

Altered Glomerular Permeability to Plasma Proteins

Abnormal excretion of protein in the urine is the hallmark of experimental and clinical glomerular disease. Whereas immune complex deposition and resulting inflammation account for abnormal permeability of the glomerular filtration barrier to proteins in glomerulonephritis, studies in rats subjected to extensive renal ablation have shown loss of glomerular barrier function to proteins of similar molecular size, yet in the apparent absence of primary immune-mediated renal injury or inflammatory response. Sieving studies using dextrans and other macromolecules in rats 7 or 14 days after five-sixths nephrectomy revealed loss of both size and charge-selectivity of the glomerular filtration barrier. Ultrastructural examination of the remnant kidneys revealed detachment of glomerular endothelial cells and visceral epithelial cells

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Figure 43-5 Scheme hypothesizing the interaction of hemodynamic and nonhemodynamic factors in the "common pathway" of mechanisms contributing to progressive nephron loss in chronic renal disease. P_{GC} , glomerular capillary hydraulic pressure; SNGFR, single-nephron glomerular filtration rate. (Reproduced from Mackenzie HS, et al: In Brenner BM [ed]: *The Kidney*, 6th ed. Philadelphia, WB Saunders, 2000.)

from the glomerular basement membrane. In addition, protein reabsorption droplets and attenuation of cytoplasm resulting in bleb formation was observed in podocytes. The authors concluded that the altered permeability may be caused, in part, by separation of endothelial cells from the glomerular basement membrane, allowing access of macromolecules, and, in part, to loss of anionic sites in the lamina rara externa, resulting in both loss of charge-selectivity and detachment of podocytes.^[460] A direct role for AII in modulating glomerular capillary permeability is suggested by the observation of marked increases in urinary protein excretion during infusion of AII in normal rats.^{[461] [462]} Whereas one group of investigators has attributed this to a direct effect of AII on the cellular components of the glomerular filtration barrier, resulting in opening of interendothelial junctions and epithelial cell disruption,^[461] others have shown that the increase in proteinuria may be accounted for almost completely by the associated hemodynamic changes, principally a reduction in Q_A and an increase in filtration fraction.^[462] On the other hand, the notion that AII may mediate changes in glomerular permeability independent of its effects on glomerular hemodynamics is supported by studies in an isolated perfused rat kidney preparation in which infusion of AII augmented urinary protein excretion and enhanced the clearance of tracer macromolecules independent of any change in filtration fraction.^[424]

Proteinuria, long considered simply a marker of glomerular injury, has been implicated in experimental studies as an effector of injury processes involved in renal disease progression, especially those resulting in tubulointerstitial fibrosis. In rats with aminonucleoside-induced nephrotic syndrome the proteinuric phase of the disease was associated with an acute interstitial nephritis, the intensity of which correlated closely with the severity of the proteinuria.^{[463] [464]} Furthermore, in an overload proteinuria model induced by daily intraperitoneal administration of bovine serum albumin to uninephrectomized rats, proximal tubule cell injury and interstitial infiltration of macrophages and lymphocytes were evident after 1 week.^[465] The severity of the proteinuria showed a positive correlation with the intensity of the infiltrate. At 4 weeks, focal areas of chronic interstitial inflammation were noted.^[465] A causative association between excessive proteinuria and interstitial inflammation has been suggested by in vitro studies of proximal tubule epithelial cells cultured in media supplemented with high concentrations of albumin, IgG, or transferrin. Cellular uptake of these proteins was observed to increase secretion of ET-1,^[466] MCP-1,^[467] and RANTES.^[468] Electrophoretic mobility shift assay of cell nucleus extracts in the latter study revealed intense activation of the transcription factor NF- κ B that was dependent on the concentration of protein in the medium. Furthermore, the liberation of these molecules was noted to be predominantly from the basolateral aspect of the cells. This would be in keeping with secretion into the renal interstitium in vivo, thereby contributing to the development of tubulointerstitial inflammation and fibrosis. It has been proposed that the mechanism for cytokine induction in renal tubule epithelial cells that ingest excessive amounts of protein resembles that operating in virus-infected cells, where accumulation of viral protein in the endoplasmic reticulum activates the transcription factor NF- κ B.^{[404] [469]} Alternatively, the increased lysosomal enzyme activity associated with protein ingestion may result in leakage of lysosomal enzymes into the cell cytoplasm causing cell injury that could then provoke reactive inflammation and scarring.^{[470] [471] [472] [473]}

The relevance of these findings to the processes occurring in vivo has been borne out by studies in rats. In the protein-overload model, the development of proteinuria at 1 week was associated with significant increases in TGF- β at both protein and mRNA levels, in interstitial as well as proximal tubule cells.^[474] Similarly, renal cortical mRNA levels encoding the macrophage chemoattractant osteopontin were increased on day 4 and immunofluorescence localized increased osteopontin staining to cortical tubules at day 7. MCP-1 and osteopontin mRNA and protein levels were elevated at 2 and 3 weeks. Furthermore, a significant effect of proteinuria on molecules involved in ECM protein turnover was observed. Although mRNA levels for various renal matrix proteins were variable, staining for the proteins in the cortical interstitium increased progressively. Levels of mRNA for the protease inhibitors PAI-1 and TIMP-1 were elevated at 2 weeks, at which time significant renal fibrosis was present.^[474] In other models of proteinuric renal disease including five-sixths nephrectomy and passive Heymann nephritis, accumulation of albumin and IgG by proximal tubule cells occurred before infiltration of the interstitium by macrophages and major histocompatibility complex-II positive

mononuclear cells. The infiltrates localized to areas where proximal tubule cells stained positive for intracellular IgG or where luminal casts were present. Furthermore, proximal tubule cells that stained positive for IgG also showed evidence of increased osteopontin production.^[475] Studies in the five-sixths nephrectomy model have suggested that tubulointerstitial injury may play an important role in the decline of GFR, especially in the late stages of progressive renal injury.^[476] By examining serial sections of remnant kidneys, the investigators were able to show that in association with a doubling in serum creatinine there was a substantial increase in the proportion of glomeruli no longer connected to glomeruli (atubular glomeruli) or connected to atrophic tubules. The majority of these glomeruli were not globally sclerosed, implying that the tubular injury was responsible for the final loss of function in these glomeruli. The authors speculate that the absorption of excess filtered protein may play an important role in this tubular injury.^[476] Earlier findings that renal function is associated more closely with tubulointerstitial injury than glomerulosclerosis in human renal disease suggest that similar mechanisms may operate in human renal disease.^{[477] [478]}

Proteins other than albumin or immunoglobulin may also play a role in the progression of chronic nephropathies. Although normally absent from tubular fluid, complement components C3 and C5b-9 neoantigen were observed along the luminal border of tubule epithelial cells in the protein overload proteinuria model.^[465] To examine the role of filtered complement in renal injury, rats with puromycin aminonucleoside nephrosis were subjected to complement depletion with cobra venom factor or inhibition of complement activation by administration of soluble recombinant human complement receptor type 1, before the onset of proteinuria. In control rats, proximal tubular degeneration, interstitial leukocyte infiltrate, and renal impairment (as assessed by inulin and *p*-aminohippurate [PAH] clearances) occurred at 7 days, together with positive staining for C3 and C5b-9 along the proximal tubule brush border. Both interventions were associated with significantly less tubulointerstitial pathology and greater clearance of PAH but not inulin, whereas the severity of the proteinuria was unaffected, suggesting that filtered complement plays a significant role in the tubulointerstitial injury associated with proteinuria.^[479] High-density (HDL) and low-density (LDL) lipoproteins have been identified in the urine, renal interstitium, and tubule cells in renal biopsy specimens of patients with nephrotic syndrome.^[480] In vitro, cultured human proximal tubule epithelial cells take up LDL and HDL.^[481] Oxidized LDL may cause tubule cell injury, and exposure of tubule epithelial cells to HDL is associated with increased synthesis of ET-1.^{[481] [482]} A role has also been proposed for compounds bound to filtered proteins such as IGF-1, which has been detected in increased amounts in the proximal tubular fluid of rats with doxorubicin nephrosis. Proximal tubule cells cultured in the presence of proximal tubular fluid from nephrotic rats exhibit enhanced cell proliferation and increased secretion of type I and type IV collagen. Both effects were inhibited by neutralizing IGF-1 receptor antibodies.^[483] In experimental models of proteinuric renal disease, filtered proteins have also been found to accumulate in the glomerular mesangium^{[460] [484] [485]} and may therefore contribute to glomerular as well as tubulointerstitial injury. Further support for this notion is derived from a meta-analysis of 57 studies of experimental CRD that found a consistent positive correlation between the severity of proteinuria and the extent of glomerulosclerosis.^[486] Lipoproteins, in particular, accumulate in the glomeruli of patients

with glomerulonephritis.^{[487] [488]} Furthermore, LDL stimulates mesangial cells to proliferate in vitro^{[489] [490]} and enhances mesangial cell synthesis of the ECM protein fibronectin.^[491] LDL exposure is also associated with increased mesangial cell mRNA levels for MCP-1^[491] and PDGF.^[490] Oxidation of LDL by mesangial cells or macrophages may enhance its toxicity.^[489] Thus, accumulation of proteins in the mesangium may stimulate a number of different mechanisms that contribute to glomerulosclerosis.

Although establishing cause-effect relationship between proteinuria and renal injury in humans is difficult, several clinical studies provide strong evidence in support of this notion. A meta-analysis of 17 clinical studies of CRD revealed a positive correlation between the severity of proteinuria and the extent of biopsy-proven glomerulosclerosis.^[486] Observations from the Modification of Diet in Renal Disease (MDRD) trial also suggest that proteinuria is an independent determinant of CRD progression: greater levels of baseline proteinuria were strongly associated with more rapid declines in GFR; and reduction of proteinuria, independent of reduction in blood pressure, was associated with lesser rates of decline in GFR. Furthermore, the degree of benefit achieved by lowering blood pressure below usual target levels was highly dependent on the level of baseline proteinuria.^[492] Similar findings were obtained in the Ramipril Efficacy In Nephropathy (REIN) trial. Higher baseline proteinuria was associated with more rapid rates of decline in GFR. In patients with pretreatment urinary protein excretion in excess of 3 g/day, treatment with ramipril reduced proteinuria to an extent that correlated inversely with the subsequent rate of decline in GFR. Treatment with other antihypertensives to achieve equivalent levels of blood pressure control did not decrease proteinuria and was associated with a higher rate of decline in GFR and an increased risk of reaching the combined end point of doubling of the baseline

serum creatinine or end-stage renal failure.^[327] A meta-analysis that included data from 1860 patients with nondiabetic CRD confirmed these findings and showed that during antihypertensive treatment the current level of proteinuria was a powerful predictor of the combined end point of doubling of baseline serum creatinine or onset of end-stage renal disease (ESRD, relative risk 5.56 for each 1.0 g/day of proteinuria).^[493] Taken together, the evidence from experimental and clinical studies provides credible support for the hypothesis that excessive filtration of proteins due to impaired glomerular permselectivity directly damages the kidney. Whether or not this is so, the close association between the severity of proteinuria and renal prognosis implies that reduction of proteinuria should be regarded as an important independent therapeutic goal in clinical strategies seeking to slow the rate of progression of CRD.