocytosed proteins remain within the endocytic vesicles as they coalesce into larger endosomes and then fuse with primary lysosomes to form secondary lysosomes. Meanwhile, the majority of the endocytosed membrane buds off the endosomes in long tubular structures [49, 50]. These dense apical tubules separate from the endosomes and recycle the membrane back to the apical surface of the cell. Kinetic studies have shown that the recycling process operates with remarkable efficiency and rapidity in the normal tubule. More than 95% of the endocytosed membrane is returned to the apical surface over a period of less than two minutes [51]. The time required to degrade endocytosed proteins, however, is longer and varies depending on protein structure [52]. It is tempting to speculate that accumulation of proteins that are difficult to degrade causes cell injury, but this remains to be tested.

Hopefully, maneuvers for blocking proximal tubule endocytosis will soon be devised. This would allow us to directly test the hypothesis that protein endocytosis causes tubular injury in glomerular disease and also potentially provide us with a means for preventing such injury. Recent studies have identified important molecular mechanisms of proximal tubule endocytosis that are potential targets for therapeutic manipulation. The first step in endocytosis is receptor binding. Remarkably, only two receptors bind the whole variety of substances now known to be taken up by endocytosis from the glomerular filtrate [53]. One of these is megalin, a member of the LDL receptor family. Most filtered proteins are endocytosed following binding to megalin alone. A smaller number must bind first to cubilin, which was initially identified as the receptor for B12-intrinsic factor complexes in the ileum but subsequently found to be an endocytic receptor in the proximal tubule. In the case of these proteins, which include albumin, the protein-cubilin complex interacts with megalin to initiate endocytosis.

A subsequent step at which endocytosis might prove subject to manipulation is endosome acidification. This process was initially shown to depend on the activity of the vacuolar H+-ATPase [54]. The required parallel ion conductivity is provided by the chloride channel CIC-5, which co-localizes with H+-ATPase in endosomes of the proximal tubule [55, 56]. The CIC-5 gene was initially identified as the locus of mutations in patients with Dent’s disease, an X-linked hereditary disorder characterized by low-molecular-weight proteinuria and also by variable hypercalciuria and nephrolithiasis [55, 57]. Recent in vitro studies suggest that both the Na+-H+ exchanger NHE3 and the vacuolar H+-ATPase participate in endocytosis. Blockade of the NHE3 prevents albumin uptake by cultured proximal tubule cells, and exposure of these cells to albumin stimulates NHE3 activity (abstract, Drumm, J Am Soc Nephrol 12:48A, 2001) [58]. Why Na+-H+ exchange as well as H+ pump activity should be required for endocytosis is not understood, but blockade of either process prevents protein endocytosis by
The potential mechanisms of tubular cell injury described here (Table 1) are supported by experimental studies. With the exception of the spread of tuft adhesions, however, their effects cannot be distinguished by morphologic examination. Moreover, we cannot block any of these mechanisms, so we cannot present assess their contribution to disease progression.

**Role of inflammation in tubular injury**

An alternate potential means of slowing progression is limiting the interstitial changes that regularly accompany tubular injury. One common feature of interstitial injury is cellular inflammation [14, 40, 59, 60]. Extensive studies have found that the inflammatory infiltrate consists of T-cells and macrophages both in human and experimental disease. Reviewers of proteinuric renal disease have regularly proposed that this infiltrate is a target for therapeutic intervention. Of course it would be best to prevent the initial glomerular injury, but failing that, it would be desirable to keep the tubules from being injured by filtered proteins, either by preventing obstruction, by limiting endocytosis, or by some other means. If this cannot be accomplished, the next best alternative might be to limit the inflammation induced by tubular injury.

The suggestion that limiting inflammation is desirable presupposes that inflammation is deleterious. At first glance this supposition might seem so obvious as to be not worth testing. Experimentally, inflammation within the kidney is most often induced by stimulating the immune system to attack some portion of the nephron. It is not surprising that in such cases the net effect of inflammation is to damage the target. But interstitial inflammation in proteinuric renal disease presents a different problem. Most studies suggest that in this setting, injured tubular cells induce inflammation by secreting chemokines and other factors, and not primarily by antigen presentation. The inflammatory infiltrate is thus not thought to be directed against a specific tubular target. Infiltrating T-cells and macrophages do have the ability to cause tubular injury, and macrophages in particular produce profibrotic substances [61]. But infiltrating cells do not always injure, and indeed might help repair, the tissues in which they accumulate [59, 62, 63]. Without experimental evidence, it is not clear that the interstitial infiltrates observed in proteinuric renal disease accelerate nephron loss.

Some experimental studies have found that anti-inflammatory drugs can limit interstitial injury and preserve renal function even when they do not reduce proteinuria [64]. In general, however, a lack of effective maneuvers to chronically suppress renal interstitial inflammation has impeded study of this issue. Results obtained with glucocorticoids and various dietary regimens have been difficult to interpret, in part because these maneuvers can alter glomerular function [64–67]. Promising results have recently been obtained in the remnant kidney and other disease models with mycophenolate mofetil [68]. It is not clear, however, that the beneficial effects of this agent are due primarily to suppression of inflammation. Genetically altered animals have provided an important new means for us to study the contribution of inflammation to renal dysfunction [63, 69]. A study of the effect of ureteral obstruction in severe combined immunodeficient (SCID) mice lacking mature T-cells provided dramatic evidence that interstitial inflammatory cells do not always accelerate injury [69]. As expected, ureteral obstruction in normal mice caused tubular injury accompanied by an interstitial infiltrate composed of T-cells and macrophages and by interstitial fibrosis. Following obstruction in SCID mice, no T-cells accumulated in the interstitium, but tubular injury, macrophage infiltration, and interstitial fibrosis proceeded unchecked. Pharmaceutical agents that block specific molecular mediators of cellular inflammation are also being developed rapidly [62]. An example of success with one of these agents has been provided by another study of ureteral obstruction in mice, which found that blockade of the chemokine receptor CCR-1 prevented infiltration of T-cells and of macrophages and also limited interstitial fibrosis in obstructed kidneys [70]. Hopefully, experiments with these new tools will clarify the role of inflammation in the progression of proteinuric glomerular disease.

**Role of interstitial fibrosis**

Along with inflammation, tubular injury in primary glomerular disease is regularly accompanied by interstitial fibrosis. The possibility that progressive fibrosis causes tubular loss has prompted widespread interest [14–16, 70, 71]. This interest has been stimulated by morphologic studies showing that the extent of interstitial fibrosis closely correlates with the degree of renal dysfunction. Again, however, as with inflammation, it is important to distinguish between association and causation (Fig. 4). Mechanisms by which fibrosis could contribute to tubular loss are easy to imagine. Fibrosis induced by tubular injury could cause constriction and occlusion of tubules which, despite injury, would otherwise continue to function. Spreading fibrosis also could lead to destruction of adjacent nephrons, either by constricting tubules or by depriving them of an adequate capillary blood supply. Addis and Oliver remarked on the potential operation of these processes, and believed that “inflammatory scarring” of the interstitium was a major cause of progressive renal dysfunction in chronic glomerulonephritis [10]. But 70 years later, we have little firm evidence of the extent to which scarring causes tubular loss. Morphologic studies have not shown convincingly
Use of the catch-all term “tubulointerstitial” to describe all changes outside the glomerulus and arterial tree reveals how little we know. Structural features associated with specific causes of nephron loss have in general not been identified. In this review, I have assumed that tubular cell injury precedes interstitial inflammation and fibrosis, but even this simple assumption might not be justified. Glomerular injury could alternatively be transmitted directly to the interstitium by the spread of inflammatory mediators through peritubular capillaries or by destruction of these capillaries [78]. Experiments employing new means of blocking tubular cell injury, inflammation, and fibrosis are eagerly awaited. These experiments should clarify the mechanisms of nephron loss and provide a basis for improved treatment to slow disease progression.

QUESTIONS AND ANSWERS

DR. NICOLAOS E. MADIAS (Executive Academic Dean, Tufts University School of Medicine, Boston, Massachusetts): Although the data you presented on serial sectioning are very convincing regarding the presence of atubular glomeruli, I wonder whether validation has also been provided by microdissection studies in experimental studies or human kidneys. Also, is there any information on the residual blood flow of these atubular glomeruli?

DR. MEYER: The question is whether there’s been proof by microdissection that there are no tubules attached to glomeruli that appear atubular in serial sections. Nobody would do the work anymore of microdissecting! And if, like Oliver, you did, you would have trouble proving that the tubules had not “broken off” from your atubular glomeruli. But I think the serial sections are spaced closely enough to convincingly establish that many glomeruli are atubular.

The second question is, how much blood flow is still going through these atubular glomeruli? Drs. Tanner and Evan showed that glomerular blood flow was reduced but present after single-nephron obstruction [5]. Blood flow has not been measured in other models, but you see red cells in the capillaries when tissue containing atubular glomeruli is not perfusion fixed. An interesting question is what happens over time. That is, do glomeruli remain perfused but atubular indefinitely?

DR. MADIAS: Is there any information on the frequency of atubular glomeruli in experimental models or clinical material of predominantly tubulointerstitial diseases as compared with glomerular/vascular diseases?

DR. MEYER: Relatively few studies have been done. Dr. Marcussen and his coworkers found atubular glomeruli in rats with cis-platinum and lithium nephrotoxicity [7, 8] and in autopsy tissue from patients with diabetic nephropathy, chronic pyelonephritis, and renal artery
steno\-sis [6]. We have found atubular glomeruli in rats with remnant kidneys and with adriamycin nephrosis [22, 23]. So atubular glomeruli appear both in “tubulointerstitial” and “glomerular” diseases. In the rat models, the prevalence of atubular glomeruli is proportional to the reduction in GFR. We have much less information about the prevalence of atubular glomeruli in human disease. And I should again emphasize that we cannot at present tell by looking at an atubular glomerulus what process caused loss of the tubule.

Dr. Madias: You indicated that atubular glomeruli have been described in lithium- and platinum-induced nephrotoxicity. What about acute postischemic renal failure?

Dr. Meyer: We have found large numbers of atubular glomeruli in rats recovering from acute ischemic renal failure [79, 80]. Presumably, some of the most severely damaged tubules do not recover, although the whole-kidney GFR returns nearly to normal. Whether glomeruli become atubular in acute renal failure in humans is not known.

Dr. John T. Harrington (Dean, Tufts University School of Medicine): I have a question about uremic cose, and bicarbonate is enormous. The amount of protein could cause tubular injury. Has there been any studies on human tissue. These studies would quantify whether these enzymes simply leaked following cell damage or whether they caused the damage. The idea that they leak from overloaded lysosomes into the cytoplasm and cause damage, suggested by Thomas Maack [81], is appealing but unproven. Another suggestion is that the metabolic work of protein reabsorption could prove excessive. I think that’s less likely. As you know, the amount of energy required to absorb sodium, glucose, and bicarbonate is enormous. The amount of protein that would be reabsorbed, even in a nephrotic patient, is comparatively modest. Unless protein reabsorption somehow consumes energy in a very extravagant way, it’s hard to imagine that it causes a cellular energy deficit. But clearly we need to learn more about the effects of increased reabsorption of filtered proteins on tubular cells.

Dr. Madias: Of the many proteins that are being filtered, do we know which ones might be injurious to the tubulointerstitium?

Dr. Meyer: Studies in cultured proximal tubule cells provide a potential means for identifying proteins that are particularly toxic. Practical limitations of these studies are that high protein concentrations can be required to produce injury and that experiments are generally of limited duration, so that the tubular cell response to proteins is assessed over hours or days and not weeks or months. Results obtained to date have been conflicting. For instance, conflicting results have been obtained as to whether transferrin is more toxic when iron is attached and whether albumin is less toxic when delipidated. It’s safe to say that at present, we haven’t clearly identified culprit proteins.

Dr. Harrington: It seems to me that we’re stuck. Does the glomerulus or the tubule drop out first? What kinds of studies would you like to do in your experimental models to answer that crucial question?

Dr. Meyer: I would first do additional serial section studies on human tissue. These studies would quantify the extent to which tubules atrophy in human proteinuric renal disease before glomeruli become globally sclerosed. This would be confirmatory and useful—you’d
find out to what extent tubules are gone when glomerular capillary surface still was available for filtration. You’d also find out to what extent tubular loss is related to occlusion—choking—of the tubular neck by material extending from tuft adhesions. I think further morphologic studies in animal models might reveal differences between tubular injury caused by obstruction, ischemia, and endocytosis of filtered substances. In short, if we looked harder at the tissue, we might get a better idea of what is destroying the tubules. But what we are really waiting for is an experimental means of interrupting the putative processes of tubular injury. For example, if endocytosis of filtered proteins causes injury, blocking endocytosis should slow tubular injury. The endocytic receptor protein megalin has been knocked out, but unfortunately the knockout mice have brain defects and grow poorly. There are dogs that do not express cubilin in the brush border and therefore do not reabsorb filtered albumin, and I expect that somebody will study renal disease progression in these animals. From such studies, we’re eventually going to find out which mechanisms contribute most to tubular injury.

DR. HARRINGTON: This patient was treated with cyclosporine on several occasions. Was this done out of desperation because we don’t have anything better? Are there well-controlled trials of cyclosporine in patients with FSGS?

DR. MEYER: The patient was diagnosed as having focal and segmental glomerulosclerosis which, as you know, is not a disease but a morphologic description. Several studies have suggested that in FSGS, cyclosporine lowers proteinuria and can slow loss of renal function. Unfortunately, proteinuria often decreases during treatment only to rebound after cyclosporine is stopped, as it initially did in the present case. An interesting question is, which cases of focal glomerular sclerosis will respond to cyclosporine? The patient we are discussing had a somewhat unusual history, in that protein was detected in his urine for more than a decade before it increased to “nephrotic” levels. I don’t think the published studies tell us whether we would expect a beneficial response to cyclosporine with this clinical history.

Another interesting question that I didn’t address is whether cyclosporine itself caused some of the tubular injury present in the second biopsy. That is, did cyclosporine reduce the arteriolar supply to some nephrons, and did these nephrons then suffer glomerular shrinkage and tubular atrophy? Again, when more than one process is active, we usually cannot tell which one caused nephron loss.

DR. HARRINGTON: This gentleman had disease for 17 years. Could you reverse his disease that many years later? That seems bizarre to me.

DR. MEYER: I think the physicians treating this patient were not hoping to reverse his disease but to reduce the proteinuria and slow disease progression. Solid evidence indicates that the magnitude of proteinuria and the rate of disease progression are related. As long as this patient only had $1^+$ or $2^+$ protein on dipstick, he received only antihypertensive medication, including ACE inhibitors. When the protein excretion rose to nephrotic levels, the treating physicians felt compelled to try further measures to limit it. There is evidence in animal models that reversal of some features of glomerular segmental sclerosis is possible [82]. But it seems very unlikely that function is ever restored in atubular glomeruli.

DR. BERTRAND L. JABER (Division of Nephrology, New England Medical Center): What is the role of apoptotic stimuli in the progression of renal disease following tubular injury by protein trafficking, and specifically, the role of the Fas/Fas ligand system in mediating these events?

DR. MEYER: Apoptosis is increased in tubular cells exposed to protein in vitro and in proximal tubules of proteinuric animals. Both mitosis and apoptosis are increased in proximal tubules when heavy proteinuria is present, that is, cell turnover is increased. I cannot comment specifically on the role of the Fas/Fas ligand system in these events. But a more general question is, can you reduce injury by interfering with apoptosis? It seems possible that apoptosis is triggered only when the cells are severely injured, and that attempts to manipulate apoptotic signals thus might not preserve tubules.

DR. DAVID CAHAN (Faulkner Hospital, Boston, Massachusetts): Do you think it’s likely that this man had primary tubular disease 20 years ago and subsequently developed heavy proteinuria that we attribute to primary glomerular disease?

DR. MEYER: It certainly seems possible. But I should point out that the first biopsy obtained in this patient did not show widespread tubular injury. So the putative initial tubular injury, if it occurred, was presumably focal.

DR. CAHAN: I used to believe that if someone had heavy proteinuria and had been taking lithium, we should consider diseases other than lithium nephropathy. The data out now indicate that people with chronic lithium nephropathy can develop heavy proteinuria. This might be another example of the evolution of tubular disease.

DR. MEYER: There are also cases, as you know, of pyelonephritis due to tuberculosis, of reflux nephropathy, and of NSAID-related nephropathy in which heavy proteinuria and focal sclerosis occur. In rats, most forms of tubular injury cause secondary focal sclerosis, including injury induced by acute radiation exposure and acute ischemia. I think we have to be somewhat careful with the human lithium and NSAID cases, because it’s guilt by association. That is, no morphologic findings have established that the original injury was initiated by these commonly used drugs. In today’s patient, it’s intriguing to wonder whether his renal disease was initiated by a
Dr. Meyer: as early recurrence after transplantation, for example, remains perfused? Dr. Khan: what causes the cases of focal sclerosis that don’t start return become atubular [79, 80]. some kind of infection. The question can be expressed, ies suggest that the nephrons in which function does not reflux in infancy that we are not aware of, or even by not return in some nephrons [86]. Our morphologic studies suggest that the nephrons in which function does not return become atubular [79, 80].

Dr. Khan: Can tubules regenerate if the glomerulus remains perfused? Dr. Meyer: My guess is that function is lost forever in the glomeruli we identify as atubular, and that their tubules can’t grow back. In these glomeruli, I think the scaffold for tubular regeneration is gone. That is, the connection of the capsular basement membrane to the tubular basement membrane is broken, and the shrunken glomerulus is sealed off by fibrosis.

Dr. Madias: This is bad news for the concept of regression of glomerulosclerosis.

Dr. Meyer: When proteinuria remits, people are seeing some evidence of reduction in the amount of interstitial expansion and some reduction in the extent of glomerular segmental scarring. Presumably, the regression of glomerular changes could improve function in glomeruli that are still connected to tubules. Regression of interstitial changes might be associated with regeneration of epithelium in atrophic tubules, but I think this remains to be demonstrated.

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