Progression and potential regression of glomerulosclerosis

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CASE PRESENTATIONS

Patient 1

A 13-year-old white boy was noted to have proteinuria, 1 g/24 hr, eight years ago on a routine physical examination for participation in school sports. The serum creatinine and blood glucose levels were normal, and serologic tests were negative. Ultrasound examination demonstrated a very small, atrophic right kidney and hypertrophy and normal echogenicity of the left kidney. A diagnosis of solitary functioning kidney was made.

The patient was treated with dietary protein restriction and an angiotensin-converting enzyme (ACE) inhibitor. At routine follow-up at age 19, the proteinuria had increased to 3.7 g/24 hr, and he was referred for further evaluation. The medical history was significant for a bilateral inguinal hernia repair at age 10 months, and a poorly documented history of vesicoureteral reflux as a child. The family history was significant for microhematuria detected in the father three months prior to this evaluation, a maternal grandmother with hypertension, and a paternal grandfather with diabetes. There was no family history of visual abnormalities or hearing loss. No hypertension had been noted at regular follow-ups. The patient had gained 25 pounds over the previous 18 months. A college student, he worked part-time in an ice cream store. He had smoked for one month one year previously and did not drink alcohol or use illicit drugs. He had not noted any peri-orbital or leg edema, shortness of breath, chest pains, hematuria, or urinary symptoms. His only medication was fosinopril, 10 mg orally each day.

The physical examination revealed a well-developed white male in no apparent distress. The blood pressure was 146/84 mm Hg; pulse, 84 beats/min; respiratory rate, 16 breaths/min; temperature, 98.6°F; weight, 215 lbs; and height, 5'9". The physical examination was unremarkable. Ultrasound tests showed an atrophic 3.4 cm echogenic right kidney, and a 12.6 cm left kidney with normal echogenicity. Laboratory evaluation revealed: serum glucose, 97 mg/dL; cholesterol, 137 mg/dL; triglycerides, 672 mg/dL; albumin, 4.1 g/dL; total protein, 7.4 g/dL; hematocrit, 46%; platelets, 361,000/mm³; serum creatinine, 1.2 mg/dL; BUN, 15 mg/dL; and normal electrolytes. Creatinine clearance was 126 mL/min.

An open renal biopsy disclosed early lesions of focal segmental glomerulosclerosis (FSGS) and glomerulomegaly, characteristic of secondary FSGS (Fig. 1). The renal changes most likely were related to his unilateral renal atrophy. He was treated with a higher dose of the same ACE inhibitor. At latest follow-up nearly two years later, the serum creatinine was 1.2 mg/dL, and proteinuria was <500 mg/24 hr.

Patient 2

A 5-year-old white girl presented 17 years ago with an 8-month history of relapsing nephrotic syndrome. The first two episodes were steroid-sensitive, but subsequent relapses were steroid-dependent, and she was admitted for renal biopsy. Laboratory tests revealed normal serum creatinine (0.8 mg/dL), hypoaalbuminemia (0.8 g/dL), proteinuria (2 g/24 hrs), negative ASO titers, and normal complement levels. Physical examination showed 3+ edema, and her blood pressure was 120/70 mm Hg.

The renal biopsy showed no segmental sclerosis in the 23 glomeruli sampled. Immunofluorescence studies were negative, and electron microscopy showed extensive foot process effacement. An initial diagnosis of minimal change disease was made. Corticosteroid treatment was continued with no response even at a higher dose (40 mg daily). Addition of a six-week course of chlorambucil resulted in remission (serum albumin, 4.3 g/dL; proteinuria, 420 mg/24 hr). She remained in remission for 18 months and had a normal serum creatinine (0.3 mg/dL) when she was 7 years old. She then had a relapse following an upper respiratory infection, which was followed by 5 relapses over the next 16 months. These relapses responded to increased prednisone dosages, one added four-week course of chlorambucil, aspirin, and diuretics. At age 8, her serum creatinine was 0.7 mg/dL, but it increased to 0.9 mg/dL at age 9.

Although she was lost to follow-up for several months, she

Key words: nephrotic syndrome, proteinuria, sclerotic lesions, end-stage renal disease, progressive kidney disease, tubulointerstitial fibrosis.

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Fig. 1. Renal biopsy from patient 1. Marked glomerulomegaly and hilar prominence of mesangial matrix and thickening of Bowman’s capsule (A), with occasional glomeruli with well-defined segmental sclerosis (B), consistent with secondary FSGS (periodic acid-Schiff, ×200, ×400).

Fig. 2. Second renal biopsy from patient 2. Advanced sclerosis in a focal and segmental pattern with proportional tubulointerstitial fibrosis typical of idiopathic FSGS (Jones’ silver stain, ×200).

was in apparent remission from her nephrotic syndrome with no edema when routine tests revealed that her serum creatinine was 6.0 mg/dL. A second renal biopsy showed advanced FSGS, typical of the idiopathic form (Fig. 2). She rapidly reached end-stage renal disease and was treated with peritoneal dialysis. Her course was complicated by multiple infections, and she underwent hemodialysis for six months. At age 11 years, she received a living related kidney transplant from her father. She became pregnant at age 19 and developed preeclampsia with proteinuria and hypertension. Her serum creatinine was 1.2 mg/dL after delivery of a healthy full-term baby boy. One year later, she had an episode of acute rejection, which responded to increased immunosuppression with a return of the serum creatinine to baseline. A renal biopsy showed tubulitis superimposed on mild-to-moderate chronic transplant nephropathy. The serum creatinine was stable one year later, but it increased to 4.1 mg/dL one year ago. A repeat biopsy of the graft showed chronic transplant nephropathy with glomerulosclerosis due to transplant glomerulopathy, without evidence of recurrent idiopathic FSGS. She was enrolled in a clinical intervention trial for treatment of chronic allograft nephropaty. Subsequent studies of the first native-kidney biopsy specimen demonstrated glomerulomegaly, with glomerular size 62% greater than age-matched control.

DISCUSSION

DR. AGNES B. FOGO (Director, Division of Renal Pathology/Electron Microscopy, Vanderbilt University Medical Center; and Professor of Pathology, Medicine, and Pediatrics, Vanderbilt University School of Medicine, Nashville, Tennessee, USA): These two cases illustrate the varied outcome in patients with glomerulosclerosis. The first patient showed only minimal sclerotic lesions despite having risk factors of unilateral renal agenesis and persistent proteinuria. In contrast, the second patient developed end-stage renal disease despite intervention when only the presence of glomerular hypertrophy heralded a sclerotic process. In the first patient, an ACE inhibitor was given even though the patient was not hypertensive; in the second patient, even aggressive treatment with corticosteroids and chlorambucil failed to prevent progressive disease. I will discuss some of the possible mechanisms contributing to progressive glomerulosclerosis, and also the possibilities of forestallment or even regression of existing sclerosis.

Progressive renal disease historically has been considered an inexorable, relentless process. Based on classic studies from more than 20 years ago, straight-line regressions with negative slopes of the reciprocal of serum creatinine have been used to predict the length of time until individual patients reach dialysis, transplantation, or death [1]. Recent studies indicate that it is time to reassess this grim picture of progression [2, 3].

Classic studies, both in animals and in humans, have shown that not only ACE inhibitors [3], but also other therapies that normalize blood pressure, slow the rate of
Mechanisms of progression

To effect regression, not only must mechanisms of progression be dampened, but remodeling of the existing sclerosis also must take place. Hypertension is a key mechanism associated with progression. Hypertension accelerates progression of chronic renal disease in humans, whether it results from or causes the renal disease. Importantly, intraglomerular pressure can be modulated differentially from systemic blood pressure. The potential impact of local glomerular hemodynamic changes on sclerosis has been extensively studied in the Munich-Wistar rat because this model has superficial glomeruli that can be directly punctured to measure glomerular blood pressures and flows. As is well known, the remnant kidney model, in which 5/6 of the total renal parenchyma is removed, results in progressive hypertension, proteinuria, and matrix expansion; focal segmental glomerulosclerosis ensues that mimics progressive injury in humans [6–8]. Anderson et al showed that ACE inhibition confers superior protection against progressive sclerosis compared with non-ACE-inhibitor antihypertensive treatment in this model [9]. Although both interventions normalized systemic pressures, only ACE inhibition decreased glomerular pressures, which remained high when systemic blood pressure was controlled to a similar degree using antihypertensive medications other than ACE inhibitors, that is, “nonspecific therapy.” This led Brenner’s group to postulate that increased glomerular capillary pressure is a key mediator of progressive renal sclerosis in a self-perpetuating vicious cycle, whereby nephron loss due to sclerosis further increases flow and pressures in the remaining glomeruli, thus augmenting and perpetuating injury [6, 9, 10]. We performed serial micropuncture studies in the same model to investigate whether hemodynamic derangement predicts the ultimate severity of sclerosis in those same punctured glomeruli. When the rats were sacrificed, the glomeruli that had been micropunctured in vivo were identified, processed, and histologically assessed for sclerosis. The sclerosis indices for the individual glomeruli then were correlated with either maximum single-nephron glomerular filtration rate (GFR), glomerular pressure, or average values for these parameters as assessed by repeated micropuncture in these very same glomeruli over the preceding weeks [11]. We found no correlation between the degree of total sclerosis and any of these hemodynamic variables in individual glomeruli. Thus, additional non-hemodynamic factors likely contribute to sclerosis.

We resurrected an old model of injury, ureteral diversion, to examine progressive injury at the single glomerular level [12]. For this model, the ureter of one kidney is diverted into the peritoneum with partial ablation of the contralateral kidney; marked glomerular hyperfusion and hyperfiltration, and increased glomerular pressure ensued. In our studies, these changes were accompanied by only minimal glomerulosclerosis and minimal glomerular enlargement. In contrast, in the 5/6 nephrectomy model (which results in glomerular hyperfiltration and hypertension comparable to the ureteral diversion model) marked glomerular enlargement occurred and was closely associated with glomerulosclerosis. These two parameters, glomerular enlargement and sclerosis, were closely correlated at an individual glomerular level in a biphasic distribution. We noted a positive correlation of glomerular enlargement and sclerosis at early phases of sclerosis (that is, as much as 50% of the tuft involved) and a negative correlation as the glomeruli progressed to end-stage sclerosis [12].

The effectiveness of ACE inhibitors in ameliorating sclerosis in the remnant kidney model was associated with inhibition of glomerular growth. This relationship confirms the correlation between glomerular growth and sclerosis [13]. However, ACE inhibition was not equally effective at all stages of fibrosis; progressive deterioration continued in glomeruli with more advanced sclerosis, whereas progression was inhibited in glomeruli at earlier stages of sclerosis. These findings suggested that different mechanisms were at work among the heterogeneously affected glomeruli, with different potentials for response to treatment.

Our next step was to determine whether glomerular hypertrophy was linked with sclerosis in humans and whether that hypertrophy is an early sign of impending sclerosis. We analyzed data from three pediatric patient groups: patients with initial biopsies consistent with minimal change disease and with a subsequent clinical course and second biopsy consistent with this diagnosis; a second patient group with similar initial biopsies and subsequent overt progression to focal segmental glomerulosclerosis (FSGS); and a third group of age-matched controls [14]. Today’s second case was one of the index patients in this study. Initial biopsies revealed significant glomerular enlargement in patients with progression to focal segmental glomerulosclerosis, whereas patients with a subsequent clinical course and a second biopsy consistent with minimal change showed normal glomerular size. Similarly, in adults with FSGS, glomeruli were larger than in those with minimal change disease [14]. These findings have now been confirmed both in pediatric and adult populations [15].
Glomerular hypertrophy is linked with development of sclerosis in other human diseases as well [15]. Abnormal glomerular growth in humans occurs with diabetes, hypoxia (as in cyanotic heart disease, sickle cell disease, and obesity-associated sleep apnea), and limited renal mass (as in transplantation, extensive removal of renal mass, and unilateral renal agenesis, a risk factor present in today’s first patient). All these conditions also have been associated with lesions of FSGS. The close association between glomerular enlargement and glomerulosclerosis likely reflects the actions of multiple factors on glomerular cells, with responses of increased matrix, hypertrophy, and proliferation often occurring in concert. Thus, increased glomerular size is a sign of a growth response to factors that often also induce increased matrix accumulation, the hallmark of sclerosis. In human diabetes and idiopathic FSGS, increased glomerular size precedes development of the sclerotic lesions. Interestingly, a recent genetic analysis of children with nephrotic syndrome found that FSGS patients have a higher prevalence of the ACE deletion (D) polymorphism, compared to the insertion (I) polymorphism, when compared with patients with minimal change disease [16]. This D allele has been associated with higher activity of the renin-angiotensin system (RAS), and this heightened activity might influence both renal growth and scarring responses. These findings suggest that minimal change disease and FSGS are distinct entities from the onset.

In vivo and in vitro experimental evidence points to the capability of many cytokines to promote growth of glomerular cells and also to increase extracellular matrix formation, thus promoting sclerosis as well as growth. Recent in vitro data based on sophisticated microchip gene array analysis demonstrate that the early fibroblast response to growth factors includes a linked response of genes implicated in cell division, wound healing, and angiogenesis [17]. These findings indicate tight linkages among growth, differentiation, and wound healing/remodeling responses.

Stimuli that have been shown in experimental conditions to promote growth and glomerulosclerosis include loss of renal mass; high-protein or high-salt diet; various hormones such as growth hormone, insulin-like growth factor, androgens, and glucocorticoids; and vasoactive molecules such as angiotensin II (Ang II) or endothelin [7, 15]. Hypertrophic stimuli accelerate sclerosis, and interventions that dampen glomerular hypertrophy also often ameliorate glomerulosclerosis. The latter include low-protein, low-salt, or low-phosphate diet, ablation of androgenic effects by castration, antagonism of growth hormone with the somatostatin analogue octreotide, genetic defects in growth hormone, and inhibition of angiotensin or endothelin.

Hypertrophic response and subsequent development of sclerosis are dependent on the genetic background, so it is likely that complex genetic traits modulate the response of glomerular cells to pathogenetic stimuli. Mice with reduced nephron number because of a radiation-induced mutation that results in approximately 50% nephron reduction in association with oligosyndactyly (Os+/+) developed severe glomerular enlargement and sclerosis when this abnormality occurred on the sclerosis-prone ROP genetic background, but not in the sclerosis-resistant C57 strain [18]. Glomerular hypertrophy was proportional to reduction of nephron mass in the former strain. In contrast, a threshold for glomerular size was observed in the C57 mice. The interplay of genetic background and response to injury is also well recognized in humans, in whom only approximately 40% of patients with type-1 diabetes mellitus develop diabetic nephropathy [19].

Age at onset of injury also modifies the kidney’s growth response to renal ablation. Animal data indicate that increased sclerosis in the young cannot be explained by hemodynamic factors or glomerular size, but rather might be related to factors unique in the young maturing kidney, which is characterized by centripetal growth and differentiation [20]. Uninephrectomy in healthy adults produces no significant sequelae. Several follow-up studies of renal transplant donors as well as individuals nephrectomized secondary to trauma suggest a benign course after unilateral nephrectomy [15, 21]. However, more extensive loss of renal mass in adult patients with bilateral tumors resulted in an increased incidence of FSGS in the remnant kidney [22]. In contrast, removal of one kidney in children, for example, because of Wilms’ tumor, has somewhat more ominous consequences: renal growth was most marked in patients who had surgery at a younger age, and increased kidney growth was associated with microalbuminuria, which developed in 11 of 34 patients [23]. Likewise, patients with unilateral renal agenesis have a significantly increased risk of proteinuria (19%), hypertension (47%), and renal insufficiency (13%), and death was ascribed to renal disease in 6 of 157 in a large series [24].

These findings suggest that the number of remnant nephrons in the congenital solitary kidney in fact might be decreased (hypogenesis) and thereby represent a more severe form of reduction in nephron population than unilateral nephrectomy. The possibility that the remnant glomeruli of the congenital solitary kidney are under greater hypertrophic stress is indeed supported by quantitative morphometric studies. The volume of each glomerulus in solitary kidneys was found to be five to six times normal, a value close to that found in oligomeganephronia, an extreme condition of decreased nephron number [25]. Decreased nephron number has been postulated to occur secondary to low birthweight [26]. Lower nephron number has been postulated to increase the risk of renal disease and to be the mecha-
nism whereby individuals with low birthweight have an increased incidence of progressive renal disease in adulthood [26]. These fewer nephrons are postulated to be under greater hemodynamic stress that contributes to progressive sclerosis. I find it interesting that glomerular size in normal African Americans is larger than that in whites and possibly reflects smaller nephron number [27]. This finding could contribute to the increased incidence of end-stage renal disease in African Americans compared to whites. Mechanisms other than hemodynamic stress that could underlie these differences in normal glomerular growth and that also relate to an increased incidence of end-stage renal disease include functional polymorphisms of genes that are involved both in renal and glomerular development and that contribute to amplified scarring mechanisms, such as the renin-angiotensin system.

In addition to the key roles of hypertension and growth factors on extracellular matrix accumulation and sclerosis, multiple other mediators have been associated with glomerular sclerosis [7, 8, 28]. The detailed discussion of these mediators is beyond the scope of this Forum, but these factors include, but are not limited to, hyperlipidemia, proteinuria, reactive oxygen species, the diabetic milieu, and infiltrating macrophages. The pattern of specific factors activated likely depends on the type of initial injury, the age of the patient when the insult occurs, and complex genetic factors, all of which modulate the process of sclerosis.

Potential regression

Considering this multiplicity of mediators of sclerosis, one can ask whether it is possible to halt or even reverse these destructive processes within the kidney. Regression of sclerosis, however, already has been accomplished in cardiovascular disease [29, 30]. Studies in animals and humans show feasibility of regression of atherosclerotic plaque, regression of coronary artery disease, and regression of left ventricular hypertrophy and fibrosis, all with associated improved cardiovascular function. Ground-breaking studies from 1970 showed resolution of atherosclerotic lesions in coronary arteries when rhesus monkeys were given diets that decreased serum cholesterol [30]. Even severe fibrotic lesions that consisted of vascular smooth muscle cell proliferation, augmented fibrosis, collagen, and abnormal endothelial reactivity improved. Myocardial fibrosis is also remediable: the increased amount of left ventricular mass due largely to collagen accumulation in the untreated spontaneously hypertensive rat was reduced with an ACE inhibitor. This improvement was associated with augmented proteolytic activity, which might have played a mechanistic role in the remodeling [31].

But results from heart, coronary artery, and aorta cannot be directly extrapolated to the sclerotic glomerulus. The glomerulus is a unique structure with complex interactions of endothelial, mesangial, and epithelial cells in forming the filtering capillary loops. Glomerulosclerosis is defined as a combination of increased matrix and obliteration of the capillary lumina. Increased matrix accumulation is the result of extracellular matrix synthesis in excess of degradation. To accomplish regression of sclerosis, these pathways have to be modified and excess extracellular matrix degraded. Most challenging is the remodeling of obliterated capillary lumina (Table 1). Depending on the cell types involved, injured cells have to be replenished, repaired, or deleted to regenerate or restore capillary loops that might result in glomerular filtration (Fig. 3). Three main obstacles have to be overcome. First, the net loss of endothelial cells must be resolved via regeneration of endothelial cells; second, there is variable mesangial cell proliferation that evolves into the sclerotic, acellular lesion, and mesangial cells must regrow; finally, the visceral epithelial cell has limited growth capacity. New glomeruli cannot be generated after full-term birth in humans, so regeneration of open loops has to occur by new capillary loop growth within injured glomeruli, with resorption of the sclerosed segments within that glomerulus. Thus, the restoration of open capillary loops is not a return to its original structure. We postulate, rather, that “regression” is mediated by new growth of capillaries that take the place of the sclerosed, reabsorbed segments. Evidence in humans and animals indicates that glomerular growth after injury can indeed be achieved by lengthening or branching of the capillary loops [32, 33]. The biologic potential of each of these processes in the glomerulus indicates that remodeling and regression of glomerulosclerosis are theoretically possible. Emerging data show that they can be accomplished in practice as well.

Cell growth versus apoptosis and differentiation. Although the sclerotic glomerulus appears acellular and “dead,” cell turnover continues; thus, even at advanced stages of sclerosis, modulation of cell growth and consequent regeneration can occur [34]. Glomerular growth is influenced by numerous cytokines and hormones, including Ang II. The angiotensin II type-1 (AT1) receptor mediates cell hypertrophy and/or hyperplasia, and is widely expressed in the kidney including in mesangial, glomerular visceral epithelial, endothelial, and vascular smooth muscle cells [35–37]. Cell growth after injury frequently is accompanied by increased apoptosis (Fig. 3). Apoptosis is induced by a variety of factors, including Ang II. This effect of Ang II is mediated by the AT2 receptor. This receptor is up-regulated in vascular smooth muscle cells in neointima at sites of injury. Because overexpression of the AT2 receptor results in decreased vascular lesions after injury, it is likely that AT2 has a role in remodeling and repair [37, 38]. Pharmacologic inhibition or absence of the AT2 receptor in null mutant
Table 1. Regression of glomerulosclerosis

<table>
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<tr>
<th>Potential</th>
<th>Obstacle</th>
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<tr>
<td>Proteases locally available (e.g., MMP-2, MMP-9, t-PA, u-PA)</td>
<td>Protease inhibitors locally expressed (e.g., TIMP-1, -2, PAI-1)</td>
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<tr>
<td>Growth factors → regeneration (e.g., VEGF)</td>
<td>Resistance to proteolysis (e.g., glycation, cross-linking)</td>
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<tr>
<td>Glomerular growth (capillary lengthening/branching)</td>
<td>Growth factors → apoptosis</td>
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<td>Growth factors → ECM (e.g., angiotensin, TGF-β)</td>
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<td>No glomerulogenesis after birth</td>
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<td>Limited growth potential of GVEC</td>
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Abbreviations are: MMP, matrix metalloproteinase; t-PA, tissue-type plasminogen activator; u-PA, urokinase-type plasminogen activator; TIMP, tissue inhibitors of metalloproteinase; PAI-1, plasminogen activator inhibitor-1; VEGF, vascular endothelial growth factor; ECM, extracellular matrix; TGF-β, transforming growth factor-β; GVEC, glomerular visceral epithelial cell.

Fig. 3. Mesangial (Mes), endothelial (Endo), and epithelial (Epi) cell interactions and response to initial injury determine the overall balance of cell growth and matrix accumulation.

mice, with resulting deficient apoptosis after injury, increases fibrosis in rodent models of renal and cardiac fibrosis [39, 40]. Apoptosis might be a healing mechanism, eliminating injured cells with minimal stimulation of immune/inflammatory mechanisms and cytokines. However, disproportionate apoptosis of cell types that cannot regenerate can have deleterious consequences.

Mesangial and endothelial cells can readily proliferate and replenish after injury. In the anti-Thy-1 model of mesangial proliferation, endothelial and mesangial cells proliferate during the spontaneous resolution phase. But when angiogenesis was pharmacologically inhibited, repair failed (abstract; Kitamura et al, J Am Soc Nephrol 9:501A, 1999). Similarly, excess apoptosis of endothelial cells was associated with sclerosis in the 5/6 nephrectomy model [41]. Numerous growth factors modulate these responses [28]. Vascular endothelial-derived growth factor (VEGF) is an endothelial cell-specific mitogen produced by glomerular visceral epithelial cells that specifically regulates physiologic and pathologic endothelial cell growth and glomerular permeability. Recent evidence implicates VEGF as a necessary survival factor for endothelial cells in vivo (abstract; Suga et al, J Am Soc Nephrol 10:561A, 1999), and inhibition of VEGF prevented repair after anti-Thy-1 injury [42].

In contrast to the strong potential of mesangial and endothelial cells to contribute to remodeling, the glomerular visceral epithelial cell is “growth challenged.” This
lack of proliferation in the normal mature glomerular visceral epithelial cell is accompanied by high expression of a cyclin-dependent kinase inhibitor, p27\textsuperscript{kip1} [43], which seems to be a rate-limiting step for the growth response of the visceral epithelial cell. Shankland recently suggested that either too much or too little proliferation of the visceral epithelial cell in response to genetic manipulation of p27\textsuperscript{kip1} is detrimental to the glomerulus [44]. Inadequate growth of the visceral epithelial cell possibly gives rise to areas of dehiscence and insudation of plasma proteins, which progress to adhesions and sclerosis [45]. Recent data showing that another cyclin-dependent kinase inhibitor, p21, appears necessary for development of injury after 5/6 nephrectomy in mice suggests the crucial importance of cell growth responses in determining the whole kidney’s response to injury [46].

Cell growth also is associated with altered cell differentiation. The mesangial cell shows α-smooth muscle actin expression in sclerotic conditions, indicating a more myofibroblast-like phenotype [47]. Mature glomerular visceral epithelial cells normally express several markers, including Wilms’ tumor (WT-1) tumor-suppressor transcription factor; a podocyte-specific tyrosine phosphatase, GLEPP-1; and synaptopodin. Dedifferentiation of visceral epithelial cells is linked to sclerosis in human collapsing glomerulopathy, a disease with severe collapse, sclerosis, and epithelial cell injury. There was loss of expression of WT-1 and other differentiation markers associated with proliferation, and perhaps apoptosis, of the visceral epithelial cell in injured segments [48]. Recently, mutations of WT-1 resulting in abnormal splice variants have been associated with the development of FSGS; thus, WT-1 might not merely be a marker of de-differentiation but also could play a role in normal control of epithelial cell function [49].

**Matrix synthesis versus degradation.** In addition to increased matrix synthesis, decreased extracellular matrix proteolysis contributes to progressive renal disease [50]. Numerous growth factors have been characterized in terms of their ability to increase matrix synthesis, including the proto-oncogenes, transforming growth factor-β (TGF-β), platelet-derived growth factor (PDGF), and vasoactive substances such as Ang II and endothelin [7]. TGF-β promotes extracellular matrix accumulation by increasing matrix production and possibly by decreasing its degradation, in part by induction of plasminogen activator inhibitor-1 (PAI-1) [51]. Both PDGF-B and Ang II increase TGF-β, and Ang II also promotes conversion of TGF-β to its active form. Plasmin might play a role in this activation [51].

In contrast to the extensive investigation of factors promoting matrix synthesis, modulators of increased matrix degradation, which could allow resolution, have not been well characterized. Several proteases and protease inhibitors are expressed in the glomerulus; thus, modulation of matrix can occur [52]. The matrix metalloproteinases (MMP) are a class of zinc-dependent proteases. Expressed in glomeruli, MMP-2 (gelatinase A) and MMP-9 (gelatinase B) have particular relevance to glomerular collagen remodeling. Both degrade collagen IV, a prominent component of glomerular basement membrane, and also proteoglycan and fibronectin, components of mesangial matrix [50]. Several tissue inhibitors of matrix metalloproteinases (TIMPs) also are present in glomeruli: TIMP-2 is produced in the glomerulus by mesangial cells and is constitutively expressed, whereas TIMP-1 is up-regulated in response to numerous injuries. TIMP-1 is induced by angiotensin in vitro and down-regulated by TGF-β; TIMP-2 is up-regulated by TGF-β and contributes to increased extracellular matrix accumulation [53].

A shift in balance of proteases and their inhibitors occurs during matrix expansion (Fig. 3). Proteolytic activity decreased during progressive renal disease in rats, and expression of TIMP increased in the puromycin model of chronic renal disease. Conversely, protection from aging-induced sclerosis in female rats was associated with increased glomerular metalloproteinase activity compared with affected males [54], and a shift towards proteolysis occurred during resolution of injury in the reversible anti-Thy-1 model of mesangial matrix expansion [55]. Treatments that slowed disease progression in puromycin nephropathy also moderated changes in protease gene expression [56].

Recent evidence has added PAI-1 to this array of matrix modulators in the kidney. Plasminogen activator inhibitor-1 inhibits not only fibrinolysis, but also proteolysis by inhibiting activation of plasminogen activators (PA). Plasmin can cleave most extracellular matrix proteins [57, 58], and both tissue-type (t-PA) and urokinase plasminogen activators (u-PA) play important roles in vascular remodeling, angiogenesis, and tumor metastasis [58]. Tissue-type plasminogen activator primarily affects fibrinolysis, whereas u-PA has less affinity for fibrin but avidly degrades matrix [58]. Normally present in very low levels in the kidney, PAI-1 is expressed in vitro in many cells, including endothelial and visceral epithelial cells [58, 59], and is increased in settings of vascular injury, whether thrombotic or fibrotic [28]. Increased PAI-1 levels, whether due to the functional 4G/4G polymorphism of the PAI-1 gene promoter, or for other causes, are associated with cardiovascular disease [60]. Diabetic patients with compound polymorphisms for both the PAI-1 4G and the ACE D allele, the latter of which increases renin-angiotensin system (RAS) activity, had increased macrovascular disease [61]. The ability of TGF-β1 to induce fibrosis also might relate to PAI-1: TGF-β1 induces PAI-1 to a greater extent than u-PA in cultured endothelial cells, and thus promotes fibrosis [62].

Our data directly link local PAI-1 expression to sites of
sclerosis, and decreased PAI-1 to resolution of sclerosis. Angiotensin II modulates fibrosis by direct effects of matrix and by activating other growth factors [7]. Also, Ang II can induce PAI-1 directly, both in vitro and in vivo [63–65], and inhibition of Ang II prevents this induction both in experimental models and in humans [66, 67]. Interactions between PAI-1 and Ang II thus could be of primary importance in fibrosis. Conversely, inhibition of the RAS could promote remodeling of sclerosis by decreasing PAI-1 and thus augmenting proteolysis. Angiotensin II and its hexapeptide metabolite, angiotensin IV, induce dose- and time-dependent increases in PAI-1 mRNA expression in vitro [63]. In vivo, this effect was mediated by Ang II and the type-1 receptor in the rat model [65]. Further, increased RAS activity mediated by exogenous infusion of physiologic amounts of Ang II or by the presence of the ACE DD polymorphism (abstract; Brown et al, Circulation 92:1-552, 1995) increases PAI-1 levels in humans with no effect on t-PA [64].

**Interactions of RAS and aldosterone.** Recent data linking aldosterone to PAI-1 induction in vitro and in vivo illustrate a further mechanism by which the renin-angiotensin system might augment sclerosis. Addition of aldosterone antagonism to angiotensin inhibition resulted in greater resolution of glomerulosclerosis in animal studies than did angiotensin inhibition alone [68]. Aldosterone antagonism alone also decreased vascular injury in the stroke-prone hypertensive rat model [69, 70]. Aldosterone increases Ang II induction of PAI-1 in vitro, and PAI-1 plasma levels correlate with aldosterone levels in patients [71]. Our recent studies show that antagonism of aldosterone with spironolactone ameliorated the development of sclerosis in the nonhypertensive sclerosis model of radiation nephropathy. This finding was not linked to effects on blood pressure or proteinuria, but was tightly associated with decreased PAI-1 [72]. Recent studies in humans have shown that PAI-1 plasma levels and activity are reduced in response to an ACE inhibitor, but not to an angiotensin type-1 receptor antagonist (AT1RA). This effect was associated with an augmented decrease in plasma aldosterone levels in the ACE inhibitor-treated patients compared with those who received an AT1RA [73]. These findings provide linkage of aldosterone to possible fibrotic mechanisms and suggest that antihypertensive treatments might have to target both the RAS and aldosterone in some settings for optimal regression of end-organ damage.

**Evidence of regression.** Inadvertent transplantation of kidneys with diabetic injury into nondiabetic recipients demonstrated resolution of mesangial expansion in humans [74]. More recent data have shown that cure of diabetes by pancreatic transplantation in patients with diabetic nephropathy also could reverse renal injury, albeit over a long period [2].

Experimental models shed light on some of the mechanisms involved in regression of glomerulosclerosis. Spontaneous resolution of mesangial matrix accumulation occurs in the anti-Thy-1 model. At one day after anti-Thy-1 injection, matrix mesangiolysis is apparent; this is followed by increased matrix at one week, more increase at two weeks, and then restoration of normal morphology by four weeks, with attendant changes in cell proliferation and increasing metalloproteinase activity [55]. Orloff et al demonstrated regression of matrix expansion in response to an intervention that cured diabetes in a rat model [75]. They assessed relative mesangial volume on renal biopsy specimens at six months after streptozotocin injection in the rat. Pancreatic transplantation was then done. Over the following one to nine months, mesangial volume expansion decreased. Pancreatic transplantation also reversed matrix expansion when the animals were treated at the very late stage, after more than 20 months of diabetes, when mesangial volume was much more expanded. One must caution that these diabetic rat models do not show true sclerosis, but rather just mesangial matrix increase, and these mechanisms of regression therefore might not be applicable to diabetic humans. However, in the chronic puromycin aminonucleoside model, a true glomerulosclerosis model in which capillary lumina are obliterated, regression of sclerosis with intervention with either an ACE inhibitor or low-protein diet was suggested. In these experiments, cohorts of animals sacrificed at different time intervals were compared. Progression occurred in almost all the untreated animals by 24 weeks. Animals treated from the onset with ACE inhibition showed less sclerosis at 24 weeks compared with treated animals that were sacrificed at 12 weeks [76]. The skeptic might argue that true regression is not absolutely demonstrated by these experiments, as lesions were not compared in the same animals. The relatively large number of animals studied at each time point makes it likely that group results are applicable to the individual animals as well. We sought to directly confirm the possibility of regression in this nonhypertensive puromycin aminonucleoside model of glomerulosclerosis, comparing biopsy-documented to autopsy-documented sclerosis in the same rats [4]. Regression of sclerosis by late-stage intervention with an ACE inhibitor or AT1RA was indeed achieved. Sclerosis, however, was very mild in this nonhypertensive model. We therefore examined whether regression could be achieved in a hypertensive model with more severe existing sclerosis, a situation more analogous to most human renal disease.

Considering the evidence that nonhemodynamic mechanisms contribute to Ang II’s effects on sclerosis, we postulated that higher doses of an Ang II inhibitor than those required to normalize blood pressure would have a greater beneficial effect on sclerosis. We assessed the severity of glomerulosclerosis by renal biopsy at eight weeks after 5/6 nephrectomy in the rat [5]. Animals were
then divided into groups that received no treatment, a "normal" dose of an ACE inhibitor (that is, minimum dose required to normalize blood pressure), or a fourfold higher dose of the ACE inhibitor. Systemic and glomerular pressures were normalized by both ACE inhibitor doses with no further hemodynamic effects at the higher dose. Sclerosis progressed in all untreated animals, with an average increase in the severity of sclerosis of 176 ± 58%. Progression of sclerosis was significantly ameliorated in animals receiving the normal ACE inhibitor dose, with a mean increase in the severity of sclerosis of only 49 ± 17%. Remarkably, sclerosis was even further ameliorated and even regressed by the fourfold higher ACE inhibitor dose, with less sclerosis at autopsy than at biopsy four weeks previously in three of the five rats. The AT1RA was similarly effective, achieving regression in one-half the rats (abstract; Nakamura et al, J Am Soc Nephrol 10:665A, 1999). Tubulointerstitial fibrosis regressed in parallel with glomerular injury.

Possible mechanisms by which these higher doses of Ang II inhibition resulted in decreased sclerosis include inhibition of Ang II's ability to increase blood pressure and increase extracellular matrix synthesis, and inhibition of other growth factors linked to Ang II, such as PDGF and TGF-β. However, to decrease existing extracellular matrix accumulation, increased matrix degradation also must have occurred. Keeping in mind the direct link between Ang II and PAI-1, we examined the potential role of PAI-1 in the regression of sclerosis. Sclerosis was similar at biopsy in both groups by study design, and was paralleled by similar PAI-1 glomerular protein expression. In individual glomeruli, PAI-1 staining correlated strongly with sclerosis (R² = 0.734, P < 0.0001). In 5/6 nephrectomy animals without treatment, PAI-1 protein was intensely expressed at autopsy and localized to sites of injury. In animals treated with AT1RA but not achieving resolution, PAI-1 expression remained prominent. In contrast, in those animals in which AT1RA produced regression of sclerosis, PAI-1 expression was virtually absent, with less staining than at biopsy in these same rats (P < 0.05). Rats with regression of sclerosis also had improved renal function, with lower serum creatinine levels in AT1RA-treated animals versus untreated rats with 5/6 nephrectomy (1.16 ± 0.12 versus 2.37 ± 0.69 mg/dL), although hypertension and proteinuria were not significantly affected (abstract; Nakamura et al, J Am Soc Nephrol 10:665A, 1999). These findings implicate inhibition of PAI-1 by high doses of AT1RA or ACE inhibition and the resulting increased matrix degradation in regression of glomerulosclerosis.

The link of PAI-1 expression and sclerosis also was demonstrated in the radiation nephropathy model, a nonhypertensive model of early endothelial injury followed by late sclerosis [67]. Plasminogen activator inhibitor-1 mRNA expression by in situ hybridization was closely associated with sites of glomerular injury, as assessed by serial action section morphologic analysis. In glomeruli, PAI-1 mRNA was localized to injured mesangial and endothelial areas, with focal expression in glomerular visceral and parietal epithelial cells. Thus, autocrine effects are implicated, as these cells also express receptors for angiotensin (AT1 and possibly AT4 receptors). Minimal PAI-1 expression was seen in intact glomeruli, whereas increasing levels of expression were present in sclerotic glomeruli or in glomeruli with a combination of sclerosis, mesangiolysis, and thrombosis. Treatment with AT1RA or an ACE inhibitor significantly inhibited the up-regulation of PAI-1 mRNA by Northern blot without affecting t-PA or u-PA expression. No changes in the expression of other growth factors were associated with endothelial injury, including PDGF, basic fibroblast growth factor, tumor necrosis factor-α, and interleukin-1β. At week 12, TGF-β mRNA levels were moderately increased, and this increase was mildly attenuated by treatment with AT1RA or an ACE inhibitor. TGF-β mRNA expression by in situ hybridization was diffuse, and no differences in mRNA expression were apparent between irradiated glomeruli with and without morphologic lesions [67].

Renal sclerosis was prevented by treatment with either an ACE inhibitor or AT1RA. Irradiated animals treated with Ang II inhibitors showed even less sclerosis than did age-matched controls; this finding indicated that Ang II inhibition even might affect the matrix accumulation that accompanies the aging process. Amelioration of age-related glomerular and vascular sclerosis also occurred in previous studies [4, 77]. We recently found that even existing age-related glomerular and vascular stenosis in the rat could be remodeled, with regression and decreased collagen content induced by starting AT1RA treatment in aging rats. This remodeling was associated with decreased PAI-1 [78].

Summary
All the data I have discussed today demonstrate that regression of biopsy-proven glomerulosclerosis can be achieved in various experimental settings, including those germane to human disease in which moderate glomerulosclerosis, proteinuria, and hypertension frequently are present at the time of diagnosis. Limited data show the feasibility of regression of injury in human diabetic nephropathy. The potential importance of the renin-angiotensin system in the spectrum of injuries leading to mesangial matrix accumulation and sclerosis is underscored by the effectiveness of therapies that aim to inhibit its manifold actions, including induction of PAI-1. Understanding of interactions of the RAS and aldosterone with PAI-1, and of the dynamic control of cell proliferation, apoptosis, and regeneration is now evolving. Ongoing studies will establish which of these recent provocative
findings from animal models are relevant to human diseases and might lead to optimal therapies to forestall progression, and perhaps even induce regression, of sclerosis.

QUESTIONS AND ANSWERS

Dr. Nicolaos E. Madias (Executive Academic Dean, Tufts University School of Medicine, Boston, Massachusetts): Thank you for your wonderful presentation. ACE inhibitors can slow the progression of chronic renal diseases, but it appears unlikely that by themselves they will be able to forestall progression in the majority of patients. Given the multiple effector mechanisms of renal injury, would you think that a multipronged approach should be explored?

Dr. Fogo: That would be the ideal way to approach chronic renal disease. In the future, patients’ genetic contributions to risk factors will be considered and maybe we will pre-emptively inhibit mechanisms that are likely to be activated in the very early stages of disease. Not much direct evidence on therapeutic combinations is available. In one small study of eight patients with proteinuria and IgA nephropathy, combined ACE inhibitor and receptor antagonist therapy yielded higher antiproteinuric effects without an additional decrease in blood pressure [79]. We are embarking on animal studies to test the combination of high doses of both these drugs.

Dr. Madias: I wanted to explore a bit further the potential role of aldosterone in renal damage. You noted that both Ang II and aldosterone induce PAI-1. Accordingly, I wonder whether there are observations on induction of PAI-1 in settings of increased mineralocorticoid but suppressed Ang II levels, such as the DOCA/salt model.

Dr. Fogo: It’s difficult to establish such interactions because of the limitations inherent in measuring these activities via serum levels rather than in the tissues. Despite the absence of an elevated plasma renin activity in many patients, ACE inhibitors impart benefit. Also, despite continued Ang II production during ACE inhibition through non-ACE mechanisms, the blood pressure remains controlled and the long-term beneficial effects of ACE inhibitors on GFR continue. In relation to aldosterone, the aldosterone effect on glomerulosclerosis does not seem to be related to hypertension, tubular effect, or sodium transport via the classic mineralocorticoid receptor. Our experiments using knockout animals devoid of angiotensinogen showed that plasma potassium in the absence of angiotensin can be a powerful stimulus to aldosterone activity [80]. When we removed sodium and potassium from the animals’ diet, they died with hypotension and very low aldosterone levels. We still don’t understand the specific mechanisms for the many interactions.

Dr. Madias: Is the ability of aldosterone to induce PAI-1 presumed to be exerted directly at the glomerular level?

Dr. Fogo: PAI-1 is expressed in many cells, including endothelium, and has a very short half-life. The hypothesis is that aldosterone exerts local direct effects that are not mediated by sodium or blood pressure effects via the classic tubular mineralocorticoid receptor.

Dr. Andrew J. King (Division of Nephrology, New England Medical Center, Boston, Massachusetts): The RALES trial might be an example of a beneficial effect of reducing aldosterone.

Dr. Fogo: Thank you for reminding me of that. This benefit is not readily explained by the classic aldosterone actions. This is analogous to the change in understanding of angiotensin effects. Fifteen to 20 years ago, only the classic actions of angiotensin, which related to sodium and water homeostasis and blood pressure, were considered. Angiotensin’s actions are now recognized to be manifold, as have the actions of the other factors we have discussed. Novel effects of aldosterone on fibrosis are now emerging.

Dr. John T. Harrington (Dean, Tufts University School of Medicine): You talked about glomerular size versus sclerosis in three groups: normals, patients with minimal change disease, and patients with focal sclerosis. Were the patients with minimal change disease and those with focal sclerosis studied at comparable times in the course of their disease? That is, did they have equal levels of proteinuria and renal function, or had the patients with minimal change disease cleared their proteinuria versus the patients with FSGS who might still have had proteinuria?

Dr. Fogo: The pediatric nephrologists would be loathe to biopsy a patient with minimal change disease but no proteinuria! These were all patients biopsied for clinical proteinuria and IgA nephropathy, combined ACE inhibitor and receptor antagonist therapy yielded higher antiproteinuric effects without an additional decrease in blood pressure [79]. We are embarking on animal studies to test the combination of high doses of both these drugs.

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Dr. Fogo: The pediatric nephrologists would be loathe to biopsy a patient with minimal change disease but no proteinuria! These were all patients biopsied for clinical reasons who had steroid-resistant or steroid-dependent nephrotic syndrome. The duration of proteinuria, the number of relapses, the preceding treatments with steroids and even, in some cases, slightly more aggressive therapy, were the same in both groups. In terms of those initial biopsies, none of them revealed sclerosis. Only later, as in our Patient 2, did the sclerosis develop. These are not the “standard” patients with minimal change disease; we do not biopsy them, just as you don’t biopsy a “typical” patient with diabetic nephropathy.

Dr. Harrington: You discussed the production of extracellular matrix in detail. Is any information available on the degree of apoptosis? Is it up-regulated, down-regulated? What, if anything, can we do about it?

Dr. Fogo: Apoptosis began to be studied with great interest in renal disease several years ago. We searched intensively for apoptosis in animal models in glomeruli that were undergoing sclerosis and found very little apoptosis. Dr. Vivette D’Agati’s group published an abstract delineating apoptosis in human biopsy specimens (abstract; Szabo et al, J Am Soc Nephrol 5:844, 1994). Of
course, proliferation as well as apoptosis were increased in proliferative forms of glomerulonephritis such as lupus and MPGN. Surprisingly, apoptosis was also present in FSGS. Our own studies in rats have shown extremely low levels of apoptosis (unpublished data). Apoptosis is a fleeting phenomenon, and determining its occurrence in a tissue section from the glomerulus is challenging. TUNEL staining is not completely specific, and morphologically apoptotic cells are rare in FSGS. The balance of cell cycle regulatory mechanisms is very important. In virtually all settings in which we find increased proliferation, we also find increased apoptosis. Elegant summaries of many of these studies have been published [44, 81].

**Dr. Andrew S. Levey (Chief, Division of Nephrology, New England Medical Center):** I need a pathology lesson. My questions relate to correlations of pathology with etiology and mechanism of injury. In your description of the two cases, I see descriptions of three types of focal sclerosis. Patient 1’s open renal biopsy shows focal segmental sclerosis and marked glomerulomegaly characteristic of secondary FSGS. Patient 2’s renal biopsy demonstrates advanced FSGS typical of the idiopathic form. The transplant renal biopsy from Patient 2 revealed glomerular sclerosis due to transplant glomerulopathy, but no evidence of recurrent idiopathic FSGS. What’s the size distribution of the glomeruli among these lesions? And what are the correlations with progression or regression of these lesions?

**Dr. Fogo:** This is one of the fun and challenging areas in pathology: to recognize a lesion and then try to dissect it, distill it, and deduce its origin and prognosis. Saying that there is a lesion of glomerular sclerosis in a focal segmental pattern is only a description, and the description doesn’t necessarily tell us what the underlying disease is. It just says that the scarpping affects some glomeruli in a segmental fashion. We look very hard for indications that this could be a secondary rather than an idiopathic process, because the two conclusions have very different treatment implications. It’s very easy to arrive at a diagnosis in a patient who has sclerosis in a focal segmental pattern but IgA deposits as well. This lesion is chronic IgA nephropathy, and the sclerosis indicates a worse prognosis. The same holds true for sclerotic lesions in lupus or membranous glomerulonephritis or in a healed crescentic glomerulonephritis—you can see the fibrocellular crescents or the immune complexes. However, the secondary FSGSs that are not immune-complex-mediated—such as those secondary to loss of a kidney very early in childhood or during development, hypertension, or transplant glomerulopathy—are more subtle lesions to analyze. In today’s first patient, the biopsy findings that suggested a secondary FSGS included thickening of Bowman’s capsule, periglomerular fibrosis, mesangial increase, very early sclerosis, and marked glomerulomegaly. The key, of course, is the history and the time course of what had happened to him. These help to correlate morphology and clinical features and to make a clinicopathologic diagnosis.

In the second patient, the first biopsy did not show signs of sclerosis. The second biopsy revealed extensive sclerosis. There were no immune complexes. The morphology in the very advanced stage of sclerosis can make it difficult to discern the underlying cause. The nonsclerotic segments in this patient had no lesions other than foot process effacement, and the sclerosis seen on light microscopy was consistent with idiopathic FSGS. Light microscopy of the transplant biopsy specimen disclosed segmental sclerosis, but she also had a basement membrane abnormality of apparent splitting, that is, reduplication of the basement membrane. This lesion is characteristic of transplant glomerulopathy and likely results from chronic endothelial injury. This injury causes expansion of the lamina rara interna, which we visualize by electron microscopy. It is virtually certain that she has transplant glomerulopathy, with the segmental sclerosis due to this injury and not due to recurrence of FSGS. The time course helps us make this diagnosis too. She had had her transplant for 10 years at that time. We know that recurrence of FSGS most often occurs within the first year, or even within the first month. In some cases, recurrent proteinuria has started while the patient was still in the operating room receiving the kidney.

In terms of glomerular size in various sclerotic lesions, it depends on the etiology. With unilateral renal agenesis, the glomeruli are very large. In oligomeganephronia, the glomeruli are even bigger. In segmental sclerosis associated with sickle cell nephropathy, Dr. Bhathena has shown the largest glomeruli demonstrated in humans [82]. We have looked at recurrent FSGS in transplants. We chose our population carefully because an adult recipient of an adult kidney normally has about a 30% increase in renal size and GFR over the first three months. The donor’s remaining kidney undergoes the same kind of changes. So there normally is glomerular growth in kidneys transplanted into adults. We therefore looked at pediatric recipients of adult kidneys, in whom glomerular enlargement generally doesn’t occur. The patients who developed recurrence of FSGS had glomerulomegaly preceding the onset of the sclerosis. The children who had FSGS or other diseases who did not develop FSGS in their transplant had no glomerulomegaly [15]. We have not looked at the issue of transplant glomerulopathy in the renal graft and glomerular size.

**Dr. Levey:** To test the effects of agents that influence all the mediators that you mentioned, of course you will need to carry out many clinical trials. But the clinical trial paradigm for progressive renal disease is unsatisfactory for comparisons of multiple agents because it requires a long follow-up period. This problem reflects our
lack of surrogate clinical markers for progression and regression. Would you accept increases or decreases in urinary protein excretion as surrogate markers of progression or regression? Are there agents in animal models in which the effects on progression are separate from the effects on proteinuria?

**Dr. Fogo:** Proteinuria is a very good marker of disease. It has even been postulated as a mediator of disease progression. Dr. Allison Eddy and coworkers have found that overload proteinuria in the rat can directly cause tubulointerstitial fibrosis and glomerular scarring [83]. However, some patients have proteinuria and do not have significant scarring, such as those with minimal change disease; this group can be either steroid-resistant or steroid-dependent. There are patients who develop sclerosis who typically don't have significant proteinuria, particularly African American patients with hypertension and renal insufficiency. In these patients, there might be a primary microvascular disease rather than a direct causal effect of hypertension in the sclerosis. Larry Hunsicker has very interesting unpublished data from the multicenter collaborative study in type-1 diabetes looking at patients who were given an ACE inhibitor and whose proteinuria did not respond to the intervention. The subanalysis of these patients revealed long-term protection of GFR compared with patients with persistent proteinuria who were receiving other medications. This effect was dissociated from the effect on proteinuria. Thus, I believe that we cannot use proteinuria as the sole criterion for response to intervention. In most cases, it is a very useful reflection of injury at the glomerular level.

**Dr. King:** Prior to the use of ACE inhibitors, it was well known that blood pressure reduction retards progression of renal failure. In your animal model of radiation nephropathy, you used an AT1 receptor blocker or an ACE inhibitor. Have you used so-called “triple” therapy or other non-Ang II-manipulating drugs? Also, what is the effect on PAI-1?

**Dr. Fogo:** I think we need to have JNC VI standards for the rats! Within so-called “normal” pressure levels it seems, at least in diabetic patients, lower is better, and the best “normal” level of blood pressure is probably a lot lower in diabetics than it is in other patients. We didn’t perform triple therapy experiments in the animals that received radiation. We have given triple therapy in other animal models, such as animals that have undergone subtotal nephrectomy. In contrast to the amelioration of sclerosis with ACE inhibitors with same blood pressure effect, triple therapy does not resolve progression of the glomerular sclerosis. Further, the effect on PAI-1 with these treatments is parallel to effects on sclerosis.

We have done some experiments in the 5/6 nephrectomy model looking at regression and comparing triple therapy with another intervention that targets peroxi-

some proliferator-activated receptor-γ (PPAR-γ). We became interested in PPAR-γ because it interacts with PAI-1 and angiotensin. We treated animals with troglitazone, a PPAR-γ agonist, with an angiotensin-receptor antagonist, or with triple therapy. Although the animals remained hypertensive when we gave them troglitazone, sclerosis was ameliorated, along with decreased PAI-1. There was no effect on progression or PAI-1 when blood pressure was normalized by triple therapy [84].

**Dr. King:** This is a follow-up to a question that Dr. Harrington asked relating to patients who have relapsing minimal change disease, or who are steroid-resistant. What is the role of corticosteroids in the progression of renal disease? As you know, Diego Garcia in a progression study suggested that corticosteroids accelerate progression in animals with 5/6 nephrectomy [85]. Do corticosteroids have any effect on PAI-1? Do you think that corticosteroids play a role in the progression from minimal change to focal glomerulosclerosis in patients?

**Dr. Fogo:** We ourselves have not pursued a corticosteroid/PAI-1 linkage. My colleagues Drs. Douglas Vaughan and Nancy Brown have in vitro studies in which they manipulated the PAI-1 gene and demonstrated a glucocorticoid-responsive element in the PAI-1 promoter, and aldosterone’s effect of increasing Ang II induction of PAI-1 also localized to this region [71]. There is potential linkage through this mechanism. We have not explored glucocorticoid-PAI-1 interactions in vivo. The difficulty comes in assessing the balance between potential benefit from the immune modulatory effects of corticosteroids versus the potential pro-fibrotic effects.

**Dr. Madias:** I want to return to the remarkable observation of regression of diabetic nephropathy following pancreatic transplantation. Why did it take so long for such a regression to occur?

**Dr. Fogo:** It is likely that these patients didn’t have a rapidly progressing disease in the first place. It takes 15 years after the onset of diabetes for one to develop the earliest stages of nephropathy. It takes several years for diabetic transplant patients to develop measurable basement membrane thickening and mesangial expansion in the graft, and 6 to 8 years for the full-blown glomerular lesion to begin to develop. If all we are doing is removing the pathologic impulse and not adding other treatments that could accelerate normal remodeling, this would be the time course you might predict by just removing the hyperglycemic environment and its effects on matrix accumulation.

**Dr. Madias:** Are there known effects of advanced glycosylated end products on metalloproteinase activity and the proteolytic pathways?

**Dr. Fogo:** The AGEs make the matrix proteins less accessible to metalloproteinas. I don’t know whether they directly decrease the activity of the matrix metalloproteinases. The animal models indicate that by decreas-
ing AGEs, aminoguanidine could ameliorate scarring. The human studies, I understand, have not only been disappointing, but several patients have developed crescentic glomerulonephritis that was ANCA-positive. Obviously, there are differences in rodents and humans. Once we can find the genetic similarities, and model human diseases more carefully, we might be able to extrapolate more directly and prudently from animals to humans. The AGEs seem to be key for both diabetes and normal aging, but aminoguanidine does not appear to be the best tool in humans for attacking this mechanism.

**Dr. Harrington:** Your first case was a patient with unilateral renal agenesis. The usual estimate is that 1 in 800 to 1000 people are born with a solitary kidney. I’ll accept the data that perhaps these patients have a slightly higher risk of subsequent focal sclerosis and progressive renal failure, but the overwhelming majority of the patients in fact do not develop progressive renal failure. What genetic or environmental factors should one look for in patients who develop focal sclerosis? What are we missing?

**Dr. Fogo:** My hypothesis is that the risk of sclerosis in the remaining kidney depends on how normal that kidney is and whether the patient has been exposed to extraordinary injuries. The spectrum of these congenital renal abnormalities seems quite broad. In many cases of unilateral renal agenesis, data suggest that the remaining kidney is not normal; these kidneys have larger glomeruli than do kidneys in patients who had nephrectomy for trauma or disease later in life. It’s possible that the remaining kidney has a forme fruste of hypoplasia and decreased nephron number or abnormal development. That partly explains why these people are at excess risk compared to people who lose a kidney to trauma or surgery in adulthood. Polymorphisms that predict increased scarring are also being identified. The ACE D polymorphism, PAI-4G and the TGF-β arginine 25 polymorphism would be of interest. Those polymorphisms could be markers of patients whom we should follow more carefully.

**Dr. Madias:** I was very interested in the capillary branching for induction of regression of glomerulosclerosis. In other systems, the endothelial nitric oxide system appears to be important for endothelial migration, vascular remodeling, and angiogenesis. Is there any evidence for a role of the endothelial nitric oxide system in glomerular remodeling?

**Dr. Fogo:** We have given L-NAME to wild type or PAI-1 knockout mice to examine the contribution of nitric oxide and PAI-1 in hypertension injury. Our data show a link between nitric oxide and PAI-1 systems in that PAI-1 knockout animals were protected from perivascular fibrosis in response to L-NAME administration (abstract, Kakita et al, *J Am Soc Nephrol* 11:335A, 2000). Another excellent candidate for modulating endothelial growth is VEGF.

**Dr. Ronald D. Perrone (Division of Nephrology, New England Medical Center):** I was intrigued by the effect of the high-dose ACE inhibition in the rat studies. You commented that the rats tolerated this well because their sympathetic and other vasoregulatory systems were intact. I think that a fair number of our patients would not tolerate it. Clearly, there are limiting side effects in some patients, particularly diabetics. Are efforts being made in drug development that would limit angiotensin blockade to the kidney?

**Dr. Fogo:** I’m not aware of such efforts. Anecdotally, I know from my clinical biopsy practice that quite a number of nephrologists are treating proteinuric patients who are difficult to manage with higher than usual doses of an ACE inhibitor or with the combination of an ACE inhibitor and an AT1 receptor antagonist and have not seen an excess of side effects, that is, hyperkalemia or hypotension. I don’t know whether those patients were selected for being low-risk patients. My clinical colleagues who work directly with patients tell me that you cannot always return blood pressure to normal with single-drug therapy with an ACE inhibitor or a receptor antagonist. It appears to me that if blood pressure control is our only goal, then we are vastly undertreating the end-organ damage and the fibrosis. We need to aim for maximal antifibrotic potential with our therapies. I would challenge us to do so and not just control blood pressure.

**Dr. Samina Khan (Nephrology Fellow, Division of Nephrology, New England Medical Center):** I was impressed with your data at the ASN meeting that showed regression of fibrosis. Are you aware of other glomerulonephritides in which reversal or regression of the fibrosis is observed, similar to the observation in diabetic patients? Also, would you address the use of antifibrotic agents in chronic graft failure, in which advanced fibrosis is a major issue and the population has a higher morbidity rate and fewer treatment options?

**Dr. Fogo:** In transplanted organs that have low-level glomerular lesions, notably, IgA deposits with mesangial expansion, these lesions can regress. An inadvertent experiment of transplanting a kidney with early diabetic nephropathy into a normal patient demonstrated that diabetic lesions can regress in that setting, too. We don’t have a lot of data on other patients because we don’t often perform repeat biopsies. You asked about antifibrotic agents. Dr. Larry Hunsicker is heading a multicenter trial in patients with chronic transplant nephropathy. One arm in this trial is treatment with an HMG CoA reductase inhibitor. The hypothesis is that this drug does not just improve hyperlipidemia, but that it has antifibrotic effects, maybe through Ras or geranyl-geranylation or other mechanisms. The second arm is an ACE inhibitor.

**Dr. Dana Miskulin (Nephrology Fellow, Division of Nephrology, New England Medical Center):** I was interested in your observations that the glomerulomegaly was
sensitive as a specific marker in patients who progressed to end-stage renal disease. Are we to think of glomerulomegaly as a pathogenetic mechanism of glomerulosclerosis?

Dr. Fogo: This has been one of the fun things that I've been involved in. This was the first human study that I did and it was developed based on experimental rat data. The relevance of the rat data to humans was gratifying. If there is glomerulomegaly, you have to exclude physiologic reasons for glomerulomegaly. Uninephrectomy, for instance, can induce glomerular growth that is physiologic and not a marker of pathologic events. Glomerular growth is not pathognomonic for events that lead to sclerosis; rather it is a marker. We have seen glomerular enlargement in adults with FSGS, and other groups have confirmed this finding in adult patients in the U.S., Korea, and Sweden [15]. Glomerular hypertrophy also has been found in preeclampsia, IgA nephropathy, and membranous glomerulonephritis, in association with scarring. In some of these diseases, the increased glomerular size is compensatory, that is, secondary to the initial glomerular injury. In idiopathic FSGS and diabetes, glomerular hypertrophy precedes sclerosis and is a marker of pathogenetic mechanisms that lead to increased matrix.

Dr. Miskulin: Isn't AIDS one setting typically associated with glomerulomegaly?

Dr. Fogo: There we have a collapsing phenotype. The glomeruli that are not collapsed are large, and the ones that are collapsed actually can be normal or smaller than normal.

Dr. Madias: Do ACE inhibitors affect TIMP expression in glomerulosclerosis?

Dr. Fogo: This was a big area of interest several years ago, and the balance between gelatinases A and B expressed in the glomerulus, and the TIMPs is key. My reason for focusing on PAI-1 is because there is a direct link to thrombosis and other areas of vascular disease. Dr. Vaughan has found that angiotensin induces TIMP-1 in vitro (unpublished data). In settings where modulation of scarring accompanies these increases in proteolytic activity, there is an imbalance of the TIMPs and matrix metalloproteinases.

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