Animal Models of FSGS: Lessons for Pathogenesis and Treatment

By Agnes B. Fogo

Glomerulosclerosis in a heterogeneous pattern, ie, focal and segmental glomerulosclerosis (FSGS), is a common endpoint in a variety of settings, including idiopathic FSGS, and scarring secondary to other renal or systemic diseases. These different causes contribute to the diverse clinical outcomes of histological focal sclerosis, and the varying histologic manifestations of sclerosis. Numerous models have been established in the rat that aim to mirror the various elements of human glomerulosclerosis. With the availability of knockout gene technology, many, but not all of these models have been translated to mouse species. This review will focus on the remnant kidney model, the podocyte injury models of puromycin aminonucleoside or adriamycin injection, and examples of newly developed genetic models, such as knockout of CD2 associated protein (CD2AP).

GLOMERULOSCLEROSIS in a heterogeneous pattern (ie, focal and segmental glomerulosclerosis [FSGS]), is a common end point in a variety of settings, including idiopathic FSGS, and scarring secondary to other renal or systemic diseases.[1,2] These different etiologies contribute to the diverse clinical outcomes of histologic focal sclerosis. Also, the broad spectrum of underlying diseases associated with histologic lesions of focal sclerosis in all likelihood reflects the presence of diverse pathogenic mechanisms leading to focal sclerosis. The morphologic appearance of sclerosis varies greatly depending on the initial insult, from the nodular scarring characteristic of diabetic nephropathy to collapsing features seen in idiopathic collapsing glomerulopathy and human immunodeficiency virus-associated nephropathy, prominent synechiae in some cases of idiopathic FSGS, or the prominent hilar localization and marked glomerular hypertrophy characteristic of secondary forms of segmental sclerosis. Such varying morphologic patterns also are seen in different experimental models (Fig 1).[3] With the podocyte injury models of adriamycin or puromycin aminonucleoside nephropathy, there are early adhesions, followed by multifocal areas of sclerosis. When the endothelial cell is injured as in radiation nephropathy, there is early thrombosis followed by organizing well-delineated segmental sclerosis.[4] In contrast, the sharply delineated areas of segmental sclerosis in the remnant kidney model develop without preceding thrombosis.

Numerous models have been established in the rat that aim to mirror the various elements of human glomerulosclerosis. With the availability of knockout gene technology, many, but not all, of these models have been translated to mouse species. The rat models have had the advantage of ease of physiologic manipulations, including direct micropuncture of the glomerulus in, for instance, the Munich Wistar rat, in which glomeruli are present on the surface. The much smaller size of the mouse makes such physiologic measurements more challenging. The possibility of direct investigation of the impact of overexpression or deletion of specific genes still renders the mouse a very attractive model. This article focuses on the remnant kidney model, the podocyte injury models of puromycin aminonucleoside or adriamycin injection, and examples of newly developed genetic models, such as knockout of CD2-associated protein (CD2AP).

REMNANT KIDNEY MODEL

The rat renal ablation model was described initially in 1932, with subsequent extensive investigation with elegant micropuncture studies by Brenner's group and others.[5,6] The removal of a large portion of renal mass results in gradually increasing proteinuria and progressive hyperperfusion, hyperfiltration, hypertrophy and focal, segmental, initially affecting the deep juxtamedullary glomeruli as in human idiopathic FSGS.[6-9] There is early glomerulosclerosis by week 4 after renal ablation by ligation, with segmental sclerosis in about 20% of glomeruli by week 8 with early tubulointerstitial fibrosis. By week 12, there is widespread glomerulosclerosis and tubulointerstitial fibrosis. Animals typically die of uremia starting at week 12 to week 16.[6,10] The model has technically been induced in

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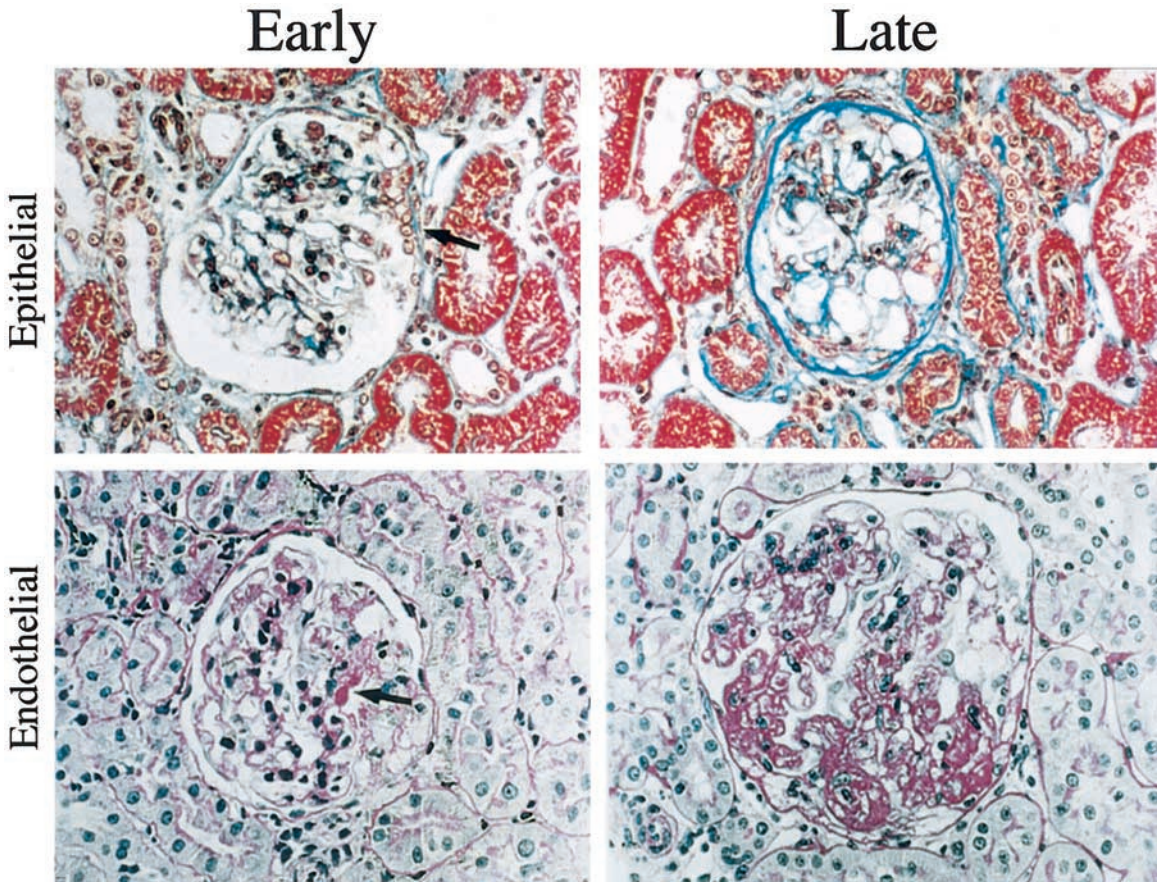

Fig 1. Different initial injuries result in differing phenotypes of sclerosis. Early endothelial injury caused by radiation nephropathy in the rat causes localized glomerular thrombosis (arrow), followed by later well-defined segmental sclerosis. Numerous adhesions (arrow) of podocytes to Bowman's capsule occur early in response to puromycin aminonucleoside, which specifically injures the visceral epithelial cell. The late consequence is multifocal segmental sclerosis. (Top panel, Masson's trichrome stain; bottom, periodic acid Schiff, all ×400).

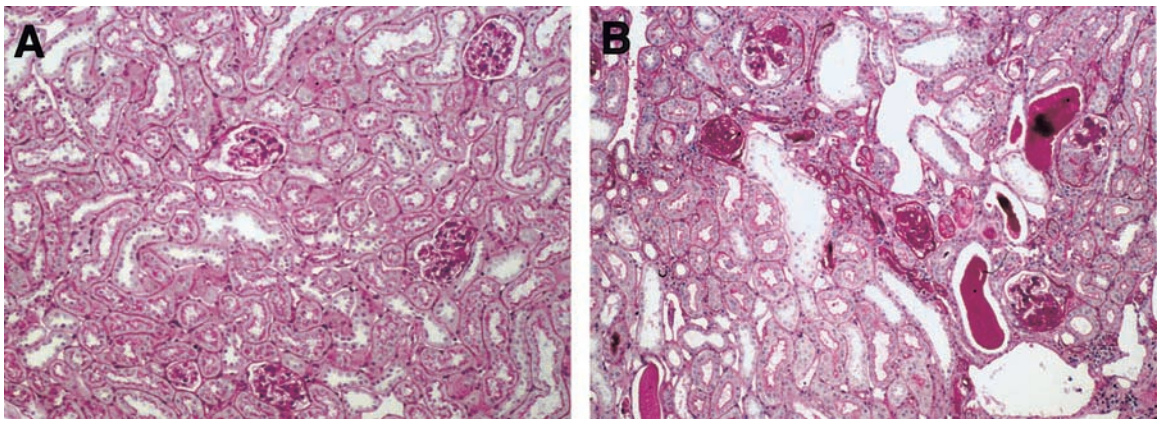

Fig 2. Variability in susceptibility to the renal ablation model in mice. (A) Minimal glomerular lesions with mesangial matrix expansion and rare sclerosis without tubulointerstitial fibrosis are seen in the C57BL6/J mouse, compared with (B) well-defined moderate to severe segmental and global sclerosis, interstitial fibrosis, tubular cystic dilatation, and atrophy in the 129 mouse. Both are shown at 10 weeks after 5/6 nephrectomy, performed by unilateral nephrectomy with cautery and ligation of renal artery branches of the contralateral kidney to ablate 5/6 of the total renal mass (periodic acid Schiff, ×100).

various ways, with removal of one kidney and ablation of two thirds of the renal mass in the contralateral kidney (ie, 5/6 nephrectomy) achieved by ligation with suture of branches of the renal artery, or polectomies, or ligation of the poles. Polectomy results in only moderate hypertension and slow development of glomerulosclerosis compared with those procedures that leave infarcted tissue in place.[11] This may, in part, relate to the marked up-regulation of components of the renin angiotensin system in the peri-infarct zone in the ligation models.[12] In the mouse, the anatomic distribution of the renal artery branches makes it difficult to achieve reproducible 5/6 nephrectomy. We have, therefore, modified our approach in the mouse to a combination of ligation of one branch of the renal artery with cautery as needed to remove additional renal mass to achieve a total of 5/6 to 7/8 nephrectomy.

After removal of renal mass, tubular and glomerular growth occur. The glomerular growth occurs primarily by lengthening of the capillaries without significant increase in diameter after loss of renal parenchyma.[13] With more extreme hemodynamic abnormalities, dilatation of capillaries may occur and contribute to increased glomerular size.[14] So-called *glomerular hypertrophy* represents both cellular hypertrophy (increase in cell size) and hyperplasia (increase in cell number). Glomerular hypertrophy can be detected biochemically and morphologically by electron microscopy as early as 2 days after 5/6 nephrectomy. Increased glomerular volume at 2 days was attributable to increased glomerular visceral epithelial cell expansion, likely hypertrophy in view of this cell's limited mitogenic capacity (see later).[15] Later increases in glomerular volume were contributed to a mesangial cell increase, likely both hypertrophy and hyperplasia. Hyperplasia predominated after 5/6 nephrectomy, whereas hypertrophy was the major growth response after uninephrectomy. The resulting glomerular growth was more marked after 5/6 nephrectomy, and was associated with later sclerosis. The patterns of gene expression were markedly different in hypertrophy versus hyperplasia, with a rapidly, transiently increased expression of the protooncogenes *c-fos*, *c-myc*, and *c-Ha-ras* in hyperplasia, with *c-fos* and *c-jun* also elevated at day 14 after 5/6 nephrectomy.[15,16] In contrast, there was a gradual, progressive increase in these protooncogenes when hypertrophy occurred after uninephrectomy. Age also affects the relative contributions of hypertrophy and hyperplasia after loss of renal mass. Although new nephrons do not form after term birth, hyperplasia is more prominent in the young and is greater after extensive nephron loss, and young animals develop more severe sclerosis after renal ablation than adult rats.[17-19]

Precise trigger(s) for initiation of growth response remain undetermined. Early work proposed that renal growth after uninephrectomy was merely in response to increased work load. However, the increased growth in the remnant kidney model in the rat occurred before increases in single nephron glomerular filtration rate. In the early 1970s the concept of renotropic factor(s) was formulated as the alternative explanation based on observations of renal growth in normal animals connected by parabiosis with an anephric partner, resurrecting observations made by Sacerdotti over a hundred years ago.[20] A possible quantitative trait locus linked to genetic modulation of the compensatory growth response has been identified in the mouse on chromosome 11. This marker maps near the genes for angiotensin I converting enzyme (ACE), growth hormone, and neural growth factor receptor, all of which have known influences on growth responses in the kidney.[21]

Glomerular cell growth responses vary after renal ablation. The mature glomerular visceral epithelial cell has limited proliferative capacity, linked to its high expression of the cyclin-dependent kinase inhibitor, $p27^{kip1}$.[22] Aberrant proliferation of the visceral epithelial cell in response to genetic manipulation of $p27^{kip1}$ is postulated to be detrimental.[23] Another cyclin-dependent kinase inhibitor, p21, appears necessary for development of injury after 5/6 nephrectomy in the 129 strain mice, pointing to the crucial importance of cell growth responses in determining response to injury.[24] The limited capability of the glomerular epithelial cell to undergo hyperplasia results in epithelial cell detachment when the glomerulus enlarges in response to injury. These denuded areas are associated with areas of hyalinosis and are believed to play a role in progressive scarring caused by injury from exudation of plasma proteins.[25] These adhesions also result in misdirected filtration, resulting in ultrafiltrate enveloping the glomerulus and dissecting along the nephron, postulated to result in fibrosis.[26]

The initial observations that single nephron function was increased after renal ablation led to further studies and the hypothesis that hyperfiltration was injurious.[6,27] It was postulated that this maladaptive change after removal of nephrons resulted in the ongoing loss of glomeruli, and thus a cycle of hyperfiltration and glomerulosclerosis was perpetuated.[27] Manipulations of hyperfiltration by feeding a low-protein diet or by giving angiotensin I converting enzyme inhibitors (ACEIs), lipid-lowering agents, or heparin were effective in ameliorating glomerular sclerosis. However, in some studies, glomerular sclerosis was decreased without altering glomerular hyperfiltration.[28] In addition, experimental maneuvers that actually increased glomerular hyperperfusion (such as thromboxane synthetase inhibitors or exercise training), not only did not accelerate progression, but actually slowed the process. Finally, glomerular sclerosis was noted to occur even in the absence of intervening hyperperfusion.[29,30]

The absence of a tight link between hyperperfusion and glomerular sclerosis shifted focus from hyperperfusion/hyperfiltration to glomerular hypertension, another element of the increased glomerular stress that follows renal ablation. Increased glomerular pressure correlated better with glomerulosclerosis. Mechanisms by which increased pressure might promote sclerosis include altered mechanical and/or shear stress. Mesangial cells in culture subjected to pulsatile mechanical stretch/relaxation cycles changed production of cytokines that affect matrix production and proliferation of glomerular cells, and altered type and amount of matrix production.[31] Many maneuvers that had been used to ameliorate matrix accumulation and thus glomerulosclerosis in vivo also affected glomerular pressures. Decreased glomerular pressure in the remnant model, in response to dietary protein restriction or antihypertensive drugs, among other interventions, was associated with slower progression.[29,30,32-34]

One study in rats examined the heterogeneous changes in function in individual nephrons after renal ablation. Glomerular filtration and pressure were assessed repeatedly by micropuncture in the same surface nephrons in the Munich Wistar rat over a 6-week period. When the single-nephron glomerular filtration rate and glomerular capillary pressure were correlated with the degree of sclerosis in the same glomerulus, no correlation was found between the levels of these hemodynamic parameters and the degree of sclerosis. Thus, the nephrons with the greatest hyperfiltration or highest glomerular capillary pressure did not show the most severe sclerosis at the end of the study, indicating that glomerular hypertension or hyperperfusion per se did not directly account for the glomerular damage.[10]

In the remnant kidney in rats, glomerular growth and sclerosis are linked. A strong biphasic correlation was found between the degree of total glomerular sclerosis and the maximum glomerular planar area, determined by serial section analysis. Severely sclerotic glomeruli showed a negative correlation with glomerular area. Development of mild to moderate sclerosis correlated closely with glomerular growth. Glomerular sclerosis and hypertrophy were decreased in parallel by ACEI, further pointing to a link between the 2 processes.[34,35] Of note, ACEI was not equally effective at all stages of glomerular growth and sclerosis: glomeruli with more advanced sclerosis showed continuous progression, whereas progression of early stage of sclerosis was inhibited. High doses of ACEI or angiotensin type 1 receptor antagonist (AT1RA), 4-fold more than the usual antihypertensive level, could even regress existing sclerosis.[36] These findings indicate that different mechanisms of cell growth and matrix accumulation are predominant among the heterogeneously affected glomeruli, with differing potential for response to treatment and remodeling.

Sclerosis is the net result of matrix synthesis exceeding matrix degradation. Extracellular matrix (ECM) dysregulation is influenced by numerous growth factors, cytokines, and chemokines, which again can be modulated by local increases in shear stress or hydraulic pressures, or by insults that include, but are not limited to, increased glomerular metabolism, heightened reactive oxygen species, altered lipid metabolism, mesangial deposition of macromolecules, and abnormal hemostasis, among others.[29,30] The beneficial effects of angiotensin inhibition and 3-hydroxy-3-methylglutaryl coenzyme reductase inhibitors, the so-called *statins*, likely reflect multiple actions on these pathologic processes. Thus, angiotensin has direct and indirect effects on pressure, ECM synthesis and degradation, thrombosis, lymphocytes, macrophages, and also stimulates other growth factors.[35-38] Statins, in addition to decreasing lipid

levels, modulate macrophages and fibrogenic responses.[39] Numerous additional factors have been implicated in the progressive scarring in the remnant kidney, including the following: platelet-derived growth factor, transforming growth factor-β, transforming growth factor-α, insulin-like growth factor-1, growth hormone, epidermal growth factor, interleukins 1 and 6, tumor necrosis factor α, basic fibroblast growth factor, endothelin and plasminogen activator inhibitor-1 (PAI-1).[29,37,38] PAI-1 inhibits not only fibrinolysis, but also proteolysis, by inhibiting activation of plasminogen activators, thereby promoting ECM accumulation. Angiotensin II directly induces PAI-1 both in vitro and in vivo, and inhibition of angiotensin decreases PAI-1 both in humans and in experimental models, including the radiation nephropathy model and remnant kidney model.[4,35,38] Decreased PAI-1 is hypothesized to be a key mechanism promoting ECM degradation, amelioration, or even regression of sclerosis.[36]

PUROMYCIN AMINONUCLEOSIDE AND ADRIAMYCIN NEPHROPATHY MODELS

Glomerular visceral epithelial cells are the primary target in many glomerular diseases, including the experimental models of adriamycin and puromycin aminonucleoside–induced nephropathies. The glomerular visceral epithelial cells are pivotal for maintenance of normal permselectivity, and are also a source of matrix in both physiologic and pathophysiologic settings. Puromycin aminonucleoside (PAN) nephropathy may be induced in the rat in various ways, with multiple intraperitoneal injections, or a single intravenous administration, with or without unilateral nephrectomy.[40-43] Depending on the exact strategy chosen and time of assessment, the model may be hypertensive or not. Micropuncture studies indicated that mild elevations in glomerular pressure do not account for sclerosis in the uninephrectomy and intraperitoneal injection variant of this model, and protection by ACE inhibitor was not linked to effects on glomerular pressures at the single glomerular level as shown by serial micropuncture studies.[43] With the single intravenous dose (50 mg/kg body weight [BWt]) model of PAN nephropathy in the rat, there is an early nephrotic phase with proteinuria peaking at approximately 10 days. The early phase of the model resembles minimal change disease, with complete effacement of foot processes. There is then apparent near resolution, followed by steady, progressive, lower-level proteinuria developing between weeks 10 and 13, associated with early segmental sclerotic lesions.[40] By week 18, about 10% to 15% of glomeruli show well-defined segmental sclerosis. The course is accelerated with uninephrectomy and multiple intraperitoneal injections (10 mg/kg BWt first dose, additional doses every 4 weeks at 40 mg/kg BWt), with well-defined glomerulosclerosis by week 8.[42,43] Adriamycin, although chemically different from puromycin aminonucleoside, causes a virtually identical lesion in the rat. Adriamycin given intravenously (2 mg/kg BWt twice with 3-wk interval) results in early phase proteinuria with focal foot process effacement shortly after the second injection, progressing over the next 2 months. Kidneys show early segmental sclerosis by week 16, progressing to widespread glomerulosclerosis with tubulointerstitial fibrosis by week 24, with some animals dying of uremia by week 28.[43,44] Adriamycin given in a single intravenous dose (5 mg/kg BWt) resulted in sclerosis by 6 months in half of the rats.[45]

The models of nephropathy induced by injection of adriamycin or puromycin aminonucleoside more recently also have been investigated in the mouse. Although most rat strains show complete susceptibility to these agents, so far only the Balb/c mouse strain has been shown to be susceptible, developing marked proteinuria and focal segmental glomerulosclerosis in response to intravenous adriamycin (dose of adriamycin, 10-11 mg/kg BWt).[46-48] Of note, these mice were resistant to PAN.[48] The mice develop overt proteinuria from day 5, with mild glomerulosclerotic lesions starting by week 4, becoming extensive with associated tubulointerstitial fibrosis by week 6.

The primary effects of puromycin aminonucleoside and adriamycin appear to be on the podocytes, suggested by morphologic changes and direct toxicity to these cells in culture.[49] Adriamycin and puromycin aminonucleoside exert oxidative damage on cell structure through a direct reduction to a semiquinone radical or by producing reactive oxygen species (ROS) via the xanthine oxidase system.[50] PAN also enhances the production of H_2O_2 in cultured podocytes,[51] though the mechanism is still unclear. Injection of ROS into the renal artery even causes acute marked, but transient, proteinuria in rats without altered GBM charge or foot process effacement.[52] To support the hypothesis

that ROS could mediate proteinuria in these models, administration of ROS scavengers significantly reduces proteinuria in PAN-injected rats or adriamycin-injected rats.[50,53] The ROS-mediated injury is associated with down-regulation and shift in polarity of α-3 integrin expression, a key molecule for maintenance of podocyte shape and adhesion.[54] Epithelial cell integrins are key modulators of cell interactions and cell-matrix binding. α-3 and α-5 integrins are decreased in areas of sclerosis.[55] Changes in these molecules may underlie the foot process effacement and altered cell-cell and cell-matrix interactions in these epithelial cell injury models. Taurine, an endogenous antioxidant, also was effective in limiting injury in PAN nephropathy, and normalized ROS-related byproducts in the kidney.[56] The protective effect of ROS scavengers on PAN-induced podocyte cytotoxicity also was seen in vitro.[51] Thus, ROS appear to mediate, in part, proteinuria and podocyte injury in PAN or adriamycin nephropathies.

In PAN nephropathy, the podocytes also show down-regulation of the podocyte proteins podoplanin and nephrin, key elements of the foot processes and slit diaphragms.[57,58] Thus, loss of these molecules could just be markers of severe podocyte injury, or even be causal in the proteinuria. In the minimal change disease phase of the PAN nephropathy model, podocytes express proliferation markers. In contrast, bromodeoxyuridine labeling of podocytes was decreased in the FSGS phase of the model, although apoptosis was increased significantly, supporting that inadequate proliferation of the podocyte contributes to development of sclerosis.[59] This concept of podocyte insufficiency also is supported in human conditions, in which decreased podocyte number relative to glomerular surface area is postulated to result in increased podocyte stress, detachment, and adhesions.[59] Podocyte depletion also was confirmed morphometrically after repeated injections of puromycin aminonucleoside.[60] The development of glomerular hypertrophy also contributes to this relative podocyte insufficiency. Significant enlargement of the glomerular tuft volume mediated by capillary lengthening preceded development of FSGS in the multiple injection PAN nephropathy model. In the later stage of PAN nephropathy, continued glomerular enlargement was caused more by an increase in capillary diameter and mesangial matrix expansion, implicating possible increased role of hemodynamic/wall tension stresses in this later phase of injury.[61]

The development of sclerosis in PAN nephropathy is associated with numerous changes of potential mediators of fibrosis. The loss of podocytes has been linked to altered expression of numerous factors, notably vascular endothelial growth factor and its receptors. Vascular endothelial growth factor is produced in the epithelial cell and acts as an endothelial cell–specific mitogen that plays a key role in both physiologic and pathologic angiogenesis and vascular permeability. In the early phase of PAN nephropathy, both vascular endothelial growth factor in podocytes and its receptor flk-1 in glomerular endothelial cells were decreased, with likely adverse effects on capillary growth and permselectivity.[62]

The tubulointerstitial fibrosis in PAN nephropathy is linked strongly to macrophage infiltration and ongoing proteinuria.[63] Proteinuria is postulated to directly up-regulate proinflammatory chemokines and fibrotic processes in the tubules. Examination of glomerular basement membrane charge and size selectivity in PAN nephropathy showed a marked increase in the number of large pores and also an increase in small-pore radius with less dramatic effects on estimated capillary wall charge.[64,65] Heparanase expression in podocytes is increased markedly in PAN nephropathy and could contribute to the loss of glomerular charge and consequent proteinuria.[66]

Although the severity of proteinuria correlates with tubulointerstitial nephritis in PAN nephropathy, PAN itself may induce tubular cell damage.[63] Further, analbuminemic rats treated with adriamycin developed the same degree of glomerular sclerosis and renal failure despite 70% reduction of proteinuria, compared with Sprague-Dawley counterparts.[67] Correlation of proteinuria and glomerulosclerosis has been shown in remnant kidney models on the whole kidney level. However, both parameters are manifest heterogeneously among the individual glomeruli in chronic renal disease. Proteinuria originates largely from the nonsclerotic rather than the sclerotic glomeruli in the remnant kidney model, shown by micropuncture study.[68] Correlation of proteinuria and glomerular sclerosis was not shown at the single nephron level. In PAN nephropathy rats, administration of AT1RA showed similar protective effect on progression of glomerular sclerosis as ACE inhibitor, even though

AT1RA had no significant antiproteinuric effect in the acute nephrotic phase.[69] Thus, proteinuria per se may not cause the progression of renal disease, and further, mechanisms underlying proteinuria and glomerular sclerosis do not appear to be identical.

Numerous chemokines are up-regulated in a complex manner in PAN nephropathy, with transient increase during early proteinuria in monocyte chemotactic protein-1 and -3 and lymphotactin, the C-C chemokine TCA3 and MIP-1 α. Macrophage infiltration is prominent at this time point. Glomerular MIP-1 β and RANTES increase slightly later, on day 10.[70] Osteopontin, which among its many functions recruits macrophages, also is up-regulated in PAN nephropathy.[71] Cytotoxic CD8+ T lymphocytes also are linked to progressive injury in the mouse model of adriamycin nephropathy, and anti-CD8 treatment protected against injury, suggesting that cells other than macrophages could contribute to progressive fibrosis.[72]

ECM dysregulation occurs in PAN nephropathy as in the remnant kidney. The accumulation of ECM in PAN nephropathy is associated with suppressed proteolytic enzymes in addition to augmented collagen synthesis. Cathepsins and metalloproteases were decreased by 4 weeks after initial puromycin injection.[73] Specific therapies antagonizing endothelin receptor A or angiotensin are linked to decreased collagen synthesis.[74] Reversal of existing PAN-induced sclerosis could even be achieved with an intervention with an ACE inhibitor, AT1RA, or low-protein diet.[69,75]

GENETIC SUSCEPTIBILITY

Genetic background greatly affects susceptibility to disease. This phenomenon is well known from rat studies in which the PVGc rat, which has a larger number of smaller glomeruli, is quite resistant to sclerosis compared with the Munich Wistar and Sprague Dawley rats. This resistance was linked to a minimal hypertrophic response to loss of nephrons.[35] Similarly, dwarf rats with a defect in growth hormone and resulting deficient growth responses are resistant to the development of glomerulosclerosis after renal ablation.[76]

In the mouse, the C57BL6/J strain is particularly resistant to induction of injury by the ablation of renal mass (Fig 2).[77] The 129 strain and Swiss Webster mice are susceptible to this model (Fogo and Ma, unpublished data).[24] This resistance does not correlate with susceptibility of development of sclerosis in the cardiovascular system: the C57BL6/J mice readily develop cardiac and aortic lesions in response to, for instance, increased pressure or high cholesterol.

The hypertrophic response, or lack thereof, and subsequent development of sclerosis are dependent on the genetic background, suggesting that complex genetic traits modulate the response of glomerular cells to pathogenic stimuli. Mice with reduced nephron number owing to a radiation-induced mutation that results in an 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 C57BL6/J strain.[78] Glomerular hypertrophy was proportional to the reduction of nephron mass in the former strain. In contrast, a threshold for glomerular size was observed in the C57BL6/J mice.

Susceptibility may be influenced by renin gene polymorphisms. Some mouse strains (eg, C57BL6/J) have one gene (*Ren-1*^{c}), whereas other strains (eg, 129), have 2 (*Ren-1*^{d} and *Ren-2*).[79] The *Ren-1* genes govern expression of renin in the kidney in the juxtaglomerular apparatuses, whereas the *Ren-2* gene controls submaxillary gland renin expression and has very low renal expression. Mice with 2 renin genes (ie, 129 strain) have 10-fold higher plasma renin activity, angiotensin-dependent hypertension, and increased blood pressure and cardiac and renal hypertrophic responses to salt compared with one renin gene mice (ie, C57BL6/J). Renin gene status thus could have a major effect on susceptibility to injuries in which the renin angiotensin aldosterone system plays a role.[79]

Most rat strains are susceptible to either puromycin aminonucleoside or adriamycin. However, the PVGc strain is resistant to PAN-induced injury.[80] Most mouse strains tested have shown resistance to both of these models. So far only the Balb/c mouse strain has been shown to be susceptible to adriamycin, and no mouse model of PAN nephropathy has been developed.[46,47]

GENETIC MODELS

Since the identification of nephrin as the responsible gene mutation in congenital nephrotic syndrome of Finnish type, numerous additional in-

sights have been gained into podocyte biology with important implications for FSGS, as reviewed elsewhere in this issue. Here we will only focus on the experimental models that have been developed so far that are relevant to these podocyte genes. The *LMX1B* gene encodes a LIM-homeodomain transcription factor and is mutated in nail patella syndrome. The knockout mouse for this gene has abnormal podocytes lacking typical slit diaphragms as discussed in detail elsewhere in this issue. Importantly, these knockout podocytes have greatly reduced CD2AP and podocin, although other podocyte genes such as nephrin, synaptopodin, ZO1, α-3 integrin, and specific laminins were preserved.[81] CD2AP also is expressed highly in the glomerulus, binds to nephrin via its C-terminal domain, and is localized to the slit diaphragm.[82,83] The knockout of CD2AP results in congenital nephrotic syndrome in mice with great similarities to congenital nephrotic syndrome of Finnish type.[82] This adapter protein was identified previously as the key facilitator of T-cell adhesion to antigen-presenting cells, enhancing CD2 T cells' clustering and anchoring. Mice lacking CD2AP have intact kidneys at birth. Foot process effacement develops early, by 1 week. Proteinuria develops at 2 weeks of age, with increased glomerular size and increased mesangial cellularity matrix by 1 week of age. By 4 weeks of age, marked mesangial expansion is present. Growth retardation starts at 3 weeks, with death by 6 to 7 weeks owing to massive proteinuria and kidney dysfunction. Interestingly, this knockout mouse with its severe lesions has a 129 background strain.

Mutations of CD2AP have not yet been identified in human disease, but acquired alterations in its expression could possibly be involved in many human proteinuric conditions. Podocin also interacts with the CD2AP and nephrin complex, possibly indicating that podocin serves in the structural organization of the slit diaphragm. This complex appears then to be anchored via α actinin-4, a mutation that causes familial focal segmental glomerulosclerosis.[84] Of note, the mutation in α actinin-4 is postulated to be a gain-of-function mutation, contrasting the loss-of-function mutation seen with nephrin and CD2AP.

Acquired disruption of some of these complex interacting podocyte molecules has been shown in PAN nephropathy. Podocalyxin, the major sialoprotein of the podocyte interacts with actin via ezrin, a cytoskeletal linker protein. This linkage occurs via the scaffold protein Na+/H+ exchanger regulator factor 2 (*Nherf2*). This podocalyxin/NHERF2/ezrin complex interaction with the actin cytoskeleton is disrupted in podocytes from animals treated with puromycin aminonucleoside, or protamine sulfate or sialidase, all resulting in dramatic loss of foot processes.[85]

An additional model of particular interest is the Buffalo/mna rat, which develops spontaneous FSGS by 2 months of age. The lesion appears to depend on a circulating factor because kidneys from other rat strains transplanted into the Buffalo/mna rat also develop proteinuria within 10 days, progressing to FSGS, whereas Buffalo/mna kidneys show regression of lesions after transplantation into healthy Lewis rats.[86] This may be of particular relevance to the human situation of recurrence of FSGS in transplants in some patients, linked to a circulating factor with effects on permeability. The Buffalo/mna rat develops epithelial cell alterations with foot process flattening and by 2 months, with development of proteinuria and focal segmental sclerosis over the ensuing months. Interestingly, the Buffalo/mna rats have lesions at the glomerular junction with the proximal tubule, the so-called *tip lesion*, early in life, but lesions extend to all parts of the glomerulus later.[3] Genetic analysis in this model has shown 2 autosomal-recessive genes with only one locus identified to date, Pur1 on chromosome 13. This region in the rat is syntenic to the region of human chromosome 1 where the *NPHS2* gene encoding podocin is located. Of great interest, some patients with FSGS recurring after transplantation have heterozygous mutations in *NPHS2*.[87]

In summary, available rat and mouse models have added invaluable information on the etiologies and pathogenesis of FSGS lesions. Bridging knowledge from unexpected glomerular lesions in knockout mice, such as the CD2AP−/−, has taught us about human disease. Conversely, developing new animal models with deficiency or mutation of genes implicated in human diseases will increase our understanding of glomerulosclerosis in humans exponentially, and provide a rationale for new therapeutic approaches.

REFERENCES

1. Schwartz MM, Korbet SM: Primary focal segmental glomerulosclerosis: Pathology, histological variants, and pathogenesis. Am J Kidney Dis 22:874-883, 1993
2. D'Agati V: The many masks of focal segmental glomerulosclerosis. Kidney Int 46:1223-1241, 1994
3. Howie AJ, Kizaki T, Beaman M, et al: Different types of segmental sclerosing glomerular lesions in six experimental models of proteinuria. J Pathol 157:141-151, 1989
4. Oikawa T, Freeman M, Lo W, et al: Modulation of plasminogen activator inhibitor-1 (PAI-1) in vivo: A new mechanism for the anti-fibrotic effect of renin-angiotensin inhibition. Kidney Int 51:164-172, 1997
5. Chanutin A, Ferris EB: Experimental renal insufficiency produced by partial nephrectomy. Arch Intern Med 49:767-787, 1932
6. Hostetter TH, Olson JL, Rennke HG, et al: Hyperfiltration in remnant nephrons: A potentially adverse response to renal ablation. Am J Physiol 241:F85-F93, 1981
7. Morrison AB, Howard RM: The functional capacity of hypertrophied nephrons: Effect of partial nephrectomy on the clearance of inulin and PAH in the rat. J Exp Med 123:829-844, 1966
8. Shimamura T, Morrison AB: A progressive glomerulosclerosis occurring in partial five-sixths nephrectomized rats. Am J Pathol 79:95-106, 1975
9. Rich AR: A hitherto undescribed vulnerability of the juxtamedullary glomeruli in lipoid nephrosis. Bull Johns Hopkins Hosp 100:173-175, 1957
10. Yoshida Y, Fogo A, Shiraga H, et al: Serial micropuncture analysis of single nephron function in the rat model of subtotal renal ablation. Kidney Int 33:855-867, 1988
11. Gretz N, Waldherr R, Strauch M: The remnant kidney model, in Gretz N, Strauch M (eds): Experimental and Genetic Rat Models of Chronic Renal Failure. Karger, Basel, 1993, pp 1-28
12. Correa-Rotter R, Hostetter TH, Manivel JC, et al: Renin expression in renal ablation. Hypertension 20:483-490, 1992
13. Nyengaard JR: Number and dimensions of rat glomerular capillaries in normal development and after nephrectomy. Kidney Int 43:1049-1057, 1993
14. Lax DS, Benstein JA, Tolbert E, et al: Effects of salt restriction on renal growth and glomerular injury in rats with remnant kidneys. Kidney Int 41:1527-1534, 1992
15. Lee GS, Nast CC, Peng SC, et al: Differential response of glomerular epithelial and mesangial cells after subtotal nephrectomy. Kidney Int 53:1389-1398, 1998
16. Kujubu DA, Norman JT, Herschman HR, et al: Primary response gene expression in renal hypertrophy and hyperplasia: Evidence for different growth initiation processes. Am J Physiol 260:F823-F827, 1991
17. Aschinberg LC, Koskimies O, Bernstein J, et al: The influence of age on the response to renal parenchymal loss. Yale J Biol Med 51:341-345, 1978
18. O'Donnell MP, Kasiske BL, Raij L, et al: Age is a determinant of the glomerular morphologic and functional responses to chronic nephron loss. J Lab Clin Med 106:308-313, 1985
19. Ikoma M, Yoshioka T, Ichikawa I, et al: Mechanism of the unique susceptibility of deep cortical glomeruli of maturing kidneys to severe focal glomerular sclerosis. Pediatr Res 28: 270-276, 1990
20. Schena FP, Strippoli GF, Wankelmuth P: Renal growth factors: Past, present and future. Am J Nephrol 19:308-312, 1999
21. Zidek V, Musilova A, Vorlieek J, et al: Quantitative trait loci for compensatory renal hypertrophy in the mouse. Biochem Biophys Res Commun 248:473-475, 1998
22. Combs HL, Shankland SJ, Setzer SV, et al: Expression of the cyclin kinase inhibitor, p27kip1, in developing and mature human kidney. Kidney Int 53:892-896, 1998
23. Shankland SJ: Cell cycle regulatory proteins in glomerular disease. Kidney Int 56:1208-1215, 1999
24. Megyesi J, Price PM, Tamayo E, et al: The lack of a functional p21(WAF1/CIP1) gene ameliorates progression to chronic renal failure. Proc Natl Acad Sci U S A 96:10830-10835, 1999
25. Fries JWU, Sandstrom DJ, Meyer TW, et al: Glomerular hypertrophy and epithelial cell injury modulate progressive glomerulosclerosis in the rat. Lab Invest 60:205-218, 1989
26. Kriz W, Hosser H, Hahnel B, et al: From segmental glomerulosclerosis to total nephron degeneration and interstitial fibrosis: A histopathological study in rat models and human glomerulopathies. Nephrol Dial Transplant 13:2781-2798, 1998
27. 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
28. Nath KA, Kren SM, Hostetter TH: Dietary protein restriction in established renal injury in the rat. Selective role of glomerular capillary pressure in progressive glomerular dysfunction. J Clin Invest 78:1199-1205, 1986
29. Fogo A, Ichikawa I: Glomerular growth promoter—the common channel to glomerular sclerosis, in Stein JH, Mitch WE (eds): Contemporary Issues in Nephrology: The Progressive Nature of Renal Disease (ed 2). New York, Churchill Livingstone, 1992, pp 23-54
30. Neuringer JR, Anderson S, Brenner BM: The role of systemic and intraglomerular hypertension, in Stein JH, Mitch WE (eds): Contemporary Issues in Nephrology—The Progressive Nature of Renal Disease (ed 2). New York, Churchill Livingstone, 1992, pp 1-21
31. Harris RC, Haralson MA, Badr KF: Continuous stretch-relaxation in culture alters mesangial cell morphology, growth characteristics, and metabolic activity. Lab Invest 66:548-554, 1992
32. Hostetter TH, Meyer TW, Rennke HG, et al: Chronic effects of dietary protein in the rat with intact and reduced renal mass. Kidney Int 30:509-517, 1986
33. Anderson S, Meyer TW, Rennke HG, et al: Control of glomerular hypertension limits glomerular injury in rats with reduced renal mass. J Clin Invest 76:612-619, 1985
34. Yoshida Y, Fogo A, Ichikawa I: Effects of anti-hypertensive drugs on glomerular morphology: A close linkage between their anti-hypertrophic and anti-sclerotic action on remnant glomeruli. Kidney Int 36:626-635, 1989
35. Fogo AB: Glomerular hypertension, abnormal glomerular growth, and progression of renal diseases. Kidney Int 57: S15-S21, 2000 (suppl 75)

36. Fogo A: Progression and potential regression of glomerulosclerosis. Nephrology Forum. Kidney Int 59:804-819, 2001
37. Fogo AB: Mesangial matrix modulation and glomerulosclerosis. Exp Nephrol 7:147-159, 1999
38. Fogo AB: The role of angiotensin II and plasminogen activator inhibitor-1 in progressive glomerulosclerosis. Am J Kidney Dis 35:179-188, 2000
39. Oda H, Keane WF: Recent advances in statins and the kidney. Kidney Int Suppl 71:S2-S5, 1999
40. Diamond JR, Karnovsky MJ: Focal and segmental glomerulosclerosis following a single intravenous dose of puromycin aminonucleoside. Am J Pathol 122:481-487, 1986
41. Anderson S, Diamond JR, Karnovsky MJ, et al: 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
42. Glasser RJ, Velosa JA, Michael AF: Experimental model of focal sclerosis. I, Relationship to protein excretion in aminonucleoside nephrosis. Lab Invest 36:519-526, 1977
43. Fogo A, Yoshida Y, Glick AD, et al: Serial micropuncture analysis of glomerular function in two rat models of glomerular sclerosis. J Clin Invest 82:322-330, 1988
44. Okuda S, Oh Y, Tsuruda H, et al: Adriamycin-induced nephropathy as a model of chronic progressive glomerular damage. Kidney Int 29:502-510, 1986
45. Bertani T, Rocchi G, Sacchi G, et al: Adriamycin-induced glomerulosclerosis in the rat. Am J Kidney Dis 7:12-19, 1986
46. Chen A, Sheu L-F, Ho Y-S, et al: Experimental focal segmental glomerulosclerosis in mice. Nephron 78:440-452, 1998
47. Wang Y, Wang YP, Tay YC, et al: Progressive adriamycin nephropathy in mice: Sequence of histologic and immunohistochemical events. Kidney Int 58:1797-1804, 2000
48. Chen A, Wei C-H, Sheu L-F, et al: Induction of proteinuria by adriamycin or bovine serum albumin in the mouse. Nephron 69:293-300, 1995
49. Fishman JA, Karnovsky MJ: Effects of the aminonucleoside of puromycin on glomerular epithelial cells in vitro. Am J Pathol 118:398-407, 1985
50. Diamond JR, Bonventre JV, Karnovsky MJ: A role for oxygen free radicals in aminonucleoside nephrosis. Kidney Int 29:478-483, 1986
51. Kawaguchi M, Yamada M, Wada H, et al: Roles of oxygen species in glomerular epithelial cell injury in vitro caused by puromycin aminonucleoside. Toxicology 72:329-340, 1992
52. Yoshioka T, Ichikawa I, Fogo A: Reactive oxygen metabolites cause massive, reversible proteinuria and glomerular sieving defect without apparent ultrastructural abnormality. J Am Soc Nephrol 2:902-912, 1991
53. Milner LS, Wei SH, Houser MT: Amelioration of glomerular injury in doxorubicin hydrochloride nephrosis by dimethylthiourea. J Lab Clin Med 118:427-434, 1991
54. Kojima K, Matsui K, Nagase M: Protection of alpha(3) integrin-mediated podocyte shape by superoxide dismutase in the puromycin aminonucleoside nephrosis rat. Am J Kidney Dis 35:1175-1185, 2000
55. Kemeny E, Mihatsch MJ, Durmuller U, et al: Podocytes lose their adhesive phenotype in focal segmental glomerulosclerosis. Clin Nephrol 43:71-83, 1995
56. Trachtman H, Del Pizzo R, Futterweit S, et al: Taurine attenuates renal disease in chronic puromycin aminonucleoside nephropathy. Am J Physiol 262:F117-F123, 1992
57. Ahola H, Wang SX, Luimula P, et al: Cloning and expression of the rat nephrin homolog. Am J Pathol 155:907-913, 1999
58. Breiteneder-Geleff S, Matsui K, Soleiman A, et al: Podoplanin, novel 43-kd membrane protein of glomerular epithelial cells, is down-regulated in puromycin nephrosis. Am J Pathol 151:1141-1152, 1997
59. Shiiki H, Sasaki Y, Nishino T, et al: Cell proliferation and apoptosis of the glomerular epithelial cells in rats with puromycin aminonucleoside nephrosis. Pathobiology 66:221-229, 1998
60. Kim YH, Goyal M, Kurnit D, et al: Podocyte depletion and glomerulosclerosis have a direct relationship in the PAN-treated rat. Kidney Int 60:957-968, 2001
61. Cahill MM, Ryan GB, Bertram JF: Biphasic glomerular hypertrophy in rats administered puromycin aminonucleoside. Kidney Int 50:768-775, 1996
62. Fan L, Wakayama T, Yokoyama S, et al: Downregulation of vascular endothelial growth factor and its receptors in the kidney in rats with puromycin aminonucleoside nephrosis. Nephron 90:95-102, 2002
63. Baboolal K, Meyer TW: Tubulointerstitial injury and impaired renal function after recovery from acute puromycin nephrosis. Am J Physiol 269:F331-F338, 1995
64. Olson JS, Rennke HG, Venkatachalam MA: Alteration in the charge and size selectivity barrier of the glomerular filter in aminonucleoside nephrosis in rats. Lab Invest 44:271-279, 1981
65. Hjalmarsson C, Ohlson M, Haraldsson B: Puromycin aminonucleoside damages the glomerular size barrier with minimal effects on charge density. Am J Physiol 281:F503-F512, 2001
66. Levidiotis V, Kanellis J, Ierino FL, et al: Increased expression of heparanase in puromycin aminonucleoside nephrosis. Kidney Int 60:1287-1296, 2001
67. Okuda S, Oochi N, Wakisaka M, et al: Albuminuria is not an aggravating factor in experimental focal glomerulosclerosis and hyalinosis. J Lab Clin Med 119:245-253, 1992
68. Yoshioka T, Shiraga H, Yoshida Y, et al: "Intact nephrons" as the primary origin of proteinuria in chronic renal disease: A study in the rat model of subtotal nephrectomy. J Clin Invest 82:1614-1623, 1988
69. Tanaka R, Kon V, Yoshioka T, et al: Angiotensin converting enzyme inhibitor modulates glomerular function and structure by distinct mechanisms. Kidney Int 45:537-543, 1994
70. Ou ZL, Natori Y, Natori Y: Transient and sequential expression of chemokine mRNA in glomeruli in puromycin aminonucleoside nephrosis. Nephron 85:254-257, 2000
71. Magil AB, Pichler RH, Johnson RJ: Osteopontin in chronic puromycin aminonucleoside nephrosis. J Am Soc Nephrol 8:1383-1390, 1997
72. Wang Y, Wang YP, Tay YC, et al: Role of CD8(+) cells in the progression of murine adriamycin nephropathy. Kidney Int 59:941-949, 2001
73. Huang S, Schaefer RM, Reisch S, et al: Suppressed activities of cathepsins and metalloproteinases in the chronic model of puromycin aminonucleoside nephrosis. Kidney Blood Press Res 22:121-127, 1999

74. Ebihara I, Nakamura T, Tomino Y, et al: Effect of a specific endothelin receptor A antagonist and an angiotensin-converting enzyme inhibitor on glomerular mRNA levels for extracellular matrix components, metalloproteinases (MMP) and a tissue inhibitor of MMP in aminonucleoside nephrosis. Nephrol Dial Transplant 12:1001-1006, 1997

75. Marinides GN, Groggel GC, Cohen AH, et al: Enalapril and low protein reverse chronic puromycin aminonucleoside nephropathy. Kidney Int 37:749-757, 1990

76. El Nahas AM, Bassett AH, Cope GH, et al: Role of growth hormone in the development of experimental renal scarring. Kidney Int 40:29-34, 1991

77. Kren S, Hostetter TH: The course of the remnant kidney model in mice. Kidney Int 56:333-337, 1999

78. Esposito C, He CJ, Striker GE, et al: Nature and severity of the glomerular response to nephron reduction is strain-dependent in mice. Am J Pathol 154:891-897, 1999

79. Wang Q, Hummler E, Nussberger J, et al: Blood pressure, cardiac, and renal responses to salt and deoxycorticosterone acetate in mice: Role of renin genes. J Am Soc Nephrol 13:1509-1516, 2002

80. Grond J, Muller EW, van Goor H, et al: Differences in puromycin aminonucleoside nephrosis in two rat strains. Kidney Int 33:524-529, 1988

81. Miner JH, Morello R, Andrews KL, et al: Transcriptional induction of slit diaphragm genes by Lmx1b is required in podocyte differentiation. J Clin Invest 109:1065-1072, 2002

82. Shih NY, Li J, Karpitskii V, et al: Congenital nephrotic syndrome in mice lacking CD2-associated protein. Science 286:312-315, 1999

83. Shih NY, Li J, Cotran R, et al: CD2AP localizes to the slit diaphragm and binds to nephrin via a novel C-terminal domain. Am J Pathol 159:2303-2308, 2001

84. Kaplan JM, Kim SH, North KN, et al: Mutations in ACTN4, encoding alpha-actinin-4, cause familial focal segmental glomerulosclerosis. Nat Genet 24:251-256, 2000

85. Takeda T, McQuistan T, Orlando RA, et al: Loss of glomerular foot processes is associated with uncoupling of podocalyxin from the actin cytoskeleton. J Clin Invest 108:289-301, 2001

86. Le Berre L, Godfrin Y, Günther E, et al: Extrarenal effects on the pathogenesis and relapse of idiopathic nephrotic syndrome in Buffalo/Mna rats. J Clin Invest 109:491-498, 2002

87. Antignac C: Genetic models: Clues for understanding the pathogenesis of idiopathic nephrotic syndrome. J Clin Invest 109:447-449, 2002