View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

Kidney International, Vol. 38 (1990), pp. 384–394

EDITORIAL REVIEW

Is glomerulosclerosis a consequence of altered glomerular permeability to macromolecules?

Background

Focal and segmental glomerulosclerosis (FSG) can be regarded as a common pathological counterpart of various diseases of the kidney associated with altered glomerular permeability to macromolecules that results in abnormal urinary protein excretion. The human diseases include steroid-resistant nephrotic syndrome, some primary glomerulonephritis, vascular diseases of the kidney, reflux nephropathy and the recently identified nephropathy associated with acquired immunodeficiency syndrome [1]. Several theories have been proposed to clarify the pathogenesis of focal and segmental glomerulosclerosis which is associated with progressive deterioration of renal function. These include changes in glomerular hemodynamics, with increased glomerular pressure and flow [2], activation of coagulation processes in glomerular microvessels [3], lipid deposition [4] and glomerular hypertrophy [5].

There is evidence for and against the role of each of these mechanisms as responsible for glomerulosclerosis, but so far no definite proof is available that any one of them fully explains the process of glomerular obsolescence.

In the present review the possibility of abnormal transit of plasma proteins through the glomerular capillary membrane being the key factor in glomerulosclerosis will be addressed. This view is based on the assumption that each of the mechanisms considered so far in the various experimental settings alters glomerular permselectivity, thus setting in motion a cascade of events ultimately leading to glomerulosclerosis.

Experimental models of altered glomerular permselective properties and evidence that proteinuria correlates with subsequent glomerulosclerosis

Renal mass ablation

When the number of nephrons is reduced in the rat by surgical ablation or infarction, glomerular capillary pressure and flow increase in the remaining nephrons [6, 7]. As a consequence the glomerular filtration rate (GFR) of intact glomeruli rises as a compensatory mechanism to keep the kidney functioning after the reduction of renal mass [7]. However, the sustained increase in glomerular capillary pressure and flow is eventually associated with glomerular injury, as documented by vacuolization of epithelial cells and foot process fusion [8]. In a few months mesangial matrix expands and endothelial cells become detached from basement membrane (GBM) in focal areas; these findings precede a process of progressive sclerosis of the glomerular tuft that leads to deterioration of renal function [9].

Of note is the fact that urinary protein excretion increases in this model—as a consequence of loss of permselective properties of the glomerular barrier—very soon after surgery or infarction and well before glomerulosclerosis develops [8]. The characteristics of altered glomerular selectivity in the remnant kidney have been further investigated by Olson et al [8] studying the fractional clearance of neutral dextrans. These authors found increased fractional clearance values for dextrans larger than 38 Å, but not in the range of 20 to 38 Å molecular radii. This is in keeping with another study [10] showing in the same model a partial loss of the size-selective properties. Besides size-selectivity, charge-selectivity may be altered in the remnant kidney as documented by a defect in the ability to restrict negatively charged macromolecules [8].

The loss of charge-selectivity in remnant kidney may also be explained by an increased number of larger pores which minimize the effect of charge on the passage of macromolecules. The mechanism of the evolution of renal disease to glomerulosclerosis proposed by studies on the ablation model is of great interest as it also applies to other models of renal injury in which the glomerular capillary pressure increases not because of renal mass reduction, but as consequence of diffuse injury to the whole nephron population. This is, for instance, the case of post-salt [11] and mineral corticoid-induced hypertension in the rat [12, 13].

In all the mentioned experimental models of glomerulosclerosis, dietary or pharmacological manipulations which limit the evolution of the disease to glomerulosclerosis also interfere with glomerular barrier permselective properties, as documented by a reduction of urinary protein excretion. Thus, low protein diet or the use of angiotensin converting enzyme inhibitors, enalapril or captopril, protects animals against renal disease progression [8, 14, 15]. This fact, besides being associated with a reduction in the number of sclerotic glomeruli, is also consistently associated with a reduction in proteinuria [8, 14, 15]. A possible interpretation of these findings is that these maneuvers may restore permselective properties of glomerular capillaries.

However, whether proteinuria is simply a marker of the extent of glomerular damage or may itself promote the subsequent evolution of the disease to sclero-hyalinosis of the glomerular structure has not been clarified so far. Findings of nearly normal glomerular epithelial cell structure and function in rats with remnant kidneys three weeks after the ablation procedure, when animals are already proteinuric, suggest that proteinuria precedes the structural glomerular epithelial cell changes detectable by electron microscopy [16]. Changes in

Received for publication November 20, 1989
and in revised form February 26, 1990
Accepted for publication March 6, 1990

© 1990 by the International Society of Nephrology

glomerular epithelial cell structure which follow a few weeks of heavy proteinuria can therefore be regarded as the consequence of increased uptake of abnormally filtered plasma proteins.

Diabetes mellitus

Both in animals and humans, diabetes mellitus is associated with elevated GFR and renal plasma flow in the early phases of the disease [17–23]. Several explanations have been put forward for the early hyperfiltration in experimental diabetes, including hyperglycemia [24, 25], loss of sensitivity of the tubuloglomerular feedback mechanism [25, 26], and possibly humoral agents such as vasodilatory prostaglandins [27, 28] and atrial natriuretic factor [29].

Independently of the mechanism(s) responsible for hyperfiltration, rats with long-standing moderate hyperglycemia develop focal and segmental glomerulosclerosis, always preceded by proteinuria [22, 23]. Because glomerular capillary pressure is increased in these animals and maneuvers that limit glomerular capillary hypertension also limit the progressive renal disease of diabetic rats, it was assumed that the alterations in glomerular membrane permeability were the consequence of changes in glomerular hemodynamics [22, 23]. However, increased glomerular capillary pressure is not a requisite for the development of glomerular injury in experimental diabetes, as demonstrated by severely hyperglycemic diabetic rats not given supplemental insulin [30, 31] that have reduced single nephron glomerular filtration rate (SNGFR) and capillary hydraulic pressure, despite persistent proteinuria.

The relation between urinary protein excretion and glomerular injury has been explored in another strain of rats, the BioBreeding/Worcester (BB/W) rats, which develop spontaneous autoimmune insulitis, insulinopenia and hyperglycemia, mimicking the syndrome of type I diabetes in humans [32, 33]. These rats develop renal hypertrophy [34, 35], progressive thickening of GBM, which is greater in rats with poor glycemic control than in rats with moderate hyperglycemia. However, unlike the majority of insulin-dependent diabetes mellitus (IDDM) patients and streptozotocin-induced diabetic rats with severe hyperglycemia, kidneys of the BB/W diabetic rats do not have mesangial expansion [34, 36]. In this strain of rats proteinuria occurs and correlates with the degree of glycemic control and GBM widening [36]. Of note, whole-animal studies of this particular strain of diabetic rats have reported no [35] or moderate [34] glomerular hyperfiltration, whether GFR was factored or not for kidney weight. However, direct measurements of the determinants of SNGFR in diabetic BB/W rats have not been performed, making difficult the comparison with the glomerular hemodynamic changes reported in the streptozotocin diabetes.

Early hyperfiltration followed by glomerular structural injury is not peculiar to streptozotocin diabetic rats but is also a feature of human diabetes mellitus [17–19]. Moreover, 30% of patients with insulin-dependent diabetes develop microalbuminuria in the early phase of the disease and subsequently overt nephropathy [17, 37, 38]. It is generally believed that early hyperfiltration is a marker of progression of the disease to overt diabetic nephropathy thus raising the question of the relationship between hemodynamic changes—and possibly the increase in glomerular capillary pressure—and the subsequent development of glomerulosclerosis [17].

At variance with abundance of data in experimental animals, there are a few studies correlating urinary protein excretion with the degree of glomerular injury in human diabetes mellitus. It is known that in man the development of microalbuminuria is a reliable marker of the evolution of the disease to overt nephropathy [39, 40], while patients with diabetes and normal urinary albumin do not develop renal disease [41, 42]. Of note, very slight elevation of blood pressure occurs in insulin dependent diabetics with normal urinary albumin excretion while high blood pressure is commonly observed in diabetics with heavy proteinuria [43]. Thus, when mean blood pressure was plotted against urinary albumin excretion rate in patients with insulin dependent diabetes with or without diabetic nephropathy and, within the latter group, with incipient or overt nephropathy a highly significant correlation was found [43]. Thus, it is not surprising that when diabetic patients develop proteinuria in a nephrotic range renal function rapidly deteriorates to end-stage renal failure [44, 45]. This has been carefully documented by Goldstein and Massry [46], who noted that in a large population of diabetics with nephrotic proteinuria end-stage renal failure rose in less than three years. The hypothesis of a beneficial effect of antihypertensive treatment on the progression of renal disease in diabetic patients with established nephropathy was recently confirmed by Parving and coworkers [47]. These authors demonstrated that the institution of an aggressive antihypertensive treatment, with reduction in mean arterial pressure from approximately 110 to 100 mm Hg, was able to reduce the rate of decline in the GFR on average from 0.9 to 0.2 ml/min/month and albuminuria from about 1000 to 500 μg/min.

Of note, the favorable effect of antihypertensive therapy on progression of the renal disease is always associated with a marked reduction of urinary protein excretion. This and others studies [48, 49] do not allow definitive conclusions, but are consistent with the possibility that antihypertensive therapy in this setting may retard the progression of diabetic nephropathy by an effect mediated by reduction in proteinuria. It is, however, of great interest that both in diabetic animals and humans maneuvers that retard glomerular structural injury also reduce proteinuria. Angiotensin converting enzyme inhibitors, that lower capillary hydraulic pressure and reduce glomerular damage in diabetic rats [23, 50] and slow the decline in renal function in human diabetic nephropathy [51, 52], also reduce urinary protein excretion rate. These findings would suggest that in diabetes, as in most other forms of glomerulosclerosis, abnormal glomerular permeability to proteins is a determinant of progressive renal disease.

This interpretation is consistent with the observation that in rats with streptozotocin-induced diabetes high-protein diet markedly increases urinary albumin excretion and is associated with a high incidence of glomerular lesions, while restriction of dietary proteins prevented both albuminuria and glomerulosclerosis [22]. Further studies [23] showed that chronic treatment with the angiotensin converting enzyme inhibitor, enalapril, had a protective effect similar to that of dietary protein restriction. In sharp contrast with the nearly exponential progression of albuminuria in untreated diabetic rats, enalapril treated animals have almost normally preserved glomerular selective properties as well as minimal glomerular structural changes, indistinguishable from those of control rats of similar age.

That glomerulosclerosis correlates with urinary protein excretion in diabetes has been very convincingly documented by the recent study of Anderson et al [50]. These authors studied the short- and long-term effects of antihypertensive therapy in experimental diabetes and found that compared to untreated diabetic animals which had albuminuria and marked glomerulosclerosis, triple antihypertensive therapy delayed but not prevented albuminuria. Triple therapy also reduced glomerulosclerosis but was less effective than captopril. In the same setting captopril reduced albuminuria to below that of control rats of the same age. Of great interest was the finding that diabetic rats given captopril also had a percentage of sclerotic glomeruli lower than age-matched control rats.

Aging

In certain strains of rats, aging is associated with progressive proteinuria and glomerulosclerosis that subsequently lead to renal insufficiency [53–55]. If the animals are fed a high-protein diet both proteinuria and renal lesions are aggravated [56, 57]. Food restriction or reduction of dietary protein reduces both proteinuria and the age-related renal disease [56, 58–60]. We have recently evaluated the time course of urinary protein excretion in aging rats exposed to diet with protein content ranging from 6% to 35% [61]. A high-protein diet, which is associated with a higher percentage of glomerular sclerotic lesions than the standard diet, was also associated with a significant increase in urinary protein excretion. By contrast low-protein diet, which protected animals from the development of late glomerulosclerosis, also reduced the amount of urinary proteins. At the onset of proteinuria no renal structural abnormalities were detected at the histological examination of the kidney in the various animal groups. A remarkable finding of this study was that three out of ten rats on standard diet (20% protein) that did not develop proteinuria, were also free of renal lesions after up to 20 months of observation. This study also addressed the important issue of whether the injurious effect of proteinuria is 'dose dependent' or is a 'threshold phenomenon'. Actually, for urinary protein excretion values lower than 60 mg/day no development of glomerulosclerosis was observed during the two months of observation, while rats with urinary protein excretion exceeding 100 mg/day for the same period had a degree of glomerulosclerosis proportional to the severity of proteinuria. That glomerulosclerosis correlated with the severity of proteinuria for values of urinary proteins above 100 mg/day in the rat has also been observed in other models [62, 63]. This and other similar studies [53, 54] indicate that proteinuria precedes the development of glomerular sclerotic lesions in aging rats. A potentially important finding of this study is the highly significant positive correlation between the degree of glomerular sclerotic lesions and the tubulointerstitial damage, that is, tubular casts and dilatation, atrophy and interstitial mononuclear cell infiltrates [61]. The association between glomerulosclerosis and tubulointerstitial lesions has been reported by others [64] in aging rats, and probably applies to other models of proteinuria and renal disease progression in the rat, as documented in the remnant kidney model and in experimental nephrosis [65–67]. In other studies [62, 68] a particular strain of rats, Munich-Wistar rats (MWF/Ztm), originally selected for their large number of superficial glomeruli, was used to correlate abnormal urinary protein excretion with glomerular hemodynamics and glomerular lesions. Urinary protein excretion in these rats is higher than in other strains. Moreover, the males had significantly higher urinary protein excretion than females at seven weeks of age, with a progressive increase that reached near 300 mg/24 hr at week 21, while females of the same age had some 20 to 30 mg/24 hr proteinuria [68]. Because in this model the glomerular damage develops spontaneously, unlike in rats undergoing renal mass reduction or given renal toxins, this may be regarded as an ideal model to study the mechanisms underlying renal disease progression. We have recently addressed whether changes in glomerular hemodynamics might explain the abnormal glomerular permeability to proteins of male MWF/Ztm rats. The results showed a 60% higher SNGFR in males than females, single nephron glomerular plasma flow (Q_A) tended to be higher although not significantly so [68], but intraglomerular capillary pressure was comparable in males and females. Although glomerular hypertension was not documented in this model, enalapril ameliorated the progression of glomerular injury by significantly reducing proteinuria and glomerulosclerosis [62]. The beneficial effect of enalapril was associated with a significant reduction of systemic blood pressure. Micropuncture studies revealed that enalapril also reduced glomerular capillary hydraulic pressure to lower than normal values by reducing afferent and efferent arteriolar resistance [62]. It appears unlikely that the reduction in glomerular capillary pressure was responsible for protecting against proteinuria in this model since untreated male and female MWF/ztm rats had comparable glomerular capillary pressure at 18 to 20 weeks of age, but glomerular damage developed only in males. In the above-mentioned study we also documented that enalapril ameliorated glomerular size-selective barrier in this model, as indicated by a significant decrease in filtration of neutral dextran macromolecules of graded sizes with effective molecular radius ranging from 26 to 64 Å [62]. The theoretical analysis of experimental data [62] documented that converting enzyme (CE) inhibition uniformly reduced the radius of all membrane pores and that this reduction was predominant for pores having radii around the mean. These data suggest that enalapril exerts a direct beneficial effect on intrinsic membrane permeability properties. The fact that the resulting reduction in urinary protein excretion was associated with a marked protection against the development of glomerular sclerotic lesions [62] would indicate that the two phenomena may be causally related.

Adriamycin and puromycin nephrosis

Adriamycin (ADR) and aminonucleoside of puromycin (PA) both induce changes in glomerular capillary selective properties in the rat, with proteinuria, overt nephrotic syndrome, and morphological features reminiscent of human "minimal change" nephropathy [69–74]. Animals given ADR or PA develop glomerulosclerosis within a few months [75–77]. As in the other models of chronic proteinuria discussed above, most of these studies documented a close relationship between tubulointerstitial changes [67, 75] and the development of FSG, and clarified that interstitial changes and casts always preceded the onset of FSG [78]. Similarly to ADR, in the rat PA induces a nephrotic syndrome with ultrastructural findings of epithelial cell damage which include foot process fusion. The disease

manifests with progressive proteinuria associated with various degrees of tubulointerstitial changes and evolution to glomerulosclerosis [66].

Measurements of intraglomerular capillary pressure in ADR or PA nephrosis have led to conflicting results so far. Fogo et al [79] have measured normal glomerular capillary hydraulic pressure during the course of ADR nephrosis, while O'Donnell et al [80] and more recently Scholey et al [67] measured an increased intraglomerular capillary pressure in ADR treated compared to non-treated animals. In ADR nephrosis Scholey et al [67] demonstrated that enalapril preserved GFR compared to the untreated animals, without beneficial effect on proteinuria and glomerulosclerosis. In another recent study Beukers et al [81] confirmed that ADR animals treated with captopril were not protected from proteinuria and glomerulosclerosis.

In PA nephrosis Fogo et al [79] found normal intraglomerular capillary pressure in the early phase of the disease. Of interest in this study is that enalapril reduced the extension of glomerulosclerosis despite the lack of glomerular hypertension. This study is challenged by the work of Anderson et al [63], who found that glomerular capillary pressure in the early phase of PA nephrosis is normal but it increases later in the course of the disease. In the latter phase the use of an angiotensin converting enzyme inhibitor lessened both abnormal urinary protein excretion and glomerulosclerosis, once again raising the possibility that the two phenomena are interdependent.

Overload proteinuria

Repeated injection of bovine serum albumin in the rat increases glomerular barrier permeability properties, inducing proteinuria as the result of the formation of large hypothetical pores through the glomerular capillary [82–86]. Efforts to document immune aggregates in the glomeruli of protein injected animals have given negative results so far, but this maneuver may induce the formation of antibodies to bovine serum albumin so the theoretical possibility that proteinuria in this model results from an immune reaction cannot be excluded. An alternative way to achieve overload proteinuria is the liver hyperplasia that follows rat pituitary tumor transplantation [87]. This raises the blood albumin level through production of adrenocorticotropic and growth hormone secretion. In turn, hyperalbuminemia promotes proteinuria and, a few weeks later, tubulointerstitial lesions and glomerular lesions develop, similar to those of ADR nephrosis and aging rats. Such changes are preceded by extensive epithelial cell abnormalities reminiscent of those found in ADR or PA nephrosis. Findings of normoalbuminemia, normalization of urinary protein excretion and reduction of tubulointerstitial changes and glomerulosclerosis after ablation of the tumor suggest a pathogenetic role of proteinuria in the development of interstitial and glomerular lesions. Whether glomerular structural damage is hemodynamically mediated or is secondary to microvascular thrombosis or tubulointerstitial inflammation cannot be established at the present moment. So far, no studies have been made on glomerular hemodynamics or permeability properties by dextran sieving curves in this model.

Analysis of the experimental data that apparently conflict with the hypothesis that glomerulosclerosis is a consequence of altered glomerular permselective properties

The different models of proteinuria so far analyzed show a clear association between changes in the glomerular permselective properties and glomerulosclerosis. However, in some other instances such a relationship appears less evident. A typical example is possibly the Heymann nephritis in which the series of published papers would indicate that glomerulosclerosis does not occur despite immune deposits on the glomerular capillary wall and proteinuria [88–92]. A critical review of the studies published during the past 15 years, however, shows that this opinion is essentially based on short-term studies which do not exceed three months. Moreover, proteinuria is generally in a range of 50 to 70 mg/day which admittedly is not high enough to promote glomerular sclerotic lesions in other models [88–93]. In the few studies showing a proteinuria in excess of 100 mg/day the animals were only followed for two to three weeks [90, 91], again making it difficult to derive any definitive conclusion. This period is too short to induce FSG, as documented in other models of proteinuria in which even four to five weeks of severe proteinuria failed to induce FSG [69, 71].

More recently, Harris and coworkers [94] have found that uninephrectomized rats injected with aminonucleoside of puromycin have nephrotic syndrome, hypercholesterolemia and focal sclerosis. Treatment with lovestatin, which reduces serum cholesterol, was followed by a lessening of glomerular sclerotic changes without reducing urinary protein excretion. They suggested that hypercholesterolemia might play an important role in the progression of glomerular diseases independently of the degree of proteinuria. However, a careful analysis of these data demonstrates that treated animals with less severe glomerular changes, still had more than 70% of glomeruli affected by moderate or severe sclerotic changes, thus supporting the view that besides hypercholesterolemia proteinuria has a major impact on glomerulosclerosis [94]. This conclusion is also consistent with the recent report of Sanfelice and coworkers [95], who found that analbuminemic diabetic rats had much higher cholesterol levels than diabetic SD animals. Despite the marked hypercholesterolemia, analbuminemic rats developed less glomerulosclerosis than hypercholesterolemic diabetic SD rats. Again, diabetic SD rats with more severe sclerotic changes had a much higher urinary protein excretion (340 ± 45 vs. 131 ± 19.5 mg/day) than hypercholesterolemic diabetic animals.

Reactive mechanisms triggered by an increased protein traffic across the glomerular capillary that may favor the process of glomerulosclerosis

Mesangial cell overload

It has been speculated that increased flux of macromolecules through the mesangium may lead to "mesangial cell overload", mesangial injury and proliferation, which in turn can induce overproduction of mesangial matrix and eventually lead to glomerulosclerosis [96, 97]. Actually mesangial cells in culture have been found to produce mesangial matrix constituents that include collagen types IV and V, laminin and fibronectin [98]. The possibility that abnormal permeability of glomerular barrier, resulting in enhanced mesangial "traffic" of macromolecules, activates mesangial cells and leads to glomerulosclero-

sis is supported by the observation that the development of focal glomerulosclerosis in rats after unilateral nephrectomy [96], as well as in PA nephrosis [97] is related to areas of increased accumulation of a colloidal carbon tracer, so that glomeruli with focal sclerosis contained significantly more carbon than normal glomeruli. It is of interest that a low-protein diet reduced mesangial accumulation of ferritin as well as structural abnormalities in the remnant kidney model [8]. Whether the increased flux of macromolecules simply results from changes in glomerular barrier permselectivity [99], or is the consequence of a delayed mesangial clearance of macromolecules has not been fully clarified. However, the fact that alterations in glomerular pemeability and proteinuria always lead to mesangial accumulation of macromolecules has been challenged by another study showing that, despite comparable degree of proteinuria, only in PA but not ADR nephrosis or Heymann nephritis, an increased glomerular uptake of aggregated IgG was demonstrated [100]. This phenomenon was inhibited by saralasin, suggesting that angiotensin II mediated mesangial uptake of macromolecules in PA nephrosis. In more general terms, the notion that an increased flux of macromolecules through the mesangial area stimulates mesangial cells to proliferate and generates mesangial matrix components has received great emphasis over the last few years. The definitive evidence that this is indeed the case is not available, and factors modulating mesangial cell function in the various models remain ill defined. Certainly more studies are needed to address this important point in order to clarify whether the abnormal traffic of macromolecules can contribute to the process of glomerular obsolescence via mesangial cell activation.

Glomerular epithelial cell damage

All the experimental models of chronic proteinuria described above, as well as their human counterparts (that is, minimal change glomerulopathy, focal and segmental sclerosis, diabetic nephropathy, and nephropathy associated with acquired immunodeficiency syndrome) have in common ultrastructural findings of severe epithelial cell damage that include vacuolization, fusion of foot processes and focal detachment of epithelial cells from the underlying basement membrane [1]. All these changes appear to be the consequence of chronic proteinuria. Schwartz, Bidani and Lewis [101] investigated the relationship between chronic proteinuria and epithelial cell structure and function in rats made proteinuric for up to eight weeks by daily injection of homologous albumin. They documented protein reabsorption droplets and vacuoles, and cytoplasmic hypertrophy but could not detect irreversible epithelial cell damage. No foot process effacement or changes in epithelial cell endocytic function were found despite the active endocytosis of filtered albumin. These results are in sharp contrast with several earlier studies showing that proteinuria causes irreversible structural and functional abnormalities in glomerular epithelial cells [84, 85, 102–104]. This discrepancy has several possible explanations. One is a different response of epithelial cells to protein load that can be maximal early in the course of a given disease, when glomerular ultrafiltrate contains large amount of proteins, and then adjusts in such a way that further increases in filtered proteins are associated with only minor and reversible changes. Since epithelial cell changes have been studied only very early in a given proteinuric condition [101], it is not surprising that studies made later in the course of the diseases gave different results.

Efforts have also been made to clarify the possible relationship between epithelial cell damage and the subsequent development of glomerulosclerosis. It has been proposed that protein-induced injury to the epithelial cell inhibits the release of heparin-like factor(s) that also have growth inhibiting effect and control the abnormal proliferation of mesangial cells after inflammatory, toxic or immunological stimuli [105–107]. Moreover, glomerular epithelial cells, in contrast to endothelial and mesangial ones, do not replicate in response to proliferative stimuli, so that when the glomerular tuft is enlarged—as in some disease processes—only part of the glomerular surface area is covered by hypertrophic podocytes [108–110]. This further contributes to foot process distortion and detachment, further increasing the traffic of macromolecules across the glomerular capillary, given the reduced resistance to convective flow at the extracellular filtration level [1]. Thus, large proteins are trapped by the lamina densa of the GBM, expanding the subendothelial space and promoting the accumulation of hyalin material [1]. Whether accumulation of this material stimulates endothelial cells to produce mesangial matrix components, thus favoring the process of focal glomerulosclerosis in a given glomerulus, is for the time being still a matter for speculation. A recent interesting line of research in this area has attributed to hypertrophy of remnant glomeruli, which developed in the remnant kidney four to six weeks after nephrectomy, a specific role as the determinant of subsequent glomerulosclerosis [5, 110, 111]. Since early hemodynamic pattern does not appear to correlate with subsequent glomerular size in these studies, it has been suggested that enhanced pressure and flow may not be the main determinants of subsequent glomerulosclerosis. Since DNA synthesis preceded increase in GFR [112], the possibility that a circulating hormonal factor is responsible for glomerular hypertrophy has been proposed. Among the heterogeneity of glomerular population a significant positive correlation has been found by Yoshida et al [113] between glomerular size and the degree of glomerulosclerosis. Glomerulosclerosis was attenuated in the above-mentioned study both by enalapril as well as by other antihypertensive therapies. Unfortunately no values of urinary protein excretion are given, but since it has been extensively documented that in remnant kidney as well as in other models enalapril has a marked antiproteinuric effect, the possibility that proteinuria mediates the process of glomerulosclerosis which predictably follows the process of remnant glomerular hypertrophy cannot be ruled out. Finally the observation that intact nephrons are the primary origin of proteinuria in subtotal nephrectomy [114], while severely damaged glomeruli contribute little to proteinuria, is consistent with the possibility that proteinuria precedes and possibly triggers the development of glomerular structural changes.

Tubulointerstitial damage

In most models of glomerulosclerosis, as well as in the majority of human diseases, the progression of proteinuric conditions to glomerulosclerosis is associated with interstitial infiltration of inflammatory cells, particularly macrophages and lymphocytes [61, 64, 65, 67, 75, 115, 116]. When the sequence of events was monitored by repeated biopsies, as in two of our recent studies on ADR nephrosis and in aging rats [61, 78], it

was documented that interstitial damage preceded the development of sclerotic lesions, thus suggesting a cause and effect type of relationship. Similarly, in another model of proteinuria in spontaneously hypertensive rats the extent of glomerulosclerosis was correlated with tubulointerstitial damage [118]. Tubulointerstitial changes have been recognized many years ago in progressive glomerulonephritis but so far have been regarded as an "epiphenomenon" of glomerular sclerotic lesions. Several recent observations may offer a new tool as for the possible significance of such changes. Thus, serial biopsies performed in aging rats and rats with ADR nephropathy indicated that the massive entry of proteins into the urinary space results in intense protein reabsorption activity of proximal tubular cells, indicated by many reabsorption droplets in the cytoplasm of proximal tubular epithelial cells [61, 78]. This event is in turn followed by the formation of proteinaceous casts at distal points that cause tubular dilatation and obstruction. In consequence of dilatation and increased tubular pressure, tubular basement membranes undergo focal breaks allowing the extravasation of Tamm-Horsfall proteins into the interstitium [78]. Evidence of loss of tubular basement membrane integrity derives from light microscopy examination and from the finding that proteins derived from the urinary space are accumulated in abnormal amount in the interstitium [78]. This may be the trigger for the interstitial inflammatory reaction which in most models precedes and possibly favors the development of glomerular sclerotic changes. Recently Davies et al [119] have shown that Tamm-Horsfall glycoprotein interacts with human polymorphonuclear leucocytes in vitro, activating the respiratory burst and cell degranulation. If confirmed in vivo such an interaction would offer an explanation for the findings of interstitial inflammating reaction seen in association with Tamm-Horsfall protein. Unfortunately only few studies have addressed the cellular characteristics of the interstitial inflammatory reactions. Eddy and Michael [120] in chronic PA and ADR nephrosis found that the cellular composition of the infiltrates was mainly macrophages and T-lymphocytes, the majority of which appear to be T cytotoxic/suppressor cells. In other experimental models as well (that is, reduction of renal mass and aging) infiltrating cells into the interstitium had morphological characteristics of lymphocytes and macrophages [61, 65]. Of interest is that in aging rats mononuclear cell infiltrates were seen in close contact with sclerotic areas of the glomeruli, and inflammatory cells appeared to penetrate the Bowman's capsule through breaks [61].

That proteinuria promotes tubular damage and interstitial inflammation emerges quite clearly in the recent paper by Eddy [121] in a model of overload proteinuria induced by repeated injections of heterologous albumin in the rat. The study demonstrates that after the onset of heavy proteinuria which follows the administration of heterologous albumin, animals showed tubular changes characterized by protein droplets in the cytoplasm of proximal tubular cells, casts and an early infiltration of macrophages and then T lymphocytes in the interstitium. The degree of inflammatory interstitial infiltration, which persisted for the entire period of proteinuria, strictly correlated with the amount of urinary proteins [121]. It has been proposed that mononuclear cell infiltration represents the response to a tubular cell damage induced by proteinuria, possibly related to the leakage of lysosomal enzymes in the cytoplasm of proximal tubular cells as a consequence of an excessive amount of readsorbed proteins. Based on the potential of inflammatory cells to release biologically active mediators of tissue injury, recent data may be taken as to indicate that lympho-monocytes and macrophages surrounding the glomerular tuft have a potentially critical role in modulating the activity of resident renal cells and particularly mesangial cells. Thus macrophage-derived products may stimulate the proliferation of mesangial cells, the synthesis of extracellular matrix, and particularly collagen, which may directly contribute to the process of glomerulosclerosis [122–125]. Besides stimulating mesangial cells to proliferate, macrophage-derived factors stimulate endothelial cells to release inflammatory mediators, including interleukin 1 (IL-1), that promotes the adherence of monocytes to endothelium and stimulates the thromboxane A_2 (TXA_2) release from platelets and possibly immune cells [126]. Interleukin 1 also induces platelet activating factor synthesis from either endothelial or mesangial cells and increases the procoagulant activity of resident glomerular cells [127]. All these are potential stimuli for platelets to release platelet-derived factors among which according to recent studies, platelet derived growth factor (PDGF), transforming growth factor β (TGF_β) and TxA_2 may contribute to the process of mesangial proliferation, extracellular matrix production and glomerular sclerosis [128–130]. In particular in vitro studies have documented that PDGF binds to specific receptors on cultured human mesangial cells and stimulates DNA synthesis and cell proliferation [128]. Whether this mechanism is responsible for the glomerular hypertrophy discussed above is still speculative. The fact that human mesangial cells express mRNA for both A and B chains of PDGF and secrete a PDGF-like protein would suggest that PGDF may perhaps act as an autocrine and paracrine growth factor for these cells [128]. Recent data have indicated that another platelet-derived protein, TGF_β, stimulates extracellular matrix production and regulates the structure of matrix proteoglycans in cultured rat mesangial cells [129]. It is rather interesting to note that TGF_β appears to be unique in this property of inducing glomerular extracellular matrix synthesis and does not share this property with other growth factors, like IL-1, PGDF or TNF. Since TGF_β is released by inflammatory cells, it is conceivable that it plays a role in glomerulonephritis and possibly participates in the process of glomerular obsolescence.

Finally a recent study has documented that TxA_2 has a direct effect on the biosynthesis of extracellular matrix proteins inducing a dose-dependent increase in laminin A, B and B_2 chains [130]. This raises the question whether TxA_2, which is released from activated platelets and macrophages during inflammatory reactions, is directly linked to the development of glomerulosclerosis and interstitial fibrosis.

Summary

Numerous studies in the last decade have set out to clarify the process of progressive deterioration of renal function that occurs in animals and humans after a critical reduction in the number of nephron units either by surgical ablation or by various diseases. Most of these studies were stimulated by Brenner, Meyer and Hostetter's provocative review on "Dietary protein intake and the progressive nature of kidney disease" published in the New England Journal of Medicine in 1982 [2]. This view focused for the first time on the possibility of a common hemodynamic pathway responsible for the pro-

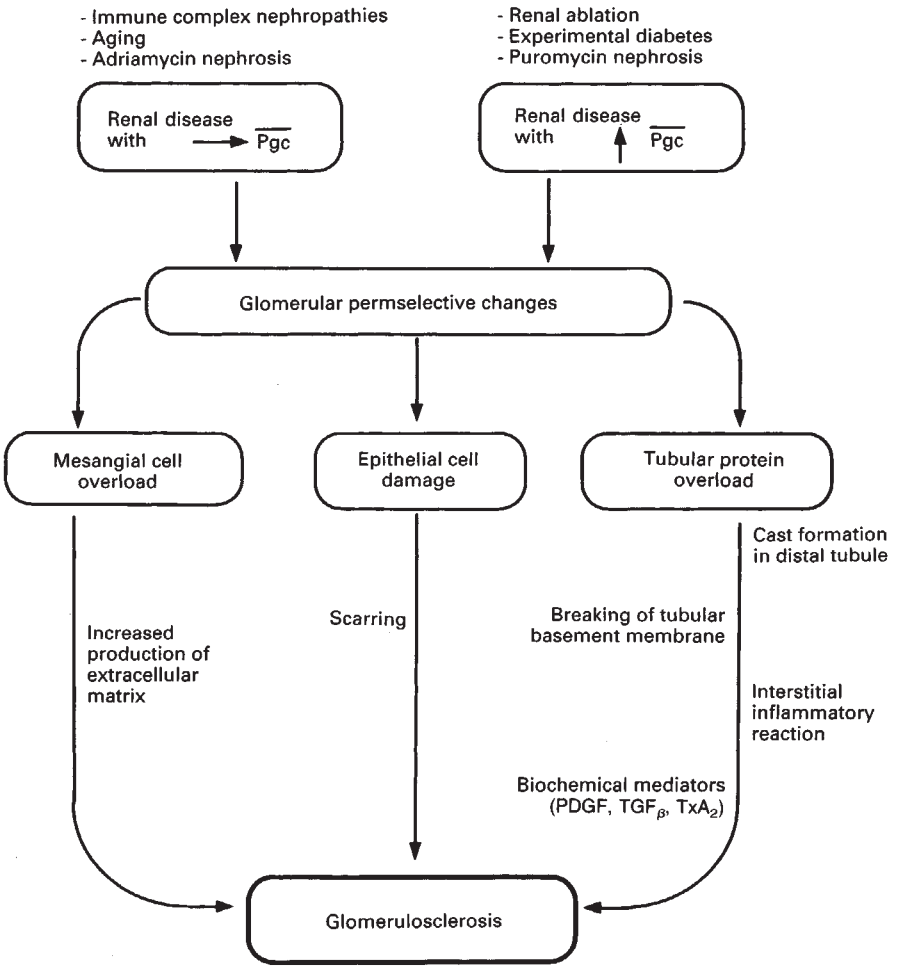

Fig. 1. *Mechanisms by which alterations in glomerular selective properties may lead to glomerulosclerosis as a result of modifications in mesangial, epithelial or tubular cell function.* This scheme applies both to renal diseases with normal or increased intraglomerular capillary pressure.

gression of renal disease independent of the initial injury. According to Brenner's group hypothesis, the fundamental lesion responsible for progressive renal insufficiency was sclerosis of the glomerular capillary tuft resulting from sustained elevation of glomerular pressures and flows in remnant glomeruli after the loss of a critical number of functioning nephrons. Most of the subsequent studies followed this original interpretation. However, since measurement of glomerular capillary pressure did not produce uniform results in the various models [67, 68, 79], some of them challenged the hypothesis of hemodynamically-mediated glomerular injury suggesting alternative mechanisms. Thus disorders of lipid metabolism [4], activation of coagulation and intraglomerular thrombosis [3], intrarenal calcium deposits [131] and glomerular hypertrophy [5], all have been claimed as key determinant for glomerulosclerosis. Indeed, in some models correction of these abnormalities prevented renal disease progression [113, 132–135]. However, none of these studies convincingly clarified the issue of a common determinant of renal disease progression. This review offers an integrated interpretation of renal disease progression, at least in rat models. We have documented that glomerular obsolescence induced by surgery, aging or some toxins share a sustained abnormality in glomerular permeability to macromolecules that is long lasting and can be regarded as the common determinant of glomerulosclerosis more than as a consequence of glomerular injury. There are at least two pathways of disruption of glomerular permselectivity in rat models of glomerulosclerosis (Fig. 1). One is through increased glomerular capillary pressures which determine a mechanical injury to the glomerular capillary barrier and operate for instance in remnant kidney or experimental diabetes [6, 7, 22, 23]. The other is initiated by toxins or immune reactants that may alter glomerular permselectivity even in the absence of increased glomerular capillary pressure [67, 79]. Once glomerular permselectivity has been disrupted, the disease progresses to end-stage renal failure by a process triggered by the exposure of renal cells to the abnormal protein load which does not depend on the initial injury. Thus, mesangial cells exposed to an abnormal protein traffic may proliferate and synthesize mesangial matrix in excessive amounts [96, 97]. Similarly, glomerular epithelial cells undergo structural and functional modifications, including focal detachment from basement membrane, with further increase of the passage of macromolecules across the glomerular capillaries [1]. This results in an increased amount of proteins in the ultrafiltrate that overwhelms proximal tubular epithelial cell reabsorption capacity [78]. The abnormal amount of proteins delivered to distal tubuli leads to the formation of casts breaking off the tubular basement membrane, and to an interstitial

inflammatory reaction [78]. Inflammatory cells surrounding the glomeruli that subsequently become sclerotic very probably play a major role in this process [61, 78]. They may do so by releasing factors that can—as has been recently documented—induce mesangial cell proliferation and promote abnormal generation of mesangial matrix [128–130]. If this interpretation is correct, maneuvers that limit abnormalities in glomerular capillary permeability will have a pivotal role in retarding renal disease progression, at least in experimental models. Whether these concepts apply to human renal disease progression is a possibility that merits appropriate investigation. With appropriate experiments the question of whether glomerulosclerosis is a consequence of alterations in membrane permselective properties may hopefully have a definitive answer in the near future, at least in animals. The most promising model appears the model of spontaneous glomerulosclerosis occurring in male MWF/ztm. The possibility to reduce both proteinuria and glomerulosclerosis with enalapril in such a model, which is devoid of glomerular capillary hypertension, represents a powerful investigative tool [62]. One can design experiments with different doses of enalapril in order to modulate urinary protein excretion values from very high to normal with some intermediate targets. Should this maneuver result in a varying degree of glomerular sclerotic lesion from 30% to almost zero with intermediate values consistent with the levels of urinary protein excretion, this would certainly contribute to validate the present hypothesis.

GIUSEPPE REMUZZI and TULLIO BERTANI
Bergamo, Italy

Acknowledgments

The authors thank Drs. Andrea Remuzzi, Norberto Perico, Carla Zoja, and Mauro Abbate for their important contribution to this manuscript and helpful suggestions and criticisms. Laura Piccoli helped to prepare the manuscript.

Reprint requests to Giuseppe Remuzzi, M.D., Mario Negri Institute for Pharmacological Research, Via Gavazzeni, 11, 24100 Bergamo, Italy.

References

1. RENNKE HG, KLEIN PS: Pathogenesis and significance of nonprimary focal and segmental glomerulosclerosis. *Am J Kidney Dis* 13:443–456, 1989
2. BRENNER BM, MEYER TW, HOSTETTER TH: Dietary protein intake and the progressive nature of kidney disease: The role of hemodynamically mediated glomerular injury in the pathogenesis of progressive glomerular sclerosis in aging, renal ablation, and intrinsic renal disease. *N Engl J Med* 307:652–659, 1982
3. KLAHR S, HEIFETS M, PURKERSON ML: The influence of anticoagulation on the progression of experimental renal disease, in *The Progressive Nature of Renal Disease*, edited by MITCH WE, BRENNER BM, STEIN JH, New York, Churchill Livingstone, 1986, p. 45
4. DIAMOND JR, KARNOVSKY MJ: Focal and segmental glomerulosclerosis: Analogies to atherosclerosis. *Kidney Int* 33:917–924, 1988
5. YOSHIDA Y, FOGO A, ICHIKAWA I: Glomerular hemodynamic changes vs. hypertrophy in experimental glomerular sclerosis. *Kidney Int* 35:654–660, 1989
6. DEEN WM, MADDOX DA, ROBERTSON CR, BRENNER BM: Dynamics of the glomerular ultrafiltration in the rat. VII. Response to reduced renal mass. *Am J Physiol* 227:556–562, 1974
7. HOSTETTER TH, OLSON JL, RENNKE HG, VENKATACHALAM MA, BRENNER BM: Hyperfiltration in remnant nephrons: A potentially adverse response to renal ablation. *Am J Physiol* 241:F85–F93, 1981
8. OLSON JL, HOSTETTER TH, RENNKE HG, BRENNER BM, VENKATACHALAM MA: Altered glomerular permselectivity and progressive sclerosis following extreme ablation of renal mass. *Kidney Int* 22:112–126, 1982
9. SHIMAMURA T, MORRISON AB: A progressive glomerulosclerosis occurring in partial five-sixths nephrectomized rats. *Am J Pathol* 79:95–106, 1975
10. ROBSON AM, MORAN J, ROOT ER, JAGER BV, SHANKEL SW, INGELFINGER JR, KIEVSTRA RA, BRICKER NS: Mechanism of proteinuria in non-glomerular disease. *Kidney Int* 16:416–429, 1979
11. AZAR S, JOHNSON MA, HERTEL B, TOBIAN L: Single-nephron pressures, flows, and resistances in hypertensive kidneys with nephrosclerosis. *Kidney Int* 12:28–40, 1977
12. DWORKIN LD, HOSTETTER TH, RENNKE HG, BRENNER BM: Hemodynamic basis for glomerular injury in rats with desoxycorticosterone-salt hypertension. *J Clin Invest* 73:1448–1461, 1984
13. MORELAND RS, WEBB RC, BOHR DF: Vascular changes in DOCA hypertension: Influence of a low protein diet. *Hypertension* 4: 11199–11201, 1982
14. ANDERSON S, MEYER TW, RENNKE HG, BRENNER BM: Control of glomerular hypertension limits glomerular injury in rats with reduced renal mass. *J Clin Invest* 76:612–619, 1985
15. ANDERSON S, RENNKE HG, BRENNER BM: Therapeutic advantage of converting enzyme inhibitors in arresting progressive renal disease associated with systemic hypertension in the rat. *J Clin Invest* 77:1993–2000, 1986
16. SCHWARTZ MM, BIDANI AK, LEWIS EJ: Glomerular epithelial cell function and pathology following extreme ablation of renal mass. *Am J Pathol* 126:315–324, 1987
17. MOGENSEN CE. Glomerular filtration rate and renal plasma flow in short-term and long-term juvenile diabetes mellitus. *Scand J Clin Lab Invest* 28:91–100, 1971
18. CHRISTIANSEN JS, GAMMELGAARD J, FRANDSEN M, PARVING H-H: Increased kidney size, glomerular filtration rate and renal plasma flow in short-term insulin-dependent diabetics. *Diabetologia* 20:451–456, 1981
19. CHRISTIANSEN JS, GAMMELGAARD S, TRONIER B, SVENDSEN PA, PARVING H-H: Kidney function and size in diabetics before and during initial insulin treatment. *Kidney Int* 21:683–688, 1982
20. HOSTETTER TH, TROY JL, BRENNER BM: Glomerular hemodynamics in experimental diabetes mellitus. *Kidney Int* 19:410–415, 1981
21. JENSEN PK, CHRISTIANSEN JS, STEVEN K, PARVING H-H: Renal function in streptozotocin-diabetic rats. *Diabetologia* 21:409–414, 1981
22. ZATZ R, MEYER TW, RENNKE HG, BRENNER BM: Predominance of hemodynamic rather than metabolic factors in the pathogenesis of diabetic glomerulopathy. *Proc Natl Acad Sci USA* 82:5963–5967, 1985
23. ZATZ R, DUNN BR, MEYER TW, ANDERSON S, RENNKE HG, BRENNER BM: Prevention of diabetic glomerulopathy by pharmacological amelioration of glomerular capillary hypertension. *J Clin Invest* 77:1925–1930, 1986
24. KASISKE BL, O'DONNELL MP, KEANE WF: Glucose-induced increases in renal hemodynamic function. Possible modulation by renal prostaglandins. *Diabetes* 34:360–364, 1985
25. WOODS LL, MIZELLE HL, HALL JE: Control of renal hemodynamics in hyperglycemia: Possible role of tubuloglomerular feedback. *Am J Physiol* 252:F65–F73, 1987
26. BLANTZ RC, PETERSON OW, GUSHAWA L, TUCKER BJ: Effect of modest hyperglycemia on tubuloglomerular feedback activity. *Kidney Int* 22 (Suppl 12):S206–S212, 1982
27. JENSEN PK, STEVEN K, BLAEHR H, CHRISTIANSEN JS, PARVING H-H: Effects of indomethacin on glomerular hemodynamics in experimental diabetes. *Kidney Int* 29:490–495, 1986
28. SCHAMBELAN M, BLAKE S, SRAER J, BENS M, NIVEZ M-P, WAHBE F: Increased prostaglandin production by glomeruli iso-

lated from rats with streptozotocin-induced diabetes mellitus. *J Clin Invest* 75:404–412, 1985
29. ORTOLA FV, BALLERMAN BJ, ANDERSON S, MENDEZ RE, BRENNER BM: Elevated plasma atrial natriuretic peptide levels in diabetic rats. Potential mediator of hyperfiltration. *J Clin Invest* 80:670–674, 1987
30. MICHELS LD, O'DONNELL MP, KEANE WF: Glomerular hemodynamic and structural correlations in long-term experimental diabetic rats. *J Lab Clin Med* 103:840–847, 1984
31. MICHELS LD, DAVIDMAN M, KEANE WF: Determinants of glomerular filtration and plasma flow in experimental diabetic rats. *J Lab Clin Med* 98:869–885, 1981
32. NAKHOODA AF, LIKE AA, CHAPEL CI: The spontaneously diabetic rat. Metabolic and morphologic studies. *Diabetes* 326:100–112, 1976
33. ROSSINI AA, MORDES JP: Animal models of diabetes. *Am J Med* 70:353–360, 1981
34. BROWN DM, STEFFES MW, THIBERT P, AZAR S, MAUER SM: Glomerular manifestations of diabetes in the BB rat. *Metabolism* 32 (Suppl 1):131–135, 1983
35. COHEN AJ, MCCARTHY DM, ROSSETTI RG: Renin secretion of the spontaneously diabetic rat. *Diabetes* 35:341–345, 1986
36. COHEN AJ, MCGILL PD, ROSSETTI RG, GUBERSKI DL, LIKE AA: Glomerulopathy in spontaneously diabetic rat. Impact of glycemic control. *Diabetes* 36:944–951, 1987
37. O'DONNELL MP, KASISKE BL, KEANE WF: Glomerular hemodynamic and structural alterations in experimental diabetes mellitus. *FASEB J* 2:2339–2347, 1988
38. MAUER SM, STEFFES MW, ELLIS EN, SUTHERLAND DER, BROWN DM, GOETZ FC: Structural-functional relationships in diabetic nephropathy. *J Clin Invest* 74:1143–1155, 1984
39. MOGENSEN CE, CHRISTENSEN CK: Predicting diabetic nephropathy in insulin dependent patients. *N Engl J Med* 311:89–93, 1984
40. VIBERTI GC, HILL RD, JARRETT RJ, ARGYROPOULOS A, MAHMUD U, KEEN H: Microalbuminuria as a predictor of clinical nephropathy in insulin-dependent diabetes mellitus. *Lancet* i: 1430–1432, 1982
41. MOGENSEN CE: A complete screening of urinary albumin concentration in an unselected diabetic out-patient clinic population (1082 patients). *Diabetic Nephropathy* 2:11–15, 1983
42. BORCH-JOHNSEN K, NISSEN H, NERUP J: Blood pressure after forty years of insulin-dependent diabetes. (abstract) *Diabetic Nephropathy* 4:11, 1984
43. MOGENSEN CE, CHRISTENSEN CK: Blood pressure changes and renal function in incipient and overt diabetic nephropathy. *Hypertension* 7 (Suppl 2):64–73, 1985
44. KNOWLES HC JR: Magnitude of the renal failure problem in diabetic patients. *Kidney Int* 4 (Suppl 1):52–57, 1974
45. KUSSMAN MJ, GOLDSTEIN HH, GLEASON PE: The clinical course of diabetic nephropathy. *JAMA* 236:1861–1867, 1976
46. GOLDSTEIN DA, MASSRY SG: Diabetic nephropathy: Clinical course and effect of hemodialysis. *Nephron* 20:286–291, 1978
47. PARVING H-H, ANDERSEN AR, SMIDT UM, SVENDSEN PA: Early and aggressive antihypertensive treatment reduced the rate of decline in kidney function in diabetic nephropathy. *Lancet* I:1175–1179, 1983
48. MOGENSEN CE: Long-term antihypertensive treatment inhibiting progression of diabetic nephropathy. *Br Med J* 285:685–688, 1982
49. MOGENSEN CE, MAUER SM, KJELLSTRAND CM: Diabetic Nephropathy, in *Disease of the Kidney*, edited by SCHRIER RW, GOTTSCHALK CW, Boston/Toronto, Little, Brown and Company, 1988, p. 2395
50. ANDERSON S, RENNKE HG, GARCIA DL, BRENNER BM: Short and long term effects of antihypertensive therapy in the diabetic rat. *Kidney Int* 36:526–536, 1989
51. HOMMEL E, PARVING HH, MATHIESEN E, EDSBERG B, NIELSEN MD, GIESE J: Effect of captopril on kidney function in insulin-dependent diabetic patients with nephropathy. *Br Med J* 293:467–470, 1986
52. BJORK S, NYBERT G, MULEC H, GRANERUS G, HERLITZ H, AURELL M: Beneficial effects of angiotensin converting enzyme inhibition on renal function in patients with diabetic nephropathy. *Br Med J* 293:471–474, 1986
53. COUSER WG, STILMAN MM: Mesangial lesions and focal glomerular sclerosis in the aging rat. *Lab Invest* 33:491–501, 1975
54. ELEMA JD, ARENDS A: Focal and segmental glomerular hyalinosis and sclerosis in the rat. *Lab Invest* 3:554–561, 1975
55. ANDERSON S, BRENNER BM: Effects of aging on the renal glomerulus. *Am J Med* 80:435–442, 1986
56. SAXTON JA JR, KIMBALL JC: Relation of nephrosis and diseases of albino rats to age to modifications of diet. *Arch Pathol* 32:951–955, 1941
57. BLATHERWICK NR, MEDLAR EM: Chronic nephritis in rats fed high protein diets. *Arch Intern Med* 59:572–596, 1937
58. BERG BN, SIMMS HS: Nutrition and longevity in the rat. II Longevity and onset of disease with different levels of food intake. *J Nutr* 71:255–258, 1960
59. JOHNSON JE, BARROWS CH: Effects of age and dietary restrictions on the kidney glomeruli of mice: Observation by scanning electron microscopy. *Anat Rec* 196:145–149, 1980
60. TUCKER SM, MASON RL, BEAUCHENE RE: Influence of diet and food restriction on kidney function of aging male rats. *J Gerontol* 31:264–269, 1976
61. BERTANI T, ZOJA C, ABBATE M, ROSSINI M, REMUZZI G: Age-related nephropathy and proteinuria in rats with intact kidneys exposed to diets with different protein content. *Lab Invest* 60:196–204, 1989
62. REMUZZI A, PUNTORIERI S, BATTAGLIA C, BERTANI T, REMUZZI G: Angiotensin converting enzyme inhibition ameliorates glomerular filtration of macromolecules and water and lessens glomerular injury in the rat. *J Clin Invest* 85:541–549, 1990
63. 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. *J Clin Invest* 82:1757–1768, 1988
64. BOLTON WK, BENTON FR, MACLAY JG, STURGILL BC: Spontaneous glomerular sclerosis in aging Sprague-Dawley rats. I. Lesions associated with mesangial IgM deposits. *Am J Pathol* 85:277–302, 1976
65. HOSTETTER TH, MEYER TW, RENNKE HG, BRENNER BM: Chronic effects of dietary protein in the rat with intact and reduced renal mass. *Kidney Int* 30:509–517, 1986
66. DIAMOND JR, ANDERSON S: Converting enzyme inhibition (CEI) lessens tubulointerstitial injury (TII) in puromycin aminonucleoside (PA) nephrosis. (abstract) *Kidney Int* 33:373, 1988
67. SCHOLEY JW, MILLER PL, RENNKE HG, MEYER TW: Effect of converting enzyme inhibition on the course of adriamycin-induced nephropathy. *Kidney Int* 36:816–822, 1989
68. REMUZZI A, PUNTORIERI S, MAZZOLENI A, REMUZZI G: Sex related differences in glomerular ultrafiltration and proteinuria in Munich-Wistar rats. *Kidney Int* 34:481–486, 1988
69. BERTANI T, POGGI A, POZZONI R, DELAINI F, SACCHI G, THOUA Y, MECCA G, REMUZZI G, DONATI MB: Adriamycin-induced nephrotic syndrome in rats: Sequence of pathologic events. *Lab Invest* 46:16–23, 1982
70. GIROUX L, SMEESTERS C, BOURY F, FAURE MP, JEAN G: Adriamycin and adriamycin-DNA nephrotoxicity in rats. *Lab Invest* 50:190–196, 1984
71. WEENING JJ, RENNKE HG: Glomerular permeability and polyanion in adriamycin nephrosis in the rat. *Kidney Int* 24:152–159, 1983
72. RYAN GB, KARNOVSKY MJ: An ultrastructural study of the mechanisms of proteinuria in aminonucleoside nephrosis. *Kidney Int* 8:219–232, 1975
73. FRENK S, ANTONOWICZ I, CRAIG JM, METCOFF J: Experimental nephrotic syndrome induced in rats by aminonucleoside. Renal lesions and body electrolyte composition. *Proc Soc Exp Biol Med* 89:424–427, 1955
74. VERNIER RL, PAPERNASTER BW, GOOD RA: Aminonucleoside nephrosis. I. Electron microscopic study of the renal lesion in rats. *J Exp Med* 109:115–126, 1959
75. BERTANI T, ROCCHI G, SACCHI G, MECCA G, REMUZZI G: Adriamycin-induced glomerulosclerosis in the rat. *Am J Kidney Dis* 7:12–19, 1986
76. OKUDA S, OH Y, TSURUDA H, ONOYAMA K, FUJIMI S, FUJISHIMA M: Adriamycin-induced nephropathy as a model of chronic progressive glomerular disease. *Kidney Int* 29:502–510, 1986

77. GLASER RJ, VELOSA JA, MICHAELS AF: Experimental model of focal sclerosis. I. Relationship to protein excretion in aminonucleoside nephrosis. *Lab Invest* 36:519–526, 1977
78. BERTANI T, CUTILLO F, ZOJA C, BROGGINI M, REMUZZI G: Tubulo-interstitial lesions mediate renal damage in adriamycin glomerulopathy. *Kidney Int* 30:488–496, 1986
79. FOGO A, YOSHIDA Y, GLICK AD, HOMMA T, ICHIKAWA I: Serial micropuncture analysis of glomerular function in two rat models of glomerular sclerosis. *J Clin Invest* 82:322–330, 1988
80. O'DONNELL MP, MICHELS L, KASISKE B, RAIJ L, KEANE W: Adriamycin-induced chronic proteinuria: A structural and functional study. *J Lab Clin Med* 106:62–67, 1985
81. BEUKERS JJB, HOEDEMAEKER PJ, WEENING JJ: A comparison of the effects of converting-enzyme inhibition and protein restriction in experimental nephrosis. *Lab Invest* 59:631–640, 1988
82. ANDERSON MS, RECANT L: Fine structural alterations in the rat kidney following intraperitoneal bovine albumin. *Am J Pathol* 40:555–569, 1962
83. FISHER ER, HELLSTROM HR: Mechanism of proteinuria: Functional and ultrastructural correlation of effects of infusion of homologous and heterologous protein (bovine serum albumin) in the rat. *Lab Invest* 11:617–637, 1962
84. DAVIES DJ, BREWER DB, HARDWICKE J: Urinary proteins and glomerular morphometry in protein overload proteinuria. *Lab Invest* 38:232–243, 1978
85. DAVIES DJ, MESSINA A, THUMWOOD CM, RYAN GB: Glomerular podocytic injury in protein overload proteinuria. *Pathol* 17:412–419, 1985
86. WEENING JJ, VAN GULDENER C, DAHA MR, KLAR N, VAN DER WAL A, PRINS FA: The pathophysiology of protein-overload proteinuria. *Am J Pathol* 129:64–73, 1987
87. MORI H, YAMASHITA H, NAKANISHI C, KOIZUMI K, MAKINO S, KISHIMOTO Y, HAYASHI Y: Proteinuria induced by transplantable rat pituitary tumor MtT SA5. Model for homologous protein-overload proteinuria. *Lab Invest* 54:636–644, 1986
88. COUSER WG, STILMANT MM, DARBY C: Autologous immune complexes nephropathy. I. Sequential study of immune complex deposition, ultrastructural changes, proteinuria, and alterations in glomerular sialoprotein. *Lab Invest* 34:23–30, 1976
89. FEENSTRA K, LEE RVD, GREBEN HA, ARENDS A, HOEDEMAEKER PHJ: Experimental glomerulonephritis in the rat induced by antibodies directed against tubular antigens. II. Influence of medication with prednisone and azathioprine: A histologic and immunohistologic study at the light microscopic and the ultrastructural level. *Lab Invest* 32:243–250, 1975
90. BOLTON WK, SPARGO BA, LEWIS EJ: Chronic autologous immune complex glomerulopathy: effect of cyproheptadine. *J Lab Clin Med* 83:695–704, 1974
91. FLEUREN GJ, HOEDEMAEKER PHJ: Triple-drug treatment of autologous immune complex glomerulonephritis. *Clin Exp Immunol* 41:218–224, 1980
92. KUPOR LR, LOWANCE DC, MCPHAUL JJ: Single and multiple drug therapy in autologous immune complex nephritis in rats. *J Lab Clin Med* 87:27–36, 1976
93. BARABAS AZ, CORNISH J, LANNIGAN R: Progressive passive Heymann nephritis: Induction of autologous antibodies to rat brush border by multiple injections of heterologous antiserum. *Clin Exp Immunol* 60:381–386, 1985
94. HARRIS KPG, PURKERSON ML, YATES J, KLAHR S: Lovastatin ameliorates the development of glomerulosclerosis and uremia in experimental nephrotic syndrome. *Am J Kidney Dis* 15:16–23, 1990
95. SANFELICE NFT, RIIBEIRO MO, PADHILA RM, SANTOS MM, ZATZ R: Limited proteinuria despite hypercholesterolemia in diabetic analbuminemic rats. (abstract) *Kidney Int* 37:519, 1990
96. GROND J. SCHILTHUIS MS, KOUDSTAAL J, ELEMA JD: Mesangial function and glomerular sclerosis in rats after unilateral nephrectomy. *Kidney Int* 22:338–343, 1982
97. GROND J, KOUDSTAAL J, ELEMA JD: Mesangial function and glomerular sclerosis in rats with aminonucleoside nephrosis. *Kidney Int* 27:405–410, 1985
98. ABRAHAMSON DR: Structure and development of the glomerular capillary wall and basement membrane. *Am J Physiol* 253:F783–F794, 1987
99. VELOSA JA, GLASSER RJ, NEVINS TE, MICHAEL AF: Experimental model of focal sclerosis. II. Correlation with immunopathologic changes, macromolecular kinetics, and polyanion loss. *Lab Invest* 36:527–534, 1977
100. KEANE WF, RAIJ L: Relationship between altered glomerular barrier permselectivity, angiotensin II and mesangial uptake of macromolecules. *Kidney Int* 25:247–252, 1984
101. SCHWARTZ MM, BIDANI AK, LEWIS EJ: Glomerular epithelial cell structure and function in chronic proteinuria induced by homologous protein-load. *Lab Invest* 55:673–679, 1986
102. BREWER DB, FILIP O: The morphometry of the glomerular epithelial cell and its foot processes after the injection of bovine serum albumin or egg albumin. *J Pathol* 120:209–220, 1976
103. DAVIES DJ, BREWER DB: Irreversible glomerular damage following heterologous serum albumin overload. *J Pathol* 123:45–52, 1977
104. MARKS MI, DRUMMOND KN: Nephropathy and persistent proteinuria after albumin administration in the rat. *Lab Invest* 23:416–420, 1970
105. PURKERSON ML, TOLLEFSEN DM, KLAHR S: N-Desulfated/acetylated heparin ameliorates the progression of renal disease in rats with subtotal renal ablation. *J Clin Invest* 81:69–74, 1988
106. CASTELLOT JJ, HOOVER RL, HARPER PA, KARNOVSKY MJ: Heparin and glomerular epithelial cell-secreted heparin-like species inhibit mesangial-cell proliferation. *Am J Pathol* 120:427–435, 1985
107. FISHMAN JA, KARNOVSKY MJ: Effects of the aminonucleoside of puromycin on glomerular epithelial cells in vitro. *Am J Pathol* 118:398–407, 1985
108. PABST R, STERZEL RB: Cell renewal of glomerular cell types in normal rats. An autoradiographic analysis. *Kidney Int* 24:626–631, 1983
109. RASCH R, NORGAARD JOR: Comparative autoradiographic studies of ^{3}H-thymidine uptake in diabetic and uninephrectomized rats. *Diabetologia* 25:280–287, 1983
110. FRIES JWU, SANDSTROM DJ, MEYER TW, RENNKE HG: Glomerular hypertrophy and epithelial cell injury modulate progressive glomerulosclerosis in the rat. *Lab Invest* 60:205–209, 1989
111. GROND J, BEUKERS JJB, SCHILTHUIS MS, WEENING JJ, ELEMA JD: Analysis of renal structural and functional features in two rat strains with a different susceptibility to glomerular sclerosis. *Lab Invest* 54:77–83, 1986
112. LOEB AL, MANDEL G, STRAW JA, BEAN BL: Increased aortic DNA synthesis precedes renal hypertension in rats. An obligatory step? *Hypertension* 8:754–761, 1986
113. YOSHIDA Y, KAWAMURA T, IKOMA M, FOGO A, ICHIKAWA I: Effects of antihypertensive drugs on glomerular morphology. *Kidney Int* 36:626–635, 1989
114. YOSHIOKA T, SHIRAGA H, YOSHIDA Y, FOGO A, GLICK AD, DEEN WM, HOYER JR, ICHIKAWA I: "Intact nephrons" as the primary origin of proteinuria in chronic renal disease. Study in the rat model of subtotal nephrectomy. *J Clin Invest* 82:1614–1623, 1988
115. SCHREINER GF, COTRAN RS, UNANUE ER: Macrophages and cellular immunity in experimental glomerulonephritis. *Springer Semin Immunopathol* 5:251–267, 1982
116. BOLTON WK, INNES DJ JR, STURGILL BC, KAISER DL: T-cells and macrophages in rapidly progressive glomerulonephritis: Clinicopathologic correlations. *Kidney Int* 32:869–876, 1987
117. STACHURA I, SI L, MADAN E, WHITESIDE T: Mononuclear cell subsets in human renal disease. Enumeration in tissue sections with monoclonal antibodies. *Clin Immunol Immunopathol* 30:362–373, 1984
118. DWORKIN LD, GROSSER M, FEINER HD, ULLIAN M, PARKER M: Renal vascular effects of antihypertensive therapy in uninephrectomized SHR. *Kidney Int* 35:790–798, 1989
119. DAVIES M, HORTON JK, THOMAS D, WILLIAMS JD: Inflammatory activation of human neutrophils (PMN) by Tamm-Horsfall glycoprotein (THG): A possible cause of interstitial nephritis. (abstract) *Kidney Int* 37:255, 1990

120. EDDY AA, MICHAEL AF: Acute tubulointerstitial nephritis associated with aminonucleoside nephrosis. *Kidney Int* 33:14–23, 1988
121. EDDY AA: Interstitial nephritis induced by protein-overload proteinuria. *Am J Pathol* 135:719–733, 1989
122. LOVETT DH, RYAN JL, STERZEL RB: Stimulation of rat mesangial cell proliferation by macrophage interleukin I. *Immunol* 131:2830–2836, 1983
123. MELCION C, LACHMAN L, KILLEN PD, MOREL-MAROGER L, STRIKER GE: Mesangial cells, effect of monocyte products on proliferation and matrix synthesis. *Transplant Proc* 141:559–564, 1982
124. YAMAMOTO T, YAOITA E, KAWASAKI K, KIHARA I: Tumor necrosis factor (TNF) augments proliferation of rat mesangial cells (RMC). (abstract) *Kidney Int* 35:367, 1989
125. DAVIES M, SHEWRING L, THOMAS G, JENNER L: Stimulation of proteoglycan (PG) synthesis in rat mesangial cells (RMC) in response to tumor necrosis factor (TNF). (abstract) *Kidney Int* 35:344, 1989
126. BEVILACQUA MP, POBER JS, WHEELER ME, COTRAN RS, GIMBRONE MA JR: Interleukin-1 acts on cultured human vascular endothelium to increase the adhesion of polymorphonuclear leukocytes monocytes and related leukocyte cell lines. *J Clin Invest* 76:2003–2008, 1985
127. CAMUSSI G, TETTA C, TURELLO E, ANDRES G: Cytokines and platelet-activating factor in the progression of glomerular injury. (abstract) *Proceedings of the 6th Capri Conference on Uremia*, September 1–4, 1989, p. 6
128. SHULTZ PJ, DICORLETO PE, SILVER BJ, ABBOUD HE: Mesangial cells express PDGF mRNAs and proliferate in response to PDGF. *Am J Physiol* 255:F674–F684, 1988
129. BORDER W, OKUDA S, LANGUINO L, RUOSLAHI E: Transforming growth factor (TGFβ) uniquely regulates production and structure of glomerular extracellular matrix proteoglycans. (abstract) *Kidney Int* 35:432, 1989
130. KLOTMAN P, BRUGGEMAN L, HASSELL J, HORIGAN E, MARTIN G, YAMADA G: Regulation of extracellular matrix by thromboxane. (abstract) *Kidney Int* 35:294, 1989
131. IBELS LS, ALFREY AC, HUFFER WE: Calcification in end-stage kidneys. *Am J Med* 71:33–37, 1981
132. KEANE WF, KASISKE BL, O'DONNELL MP: Hyperlipidemia and the progression of renal disease. *Am J Clin Nutr* 47:157–160, 1988
133. HEIFETS M, DAVIS TA, TEGTMEYER E, KLAHR S: Exercise training ameliorates progressive renal disease in rats with subtotal nephrectomy. *Kidney Int* 32:815–820, 1987
134. PURKERSON ML, JOIST JH, GREENBERG JM, KAY D, HOFFSTEN PE, KLAHR S: Inhibition by anticoagulant drugs of the progressive hypertension and uremia associated with renal infarction in rats. *Thromb Res* 26:227–240, 1982
135. OLSON JL: Role of heparin as a protective agent following reduction of renal mass. *Kidney Int* 25:376–382, 1984