The Renal Tubule in the Progression of Chronic Renal Failure: Not an Innocent Bystander

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Introduction

The development of progressive renal dysfunction even after the apparent resolution of an injurious condition is observed in a large number of renal diseases. A great deal of research has focused on changes in glomerular structure and function that occur in the setting of reduced renal mass as being primarily responsible for perpetuating renal disease progression. In particular, increased glomerular capillary hydraulic pressure and glomerular hypertrophy have both been linked to the development of glomerular sclerosis and proteinuria. While considerable attention continues to be focused on the role of the glomerulus, increasing attention is now being paid to the role of the tubulointerstitium in mediating progressive renal failure. It has been known for quite some time that the impairment in renal function in primary glomerular diseases correlates better with the extent of tubulointerstitial damage than with the degree of glomerular damage. Just how progressive tubulointerstitial injury evolves when the initial injury is targeted to the glomerulus is still unknown. What has become clear however is that tubulointerstitial disease can no longer be viewed solely as an ischemic sequela of glomerular sclerosis. Rather, the development of tubulointerstitial disease in association with primary glomerular injury is the result of a complex process involving interactions between the renal tubular cell, infiltrating inflammatory cells, and a variety of cytokines.

The Tubulointerstitium as a Predictor of Renal Function and Outcome

The renal tubules and vasculature comprise greater than 80% of the renal cortex. As a result, it is not surprising that the extent of tubulointerstitial disease is highly correlated with both renal function and disease progression. One of the earliest reports to comment on the relationship between tubulointerstitial disease and renal function involved 50 patients with persistent glomerulonephritis. In this report, the degree of tubular atrophy was demonstrated to be a better predictor of the serum creatinine concentration and creatinine clearance than were histologic changes in the glomerulus (1). Similar findings were reported in a subsequent study of 70 patients with variety of renal diagnosis (2). The severity of tubulointerstitial disease was inversely correlated to the glomerular filtration rate as measured by inulin clearance while the correlation between renal function and glomerular pathology was poor. More recently, these observations have been confirmed in an analysis of

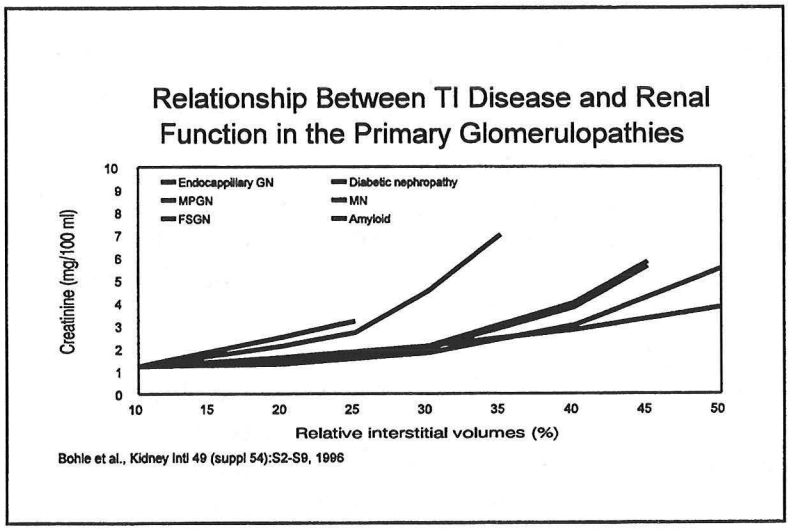

Figure 1

over 2000 renal biopsy cases that included patients with glomerulonephritis, diabetic nephropathy, and amyloidosis (3) (Figure 1). In this study, there was a significantly positive correlation between the relative volume of the cortical interstitium and the serum creatinine concentration. In patients with diabetic glomerulosclerosis and renal amyloidosis, a negative correlation was noted between the relative cortical capillary volume and the serum creatinine concentration. In those cases in which disease was confined solely to the glomerulus, no matter how severe, the serum creatinine concentration tended to be normal. By contrast, even mild disease of the glomerulus was accompanied by an elevated serum creatinine concentration if interstitial fibrosis was present.

The degree of tubulointerstitial disease has also been found to be correlated with the eventual development of end stage renal disease. Such a correlation has been demonstrated in a variety of renal diseases to include membranoproliferative glomerulonephritis type 1 (MPGN) (4), membranous glomerulopathy (5), focal and segmental glomerulosclerosis (FSGS) (6), and IgA nephropathy (7). Even in diabetic patients with varying degrees of glomerulosclerosis, the 5 and 10 year renal survival rates are highest for those patients in whom the renal tubules and renal cortical interstitium are of normal appearance (8). The importance of tubulointerstitial disease in predicting renal outcome was recently confirmed in a retrospective analysis of the most recent literature examining patients with IgA nephropathy, membranous glomerulopathy, and MPGN (Figure 2). In this review, cellular infiltration and fibrosis of the tubulointerstitium and not glomerular damage emerged as the histologic factor that most strongly and consistently predicted poor outcome in all three glomerular diseases (9).

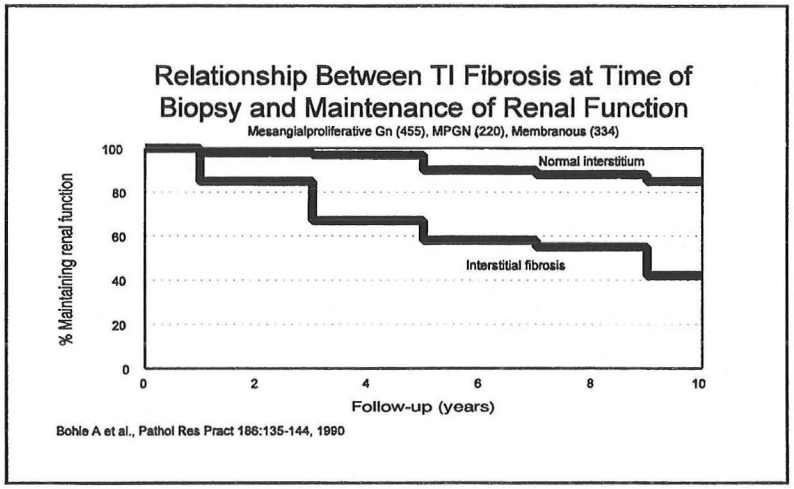

Figure 2

Studies in experimental animals have also shown that progressive glomerulosclerosis and renal insufficiency are seldom observed in the absence of tubulointerstitial scarring. The anti-thy 1 model of glomerulonephritis is produced by administering an antibody directed against an antigen on the surface of mesangial cells within the glomerulus. Depending on the protocol used, infusion of the antibody results in mesangial cell lysis which is followed by proliferation and eventual glomerulosclerosis. In this model, the development of glomerulosclerosis is reversible in the absence of tubulointerstitial disease but is progressive when lesions are present (10,11). Similar, observations have been made in experimental models of the nephrotic syndrome (12,13). In the remnant kidney model a great deal of attention has focused on the role of glomerular

hemodynamic changes and glomerular hypertrophy in mediating the development of glomeruloscelrosis. However, even in this model, tubulointerstitial changes, including interstitial infiltration, edema and fibrosis, often proceed and exceed glomerular scarring. These observations support the idea that the severity of tubulointerstitial injury is the major determinant as to whether an insult to the kidney will result in progression to end-stage-renal disease or will culminate in the reestablishment of normal kidney function.

The mechanism by which tubulointerstitial lesions develop in the setting of glomerular disease is not entirely clear. Traditionally, tubulointerstitial lesions were simply viewed as the inevitable sequelae of glomerulosclerosis. According to this view, vascular sclerosis and diminished blood flow through a contracted glomerular capillary bed would result in ischemic-induced tubular atrophy and development of interstitial fibrosis. However, as detailed in the remainder of this paper, a number of observations suggest that tubulointerstitial disease is much more than just the ischemic sequelae of glomerular demise.

One such observation is the frequent presence of chronic inflammatory cells in the interstitium during the development of tubulointerstitial scarring. This infiltration of leukocytes into the interstitium is characteristic of a wide variety of renal diseases including not only proliferative immune-mediated glomerular diseases (lupus nephritis, acute postinfectious glomerulonephritis, and IgA nephropathy) but also nonproliferative glomerular diseases such a membranous glomerulopathy, FSGS, and diabetic nephropathy (6,8,14-18) (Figure 3). The cellular infiltrate is usually dominated by T lymphocytes but monocytes are also increased in number. In general, there are equal numbers of CD4 and CD8 (+) cells although there are cases in which one cell type may predominate. These cells have been shown to express surface markers indicative of activation such as HLA class II antigens, intercellular adhesion molecule-1 (ICAM-1), and the interleukin-2 receptor. A similar influx of chronic inflammatory cells has been observed in a diverse array of experimental models of renal injury to include the nephrotic syndrome (12,13), renal ablation (19), ureteral obstruction (20), and chronic infusion of angiotensin II (21).

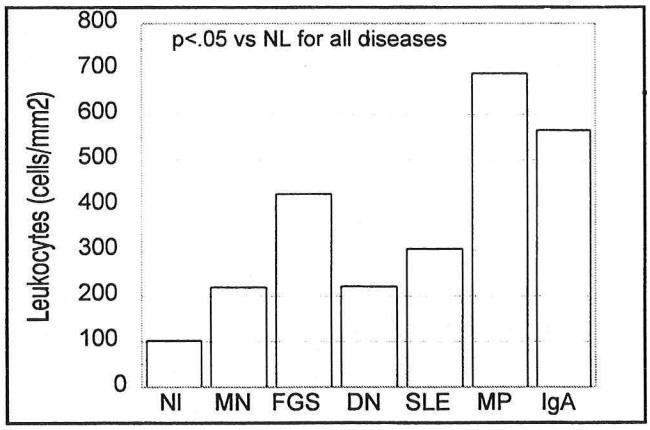

Figure 3. Interstitial inflammatory cells in proliferative and nonproliferative glomerular disease (KI 31:964,1987).

The magnitude of the interstitial cellular infiltrates has been shown to be inversely related to renal function and directly related to eventual renal outcome (14,16). In a recent study of patients with IgA nephropathy, the mean number of infiltrating T lymphocytes and monocytes was found to be positively correlated with the serum creatinine concentration at the time of renal

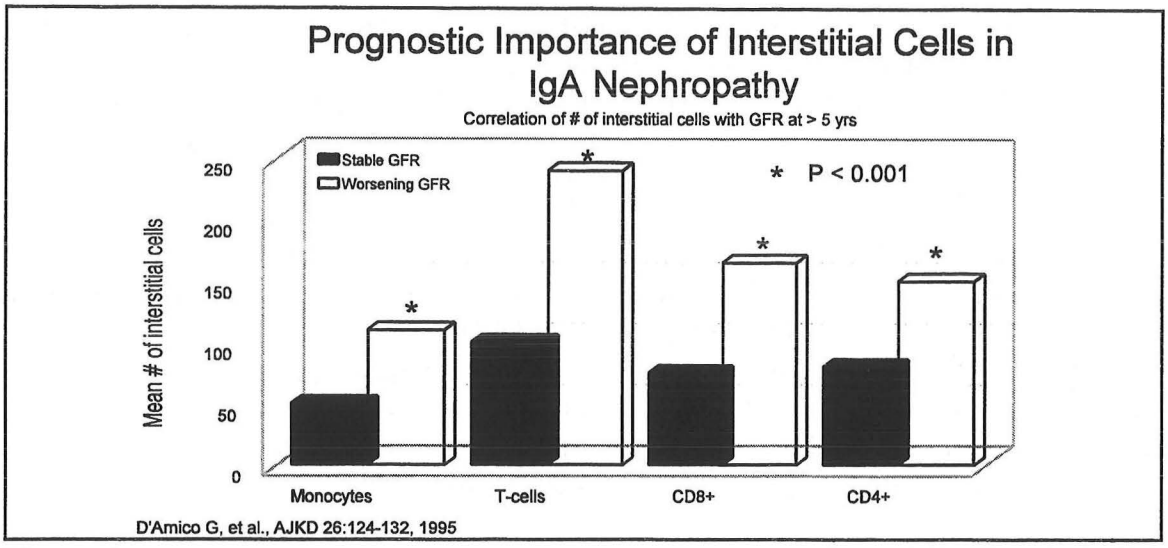

Figure 4

biopsy (14). Those patients with the greatest cellular infiltrate went on to show a progressive deterioration in renal function no matter what their serum creatinine levels were at the time of the biopsy (Figure 4). This same significant correlation between renal outcome and the number of interstitial leukocytes has been reported in patients with membranous glomerulopathy (22) and lupus nephritis (23).

The Role of the Renal Tubular Cell in Tubulointerstitial Injury

The influx of activated leukocytes into the renal interstitum is likely to be in response to an injury to some component of the tubulointerstitium. Increasing evidence suggests that renal tubular cells are not just an innocent bystander in this process but rather play an active role in the recruitment of these inflammatory cells.

In several models of renal injury renal tubular cells have been shown capable of secreting a variety of cytokines that have both chemoattractant and pro-inflammatory properties (Figure 5). In human cultured cortical epithelial cells exposed to gamma interferon, monocyte chemoattractant protein-1 (MCP-1) is secreted (24). This cytokine has been shown to attract T-lymphocytes as well as cells of the monocyte/macrophage lineage (25). In addition to stimulating chemotaxis, MCP-1 also activates monocytes to secrete cytokines and express cell surface adhesion molecules (26). In renal biopsy material taken from patients with FSGS, membranous glomerulopathy, and IgA nephropathy, there is increased tubular expression of MCP-1 as compared to tissue obtained from normal controls (27). A recent study examining renal biopsy material in patients with IgA nephropathy demonstrated a strict correlation between the amount of MCP-1 gene and protein expression in cortical tubular epithelial cells and the number of monocytes infiltrating the interstitium and the degree of tubulointerstitial damage (28). Osteopontin is a glycoprotein with chemoattractant properties that has also been shown to be synthesized in renal tubular cells

(29,30). In animal models of immune injury such as the anti-Thy-1 model of glomerulonephritis and passive Heymann nephritis as well as in a toxic model of renal injury, puromycin aminonucleoside, there is increased tubular expression of osteopontin mRNA (30). In these models, staining for osteopontin protein in proximal tubules is preceded by morphologic evidence of tubular damage and followed by an influx of monocytes into the interstitial space. In addition, the amount of osteopontin mRNA and protein expression correlates closely with the degree of tubulointerstitial damage. In a model of renal injury induced by the constant infusion of AII, increased osteopontin protein and mRNA levels were also detected in renal tubular cells prior to the interstitial influx of monocytes (31). A third chemotactic factor has been demonstrated in cultured rat tubular epithelial cells. Upon exposure to bovine serum albumin, these cells secrete a novel lipid that possesses chemotactic activity for monocytes (32).

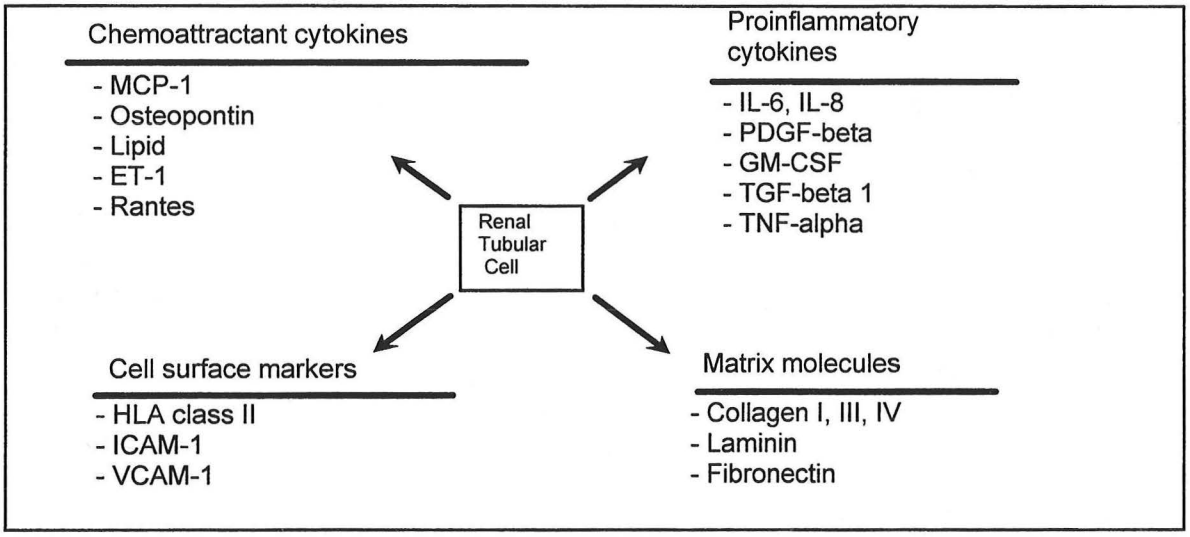

Figure 5

A number of pro-inflammatory molecules have also been shown to be produced by renal tubular cells. In tubular cells grown from human biopsy material, expression of mRNA for interleukin-6, granulocyte-macrophage colony-stimulating factor (GM-CSF), and platelet-derived growth factor-B (PDGF-B) has been demonstrated (33). The amount of mRNA for GM-CSF and PDGF-B is greater in tubular cells obtained from diseased and fibrotic kidneys in comparison to nondiseased and nonfibrotic kidneys (34). In the rat there is detectable baseline expression of PDGF-B in renal tubules (19). Following 5/6-nephrectomy, the tubular expression of PDGF mRNA and protein is markedly increased particularly in areas of inflammatory infiltrates (19). Increased tubular expression of PDGF mRNA has also been demonstrated in rats subjected to continuous infusion of AII (21). Endothelin-1 (ET-1) is produced in renal tubular cells (35,36). This protein is chemotactic for blood monocytes and can bind to specific receptors on monocytes/macrophages and stimulate proinflammatory cytokine secretion (37,38). In various animal models of proteinuric progressive renal failure increased renal ET-1 gene expression or increased urinary excretion of ET-1 has been found to correlate with renal structural damage (39,40). Other cytokines expressed by renal tubular cells include Rantes, TGF-β1, tumor necrosis

factor-α (TNF-α), and IL-8 (41-45).

In addition to secreting substances that can recruit and activate inflammatory cells, renal tubular cells can also express a number of cell surface markers that enable them to interact with interstitial leukocytes. Increased expression of HLA class II antigens, ICAM-1, and VCAM-1 have all been described on renal tubular cells (46-50). The expression of these molecules have been induced after tubular cells are exposed to a variety of proinflammatory cytokines as well as after chemical or ischemic injury (14). In renal biopsy material taken from patients with a variety of glomerular diseases, increased tubular expression of HLA class II antigens and ICAM-1 is mainly observed in disease states that are typically accompanied by interstitial cellular infiltrates (49,50). The expression of HLA class II antigens and adhesion molecules could potentially allow renal tubular cells to act as antigen-presenting cells to interstitial lymphocytes and thereby amplify the inflammatory response within the tubulointerstitium (48,51,52). A coordinated upregulation of a variety of selectins, integrins, and members of the immunoglobulin superfamily in the tubulointerstitium has recently been demonstrated in renal biopsy material taken from humans with a variety of renal diseases (53). Increased expression of these markers was associated with interstitial fibrosis and tubular atrophy irrespective of the underlying diagnosis suggesting that adhesion molecule expression may be involved in the pathogenesis of tubulointerstitial injury.

Finally, there is evidence that renal tubular cells are also directly involved in the production of matrix proteins. These cells have been shown to produce a number of extracellular matrix molecules such as collagens type I, III, and IV, laminin, and fibronectin (54-56). There is also evidence to suggest that renal tubular cells may be capable of transforming into activated fibroblasts thus contributing directly to the development of interstitial fibrosis (57,58).

Proteinuria-Induced Activation and/or Injury of the Renal Tubular Cell

Having established that tubulointerstitial disease is an important determinant of renal outcome in a variety of glomerular diseases and that renal tubular cells, if injured, possess the machinery required to participate in the development and progression of interstitial disease, the question then arises as to what factors initiate tubular cell injury in the setting of glomerular disease. One such factor that is receiving a great deal of attention is the role of proteinuria and its effects on the renal tubular cell.

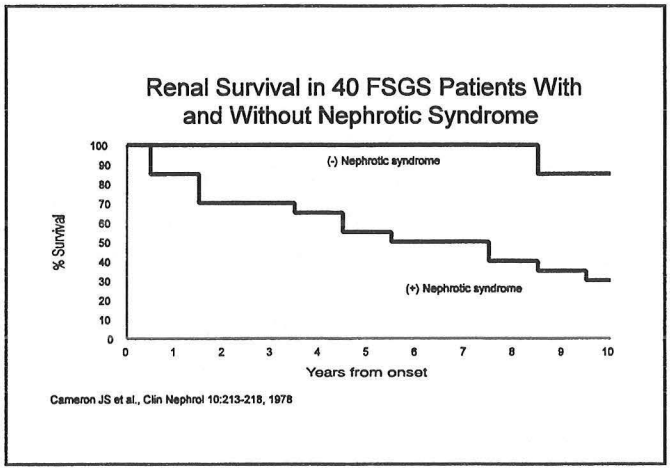

Figure 6

A casual role for proteinuria in the development of interstitial disease comes from studies correlating the rate of

progression of renal failure to the degree of proteinuria. In 40 consecutive patients with FSGS followed for up to 16 years, patients presenting with nephrotic syndrome had a worse prognosis than those presenting with less proteinuria (59) (Figure 6). Similarly, 41% of patients with membranous glomerulopathy who had nephrotic syndrome developed progressive renal failure over a mean follow up of 54.8 months compared to none of those presenting with less than nephrotic range proteinuria (60). This same correlation has been demonstrated in other human renal diseases such as IgA nephropathy (61) and MPGN type-1 (62). In the recently completed Modification of Diet in Renal Disease (MDRD) study, a higher baseline urinary protein excretion was also associated with a more rapid decline in the glomerular filtration rate (63).

The degree of proteinuria has also been correlated to the severity of interstitial disease as determined histologically. In several studies of patients with IgA nephropathy, the extent of interstitial fibrosis or the degree of tubular atrophy has been found to be significantly correlated to the degree of proteinuria (64-66). In type 1 diabetes and nephropathy, patients with more than 400 mg/24 hours of urinary albumin excretion had more severe interstitial disease than those with less albuminuria (67,68). A similar correlation between the degree of proteinuria and histologic damage has been observed in patients with membranous glomerulopathy and systemic lupus erythematosus (5,69,70).

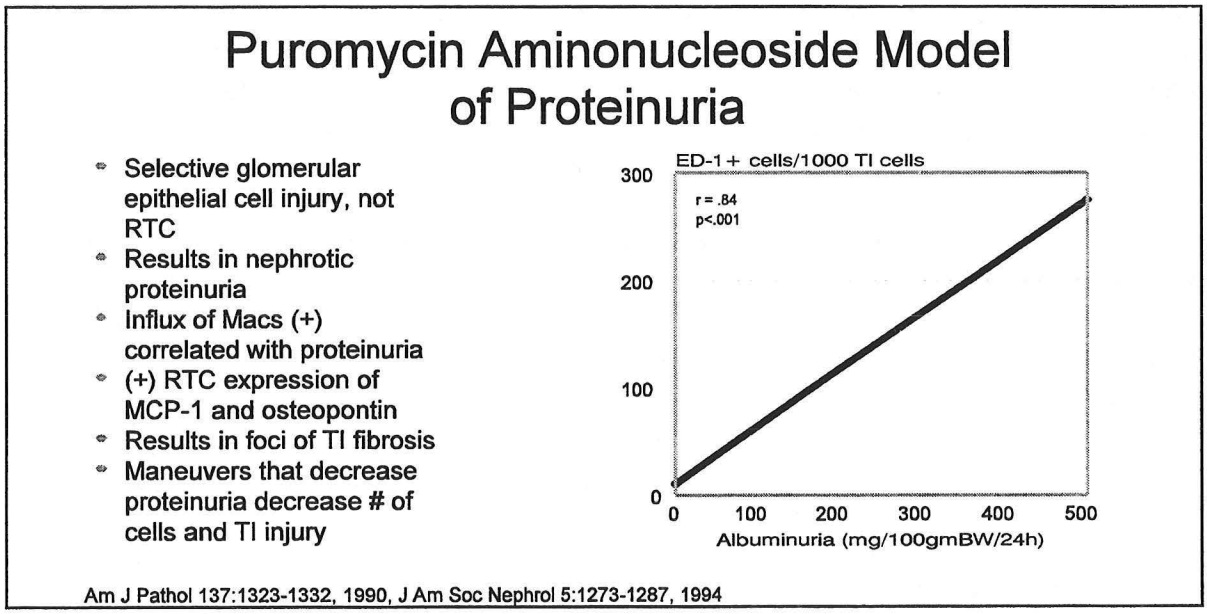

Figure 7

Studies in experimental animals have provided evidence that proteinuria may be an important factor in the initiation of tubulointerstitial disease. One such model is nephrosis induced by the administration of puromycin aminonucleoside (PAN) (71,72). In PAN nephrosis, a single dose of PAN results in selective toxicity to glomerular epithelial cells and causes the development of nephrotic range proteinuria. The proteinuria worsens up to 14 days and then returns to normal.

In association with the proteinuria, there is an influx of chronic inflammatory cells into the interstitium. The severity of the interstitial infiltrate is closely related to the degree of proteinuria (Figure 7). There is a significant increase in the mRNA for MCP-1 as well as osteopontin in tubular epithelial cells. The mRNA for genes encoding extracellular matrix proteins are also significantly increased in association with the proteinuria and the interstitial infiltrate. The model is also characterized by foci of tubulointerstitial fibrosis. Maneuvers that decrease the amount of proteinuria are associated with less inflammatory infiltrate and afford histologic protection. A second model is referred to as protein overload nephropathy and is induced in rats by the peritoneal injection of large amounts of bovine serum albumin (29,73). These animals develop renal injury that is initially restricted to the glomerulus and is manifested by heavy proteinuria. There is no evidence of immune complex formation in either the glomerulus or the interstitium. In association with increasing proteinuria there is an influx of inflammatory cells into the interstitium. Similar to the PAN model of nephrosis, there is increased mRNA expression for MCP-1 and osteopontin in renal tubular cells. Increased matrix protein synthesis and altered matrix degradation and remodeling contribute to the development of interstitial fibrosis. It has been proposed that these two models are examples of primary glomerular injury in which the development of tubulointerstitial disease is a secondary event. The cause of tubulointerstitial disease is thought to be the result of proteinuria-induced tubular injury resulting in the development of an inflammatory response and ultimately fibrosis within the interstitium. A third model which is in keeping with this theory is the development of interstitial fibrosis in the Milan normotensive rat (74). In these animals, damage to the podocyte is associated with the onset of proteinuria. Subsequently there is increased tubular expression of ostepontin that is followed by a monocyte/ macrophage influx. In association with these later changes, there is a progressive accumulation of various extracellular matrix proteins.

A number of different mechanisms can potentially explain the development of renal tubular injury in the setting of glomerular proteinuria (Table 1). One mechanism is immunologic in nature. During states of glomerular proteinuria, potentially damaging antibodies may be filtered into the urinary space where they may interact with tubular antigens present on the apical surface (74). In rats immunized with Heymann antigens there is binding of antibody to brush border antigens that occurs during the heavy phase of proteinuria (75,76). Increased mitotic activity in these tubular cells is suggestive that such antibody binding is injurious in nature. A more nonspecific type of toxicity may relate just to the severity of the proteinuria itself. Normally the proximal tubule attempts to cope with an increased load of protein by increased reabsorption and subsequent degradation in lysozomes. It has been proposed that this process can be

Table 1. Mechanisms by which proteinuria can activate or injure the renal tubular cell.

- Lysosomal swelling and rupture
- Intratubular casts and obstruction
- Filtered Ig, cytokines
- Exacerbate ischemic injury
- Filtered iron and transferrin
- Complement activation
- RTC secretion of matrix
- RTC secretion of chemotactic factors
- Hyperlipidemia

overwhelmed to the point of lysosomal swelling and rupture resulting in the cytoplasmic release of injurious lysosomal enzymes (77). Massive proteinuria may also injure the tubule by forming proteinaceous casts and causing tubular obstruction (78).

Proteinuria may exacerbate ischemic injury to the renal tubular cell. Infiltrating macrophages and renal tubular cells can both secrete vasoactive substances such as endothelin and nitric oxide (79-82). These vasoactive compounds may alter regional blood flow within the interstitium in such a way that the tubular cell is rendered susceptible to hypoxic injury. The additional energy required to reabsorb and digest large amounts of protein could then result in damage to these already stressed cells. In this regard, infusion of low molecular weight proteins into animals first subjected to renal artery occlusion or hemorrhagic shock results in worse tubular injury than when similar infusions are performed in nonischemic animals (83,84). Worsening tubular ischemia could exacerbate interstitial injury through immune-mediated events. Ischemia-induced tubular expression of HLA class II antigens would provide these cells the capability of presenting antigens directly to infiltrating lymphocytes (85,86).

Another mechanism whereby proteinuria may cause ischemic tubular injury is by predisposing to the generation of reactive oxygen metabolites. The excretion of the iron carrier protein, transferrin, into the urine can lead to the release of iron particularly as the pH of the fluid becomes more acidic. Iron is capable of catalyzing the Haber-Weiss reaction leading to the generation of hydroxyl radicals. In addition, iron has been shown to alter several aspects of renal tubular epithelial function (87). In the nephrotoxic serum nephritis model, animals fed a diet that was deficient in iron were shown to develop significantly less renal interstitial injury than iron replete control animals (88). A similar relationship between tubular iron accumulation and the generation of reactive oxygen species and tubular damage has also been noted in the rat remnant kidney model (89). In addition to ischemic injury, iron may also contribute to the intensity of interstitial inflammation. In preliminary studies, the iron carrier protein, transferrin, has been shown to induce a dose dependent increase in MCP-1 mRNA expression in renal tubular cells (90).

Increased glomerular permeability may allow circulating complement components to enter the tubular fluid potentially resulting in tubular injury. Complement can effect the renal tubule through its ability to act as a chemoattractant, stimulate cytokine release, cause cell lysis, and stimulate matrix synthesis (91,92). Increased urinary excretion of the C5b-9 complex has been demonstrated in patients with membranous glomerulopathy, diabetic nephropathy, and FSGS where it has been shown to correlate with the degree of proteinuria (93,94). In patients with membranous glomerulopathy, formation of the C5b-9 complex is thought to occur in the glomerulus where it plays a role in injuring the glomerular epithelium. By contrast the C5b-9 complex has not been demonstrated in the glomerulus of patients with FSGS and diabetic nephropathy raising the possibility that its formation occurs in the tubular fluid. In this regard, the brush border of the proximal tubule can activate complement via the alternative pathway (95). In addition the renal tubular cell has been shown capable of synthesizing several components of complement (96-98). The mRNA for C4 is constitutively expressed in proximal epithelial cells.

By contrast, in situ hybridization studies have identified the mRNA for C3 and factor B in the tubules of diseased kidneys usually restricted to areas of interstitial inflammation (97,98). Another mechanism whereby complement may become activated by tubular cells is by ammoniagenesis. In the setting of proteinuria urinary ammonia is increased possibly as a result of increased protein catabolism in the proximal tubule (99). Such increases in ammonia concentration can lead to activation of complement (100). In the rat remnant kidney model administration of sodium bicarbonate to decrease ammoniagenesis led to a reduction in the level of proteinuria and decrease in interstitial damage (101). In addition, those animals treated with bicarbonate had less peritubular deposition of C3 and C5b-9.

Recent studies suggests that renal tubular cells may directly contribute to the scarring process in the interstitium once exposed to excess tubular protein. In one study, application of serum proteins of a molecular weight normally found in the setting of glomerular proteinuria to the apical surface of cultured renal tubular cells resulted in increased basolateral secretion of the matrix protein fibronectin (56). There was also increased secretion of PDGF as well as increased release of lactate dehydrogenase suggesting a degree of cytotoxicity. Another study in cultured tubular cells showed that addition of albumin to the culture media induced the expression of an integrin not normally found in basal conditions (102). These results were confirmed in kidney biopsy material taken from patients with a variety of proteinuric glomerular diseases in which increased tubular expression of this same integrin was found. It is possible that such remodeling of the adhesive apparatus of tubular cells in response to proteinuria may play a role in the development of the interstitial fibrotic process.

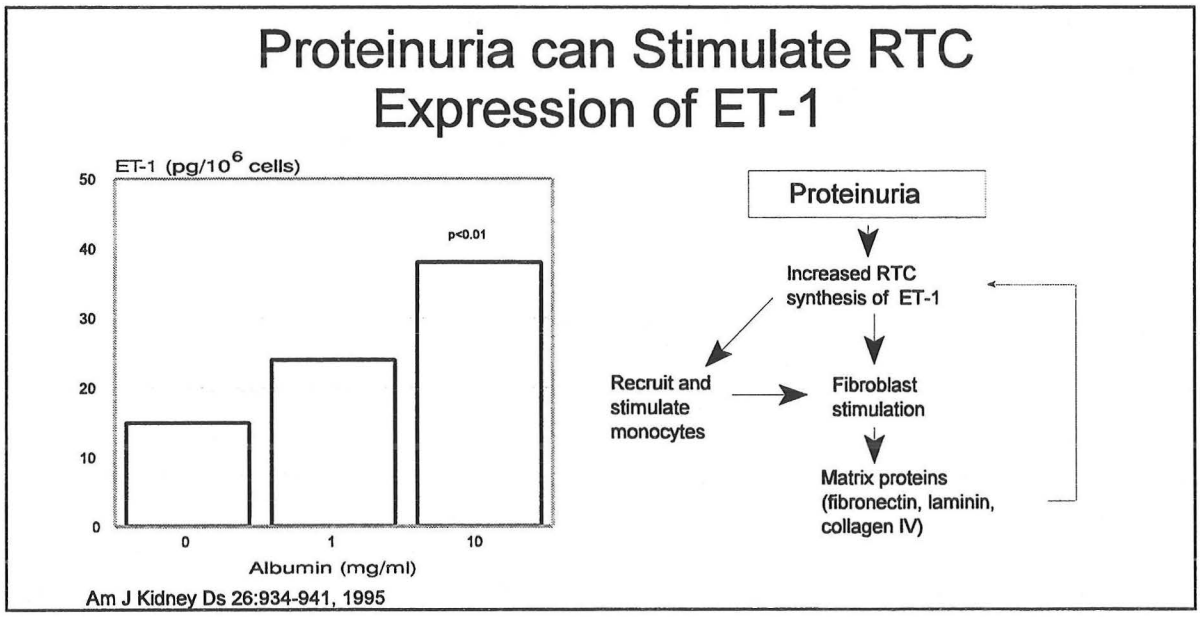

Figure 8

There is also evidence that proteins present in the urine and subsequently processed by renal tubular cells cause the release of factors that can directly participate in interstitial

inflammation. Renal tubular cells incubated with bovine serum albumin that contains fatty acids release a lipid factor that is highly chemotactic for macrophages (32). This factor has a chromatographic pattern identical to a lipid factor extracted from urine of rats with experimental protein overload proteinuria. Incubation of cultured proximal tubular cells with albumin has also been shown to induce a dose-dependent release of endothelin-1 (ET-1) (35) (Figure 8). In these studies the release of ET-1 is directed towards the basolateral side of the cell. Accumulation of ET-1 within the interstitium can contribute to the influx of blood monocytes and stimulate these cells to secrete proinflammatory cytokines (37,38). In addition, ET-1 can bind to receptors on interstitial fibroblasts causing these cells to proliferate and synthesize matrix proteins (103,104). In turn, matrix proteins such as fibronectin, collagen IV, and laminin have been shown to induce ET-1 synthesis in cultured tubular cells thus providing an amplification loop in the fibrotic process (103).

A more indirect way in which nephrotic proteinuria may lead to interstitial disease is through the development of hyperlipidemia. In several animal models, hyperlipidemia has been shown to play a role in progressive renal injury (105). Most of these studies have focused on how lipid disorders modulate glomerular injury. LDL can interact with mesangial cells to stimulate cellular proliferation and induce the synthesis of cytokines that are chemoattractant for macrophages. Both macrophages and mesangial cells can produce oxidized LDL which along with native LDL stimulates mesangial cells to produce extracellular matrix (105,106). There are now experimental observations to suggest that lipoproteins may also exert similar effects on the renal tubular cell thus contributing to progressive tubulointerstitial injury. In the protein overload model of glomerular injury, rats fed a diet enriched with cholesterol developed more evidence of tubular injury characterized by a higher mitotic rate and a significantly greater number of dilated and cystic tubules as compared to animals maintained on a regular rat chow (107). In the rat model of mesangialproliferative glomerulonephritis, diet-induced hyperlipidemia was associated with a greater influx of monocytes into the interstitium as compared to normolipidemic animals (107). In addition, the hyperlipidemic animals developed significantly greater amounts of tubular atrophy and interstitial fibrosis. Similar results have been found in rats with adriamycin-induced nephrosis and in the rat remnant kidney model (108). Even in animals without pre-existing glomerular disease, hyperlipidemia is associated with an interstitial mononuclear infiltrate that is localized around slightly ectatic tubules with thickened basement membranes (107).

Mechanisms Linking Renal Tubular Cell Activation/Injury and Tubulointerstitial Fibrosis

The final step in the process of tubulointerstitial damage is the development of interstitial fibrosis. During the earliest phases of this process, interstitial fibroblasts begin to undergo phenotypic changes including the acquisition of smooth muscle cell and mesenchymal markers. Such markers include the expression of α smooth muscle actin and intermediate filament proteins such as vimentin and desmin (109). This phenotypic change into what has been called a myofibroblast is thought to represent evidence of activation or injury. These cells contribute to the development of interstitial fibrosis by secreting extracellular matrix and possibly by elaborating inhibitors of matrix breakdown (109). In both experimental and clinical

nephropathies, there is a marked proliferation of myofibroblasts which tend to localize in areas of increased collagen content (21,110-113). Another molecule that is only expressed on myofibroblasts and not resting fibroblasts is SPARC (secreted protein acidic and rich in cysteine) (114). As with the other markers of fibroblast activation, increased expression of SPARC is primarily found on myofibroblasts at sites of tubulointerstitial injury and fibrosis.

In both experimental and clinical glomerular disease, the presence of interstitial myofibroblasts has been associated with a poor prognosis. In the rat model of nephrotoxic nephritis, a strong predictive value was found for the presence of these cells and the late development of interstitial fibrosis (115). In patients with IgA nephropathy, staining of renal biopsy material for the presence of interstitial myofibroblasts is predictive of a more rapid decline in renal function (112,113) (Figure 9). Similar results have been reported in patients with chronic mesangiocapillary glomerulonephritis where the number of interstitial cells expressing vimentin predicted the development of tubulointerstitial fibrosis (109).

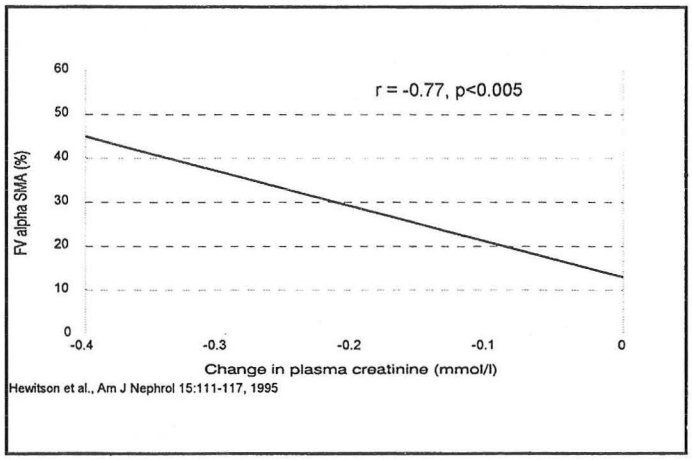

Figure 9. Relationship between fractional volume of αsmooth muscle actin (+) cells and change in creatinine over time.

The cell of origin for interstitial myofibroblasts remains unknown. In addition to resident interstitial fibroblasts, there is some evidence that renal tubular cells may undergo transdifferentiation into fibroblastic cells. In the murine model for antitubular basement membrane disease, some tubular cells acquire the ability to express a protein specific to fibroblasts (57). Overexpression of this protein in tubular cells causes the cells to convert from their epithelial phenotype to a mesenchymal phenotype. In other studies, positive staining for the mesenchymal marker vimentin has been demonstrated in tubular cells in animals after exposure to nephrotoxins as well as in atrophic tubules from humans with scarred kidneys (58,116). There is also evidence that perivascular cells may be able to undergo this transformation and secrete collagen. In the rat model of anti-GBM nephritis perivascular adventitial cells demonstrated increased expression of procollagen α1(I) transcripts (117). The ability of perivascular cells to secrete collagen could provide a link between vascular injury as might occur in hypertension and the development of tubulointerstitial fibrosis.

The activation of myofibroblasts can result from cytokines secreted by infiltrating mononuclear cells as well as renal tubular cells. Factors secreted by infiltrating monocytes that have been implicated in the activation of myofibroblasts include TGF-β1, PDGF, basic fibroblast

growth factor, IL-6, IL-1 and TNF-α (78). Proximal tubular cells can also be stimulated to secrete many of these same cytokines (14,78) . Receptors for growth factors have been demonstrated on fibroblasts and myofibroblasts and are therefore are able to respond to these cytokines by activation, proliferation, and extracellular matrix synthesis. PDGF and TGF-β1 are the growth factors that have received the greatest attention in transforming fibroblasts to myofibroblasts.

The receptor for PDGF is constitutively expressed on cortical interstitial fibroblasts (118). Studies in human renal injury using techniques of immunohistochemistry and in situ hybridization for detection of mRNA have shown evidence of increased interstitial production of this receptor during interstitial injury (119,120). Using these same techniques in the rat 5/6 nephrectomy model, increased mRNA and protein expression for PDGF was demonstrated in tubules and in the interstitium at a time that interstitial cells were expressing markers indicative of myofibroblast transformation (19). PDGF mRNA is also expressed at sites of interstitial cell proliferation and fibrosis in renal injury induced by prolonged infusion of angiotensin II (21). Exogenous infusion of PDGF into normal rats induces tubulointerstitial fibroblast proliferation with subsequent differentiation of these cells into myofibroblasts as evidenced by the expression of α smooth muscle actin. In association with these changes there is increased collagen III accumulation (121). These observations suggest that increased production of PDGF by renal tubular cells and mononuclear cells in areas of tubulointerstitial injury may play an important role in mediating fibroblast migration, proliferation, and increase matrix production (Figure 10).

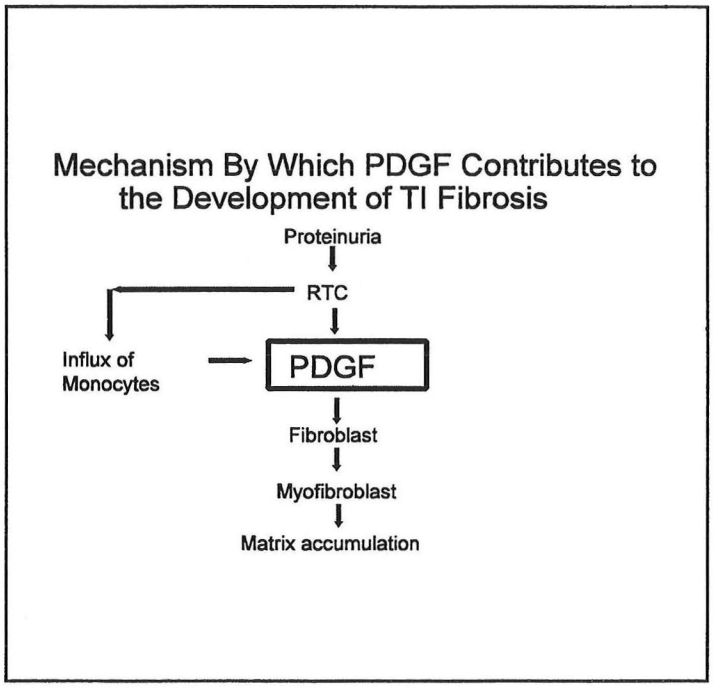

Figure 10

One of the most widely studied cytokines associated with interstitial fibrosis is TGF-β1. In several models of primary glomerular disease associated with the development of interstitial damage, there is increased expression of the mRNA for TGF-β1 (122). The source of this increased expression is primarily infiltrating monocytes as well as renal tubular cells (29,42,122,123). TGF-β1 elicits a variety of responses that can contribute to the development of interstitial fibrosis (124-126). TGF-β1 contributes to extracellular matrix accumulation by stimulating the production of collagens type I, III, and IV and fibronectin in renal fibroblasts. At

the same time, TGF-β1 inhibits matrix degradation by directly inhibiting the production of matrix-degrading metalloproteinase enzymes. These enzymes are further inhibited by the effects of TGF-β1 to up-regulate metalloproteinase inhibitors such as the tissue inhibitor of metalloproteinase-1 (TIMP-1) and plasminogen activator inhibitor (PAI). TGF-β1 secreted by renal tubular cells or monocytes can also stimulate myofibroblasts to secrete additional TGF-β1 thus providing an amplification loop in the fibrogenic process. Thus, the net effect of TGF-β1 is to tip the balance of matrix synthesis and degradation toward the accumulation of matrix proteins (Figure 11).

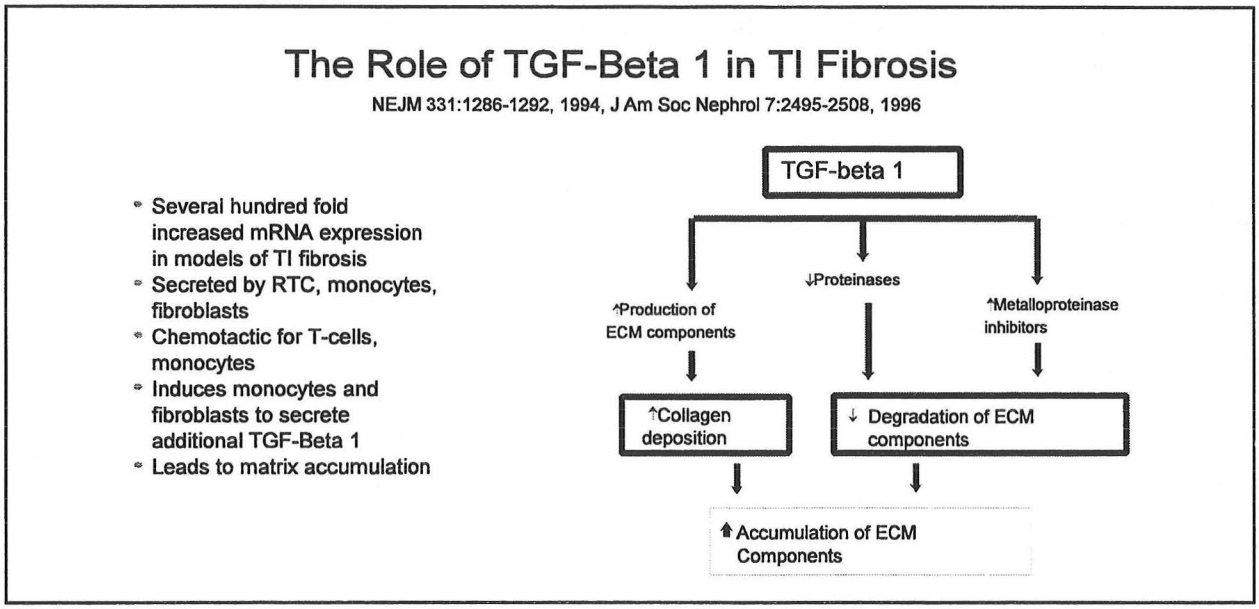

Figure 11

In addition to contributing to the development of interstitial fibrosis, activated myofibroblasts may also play a role in the development of glomerulosclerosis. These cells may migrate into the glomerular tuft through holes and adhesions within Bowmans capsule (127). It has been suggested that such breaks in the integrity of the glomerular capsule may be induced by the periglomerular inflammatory cells often associated with renal scarring. A trail of myofibroblasts has been described moving from the interstitium into Bowmans space in crescentic glomerulonephritis (111). Such infiltration of myofibroblasts would explain the appearance of collagen types normally restricted to the interstitium in sclerosed glomeruli of experimental animals and humans. Interstitial myofibroblasts could be attracted to glomeruli as a result of growth factors synthesized within the glomerulus. In rabbits with glomerulonephritis, proliferating perivascular cells (a cell type described as a precursor of myofibroblasts) have been shown to migrate into the interstitium as well as the inflamed glomerulus ultimately contributing to the development of sclerosis in both compartments (117). Such a scenario in which cells of vascular origin directly contribute to both interstitial and glomerular injury would potentially explain the development of interstitial fibrosis and glomerulosclerosis in hypertension.

Effects of ACE Inhibitors and Low Protein Diet on Development of TI Disease

In clinical trials of patients with chronic renal failure, angiotensin converting enzyme (ACE) inhibitors and protein restricted diets have both been shown effective in slowing the rate at which renal function declines. In diabetic patients, randomized multicenter trials have shown that ACE inhibitors have renoprotective effects in those with already overt nephropathy as well as in those with incipient nephropathy (128-130). These agents have also been shown protective in non-diabetic chronic renal failure (131). Similarly, dietary protein restriction has been shown effective in retarding the progression of chronic renal failure in diabetic and nondiabetic renal disease (132-134). Based on numerous studies in experimental animals, the beneficial effects of these interventions has been attributed to favorable effects on glomerular function and structure (135) (Table 2). There is now accumulating evidence to suggest that the renoprotective effects of these interventions may also relate to their ability to interfere in the mechanisms responsible for the development of tubulointerstitial disease. In this regard, most studies have shown that renoprotection observed with ACE inhibitors and low protein diet is generally accompanied by a decrease in proteinuria (128,130-132). Based on the above discussion, this decrease in proteinuria may be more than just a marker of improved glomerular function and, in fact, may be a primary event in explaining the renoprotective effect associated with these interventions.

Table 2. Effects of CEI on glomerular function and structure that may explain renal protective effect.

• Decrease systemic blood pressure
• Reduce intraglomerular pressure
• Limit glomerular hypertrophy
• Improve glomerular capillary filtration barrier
• Decrease serum lipids

ACE inhibitors may limit the development of tubulointerstitial disease through a variety of mechanisms. These agents are known to have a significant antiproteinuric effect. In both experimental models and human studies, the renoprotection afforded by ACE inhibitors has typically been accompanied by a significant decrease in proteinuria (136-137) (Figure 12). By reducing the amount of protein in tubular fluid, there should be a decrease in the tubular expression and synthesis of vasoactive and inflammatory molecules that are upregulated by protein overload. For example, in the animal model of passive Heymann nephritis, the development of proteinuria and interstitial disease is associated with increased staining for ET-1 in tubular cells (103). By comparison, animals treated with a combination of an ACE inhibitor and an angiotensin II receptor antagonist do not develop proteinuria and have tubular expression of ET-1 comparable to control.

ACE inhibitors may also help preserve the integrity of the tubulointerstitium by directly inhibiting the synthesis of AII. AII has been shown to have a number of effects that can directly contribute to the development of tubulointerstitial fibrosis. Systemic infusion of AII into healthy

rats results in an increased tubular expression of both the protein and mRNA for osteopontin (21,138). Following the expression of this protein there is a monocyte/macrophage influx into the interstitium that is subsequently followed by increased synthesis of fibronectin and collagen. AII has been shown to directly stimulate renal interstitial fibroblasts to proliferate and to increase the synthesis of matrix proteins like collagen and fibronectin (139). AII can further enhance matrix production through its ability to stimulate the release of other cytokines. For example, AII has been shown to stimulate TGF-β1 production in renal tubular cells and upregulate TGF-β1 expression in fibroblasts (122,140).

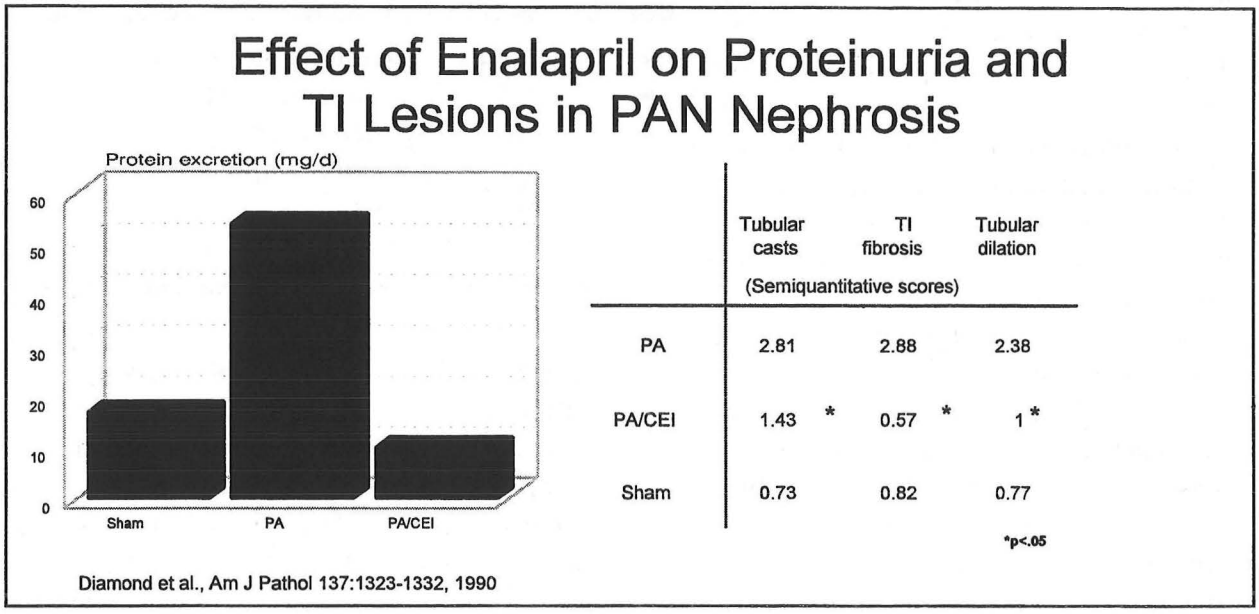

Figure 12

Renal tubular cells possess the complete machinery of substrates and enzymes required for the synthesis of AII (139). While there is little evidence that the systemic renin-angiotensin system is activated in most chronic renal disease, multiple lines of evidence suggest that the intrarenal system may play an important role in progressive renal failure (141). ACE inhibitors may limit the development of tubulointerstitial disease by inhibiting the effects of locally produced AII. In a rat model of chronic glomerulonephritis, serum ACE activity in nephritic animals did not differ from controls (142). By contrast, ACE activity measured in the brush border of nephritic animals was markedly elevated. In addition, when these measurements were performed in animals treated with an ACE inhibitor, there was a marked inhibition of ACE activity in the cortex. In association with this decrease in activity, the gene expression for fibronectin and collagens I, III, and IV in the renal cortex was also markedly decreased. Treated animals developed less proteinuria and morphologically had less tubular and interstitial lesions. Similarly, in rats with chronic PAN nephrosis, treatment with enalapril resulted in significantly less interstitial fibrosis as compared to control animals (143). This same drug has been shown to attenuate the development of interstitial fibrosis in the rat remnant kidney model and in the aging mouse (144,145). In addition, rats treated with a combination of an ACE inhibitor and an

angiotensin II receptor develop less cyclosporin-induced interstitial disease (146).

There is evidence that the renin-angiotensin system participates in the events that characterize interstitial injury in the model of chronic ureteral obstruction. Histologically, this model is characterized by the development of an interstitial cellular infiltrate and marked interstitial fibrosis. The sequence of events in the development of these lesions has been extensively studies in the rat model of unilateral ureteral obstruction (20,147-151). Within hours of ureteral ligation increased mRNA for MCP-1, osteopontin, and ICAM-1 can be detected in renal tubular cells. Subsequently, there is an influx of macrophages into the interstitium. These cells are thought to be, in part, the source of increased TGF-β1 expression within the kidney. In association with increased TGF-β1, the mRNA levels for TIMP-1 also begin to increase. Interstitial fibroblasts begin to transform into myofibroblasts as evidenced by increased expression of α smooth muscle actin and the interstitial matrix progressively expands. Administration of ACE inhibitors at the time of ureteral ligation or several days later have been shown to markedly attenuate these changes and limit the development of interstitial fibrosis (150,151). The AII receptor antagonists are also effective in limiting interstitial injury in this model (150). As with the ACE inhibitors, the AII receptor blockers decrease the mRNA for TGF-beta-1 and decrease the amount of interstitial fibrosis. However, unlike ACE inhibitors, these agents do not decrease the influx of mononuclear cells into the interstitium.

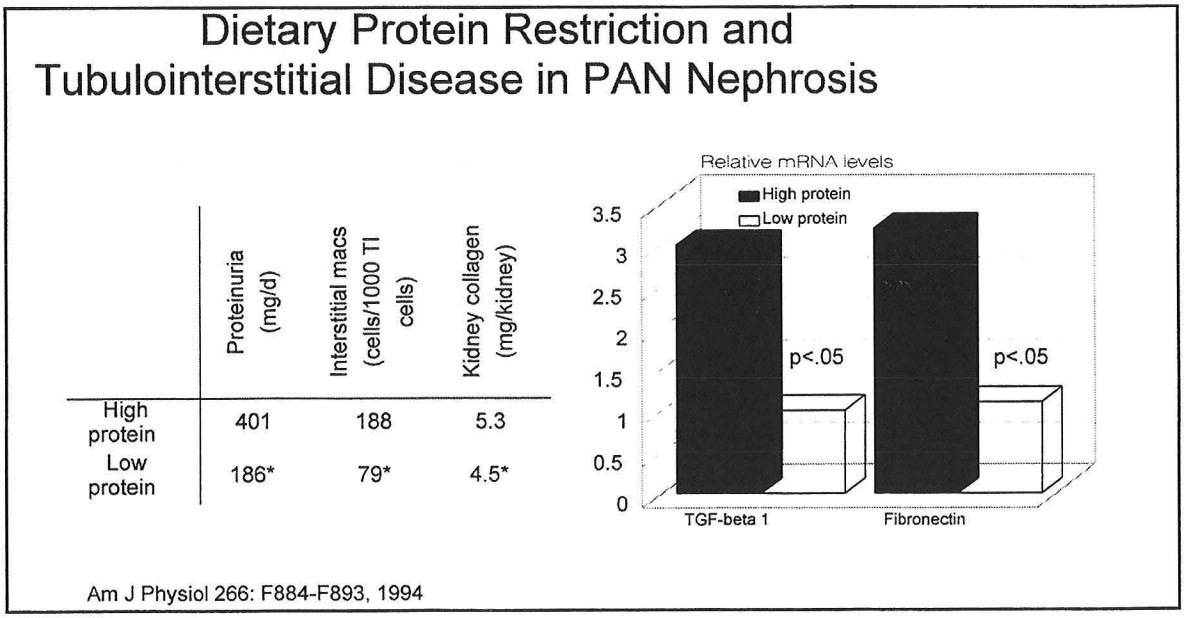

Figure 13

Another mechanism whereby ACE inhibitors may limit tubulointerstitial damage is through their effects on decreasing serum lipid concentrations. These drugs can decrease lipid levels as a result of their antiproteinuric effect (152). In one study there was a 15% decrease in total and LDL cholesterol levels. Along these lines, there is convincing evidence in experimental

animals and suggestive evidence in human studies that treatment of hyperlipidemia may slow the deterioration of chronic renal failure (105).

The ability of dietary protein restriction to preserve renal function may also be related to decreased damage of the tubulointerstitium (Figure 13). As with ACE inhibitors, a decrease in dietary protein intake is associated with an antiproteinuric effect (132). In rats with PAN nephrosis, administration of a low protein diet leads to a reduction in the number of interstitial macrophages (42). In association with the decreased inflammatory response, there is normalization of TGF-beta-1 levels and a significant decrease in several extracellular matrix proteins. Low protein diet has also been shown to attenuate increased PDGF gene expression in this same model (153).

References

1. Risdon RA, Sloper JC, De Wardener H: Relationship Between Renal Function and Histological Changes Found In Renal-Biopsy Specimens From Patients With Persistent Glomerular Nephritis. The Lancet 2:363-366, 1968.

2. Schainuck LI, Striker GE, Cutler RE, Benditt EP: Structural-Functional Correlations In Renal Disease. Human Pathology 1:631-641, 1970.

3. Bohle A, Muller GA, Wehrmann M, Mackensen-Haen S, Xiao J: Pathogenesis of Chronic Renal Failure in the Primary Glomerulopathies, Renal Vasculopathies, and Chronic Interstitial Nephritides. Kidney International 49 (Suppl 54):S-2-S-9, 1996.

4. Schmitt H, Bohle A, Reineke T, Mayer-Eichberger D, Vogl W: Long-Term Prognosis of Membranoproliferative Glomerulonephritis Type I. Nephron 55:242-250, 1990.

5. Wehrmann M, Bohle A, Bogenschutz O, Eissele R, Freislederer A, Ohlschlegel C, Schumm G, Batz C, Gartner H: Long-Term Prognosis of Chronic Idiopathic Membranous Glomerulonephritis. Clinical Nephrology 31:67-76, 1989.

6. Wehrmann M, Bohle A, Held H, Schumm G, Kendziorra H, Pressler H: Long-Term Prognosis of Focal Sclerosing Glomerulonephritis. An Analysis of 250 Cases With Particular Regard to Tubulointerstitial Changes. Clinical Nephrology 33:115-122, 1990.

7. Abe S, Amagasaki Y, Iyori S, Konishi K, Kato E, Sakaguchi H: Significance of Tubulointerstitial Lesions In Biopsy Specimens of Glomerulonephritis Patients. American Journal of Nephrology 9:30-37, 1989.

8. Bohle A, Wehrmann M, Bogenschutz O, Batz C, Muller CA: The Pathogenesis of Chronic Renal Failure In Diabetic Nephropathy. Path Res Pract 187:251-259, 1991.

9. D'Amico G: Influence of Clinical and Histological Features on Actuarial Renal Survival in Adults Patients With Idiopathic IgA Nephropathy, Membranous Nephropathy, and Membranoproliferative Glomerulonephritis: Survey of the Recent Literature. American Journal of Kidney Diseases 20:315-323, 1992.

10. Okuda S, Languino LR, Ruoslahti E, Border WA: Elevated Expression of Transforming Growth Factor-β and Proteoglycan Production in Experimental Glomerulonephritis. Journal of Clinical Investigation 86:453-462, 1990.

11. Yamamoto T, Noble NA, Miller DE, Border WA: Sustained Expression of TGF-β1 Underlies Development of Progressive Kidney Fibrosis. Kidney International 45:916-927, 1994.

12. Bertani T, Cutillo F, Zoja C, Broggini M, Remuzzi G: Tubulo-Interstitial Lesions Mediate Renal Damage in Adriamycin Glomerulopathy. Kidney International 30:488-496, 1986.

13. Anderson S, Diamond JR, Karnovsky MJ, Brenner BM: Mechanisms Underlying Transition From Acute Glomerular Injury To Late Glomerular Sclerosis In a Rat Model of Nephrotic Syndrome. Journal of Clinical Investigation 82:1757-1768, 1988.

14. D-Amico G, Ferrario F, Rastaldi MP: Tubulointerstitial Damage in Glomerular Diseases: Its Role in the Progression of Renal Damage. American Journal of Kidney Diseases 26:124-132, 1995.

15. Stein-Oakley AN, Maguire JA, Dowling J, Perry G, Thomson NM: Altered Expression of Fibrogenic Growth Factors in IgA Nephropathy and Focal and Segmental Glomerulosclerosis. Kidney International 51:195-204, 1997.

16. Hooke DH, Gee DC, Atkins RC: Leukocyte Analysis Using Monoclonal Antibodies in Human Glomerulonephritis. Kidney International 31:964-972, 1987.

17. Brunati C, Brando B, Confalonieri R, Belli LS, Lavagni MG, Minetti L: Immunophenotyping of Mononuclear Cell Infiltrates Associated With Renal Disease. Clinical Nephrology 26:15-20, 1986.

18. Boucher A, Droz D, Adafer E, Noel L: Characterization of Mononuclear Cell Subsets in Renal Cellular Interstitial Infiltrates. Kidney International 29:1043-1049, 1986.

19. Kliem V, Johnson RJ, Alpers CE, Yoshimura A, Couser WG, Koch KM, Floege J: Mechanisms Involved in the Pathogenesis of Tubulointerstitial Fibrosis in 5/6-Nephrectomized Rats. Kidney International 49:666-678, 1996.

20. Diamond JR, Kees-Folts D, Ding G, Frye JE, Restrepo NC: Macrophages, Monocyte Chemattractant Peptide-1, and TGF-β1 in Experimental Hydronephrosis. American Journal of Physiology 266:F926-F933, 1994.

21. Johnson RJ, Alpers CE, et al: Renal Injury From Angioensin II-Mediated Hypertension. Hypertension 19:464-474, 1992.

22. Alexopoulos E, Seron D, Hartley RB, Nolasco F, Cameron S: Immune Mechanisms In Idiopathic Membranous Nephropathy: The Role of the Interstitial Infiltrates. American Journal of Kidney Diseases 13:404-412, 1989.

23. Alexopoulos E, Seron D, Hartley RB, Cameron JS: Lupus Nephritis: Correlation of Interstitial Cells With Glomerular Function. Kidney International 37:100-109, 1990.

24. Schmouder RL, Strieter RM, Kunkel SL: Interferon-g Regulation of Human Renal Cortical Epithelial Cell-Derived Monocyte Chemotactic Peptide-1. Kidney International 44:43-49, 1993.

25. Taub DD, Proost P, Murphy WJ, Anver M, Longo DL, Van Damme J, Oppenheim JJ: Monocyte Chemotactic Protein-1 (MCP-1), -2, and -3 Are Chemotactic For Human T Lymphocytes. The Journal of Clinical Investigation 95:1370-1376, 1995.

26. Jiang Y, Beller DI, Frendl G, Graves DT: Monocyte Chemoattractant Protein-1 Regulates Adhesion Molecule Expression and Cytokine Production in Human Monocytes. The Journal of Immunology 148:2423-2428, 1992.

27. Prodjosudjadi W, Gerritsma JSJ, Klar-Mohamad N, Gerritsen AF, Bruijn JA, Daha MR, Van es LA: Production and Cytokine-Mediated Regulation of Monocyte Chemoattractant Protein-1 By Human Proximal Tubular Epithelial Cells. Kidney International 48:1477-1486, 1995.

28. Grandaliano G, Gesualdo L, Ranieri E, Monno R, Montinaro V, Marra G, Schena FP: Monocyte Chemotactic Peptide-1 Expression In Acute and Chronic Human Nephritides: A Pathogenetic Role In Interstitial Monocytes Recruitment. JASN 7:906-913, 1996.

29. Eddy AA, Giachelli CM: Renal Expression of Genes That Promote Interstitial Inflammation and Fibrosis in Rats With Protein-Overload Proteinuria. Kidney International 47:1546-1557, 1995.

30. Pichler R, Giachelli CM, Lombardi D, Pippin J, Gordon K, Alpers CE, Schwartz SM, Johnson RJ: Tubulointerstitial Disease in Glomerulonephritis. Potential Role of Osteopontin (Uropontin). American Journal of Pathology 144:915-926, 1994.

31. Giachelli CM, Pichler R, Lombardi D, Denhardt DT, Alpers CE, Schwartz SM, Johnson RJ: Osteopontin Expression In Angiotensin II-Induced Tubulointerstitial Nephritis. Kidney International 45:515-524, 1994.

32. Kees-Folts D, Sadow JL, Schreiner GF: Tubular Catabolism of Albumin Is Associated With The Release Of An Antiinflammatory Lipid. Kidney International 45:1697-1709, 1994.

33. Frank J,Engler-Blum G, Rodemann H, Muller GA: Human Renal Tubular As A Cytokine Source: PDGF-b, GM-CSF, and IL-6 mRNA Expression *In Vitro*. Experimental Nephrology 1:26-35, 1993.

34. Burton CJ, Walls J: Proximal Tubular Cell, Proteinuria And Tubulo-Interstitial Scarring. Nephron 68:287-293, 1994.

35. Zoja C, Morigi M, Figliuzzi M, Bruzzi I, Oldroyd S, Benigni A, Ronco P, Remuzzi G: Proximal Tubular Cell Synthesis and Secretion of Endothelin-1 On Challenge with Albumin And

Other Proteins. AJKD 26:934-941, 1995.

36. Ong ACM, Jowett TP, Moorhead JF, Owen JS: Human High Density Lipoproteins Stimulate Endothelin-1 Release By Cultured Human Renal Proximal Tubular Cells. Kidney International 46:1315-1321, 1994.

37. Hanggono T, Rao GS: Chemotaxis of Human Blood Monocytes Toward Endothelin-1 And The Influence Of Calcium Channel Blockers. Biochem & Biophys Res Comm 189:994-1000, 1992.

38. Benigni A, Remuzzi G: Glomerular Protein Trafficking And Progression Of Renal Disease To Terminal Uremia. Seminars in Nephrology 16:151-159, 1996.

39. Orisio S, Benigni A, Bruzzi I, Corna D, Perico N, Zoja C, Benatti L, Remuzzi G: Renal Endothelin Gene Expression Is Increased In Remnant Kidney And Correlates With Disease Progression. Kidney International 43:354-358, 1993.

40. Zoja C Corna D, Bruzzi I, Foglierri C, Bertani T, Remuzzi G, Benigni A: Passive Heymann Nephritis: Evidence That Angiotensin Converting Enzyme Inhibition Reduces Proteinuria And Retards Renal Structural Injury. Exp Nephrol 4:213-221, 1996.

41. Heeger P, Wolf G, Meyers C, Sun MJ, O'Farrell SC, Krensky AM, Neilson EG: Isolation And Characterization of cDNA From Renal Tubular Epithelium Encoding Murine Rantes. Kidney International 41:220-225, 1992.

42. Eddy AA: Protein Restriction Reduces Transforming Growth Factor-β And Interstitial Fibrosis In Nephrotic Syndrome. Amer J Physiol 266:F884-F893, 1994.

43. Jevnikar AM, Brennan DC, Singer GG, Heng JE, Maslinski W, Wuthrich RP, Glimcher LH, Kelley VER: Stimulated Kidney Tubular Epithelial Cells Express Membrane Associated And Secreted TNFα. Kidney International 40:203-211, 1991.

44. Wuthrich RP, Glimcher LH, Yui MA, Jevnikar AM, Dumas SE, Kelley VE: MHC Class II, Antigen Presentation and Tumor Necrosis Factor In Renal Tubular Epithelial Cells. Kidney International 37:783-792, 1990.

45. Schmouder RL, Strieter RM, Wiggins RC, Chensue SW, Kunkel SL: I*n Vitro* And *In Vivo* Interleukin-8 Production in Human Renal Cortical Epithelia. Kidney International 41:191-198, 1992.

46. Brady H: Leukocyte Adhesion Molecules And Kidney Diseases. Kidney International 45:1285-1300, 1994.

47. Jevnikar AM, Wuthrich RP, Takei F, Xu H, Brennan DC, Glimcher LH, Rubin-Kelley VE: Differing Regulation And Function of ICAM-1 And Class II Antigens On Renal Tubular Cells. Kidney International 38:417-425, 1990.

48. Kelley VR, Diaz-Gallo C, Jevnikar AM, Singer GG: Renal Tubular Epithelial and T Cell Interactions In Autoimmune Renal Disease. Kidney International 43 (Suppl 39):S108-S115, 1993.

49. Muller GA, Markovic-Lipkovski J, Muller CA: Intracellular Adhesion Molecule-1 Expression In Human Kidneys With Glomerulonephritis. Clinical Nephrology 36:203-208, 1991.

50. Muller GA, Markovic-Lipkovski J, Rodemann HP: The Progression of Renal Diseases: On The Pathogenesis of Renal Interstitial Fibrosis. Klim Wochenschr 69:576-586, 1991.

51. Hagerty DT, Allen PM: Processing And Presentation of Self and Foreign Antigens By The Renal Proximal Tubule. The Journal of Immunology 148:2324-2330, 1992.

52. Kelly CJ, Roth DA, Meyers CM: Immune Recognition And Response To The Renal Interstitium. Kidney International 31:518-530, 1991.

53. Roy-Chaudhury P, Wu B, King G, Campbell M, Macleod AM, Haites NE, Simpson JG, Power DA: Adhesion Molecule Interactions In Human Glomerulonephritis Importance Of The Tubulointerstitium. Kidney International 49:127-134, 1996.

54. Haverty TP, Kelly CJ, Hines WH, Amenta PS, Watanabe M, Harper RA, Kefalides NA, Neilson EG: Characterization Of A Renal Tubular Epithelial Cell Line Which Secretes The Autologous Target Antigen Of Autoimmune Experimental Interstitial Nephritis. Journal of Cell Biology 107:1359-1368, 1988.

55. Creely JJ, DiMari SJ, Howe AM, Haralson MA: Effects Of Transforming Growth Factor-β On Collagen Synthesis By Normal Rat Kidney Epithelial Cells. American Journal of Pathology 140: 45-55, 1992.

56. Burton CJ, Combe C, Walls J, Harris KPG: Fibronectin Production By Human Tubular Cells: The Effect Of Apical Protein. Kidney International 50:760-767, 1996.

57. Strutz F, Okada H, Lo CW, Danoff T, Carone RL, Tomaszewski JE, Neilson EG: Identification And Characterization Of A Fibroblast Marker: FSP1. Journal of Cell Biology 130:393-405, 1995.

58. Nadasdy T, Laszik Z, Blick KE, Johnson DL, Silva FG: Tubular Atrophy In The End-Stage Kidney: A Lectin And Immunohistochemical Study. Hum Pathol 25:22-28, 1994.

59. Cameron JS, Turner DR, Ogg CS, Chantler C, Williams DG: The Long-Term Prognosis Of

Patients With Focal Segmental Glomerulosclerosis. Clinical Nephrology 10:213-218, 1978.

60. Erwin DT, Donadio Jr. JV, Holley KE: The Clinical Course Of Idiopathic Membranous Nephropathy. Mayo Clinic Proceedings 48:697-712, 1973.

61. D'Amico G, Minetti L, Ponticelli C, Fellin G, Ferrario F, Di Belgioioso GB, Imbasciati E, Ragni A, Bertoli S, Fogazzi G, et al: Prognostic Indicators In Idiopathic IgA Mesangial Nephropathy. Quarterly Journal of Medicine 59:363-378, 1986.

62. Cameron JS, Turner DR, Heaton J, Williams DG, Ogg CS, Chantler C, Haycock GB, Hicks J: Idiopathic Mesangiocapillary Glomerulonephritis. The American Journal of Medicine 74:175-192, 1983.

63. Klahr S, Levey AS, Beck GJ, Caggiula AW, Hunsicker L, Kusek JW, Striker G: The Effects of Dietary Protein Restriction And Blood-Pressure Control On The Progression Of Chronic Renal Disease. NEJM 330:877-884, 1994.

64. Croker BP, Dawson DV, Sanfilippo F: IgA Nephropathy: Correlation Of Clinical And histologic Features. Laboratory Investigation 48:19-24, 1983.

65. Okada K, Funai M, Kawakami K, Kagami S, Yano I, Kuroda Y: IgA Nephropathy In Japanese Children And Adults: A Comparative Study Of Clinicopathological Features. Am J Nephrol 10:191-197, 1990.

66. Widstam-Attorps U, Berg U, Bohman S, Lefvert A: Proteinuria And Renal Function In Relation To Renal Morphology. Clinical Nephrology 38:245-253, 1992.

67. Mauer SM, Steffes MW, Ellis EN, Sutherland DER, Brown DM, Goetz FC: Structural-Functional Relationships In Diabetic Nephropathy. Journal of Clinical Investigation 74:1143-1155, 1984.

68. Lane PH, Steffes MW, Fioretto P, Mauer SM: Renal Interstitial Expansion In Insulin-Dependent Diabetes Mellitus. Kidney International 43:661-667, 1993.

69. Appel G: Renal Involvement In Systemic Lupus. Medicine 57:371-410, 1978.

70. Magil AB: Tubulointerstitial Lesions In Human Membranous Glomerulonephritis: Relationship To Proteinuria. Amer J Kid Dis 25:375-379, 1995.

71. Diamond JR, Anderson S: Irreversible Tubulointerstitial Damage Associated With Chronic Aminonucleoside Nephrosis: Amelioration By Angiotensin I Converting Enzyme Inhibition. Am J Pathology 137:1323-1332, 1990.

72. Eddy AA, McCulloch L, Liu E, Adams J: A Relationship Between Proteinuria And Acute Tubulointerstitial Disease In Rats With Experimental Nephrotic Syndrome. Am J Pathology 138:1111-1123, 1991.

73. Eddy AA: Interstitial Nephritis Induced By Protein-Overload Proteinuria. Am J Pathology 135:719-733, 1989.

74. Floege J, Hackmann B, Kliem V, Kriz W, Alpers CE, Johnson RJ, Kuhn KW, Koch K, Brunkhorst R: Age-Related Glomerulosclerosis And Interstitial Fibrosis In Milan Normotensive Rats: A Podocyte Disease. Kidney International 51:230-243, 1997.

75. Eddy AA, Ho GC, Thorner PS: The Contribution of Antibody-Mediated Cytotoxicity And Immune-Complex Formation To Tubulointerstitial Disease In Passive Heymann Nephritis. Clinical Immunology and Immunopathology 62:42-55, 1992.

76. Mendrick DL, Noble B, Brentjens JR, Andres GA: Antibody-Mediated Injury To Proximal Tubules In Heymann Nephritis. Kidney International 18:328-343, 1980.

77. Brodkin M, Noble B: Antibody-Mediated Proliferation Of Proximal Tubule Cells. Clin Exp Immunol 71:107-112, 1988.

78. Maack T, Park CH, Camergo M: Renal Filtration, Transport, And Metabolism Of Proteins, In: The Kidney: Physiology And Pathophysiology. (2nd ed.). New York, Raven Press, 1992, pp 3005-3038

79. Eddy AA: Experimental Insights Into The Tubulointerstitial Disease Accompanying Primary Glomerular Lesions. JASN 5:1273-1287, 1994.

80. Ehrenreich H, Anderson RW, Fox CH, Rieckmann P, Hoffman GS, Travis WD, Coligan JE, Kehrl JH, Fauci AS: Endothelins, Peptides With Potent Vasoactive Properties, Are Produced By Human Macrophages. J Exp Med 172:1741-1748, 1990.

81. Kohan DE: Endothelin Synthesis By Rabbit Renal Tubule Cells. Am J Physiol 261:F221-F226, 1991.

82. Ishii K, Chang B, Kerwin Jr. JF, Wagenaar FL, Huang Z, Murad F: Formation Of Endothelium-Derived Relaxing Factor In Porcine Kidney Epithelial LLC-PK_1 Cells: An Intra-and Intercellular Messenger For Activation Of Soluble Guanylate Cyclase. J Pharm and Exp Therap 256:38-43, 1991.

83. Zager RA, Teubner EJ, Adler S: Low Molecular Weight Proteinuria Exacerbates Experimental Ischemic Renal Injury. Laboratory Investigation 56:180-188, 1987.

84. Zager RA: Myoglobin Depletes Renal Adenine Nucleotide Pools In The Presence And Absence Of Shock. Kidney International 39:111-119, 1991.

85. Shoskes DA, Parfrey NA, Halloran PF: Increased Major Histocompatibility Complex Antigen Expression In Unilateral Ischemic Acute Tubular Necrosis In The Mouse. Transplantation 49:201-207, 1990.

86. Shackleton C: Effect Of Recovery From Ischemic Injury On Class I And Class II MHC Antigen Expression. Transplantation 49:641-644, 1990.

87. Sponsel HT, Alfrey AC, Hammond WS, Durr JA, Ray C, Anderson RJ: Effect Of Iron On Renal Tubular Epithelial Cells. Kidney International 50:436-444, 1996.

88. Alfrey AC, Froment DH, Hammond WS: Role Of Iron In The Tubulo-Interstitial Injury In Nephrotoxic Serum Nephritis. Kidney International 36:753-759, 1989.

89. Nankivell BJ, Tay Y, Boadle RA, Harris DCH: Dietary Protein Alters Tubular Iron Accumulation After Partial Nephrectomy. Kidney International 45:1006-1013, 1994.

90. Harris D, Chen J: Monocyte Chemoattractant Protein-1 (MCP-1) mRNA Expression In Response To Protein In Rat Proximal Tubule Cells In Culture. JASN 6:1015, 1995.

91. Sacks SH, Zhou W, Sheerin NS: Complement Synthesis In The Injured Kidney: Does It Have A Role In Immune Complex Glomerulonephritis? JASN 7:2314-2319, 1996.

92. Halperin JA, Taratuska A, Nicholson-Weller A: Terminal Complement Complex C5b-9 Stimulates Mitogenesis in 3T3 Cells. J Clin Invest 91:1974-1978, 1993.

93. Schulze M, Donadio JV, Pruchno CJ, Baker PJ, Johnson RJ, Stahl RAK, Watkins S, Martin DC, Wurzner R, Gotze O, et al: Elevated Urinary Excretion Of The C5b-9 Complex In Membranous Nephropathy. Kidney International 40:533-538, 1991.

94. Ogrodowski JL, Hebert LA, Sedmak D, Cosio FG, Tamerius J, Kolb W: Measurement Of SC5b-9 In Urine In Patients With The Nephrotic Syndrome. Kidney International 40:1141-1147, 1991.

95. Camussi G, Rotunno M, Segoloni G, Brentjens JR, Andres GA: *In Vitro* Alternative Pathway Activation Of Complement By The Brush Border Of Proximal Tubules Of Normal Rat Kidney. The Journal of Immunology 128:1659-1663, 1982.

96. Brooimans RA, Stegmann APA, van Dorp WT, van der Ark AAJ, van der Woude FJ, Van es LA, Daha MR: Interleukin 2 Mediates Stimulation Of Complement C3 Biosynthesis In Human Proximal Tubular Epithelial Cells. J Clin Invest 88:379-384, 1991.

97. Welch TR, Beischel LS, Witte DP: Differential Expression Of Complement C3 And C4 In The Human Kidney. J Clin Invest 92:1451-1458, 1993.

98. Welch TR, Beischel LS, Frenzke M, Witte D: Regulated Expression Of Complement Factor B In The Human Kidney. Kidney International 50:521-525, 1996.

99. Rustom R, Jackson MJ, Critchley M, Bone JM: Tubular Metabolism Of Aprotinin ^{99m}Tc And Urinary Ammonia: Effects Of Proteinuria. Miner Electrolyte Metab 18:108-112, 1992.

100. Nath KA, Hostetter MK, Hostetter TH: Ammonia-Complement Interaction In The Pathogenesis Of Progressive Renal Injury. Kidney International 36 (Suppl 27):S-52-S-54, 1989.

101. Nath KA, Hostetter MK, Hostetter TH: Pathophysiology Of Chronic Tubulo-Interstitial Disease In Rats: Interaction Of Dietary Acid Load, Ammonia, And Complement Component C3. J Clin Invest 76:667-675, 1985.

102. Peruzzi L, Trusolino L, Amore A, Gianoglio B, Cirina P, Basso G, Emancipator SN, Marchisio PC, Coppo R: Tubulointerstitial Responses In The Progression Of Glomerular Diseases: Albuminuria Modulates $\alpha_v\beta_5$ Integrin. Kidney International 50:1310-1320, 1996.

103. Remuzzi G, Giuseppe R, Ruggenenti P, Benigni A: Understanding The Nature Of Renal Disease Progression. Kidney International 51:2-15, 1997.

104. Kohan DE: Endothelins In The Normal And Diseased Kidney. Am J Kid Dis 29:2-26, 1997.

105. Keane WF: Lipids And The Kidney. Kidney International 46:910-920, 1994.

106. Lee HS, Kim BC, Kim YS, Choi KH, Chung HK: Involvement Of Oxidation In LDL-Induced Collagen Gene Regulation In Mesangial Cells. Kidney International 50:1582-1590, 1996.

107. Grone H, Hohbach J, Grone EF: Modulation Of Glomerular Sclerosis And Interstitial Fibrosis By Native And Modified Lipoproteins. Kidney International 49 (Suppl 54):S-18-S-22, 1996.

108. Eddy AA: Expression Of Genes That Promote Renal Interstitial Fibrosis In Rats With Proteinuria. Kidney International 49 (Suppl 54):S-49-S-54, 1996.

109. El Nahas AM, Muchaneta-Kubara EC, Zhang GZ, Adam A, Goumenos D: Phenotypic Modulation Of Renal Cells During Experimental And Clinical Renal Scarring. Kidney International 49 (Suppl 54):S-23-S-27, 1996.

110. Diamond JR, van Goor H, Ding G, Engelmyer E: Myofibroblasts In Experimental

Hydronephrosis. Am J Pathol 146:121-129, 1995.

111. Alpers CE, Hudkins KL, Floege J, Johnson RJ: Human Renal Cortical Interstitial Cells With Some Features Of Smooth Muscle Cells Participate In Tubulointerstitial And Crescentic Glomerular Injury. JASN 5:201-210, 1994.

112. Goumenos DS, Brown CB, Shortland J, El Nahas AM: Myofibroblasts, Predictors Of Progression Of Mesangial IgA Nephropathy ? Nephrol Dial Transpl 9:1418-1425, 1994.

113. Hewitson TD, Becker GJ: Interstitial Myofibroblasts In IgA Glomerulonephritis. Am J Nephrol 15:111-117, 1995.

114. Pichler RH, Hugo C, Shankland SJ, Reed MJ, Bassuk JA, Andoh TF, Lombardi DM, Schwartz SM, Bennett WM, Alpers CE, et al: SPARC Is Expressed In Renal Interstitial Fibrosis And In Renal Vascular Injury. Kidney International 50:1978-1989, 1996.

115. Zhang G, Moorhead PJ, El Nahas AM: Myofibroblasts And The Progression Of Experimental Glomerulonephritis. Exp Nephrol 3:308-318, 1995.

116. Nouwen EJ, et al: Hyperplasia, Hypertrophy, And Phenotypic Alterations In The Distal Nephron After Acute Proximal Tubular Injury In The Rat. Laboratory Investigation 70:479-493,1994.

117. Wiggins R, Goyal M, Merritt S, Killen PD: Vascular Adventitial Cell Expression Of Collagen I Messenger Ribonucleic Acid In Antiglomerular Basement Membrane Antibody-Induced Crescentic Nephritis In The Rabbit. Laboratory Investigation 68:557-565, 1993.

118. Alpers CE, Seifert RA, Hudkins KL, Johnson RJ, Bowen-Pope DF: PDGF-Receptor Localizes To Mesangial, Parietal Epithelial, And Interstitial Cells In Human And Primate Kidneys. Kidney International 43:286-294, 1993.

119. Gesualdo L, Di Paolo S, Miani S, Pinzani M, Grappone C, Ranieri E, Pannarale G, Schena FP: Expression Of Platelet-Derived Growth Factor Receptors In Normal And Diseased Human Kidney. J Clin Invest 94:50-58, 1994.

120. Fellstrom B, Klareskog L, Heldin CH, Larsson E, Ronnstrand L, Terracio L, Tufveson G, Wahlberg J, Rubin K: Platelet-Derived Growth Factor Receptors In The Kidney - Upregulated Expression In Inflammation. Kidney International 36:1099-1102, 1989.

121. Tang WW, Ulich TR, Lacey DL, Hill DC, Qi M, Kaufman SA, Van GY, Tarpley JE, Yee JS: Platelet-Derived Growth Factor-BB Induces Renal Tubulointerstitial Myofibroblast Formation And Tubulointerstitial Fibrosis. Am J Pathol 148:1169-1180, 1996.

122. Eddy AA: Molecular Insights Into Renal Interstitial Fibrosis. JASN 7:2495-2508, 1996.

123.Eddy AA: Interstitial Inflammation And Fibrosis In Rats With Diet-Induced Hypercholesterolemia. Kidney International 50:1139-1149, 1996.

124. Border W, Noble N: Transforming Growth Factor β In Tissue Fibrosis. NEJM 331:1286-1292, 1994.

125. Sharma K, Ziyadeh FN: The Emerging Role of Transforming Growth Factor-β In Kidney Diseases. Am J Physiol 266:F829-F842, 1994.

126. Alvarez RJ, Sun MJ, Haverty TP, Iozzo RV, Myers JC, Neilson EG: Biosynthetic And Proliferative Characteristics Of Tubulointerstitial Fibroblasts Probed With Paracrine Cytokines. Kidney International 41:14-23, 1992.

127. Atkins RC, Nikolic-Paterson DJ, Song Q, Lan HY: Modulators Of Crescentic Glomerulonephritis. JASN 7:2271-2278, 1996.

128. Lewis EJ, Hunsicker LG, Bain RP, Rohde RD: The Effect Of Angiotensin-Converting-Enzyme Inhibition On Diabetic Nephropathy. NEJM 329:1456-1462, 1993.

129. Viberti G, Morgenen CE, Groop LC, Pauls JF: Effect Of Captopril On Progression To Clinical Proteinuria In Patients With Insulin-Dependent Diabetes Mellitus And Microalbuminuria. JAMA 271:275-279, 1994.

130. Bakris GL, Copley JB, Vicknair N, Sadler R, Leurgans S: Calcium Channel Blockers Versus Other Antihypertensive Therapies On Progression Of NIDDM Associated Nephropathy. Kidney International 50:1641-1650, 1996.

131. Maschio G, Alberti D, Janin G, Locatelli F, Mann JFE, Motolese M, Ponticelli C, Ritz E, Zucchelli P: Effect Of The Angiotensin-Converting-Enzyme Inhibitor Benazepril On The Progression Of Chronic Renal Insufficiency. NEJM 334:939-945, 1996.

132. Zeller K, Whittaker E, Sullivan L, Raskin P, Jacobson HR: Effect Of Restricting Dietary Protein On The Progession Of Renal Failure In Patients With Insulin-Dependent Diabetes Mellitus. NEJM 324:78-84, 1991.

133. Fouque D, Laville M, Boissel JP, Chifflet R, Labeeuw M, Zech PY: Controlled Low Protein Diets In Chronic Renal Insufficiency: Meta-Analysis. Brit Med J 304:216-220, 1992.

134. Pedrini MT, Lewey AS, Lau J, Chalmer TC, Wang PH: The Effect Of Dietary Protein Restriction On The Progression Of Diabetic And Nondiabetic Renal Diseases: A Meta-Analysis.

Ann Intern Med 124:627-632, 1996.

135. Brenner BM, Lawler EV, Mackenzie HS: The Hyperfiltration Theory: A Paradigm Shift In Nephrology. Kidney International 49:1774-1777, 1996.

136. ter Wee PM, Epstein M: Angiotensin-Converting Enzyme Inhibitors And Progression Of Nondiabetic Chronic Renal Disease. Arch Intern Med 153:1749-1759, 1993.

137. Kasiske BL, Kalil RSN, Ma JZ, Liao M, Keane WF: Effect Of Antihypertensive Therapy On The Kidney In Patients With Diabetes: A Meta-Regression Analysis. Ann Intern Med 118:129-138, 1993.

138. Giachelli CM, Pichler R, Lombardi D, Denhardt DT, Alpers CE, Schwartz SM, Johnson RJ: Osteopontin Expression In Angiotensin II-Induced Tubulointerstitial Nephritis. Kidney International 45:515-524, 1994.

139. Egido J: Vasoactive Hormones And Renal Sclerosis. Kidney International 49:578-597, 1996.

140. Wolf G, Mueller E, Stahl RAK, Ziyadeh FN: Angiotensin II-Induced Hypertrophpy Of Cultured Murine Proximal Tubular Cells Is Mediated by Endogenous Transforming Growth Factor-β. J Clin Invest 92:1366-1372, 1993.

141. Rosenberg ME, Smith LJ, Correa-Rotter R, Hostetter TH: The Paradox Of The Renin-Angiotensin System In Chronic Renal Disease. Kidney International 45:403-410, 1994.

142. Ruiz-Ortega M, Gonzalez S, Seron D, Condom E, Bustos C, Largo R, Gonnzalez E, Ortiz A, Egido J: ACE Inhibition Reduces Proteinuria, Glomerular Lesions And Extracellular Matrix

Production In a Normotensive Rat Model Of Immune Complex Nephritis. Kidney International 48:1778-1791, 1995.

143. Diamond JR, Anderson S: Irreversible Tubulointerstitial Damage Associated With Chronic Aminonucleoside Nephrosis. Am J Pathol 137:1323-1332, 1990.

144. Norman JT, Gallego C, Bridgeman DA, Fogo A, Fine LG: Enalapril Ameliorates Interstitial Fibrosis In The Remnant Kidney Of The Rat. JASN 3:746, 1992.

145. Inserra F, Romano LA, de Cavanagh EMV, Ercole L, Ferder LF, Gomez RA: Renal Interstitial Sclerosis In Aging: Effects Of Enalapril And Nifedipine. JASN 7:676-680, 1996.

146. Bennett WM, DeMattos A, Meyer MM, Andoh T, Barry JM: Chronic Cyclosporine Nephropathy: The Achilles' Heel Of Immunosuppressive Therapy. Kidney International 50:1089-1100, 1996.

147. Diamond JR: Macrophages And Progressive Renal Disease In Experimental Hydronephrosis. AJKD 26:133-140, 1995.

148. Diamond JR, Kees-Folts D, Ricardo SD, Pruznak A, Eufemio M: Early And Persistent Up-Regulated Expression Of Renal Cortical Osteopontin In Experimental Hydronephrosis. Am J Pathol 146:1455-1466, 1995.

149. Ricardo SD, Levinson ME, DeJoseph MR, Diamond JR: Expression Of Adhesion Molecules In Rat Renal Cortex During Experimental Hydronephrosis. Kidney International 50:2002-2010, 1996.

150. Klahr S, Ishidoya S, Morrissey J: Role Of Angiotensin II In The Tubulointerstitial Fibrosis Of Obstructive Nephropathy. AJKD 26:141-146, 1995.

151. Ishidoya S, Morrissey J, McCracken R, Klahr S: Delayed Treatment With Enalapril Halts Tubulointerstitial Fibrosis In Rats Wtih Obstructive Nephropathy. Kidney International 49:1110-1119, 1996.

152. Keilani T, Schlueter WA, Levin ML, Batlle DC: Improvement Of Lipid Abnormalities Associated With Proteinuria Using Fosinopril, An Angiotensin-Converting Enzyme Inhibitor. Ann Intern Med 118:246-254, 1993.

153. Fukui M, Nakamura T, Ebihara I, Nagaoka I, Tomino Y, Koide H: Low-Protein Diet Antenuates Increased Gene expression Of Platelet-Derived Growth Factor And Transforming Growth Factor-β In Experimental Glomerular Sclerosis. J Lab Clin Med 121:224-234, 1993.