

# **Immune and Inflammatory Glomerular Injury**

**Terry Cook**

It is now over a century since the first animal model of glomerulonephritis was established by Lindemann [1] who injected rabbits with heterologous antiserum to rabbit kidney raised in guinea pigs. In recent years there has been increasing opposition to the use of animals for research with an escalation in damage to property and violent attacks on researchers by those opposed to such research. This has been a major problem in the UK and is an increasing problem in the US [2]. There is therefore a need for scientists who carry out animal research to demonstrate that the inevitable discomfort and harm which is suffered by experimental animals is reduced as far as possible by good experimental design and, most importantly, is justified by the benefits it brings in understanding and treating human disease. In this talk I will discuss models that have been used in studying immune and inflammatory glomerular injury and ask;

1. How representative they are of human disease
2. What are the key insights they have given into pathogenesis
3. How has this been translated into treatment
4. Are we in danger of being misled by reliance on animal models

Although other animals such as rabbits and sheep have been studied most experiments on glomerulonephritis have been performed in rats or mice. There is a clear advantage to performing experiments in mice because of the availability of gene targeted mice and therefore there is a need for good models of human disease that can be used in mice.

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From Bedside to Bench and Back Again: What Animal Models Teach Us  
About Renal Disease and What They Don't

Paradigms in Diabetic Nephropathy (DN)  
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1. Animal models of DN can be useful, even if imperfect, if they generate testable concepts in humans.
2. In order to be useful, these models:
  - a. Need to reflect components of the structural, functional and natural history, human conditions which are well understood.
  - b. Should recapitulate the order of the human disorder so that the highly disease specific processes involved in the genesis of the early lesions are not confused with the progression promoters associated with advanced injury, which share commonalities among multiple renal disorders.
3. An example of a concept that has been distorted, in the end, by the interpretations provided to animal models of DN (i.e., hyperfiltration/capillary hypertension/renin angiotensin system blockade) resulting in inappropriate extrapolation to humans will be provided.
4. A review of human diabetic nephropathy, natural history, and structural functional relationships and examples of useful animal models will be presented.

**The Histology of Progressive Diabetic Nephropathy in Humans**

The constellation of the renal structural lesions occurring in diabetes is unique, although many of these lesions can be individually observed in other renal disorders. The morphologic lesions in type 1 diabetes (T1DM), predominantly affect the glomeruli, with thickening of glomerular basement membrane (GBM) and mesangial expansion, although also the podocytes, renal tubules, interstitium and arterioles undergo substantial changes, especially at later stages of disease (1-5).

GBM thickening, the first measurable change, has been detected as early as 1.5 to 2.5 years after the onset of T1DM (6, 7). Thickening of tubular basement membrane (TBM) closely parallels that of GBM thickening (3). Mesangial expansion, predominantly due to an increase in mesangial matrix, develops later although an increase in the matrix component of the mesangium can be detected as early as 5-7 years after the onset of diabetes (8-11). While GBM thickening may develop steadily over time, mesangial expansion has a more asymptotic relationship with T1DM duration (Steinke J, Mauer M, unpublished observations). However, when renal insufficiency occurs, marked mesangial expansion and increased GBM width are present in virtually all T1DM patients (9-10). Diffuse mesangial expansion, commonly termed diffuse diabetic glomerulosclerosis, can be associated with nodular lesions consisting of areas of marked mesangial expansion forming large round fibrillar mesangial zones with palisading of mesangial

nuclei around the periphery of the nodule and compression of the associated glomerular capillaries (Kimmelstiel-Wilson nodules). Both mesangial expansion and GBM and TBM thickening are a consequence of extracellular matrix (ECM) accumulation, with increased deposition of types IV and VI collagen, laminin and fibronectin (12-13). In contrast, initial interstitial expansion is primarily due to an increase in the cellular component of this renal compartment (14); increase in fibrillar collagen is measurable only in patients with advanced disease (14).

Afferent and efferent arteriolar hyalinosis may be present within a few years after diabetes onset (5) and this vascular lesion contributes to ischemic global glomerular sclerosis. Similar lesions may occur in the glomerular subendothelial space (hyaline caps) and along the parietal surface of Bowman's capsule (capsular drops).

Abnormalities of the glomerular-tubular junction (GTJA) occur as late manifestations of the disease (15) predominantly in patients with proteinuria, and rarely at earlier stages (16). These manifest with focal adhesions, obstruction of the proximal tubular take-off from the glomerulus detachment of the tubule from the glomerulus (atubular glomerulus). These focal segmental glomerulosclerosis (FSGS) lesions have a marked predilection for the GTJ and are uncommon at other locations. The lesions at the GTJ are inversely correlated with GFR (15, 16) and probably contribute to the loss of renal function in proteinuric diabetic patients.

These various lesions of diabetic nephropathy progress at varying rates within and between T1DM patients, and, as discussed below, this is even more the case in type 2 diabetes (T2DM). For example, GBM width and Vv (Mes/glom) are significantly but not very precisely correlated with one another; with some patients have relatively marked GBM thickening without much mesangial expansion and others the contrary (9). Marked renal extracellular basement membrane accumulation resulting in extreme mesangial expansion and GBM thickening are present in the vast majority of T1DM patients who develop overt diabetic nephropathy (DN) manifesting as proteinuria, hypertension, and declining GFR (8, 17). Ultimately, focal and global glomerulosclerosis, tubular atrophy, interstitial expansion and GTJA facilitate this downward spiral. However, tubulo-interstitial lesions and GTJA contribute only  $\approx 10-15\%$  to functional loss in T1DM patients whose GFR is above  $40 \text{ ml/min/1.73m}^2$  (16). Tubulo-interstitial disease may be more important in the progression from moderate renal insufficiency to end-stage renal disease (ESRD) (18), but it is probably a mistake to extrapolate this to earlier stages of DN progression. The situation in T2DM is more complex. The real frequency of non-diabetic renal diseases among patients with T2DM and proteinuria is difficult to assess in studies of which patients biopsied for clinical reasons because of selection bias towards atypical cases (19-24).

Research renal biopsies in a large cohort of T2DM patients with microalbuminuria (MA) and proteinuria and described marked heterogeneity in renal structure among these patients; in fact, only a minority subset had DN patterns typical of those seen in T1DM patients; the remaining had mild or absent diabetic glomerulopathy with or without tubulo-interstitial, arteriolar and global glomerulosclerosis changes (25). Less than 10% of our proteinuria patients had non-diabetic renal diseases. Based on these observations, we proposed a classification system which included 3 major categories (25):

*Category C I: Normal or near normal renal structure.* These patients (35% of MA and 15% of proteinuria) had normal renal biopsies or showed very mild glomerular, tubular, interstitial and/or vascular changes.

*Category C II: Typical diabetic nephropathology.* These patients (30% of MA and 50% of proteinuria) had established diabetic lesions with an approximately balanced severity of glomerular, tubulo-interstitial and arteriolar changes, a picture typical of that seen in most T1DM patients with obvious light microscopic DN changes.

*Category C III: Atypical patterns of renal injury.* These patients (35% of MA and proteinuria) had relatively mild diabetic glomerular changes considering disproportionately severe: (a) Tubular atrophy, TBM thickening and reduplication and interstitial fibrosis (tubulo-interstitial lesions). (b) Advanced glomerular arteriolar hyalinosis commonly associated with atherosclerosis of larger vessels. (c) Global glomerular sclerosis. In C III group these patterns were present in all possible combinations. More recently, examining the associations of albumin excretion rates (AER) and electron microscopic morphometrically quantitated DN lesions, we could mathematically define a spatial cluster of structural/functional relationships which contained the T1DM patients. About 1/3 of the T2DM fell outside of this cluster because of MA or proteinuria despite a paucity of diabetic glomerulopathy lesions (26). These objective data largely confirm the more subjective categorical classifications.

Thus, hyperglycaemia may cause different patterns of renal injury in T1DM compared to T2DM patients. Alternatively, the disproportionate tubulo-interstitial, glomerulosclerotic and vascular changes of T2DM could also be related to aging, atherosclerosis and systemic hypertension. The natural history of MA and proteinuria T2DM patients with minimal or no renal lesions is not yet well understood, however, GFR loss in the relatively short-term (about 3 years), is largely confined to T2DM research patients with mesangial expansion (27).

### **Morphometric analysis and structural-functional relationships**

The critical lesion in T1DM is mesangial expansion, morphometrically termed mesangial fractional volume [ $V_v(\text{Mes}/\text{glom})$ ] (the fraction of the cross-sectional area of the glomerular tuft made up by mesangium); this is the electron microscopically estimated structural parameter that best correlates with all functional parameters in T1DM (9, 17). Indeed, a highly significant inverse correlation exists between  $V_v(\text{Mes}/\text{glom})$  and GFR (9, 15-17); when mesangium expands it restricts and distorts glomerular capillaries and diminishes capillary filtration surface (9), which is strongly directly related to  $V_v(\text{Mes}/\text{glom})$  and inversely to GFR (28).  $V_v(\text{Mes}/\text{glom})$  is also related to AER (9, 15-17, 29) and blood pressure levels (30). In contrast, GBM thickening is closely related to AER and less so to GFR or hypertension, suggesting that this lesion is a closer surrogate to the pathogenesis of albuminuria. Interstitial expansion and percentage of global sclerosis are also directly related to proteinuria, hypertension and inversely to GFR (4, 5, 9, 15, 16). Progression from normoalbuminuria (NA) to MA and from MA to proteinuria is primarily related to progressive mesangial expansion (11) with no significant progression in interstitial fibrosis or GBM thickening over the 5 years of this study. These data may initially seem contradictory to recent studies describing that greater GBM width at baseline biopsy was predictive of AER after 5 or 6 years of follow-up (31, 32). However, given the linear course of GBM thickening *vs.* the non-linear trajectory of mesangial expansion, it is not surprising that GBM width, a strong correlate of AER, is a better predictor of DN risk while mesangial

expansion, through its intimate relationship with filtration surface, better defines the clinical course of those destined to develop severe diabetic kidney disease. Although an increase in AER to the MA range is usually considered the first clinical expression of DN, some long-term T1DM patients have reduced GFR as initial indicator of renal disease (33). This situation has also been seen in T2DM patients (34).

As alluded to above, through much of the natural history of DN lesions develop in complete clinical silence. When persistent MA and proteinuria supervene, lesions are often far advanced and loss of GFR may then progress relatively rapidly toward ESRD. This typical clinical story is best described by non-linear analyses of structural-functional relationships (16). Using simple linear regression models, glomerular structural variables explained about 65% of AER and 35% of GFR variability among T1DM patients (17). However, using piecewise (spline) regression models, glomerular structural variables alone, GBM width, [Vv(Mes/glom)], and total filtration surface per glomerulus or TFS], explained 95% of variability in AER ranging from NA to proteinuria. These same glomerular structures, however, explained only 78% of GFR variability in this study, and this increased to 92% with the addition of indices of GTJA and interstitial expansion (16).

In summary, most of the AER and GFR changes in T1DM are explained by diabetic glomerulopathy lesions and these structural-functional relationships are largely driven by patients with more advanced lesions and clinical functional abnormalities while structure is highly variable (from virtually none to moderate severity) in patients without functional abnormalities. In the end, as in other slowly progressive renal diseases, clinical findings in DN may, at least in part, reflect the lesions outstripping of renal compensatory capacities and this may be mirrored in the non-linear analyses described above.

### **Reversibility of diabetic nephropathy lesions**

Pancreas transplantation offers the opportunity to test the effects of long-term normoglycemia to prevent, halt or reverse DN lesions. GBM and TBM widths were decreased after 10-years of normoglycemia, returning to normal values in most patients (35). Vv(Mes/glom) and mesangial matrix fractional volume [Vv(MM/glom)] were also lower at 10 years than at baseline or 5 years (35). Light microscopic observations revealed a remarkable amelioration of glomerular structure, including the total disappearance of Kimmelstiel-Wilson nodular lesions and reopening of glomerular capillaries previously compressed by mesangial expansion (35). These findings call for further studies aimed at identifying the molecular and cellular mechanisms involved in these healing processes which could provide new directions in the treatment of DN.

### **References**

1. Mauer M, Fioretto P, Woredek Y, et al: Diabetic nephropathy, in Schrier RW (ed): Disease of the Kidney and Urinary Tract, Philadelphia, Lippincott Williams and Wilkins, 2001, pp 2083-2127.
2. Bell ET: Renal vascular disease in diabetes mellitus. *Diabetes* 2:376-389, 1953.
3. Brito P, Fioretto P, Drummund K, et al: Proximal tubular basement membrane width in insulin-dependent diabetes mellitus. *Kidney Int* 53:754-761, 1998.

4. Lane PH, Steffes MW, Fioretto P, et al: Renal interstitial expansion in insulin-dependent diabetes mellitus. *Kidney Int* 43:661-667, 1993.
5. Harris RD, Steffes MW, Bilous RW, et al: Global glomerular sclerosis and glomerular arteriolar hyalinosis in insulin-dependent diabetes. *Kidney Int* 40:107-114, 1991.
6. Østerby R: Early phases in the development of diabetic glomerulopathy. *Acta Med Scand* 475:1-7, 1975.
7. Østerby R: Morphometric studies of the peripheral glomerular basement membrane in early juvenile diabetes I. Development of initial basement membrane thickening. *Diabetologia* 8:84-92, 1972.
8. Fioretto P, Steffes MW, Mauer SM: Glomerular structure in non-proteinuric insulin-dependent diabetic patients with various levels of albuminuria. *Diabetes* 43:1358-1364, 1994.
9. Mauer SM, Steffes MW, Ellis EN, et al: Structural functional relationships in diabetic nephropathy. *J Clin Invest* 74:1143-1155, 1984.
10. Østerby R, Andersen AR, Gundersen HJ: Quantitative studies of glomerular ultrastructure in type 1 diabetics with incipient nephropathy. *Diabet Nephropathy* 3:95, 1984.
11. Fioretto P, Steffes MW, Sutherland DER, et al: Sequential renal biopsies in IDDM patients: Structural factors associated with clinical progression. *Kidney Int* 48:1929-1935, 1995.
12. Falk RJ, Scheinman JI, Mauer SM, et al: Polyantigenic expansion of basement membrane constituents in diabetic nephropathy. *Diabetes* 32:34, 1983.
13. Kim Y, Kleppel MM, Butkowski R, et al: Differential expression of basement membrane collagen chains in diabetic nephropathy. *Am J Pathol* 138:413, 1991.
14. Katz A, Caramori ML, Sisson-Ross S, et al: An increase in the cell component of the cortical interstitium antedates interstitial fibrosis in type 1 diabetic patients. *Kidney Int* 61:2058-2066, 2002.
15. Najafian B, Kim Y, Crosson JT, et al: Atubular glomeruli and glomerulotubular junction abnormalities in diabetic nephropathy. *J Am Soc Nephrol* 14:908-917, 2003.
16. Najafian B, Crosson JT, Kim Y, et al: Glomerulotubular junction abnormalities are associated with proteinuria in type 1 diabetes. *J Am Soc Nephrol* 17:S53-S60, 2006.
17. Caramori ML, Kim Y, Huang C, et al: Cellular basis of diabetic nephropathy: 1. Study design and renal structural-functional relationships in patients with long-standing diabetes. *Diabetes* 51:506-513, 2002.

18. Bader R, Bader H, Grund KE, et al: Structure and function of the kidney in diabetic glomerulosclerosis. Correlations between morphological and functional parameters. *Pathol Res Pract* 167:204-216, 1980.
19. Parving H-H, Gall M-A, Skøtt P, et al: Prevalence and causes of albuminuria in non-insulin-dependent diabetic patients. *Kidney Int* 41:758-762, 1992.
20. Lipkin GW: More than one kind of type 2 diabetes with renal disease do not have diabetic nephropathy. *J Am Soc Nephrol* 5:37, 1994.
21. Gambará V, Mecca G, Remuzzi G, et al: Heterogeneous nature of renal lesions in type II diabetes. *J Am Soc Nephrol* 3:1458-1466, 1993.
22. Ruggenti P, Gambará V, Perna A, et al: The nephropathy of non-insulin dependent diabetes: predictors of outcome relative to diverse patterns of renal injury. *J Am Soc Nephrol* 9:2336-2343, 1998.
23. Olsen S, Mogensen CE: Non-diabetic renal disease in NIDDM proteinuric patients may be rare in biopsies from clinical practice. *Diabetologia* 39:1638-1645, 1996.
24. Mazzucco G, Bertani T, Fortunato M: Different patterns of renal damage in type 2 diabetes mellitus: a multicentric study on 393 biopsies. *Am J Kid Dis* 39:713-720, 2002.
25. Fioretto P, Mauer M, Brocco E, et al: Patterns of renal injury in type 2 (non-insulin dependent) diabetic patients with microalbuminuria. *Diabetologia* 39:1569-1576, 1996.
26. Najafian B, Caramori ML, Mauer M, et al: Clustering of type 1 and type 2 diabetic patients based on diabetic nephropathy structural-functional relationships. *J Am Soc Nephrol* 16:679A, 2005 (abstr).
27. Nosadini R, Velussi M, Brocco E, et al: Course of renal function in type 2 diabetic patients with abnormalities of albumin excretion rate. *Diabetes* 49:476-484, 2000.
28. Ellis EN, Steffes MW, Goetz FC, et al: Glomerular filtration surface in type 1 diabetes mellitus. *Kidney Int* 29:889, 1986.
29. Chavers BM, Bilous RW, Ellis EN, et al: Glomerular lesions and urinary albumin excretion rate in type 1 diabetic patients without overt proteinuria. *New Engl J Med* 320:966-970, 1989.
30. Mauer SM, Sutherland DER, Steffes MW: Relationships of systemic blood pressure to nephropathy in insulin-dependent diabetes mellitus. *Kidney Int* 41:736, 1992.
31. Bangstad HJ, Østerby R, Hartmann A, et al: Severity of glomerulopathy predicts long-term urinary albumin excretion rate in patients with type 1 diabetes and microalbuminuria. *Diabetes Care* 22:314-319, 1999.

32. Steinke JM, Sinaiko AR, Kramer MS, *et al* for the International Diabetic Nephropathy Study Group: The early natural history of nephropathy in type 1 diabetes. III. Predictors of five-year urinary albumin excretion rate patterns in initially normoalbuminuric patients. *Diabetes* 54(7):2164-2171, 2005.
33. Caramori ML, Fioretto P, Mauer M. Low glomerular filtration rate in normoalbuminuric type 1 diabetic patients: an indicator of more advanced glomerular lesions. *Diabetes* 52:1036-1040, 2003.
34. MacIsaac RJ, Tsalamandris C, Panagiotopoulos S, *et al*: Normoalbuminuric renal insufficiency in type 2 diabetes. *Diabetes Care* 27:195-200, 2004.
35. Fioretto P, Steffes MW, Sutherland DER, *et al*: Reversal of lesions of diabetic nephropathy after pancreas transplantation. *New Engl J Med* 339:69-75, 1998.

# **Podocyte Injury: Lessons Learned From Animal Models**

## **A Play in Five Acts**

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Just a decade ago, our knowledge of podocyte injury in animal models was rudimentary. We recognized 2 major paradigms of irreversible podocyte injury leading to focal segmental glomerulosclerosis (FSGS). The first is direct podocyte injury due to exposure to such cell toxins as puromycin aminonucleoside and adriamycin/doxorubicin (1). These toxic models fostered the concept of primary podocyte injury in the pathogenesis of foot process effacement and glomerulosclerosis. By contrast, renal ablation models pointed to glomerular hypertension (elevated glomerular capillary pressures and flow rates) as the primary pathophysiologic process in the course of adaptive responses to reduced number of functioning nephrons or other glomerular stress, in turn causing secondary podocyte injury (2). These basic paradigms form the conceptual dichotomy between “primary” and “secondary” (post-adaptive) FSGS. Only in the past few years has the molecular basis for these pathologic alterations been elucidated through a greater understanding of podocyte biology.

### **ACT 1: SEEING IS BELIEVING**

#### **ULTRASTRUCTURAL STUDIES PROVIDE MECHANISTIC INSIGHTS**

Initial insights into the podocyte injury in proteinuric conditions came from meticulous 3-dimensional ultrastructural observations using scanning electron microscopy. Studies by Inokuchi et al. in puromycin nephropathy elucidated that foot process effacement consists of a distinctive and predictable change in podocyte shape (3). The foot processes, which are analogous to specialized lamellipodia in other biologic systems, spread and fan out, incriminating the actin cytoskeleton in the effacement process. This simple, but elegant, observation laid the groundwork for elucidation of specific actin-associated podocyte proteins that may be operant in foot process maintenance.

By contrast, seminal ultrastructural observations by Nagata and Kriz in an ablation model of uninephrectomy in the young rat showed remarkably different podocyte alterations (4). As the tuft hypertrophied in response to the reduced number of functioning nephrons, podocyte cell number did not increase. Podocyte cell bodies were forced to stretch to cover a much larger surface area and serve many more glomerular capillaries. Cell bodies hypertrophy and become attenuated into cytoplasmic sheets. Primary processes thin out and extend to remote capillaries. Filtrate is now delivered into the subcellbody space, causing bulging of the cytoplasmic sheets and formation of pseudocysts, under which foot processes are largely preserved. The formation of denuded patches of GBM owing to podocyte detachment and the apposition of distended podocyte cell bodies to Bowman's capsule form the nidus of the segmental sclerotic lesion. In this model, podocyte insufficiency and maladaptive responses develop into irreversible structural lesions.

## **ACT 2: GOING, GOING, GONE... THE ROLE OF PODOCYTE DEPLETION**

In both toxic and adaptive models of FSGS, a central role for podocyte loss has been proposed. Podocyte depletion has been identified in many human glomerular diseases, including diabetic nephropathy, focal segmental glomerulosclerosis and IgA nephropathy (5, 6). In human disease, podocytes can be detected in the urine and the reduction in podocyte cell number correlates with the degree of proteinuria and severity of sclerosis (7).

This mechanism has been validated in several ingenious models of targeted podocyte cell death (8-10). Using a transgenic rat strain in which the human diphtheria toxin (DT) receptor is specifically expressed in podocytes driven by the podocin promoter, Wiggins and coworkers were able to produce different stages of glomerular injury depending on the percentage of podocytes depleted after injection of DT, consistent with a dose response (8). Over 40% podocyte depletion produced segmental and global glomerulosclerosis with high grade proteinuria and reduced renal function. In analogous experiments, Ichikawa and colleagues engineered a mouse model of glomerular sclerosis by selectively expressing human CD25 in podocytes (9,10). Injection of anti-Tac (Fv)-PE38 (LMB2) immunotoxin induced progressive proteinuria and glomerulosclerosis in a dose dependent fashion. By permanently labeling the podocyte lineage with lacZ, the investigators could determine their fate. The number of lacZ stained podocytes progressively declined as parietal epithelial cells avidly proliferated to cover the denuded tuft, resembling collapsing FSGS. These studies demonstrate the central role of podocyte depletion in the process of glomerular sclerosis. If the initial insult is of sufficient impact, there may be spreading of sclerosis to adjacent segments after the insult has been withdrawn. This process suggests a vicious cycle of local spreading of sclerosis, incriminating toxic substances secreted in a paracrine or autocrine fashion (such as TGF $\beta$ , AII, MIF) or reduction in survival factors (such as VEGF) (11).

## **ACT 3: PROOF OF CONCEPT: ANIMAL MODELS AND THE GENETIC BASIS OF FSGS**

Great advances have been made in our understanding of the genetic basis for MCD and FSGS. A number of critical podocyte proteins have been identified to be mutated or deficient in human forms of congenital nephrotic syndrome or inherited FSGS (12-24). Many of these proteins were identified first by positional cloning in affected families. Others were identified serendipitously while studying other disease systems. The critical role of these proteins in the mediation of FSGS was later validated in experimental models, including knock-out models or transgenic models expressing mutant proteins (25-31). The animal models provided proof of concept that deletion of a particular gene was sufficient to cause proteinuria or FSGS. A number of other podocyte proteins produce FSGS in null mice or conditional knock-outs, although a role in human disease has not yet been identified (32-35). The responsible genes encode proteins that are located in various subcellular domains of the podocyte, including membrane-associated (slit diaphragm, basal membrane), nuclear (transcription factors and chromatin bundling proteins), and cytosolic (associated with the actin cytoskeleton or cell energetics). These genes are listed in the table below. An asterisk marks the human disease genes that have been validated in animal models.

A unifying concept in all these models is the central role of the actin cytoskeleton in coordinating cell signaling from the various membrane compartments. Interference with cell signaling may promote the stereotypic response of foot process effacement common to all these conditions (36). This hypothesis is supported by evidence that the slit diaphragm is a mechanosensor that serves as a platform for signal transduction (37).

<b>HUMAN GENE PRODUCTS</b>	<b>GENE</b>	<b>INHERITANCE</b>	<b>CHROMOSOME</b>
<b>Slit Diaphragm proteins</b>			
Nephrin*	NPHS1	AR	19q13.1
Podocin*	NPHS2	AR	1q25-31
CD2 associated protein*	CD2AP	AD	6p12
Transient Receptor Cation 6	TRPC6	AD	11q21-22
<b>Cytosolic proteins</b>			
Alpha-actinin 4*	ACTN4	AD	19q13
Phospholipase C $\epsilon$ 1	PLCE1	AR	10q23-24
<b>Basal Membrane proteins</b>			
Laminin $\beta$ 2*	LAMB2	AR (Pierson syndrome)	3p21
Beta 4 integrin*	ITGB4	AR (Epidermolysis bullosa)	17q11
<b>Nuclear Proteins</b>			
Wilm's tumor 1	WT1	AD (DMS, Frasier syndrome)	11p13
Chromatin bundling protein	SMARCAL1	AD (Schimke syndrome)	2q34-36
<b>Mitochondrial Products</b>			
Mitochondrial tRNA <sup>Leu</sup>	mtDNA-A3243G	Maternal	mtDNA

**MOUSE GENE MUTANTS**  
**(Role in human disease unknown)**

**Slit Diaphragm Proteins**

Neph1  
 Fyn  
 FAT1

**Actin Associated Proteins**

Nck 1/2

**ACT 4: MORE IS NOT NECESSARILY BETTER:**

**PODOCYTE PROLIFERATION AND DYSREGULATION IN COLLAPSING FSGS**

The mature podocyte is a post-mitotic cell. Podocytes can undergo DNA synthesis to a limited degree but do not proliferate because they arrest in the G2/M phase of the cell cycle (38). Findings in human collapsing FSGS suggest that podocytes may exhibit rare replicative capacity under select conditions, producing the glomerular pseudocrescents typical of this variant (39). This has been difficult to prove without lineage specific markers, and there is increasing evidence that parietal epithelial cells contribute to the glomerular epithelial cell proliferation (40). In collapsing FSGS, the podocytes downregulate their mature podocyte markers (WT-1,

synaptopodin, podocalyxin, GLEPP-1), express KI-67 and enter the cell cycle (39, 41). A similar podocyte phenotype has been identified in primary collapsing glomerulopathy and human HIV-associated nephropathy, as well as an HIV transgenic model (39, 42). A decrease in the CDK inhibitor, p27 and increase in cyclin D1 underlie the proliferative phenotype (43, 44).

Evidence in human HIVAN supports a direct viral infection of renal parenchymal cells, rather than a systemic or indirect immune dysregulation by the HIV virus. Cohen et al in 1989 were the first to report the detection of HIV-1 in renal epithelial cells by DNA in situ hybridization (45). In 2000, Bruggeman et al. detected HIV-1 in renal epithelial cells of patients with HIVAN by RNA in situ hybridization (46). These findings were confirmed using DNA in situ hybridization and by application of riboprobes specific for both the *nef* and *gag* genes. Virus was identified in renal tubular cells, often involving many contiguous cells in individual tubular profiles, as well as podocytes, parietal epithelial cells, and some interstitial leukocytes (46). In one particularly illustrative case treated with highly active anti-retroviral therapy (HAART), virus persisted in the tubular epithelium as determined by RNA in situ hybridization, even after viral load in the peripheral blood had become undetectable and renal histology had improved (47). The ability of the kidney to serve as a reservoir for HIV-1 was later confirmed by Marras et al using laser capture microdissection to characterize the HIV-1 quasi-species present in tubular epithelium (48). Comparison of the envelope sequences from renal tubular epithelial cells and peripheral blood leukocytes in individual patients showed variations in the HIV-1 envelope sequences in tubular epithelium compared to blood, indicating that the kidney is able to support viral replication and quasispecies evolution as a separate compartment.

Several animal models have provided insights into disease pathogenesis. One of the first models, Tg26, was established in transgenic mice containing a replication-defective HIV-1 construct that lacks the *gag* and *pol* genes and is expressed under the control of the long terminal repeat (LTR) viral promoter (49). In this model, which closely recapitulates the morphologic features of the human HIVAN, viral transgene was expressed in glomerular and tubular epithelial cells (50). Cross-transplantation of kidneys between Tg26 and WT mice showed that renal transgene expression was required for the development of nephropathy (50). A similar model has been established in rats using the same transgene construct (51). Hanna et al. generated several different transgenic lines with mutations in one or more of the HIV genes, and found that *nef* was necessary and sufficient to produce the renal phenotype (52). In vitro studies in cultured podocytes suggest that *nef*-induced activation of Stat3 and Ras-MAPK1,2 via Src-dependent pathways is responsible for podocyte proliferation and dedifferentiation (53). Transgenic expression of nonstructural HIV-1 genes (*vif*, *vpr*, *nef*, spliced forms of *tat* and *rev*, but not *vpu*) selectively in podocytes using the nephrin promoter in mice with FVB/N genetic background results in podocyte injury, glomerulosclerosis and tubular microcyst formation (54). Podocyte specific expression of *nef* and *vpr* in a double-transgenic murine model recapitulated the severe morphologic and functional features of human HIVAN, suggesting a synergistic interaction of these proteins (55). In the Tg26 model of HIVAN, inhibition of podocyte proliferation using a CDK2 inhibitor reduced proteinuria and glomerulosclerosis (56).

In HIVAN and idiopathic collapsing glomerulopathy, the proliferating podocytes have an undifferentiated phenotype, leading to functional podocyte insufficiency, defective podocyte adhesion, and shedding of podocytes into the urine. Over the long-term, collapsing glomerulopathy is likely to lead to progressive podocyte depletion, as in other models of FSGS.

**ACT 5: THE MISSING LINK  
HOW PODOCYTE INJURY PROMOTES SCLEROSIS**

Conventional wisdom suggests that foot process effacement, if reversed can lead to restoration of glomerular architecture (as in steroid responsive MCD). The failure of reparative mechanisms promotes persistent proteinuria and the development of glomerulosclerosis. Precisely how podocyte injury promotes glomerulosclerosis is poorly understood, but podocyte loss is emerging as a central pathomechanism. Evidence from animal models suggests that critical perturbations in the balance between pro-apoptotic and anti-apoptotic factors promote podocyte depletion and progressive glomerulosclerosis (Reviewed in 38). For example, toxins such as puromycin and adriamycin induce podocyte production of ROS, leading to podocyte DNA damage, apoptosis, and GBM protein peroxidation. Mechanical stretch can promote podocyte hypertrophy and apoptosis (57). Excessive protein trafficking through the podocyte itself generates ER stress and podocyte injury (58). Many of the pro-apoptotic factors listed below (such as AII and TGFβ) also possess prosclerotic properties, providing a link to sclerosis.

The TGFβ1 transgenic mouse is a particularly valuable model that has shed mechanistic insights into the inter-relationship between podocyte apoptosis and glomerulosclerosis. (59). Podocytes expressing TGFβ1 undergo apoptosis associated with marked upregulation of Smad7. TGFβ1 and Smad7 promote podocyte apoptosis through different mechanisms. TGFβ1 induces apoptosis by activation of mitogen-activating protein (MAP) kinase p38 and classic effector caspase-3, whereas TGFβ-inducible Smad7 inhibits signaling by the cell survival factor NF-kB. In this model, podocyte depletion through apoptotic pathways leads to progressive FSGS.

**Balance of Factors Influencing Podocyte Depletion (adapted from Shankland, ref 38)**

<b>Podocyte pro-apoptotic factors</b>	<b>Pro-survival (anti-apoptotic) factors</b>
Angiotensin II	Cyclin 1
AT1 Receptor	Nephrin
TGF-β	CD2AP
Cyclosporine	Dexamethasone
SMAD7	Bcl-2
ROS	Cell-cell contact
Detachment	VEGF
↓ p21	Collagen via Ras-ERK signaling
↓ p27	Focal adhesion kinase
Stress-tension	Hepatocyte growth factor
bFGF	Insulin-like growth factor
Lytic C5b-9	
p53	
Hyperglycemia	

## References

1. Ryan GB, Karnovsky MJ: An ultrastructural study of the mechanisms of proteinuria in aminonucleoside nephrosis. *Kidney Int* 8: 219-232, 1975.
2. Olson JL, Hostetter TH, Rennke HG, Brenner BM, Venkatachalam MA: Altered glomerular permselectivity and progressive sclerosis following extreme ablation of renal mass. *Kidney Int* 22:112-126, 1982.
3. Inokuchi S, Shirato I, Kobayashi N, Koide H, Tomino Y, Sakai T: Re-evaluation of foot process effacement in acute puromycin aminonucleoside nephrosis. *Kidney Int* 50: 1278-1287, 1996.
4. Nagata M and Kriz W: Glomerular damage after uninephrectomy in young rats. II. Mechanical stress on podocytes as a pathway to sclerosis. *Kidney Int* 42: 148-160, 1992.
5. Meyer TW, Bennett PH, Nelson RG: Podocyte number predicts long-term urinary albumin excretion in Pima Indians with type II diabetes and microalbuminuria. *Diabetologia* 42: 1341-1344, 1999.
6. Lemley KV, Lafayette RA, Safai M, Derby G, Blouch K, Squarer A, Myers BD: Podocytopenia and disease severity in IgA nephropathy. *Kidney Int* 61: 1475-1485, 2002.
7. Hara M, Yanagihara T, Kihara I: Urinary podocytes in primary focal segmental glomerulosclerosis. *Nephron* 89:342-347, 2001.
8. Wharram BL, Goyal M, Wiggins J, Sanden SK, Hussain S, Filipiak WE, Saunders TL, Dysko RC, Kohno K, Holzman LB, Wiggins RC: Podocyte depletion causes glomerulosclerosis: Diphtheria toxin-induced podocyte depletion in rats expressing human diphtheria toxin receptor transgene. *J Am Soc Nephrol* 16: 2941-2952, 2005.
9. Matsusaka T, Xin J, Niwa S, Kobayashi K, Akatsuka A, Hashizume H, Wang QC, Pastan I, Fogo AB, Ichikawa I: Genetic engineering of glomerular sclerosis in the mouse via control of onset and severity of podocyte specific injury. *J Am Soc Nephrol* 16:1013-1023, 2005.
10. Asano T, Niimura F, Pastan I, Fogo AB, Ichikawa I, Matsusaka T: Permanent genetic tagging of podocytes: Fate of injured podocytes in a mouse model of glomerular sclerosis. *J Am Soc Nephrol* 16: 2257-2262, 2005.
11. Ichikawa I, Ma J, Motojima M, Matsusaka T: Podocyte damage damages podocytes: autonomous vicious cycle that drives local spread of glomerular sclerosis. *Curr Opin Nephrol Hypertens* 14: 205-210, 2005.
12. Tryggvason K, Patrakka J, Wartiovaara J: Hereditary proteinuria syndromes and mechanisms of proteinuria. *N Eng J Med* 354: 1387-1401, 2006.
13. Kestila M, Lenkkeri U, Mannikko M et al. Positionally cloned gene for a novel glomerular protein - nephrin - is mutated in congenital nephrotic syndrome. *Mol Cell* 1: 575-82, 1998.
14. Boute N, Gribouval O, Roselli S, et al: NPHS2, encoding the glomerular protein podocin, is mutated in autosomal recessive steroid-resistant nephrotic syndrome. *Nat Genet* 24: 349-354, 2000.
15. Koziell A, Grech V, Hussain S, et al: Genotype/phenotype correlations of NPHS1 and NPHS2 mutations in nephrotic syndrome advocate a functional inter-relationship in glomerular filtration. *Hum Mol Genet.* 11: 379-88, 2002.
16. Kim JM, Wu H, Green G, et al.: CD2-associated protein haploinsufficiency is linked to glomerular disease susceptibility. *Science.* 300:1298-300, 2003.

17. Winn MP, Conlon PJ, Lynn KL, et al.: A mutation in the TRPC6 cation channel causes familial focal segmental glomerulosclerosis. *Science* 308: 1801-1804, 2005.
18. 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
19. Hinkes B, Wiggins RC, Gbadegesin R et al. Positional cloning uncovers mutations in PLC $\epsilon$ 1 responsible for a nephrotic syndrome variant that may be reversible. *Nat Genet* 38: 1397-1405, 2006.
20. Denamur E, Bocquet N, Mougenot B, et al: Mother-to-child transmitted WT1 splice-site mutation is responsible for distinct glomerular diseases. *J Am Soc Nephrol* 10: 2219-2223, 1999
21. Lucke T, Billing H, Sloan EA, et al.: Schimke-immuno-osseous dysplasia: new mutation with weak genotype-phenotype correlation in siblings. *Am J Med Genet A*. 135: 202-205, 2005.
22. Guery B, Choukroun G, Noel LH, et al: The spectrum of systemic involvement in adults presenting with renal lesion and tRNA (Leu) gene mutation. *J Am Soc Nephrol* 14: 2099-2108, 2003
23. Kambham N., Tanji N., Seigle RL et al: Congenital focal segmental glomerulosclerosis associated with  $\beta$ 4 integrin mutation and epidermolysis bullosa. *Am J Kid Dis* 36: 190-196, 2000
24. Zenker M, Aigner T, Wendler O, et al. Human laminin beta2 deficiency causes congenital nephrosis with mesangial sclerosis and distinct eye abnormalities. *Hum Mol Genet* 13: 2625-2632, 2004.
25. Putaala H, Soininen R, Kilpelainen P, Wartiovaara J, Tryggvason K : The murine nephrin gene is specifically expressed in kidney, brain and pancreas : inactivation of the gene leads to massive proteinuria and neonatal death. *Hum. Mol. Genet* 10:1-8, 2001.
26. Roselli S, Heidet L, Sich M, et al. Early glomerular filtration defect and severe renal disease in podocin-deficient mice. *Mol Cell Biol*. 24: 550-560, 2004.
27. Shih NY, Li J, Karpitskii V et al. Congenital nephrotic syndrome in mice lacking CD2-associated protein. *Science* 286: 312-315, 1999.
28. Michaud JL, Lemieux LI, Dube M. Et al. Focal and segmental glomerulosclerosis in mice with podocyte-specific expression of mutant alpha-actinin-4. *J Am Soc Nephrol* 14: 1200-1211, 2003.
29. Kos CH, Le TC, Sinha S et al. Mice deficient in alpha-actinin-4 have severe glomerular disease. *J Clin Invest* 111: 1683-1690, 2003.
30. Kreidberg JA et al: Alpha 3 beta 1 integrin has a crucial role in kidney and lung organogenesis. *Development* 122: 3537-3547, 1999.
31. Jarad G, Cunningham J, Shaw AS, Miner JH: Proteinuria precedes podocyte abnormalities in Lamb2 $^{-/-}$  mice, implicating the glomerular basement membrane as an albumin barrier. *J Clin Invest* 116: 2272-2279, 2006.
32. Donovan DB, Freed DD, Vogel H et al. Proteinuria and perinatal lethality in mice lacking NEPH1, a novel protein with homology to NEPHRIN. *Mol Cell Biol* 21: 4829-4836, 2001.
33. Ciani L, Patel A, Allen ND, French-Constant C: Mice lacking the giant protocadherin mFAT1 exhibit renal slit junction abnormalities and a partially penetrant cyclopia and anophthalmia phenotype. *Mol Cell Biol* 23: 3575-3582, 2003.

34. Huber TB, Kwok C, Wu H et al. Bigenic mouse models of focal segmental glomerulosclerosis involving pairwise interaction of CD2AP, Fyn and synaptopodin. *J Clin Invest* 116: 1337-1345, 2006.
35. Jones N, Blasutig IM, Eremina V et al. Nck adaptor proteins link nephrin to the actin cytoskeleton of kidney podocytes. *Nature* 440: 818-823, 2006.
36. Kerjaschki D. Caught flat-footed: podocyte damage and the molecular bases of focal glomerulosclerosis. *J Clin Invest* 108: 1583-1587, 2001.
37. Benzing T: Signaling at the slit diaphragm. *J Am Soc Nephrol* 15: 1382-1391, 2004.
38. Shankland SJ. The podocyte's response to injury: Role in proteinuria and glomerulosclerosis. *Kidney Int* 69: 2131-2147, 2006.
39. Barisoni L, Kriz W, Mundel P, D'Agati V. The dysregulated podocyte phenotype; a novel concept in the pathogenesis of collapsing idiopathic focal segmental glomerulosclerosis and HIV-associated nephropathy. *J Am Soc Nephrol* 10: 51-61, 1999.
40. Dijkman HB, Weening JJ, Smeets B et al : Proliferating cells in HIV and pamidronate-associated collapsing focal segmental glomerulosclerosis are parietal epithelial cells. *Kidney Int* 70: 338-344, 2006. .
41. Bariety J, Bruneval P, Hill G, et al: Post-transplantation relapse of FSGS is characterized by glomerular epithelial cell transdifferentiation. *J Am Soc Nephrol* 12: 261-274, 2001.
42. Barisoni L, Bruggeman LA, Mundel P, et al.: HIV-1 induces renal epithelial dedifferentiation in a transgenic model of HIV-associated nephropathy. *Kidney Int* 58: 173-181, 2000.
43. Shankland SJ, Eitner F, Hudkins KL et al. Differential expression of cyclin-dependent kinase inhibitors in human glomerular disease: role in podocyte proliferation and maturation. *Kidney Int* 58: 674-683, 2000.
44. Barisoni L, Mokrzycki M, Sablay L, et al: Podocyte cell cycle regulation and proliferation in collapsing glomerulopathies. *Kidney Int* 58: 137-143, 2000.
45. Cohen AH, Sun NC, Shapshak P, Imagawa DT: Demonstration of human immunodeficiency virus in renal epithelium in HIV-associated nephropathy. *Modern Pathol* 2: 125-128, 1989.
46. Bruggeman LA, Ross MD, Tanji N, et al.: Renal epithelium is a previously unrecognized site of HIV-1 infection. *J Am Soc Nephrol* 11: 2079-2087, 2000.
47. Winston JA, Bruggeman LA, Ross MD, et al. : Nephropathy and establishment of a renal reservoir of HIV type 1 during primary infection. *N Engl J Med* 344: 1979-1984, 2001.
48. Marras D, Bruggeman LA, Gao F, et al.: Replication and compartmentalization of HIV-1 in kidney epithelium of patients with HIV-associated nephropathy. *Nat Med* 8: 522-526, 2002.
49. Kopp JB, Klotman ME, Adler SH, et al.: Progressive glomerulosclerosis and enhanced renal accumulation of basement membrane components in mice transgenic for human immunodeficiency virus type 1 genes. *Proc Natl Acad Sci USA* 89: 1577-1581, 1992.
50. Bruggeman LA, Dikman S, Meng C, et al.: Nephropathy in human immunodeficiency virus-1 transgenic mice is due to renal transgene expression. *J Clin Invest* 100: 84-92, 1997.
51. Reid W, Sadowska M, Denaro F, et al.: An HIV-1 transgenic rat that develops HIV-related pathology and immunologic dysfunction. *Proc Natl Acad Sci USA* 98: 9271-9276, 2001.

52. Hanna Z, Weng X, Kay DG, et al.: The pathogenicity of human immunodeficiency virus (HIV) type 1 Nef in CD4C/HIV transgenic mice is abolished by mutation of its SH3-binding domain, and disease development is delayed in the absence of Hck. *J Virol* 75: 9378-9392, 2001.
53. He JC, Husain M, Sunamoto M, et al.: Nef stimulates proliferation of glomerular podocytes through activation of Src-dependent Stat3 and MAPK1,2 pathways. *J Clin Invest* 114: 643-651, 2004.
54. Zhong J, Zuo Y, Ma J et al. Expression of HIV-1 genes in podocytes alone can lead to the full spectrum of HIV-1 associated nephropathy. *Kidney Int* 68: 1048-1060, 2005.
55. Zuo Y, Matsusaka T, Zhong J et al. HIV-1 genes synergistically damage podocytes, leading to glomerulosclerosis. *J Am Soc Nephrol* 17: 2832-2843, 2006.
56. Gherardi D, D'Agati V, Chu TH, et al.: Reversal of collapsing glomerulopathy in mice with the cyclin-dependent kinase inhibitor CYC202. *J Am Soc Nephrol* 15: 1212-1222, 2004.
57. Durvasula RV, Petermann AT, Kiromura K et al. Activation of a local tissue angiotensin system in podocytes by mechanical strain. *Kidney Int* 65: 30-39, 2004.
58. Inagi R, Nangaku M, Onogi H et al. Involvement of endoplasmic reticulum (ER) stress in podocyte injury induced by excessive protein accumulation. *J Am Soc Nephrol* 68: 2639-2650, 2005.
59. Schiffer M, Bitzer M, Roberts ISD et al. Apoptosis in podocytes induced by TGF $\beta$  and Smad7. *J Clin Invest* 108: 807-816, 2001.

## **RENAL FIBROSIS AND PROGRESSIVE RENAL INJURY – WHAT WE CAN LEARN FROM ANIMAL MODELS**

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### **Introduction**

Human chronic kidney disease (CKD) is characterized by progressive azotemia, culminating in uremia, and often accompanied by proteinuria and hypertension. Morphologically, this functional deterioration is due to lesions affecting ultimately all compartments of the kidney, including glomeruli, vessels, tubules and interstitium. In addition to these chronic sclerotic changes, there may also be disease-specific findings that are evident even at advanced stages. A common vicious cycle has been proposed for the usually inexorable progression of human CKD, where remaining nephrons not destroyed by the initial disease process undergo ultimately maladaptive changes, furthering progressive injury. Thus, the varying human conditions resulting in CKD may have many non-specific scarring changes in addition to specific lesions related to the initial disease process. This relentless progression of CKD with loss of GFR is a key characteristic that should be reproduced in experimental animal models, when the goal is to test interventions and their efficacy in altering the course of CKD.

### **Selected Animal Models Relevant to Human Disease**

Other speakers will focus on immune and inflammatory glomerular injury and various injuries of the podocyte and details of diabetic nephropathy. In this review, I will briefly mention some aspects of diabetic nephropathy, and then focus on non-immune progressive disease models that manifest with predominantly tubulointerstitial fibrosis or glomerulosclerosis, including hypertensive and non-hypertensive models.

### **Diabetic Nephropathy**

As will be discussed in detail elsewhere, human diabetic nephropathy is clinically characterized by progression from microalbuminuria to proteinuria and slow progression to ESRD. The key morphological findings, which should be present in a useful animal model of this process, include mesangial matrix expansion, with or without Kimmelstiel-Wilson nodules, GBM thickening, arteriolar hyalinization, and importantly tubulointerstitial fibrosis. These lesions should then result in renal dysfunction that is progressive with accompanying proteinuria to mirror human disease. Multiple models of diabetic nephropathy in rodents have been investigated, most with only minor changes of mild mesangial expansion and occasional sclerosis. These include destruction of pancreatic beta cells by streptozotocin injection to model type 1 diabetes, or genetic strains such as obese Zucker rats or the leptin receptor deficient db/db mice as models of type 2 diabetes. However, these rodent models have been lacking in some of the key characteristics of human diabetic nephropathy, most notably sclerosis, tubulointerstitial fibrosis and progressive uremia, and thus the NIH funded a multi-center consortium, the Animal Models of Diabetic Complications Consortium (AMDCC). The goal of the AMDCC was to create a better mouse model for study of diabetes and its complications.

Briefly, we examined response of multiple mice strains to injected streptozotocin, and found great variability both in hyperglycemic response, microalbuminuria and renal morphology, with most severe lesions being seen in KK/HIJ mice. Of note, C57BL/6J mice, a common background strain for numerous transgenic and knockout mice, were quite resistant to injury. Numerous manipulations of superimposed genetic abnormalities have been investigated in these various strains. Our preliminary data of AT1a  $-/-$  mice show worse diabetic nephropathy than in wild type, and our more recent preliminary data indicate that even endothelial cell-specific knockout of the AT1a receptor results in paradoxically worse injury. Recent data

indicate that superimposing deficiency of endothelial cell nitric oxide synthase (eNOS) on the db/db mice also worsens injury. These mice had arteriolar hyaline, GMB thickening, mesangial expansion and occasional nodules. Importantly, these mice also showed dramatic albuminuria and decreased GFR to less than half of that seen in eNOS (+/+) db/db mice. Thus, these mice provide a robust and useful model of diabetic nephropathy.

### **Tubulointerstitial Fibrosis**

Tubulointerstitial fibrosis is well modeled by inducing unilateral ureteral obstruction, which is effective in all background strains of mice tested and in rats. Although the mode of initial injury does not mirror common causes of tubulointerstitial fibrosis in humans, the model has numerous advantages. These include that the contralateral non-obstructed kidney can serve as an internal control, that animals do not develop hypertension or progressive uremia such that these systemic factors do not obfuscate basic intrarenal mechanisms of progressive fibrosis, and the lack of strain dependence of injury. In addition, the onset of injury is fairly rapid, with early changes of fibrosis occurring within several days, being well established by five days, and quite advanced by 10-14 days. We and others have used this model extensively to examine mechanisms of tubulointerstitial fibrosis in various transgenic mice and knockout mice with added pharmacological intervention. For instance, in one such series of studies, we investigated the dependence of tubulointerstitial injury on TGF $\beta$ , using  $\beta 6^{-/-}$  mice. The heterodimeric integrin  $\alpha_v\beta 6$  is expressed in epithelia in skin, lung and in the kidney. It can cleave TGF $\beta$  from latency-associated peptide, and thus serves as one mechanism for local activation of TGF $\beta$ . Interestingly,  $\beta 6^{-/-}$  mice were completely protected from fibrosis induced by UUO. However, superimposing exposure to angiotensin or aldosterone, commonly associated with renal fibrosis in numerous models, restored fibrosis to these mice. This fibrosis was not associated with activation of TGF $\beta$  by other means, as seen by lack of phosphoSmad 2, but was associated with increased plasminogen activator inhibitor-1 (PAI-1). In other studies, separate manipulation of parenchymal versus bone marrow-derived cells has been done in this and also other models by creating chimeric mice using bone marrow transplant, allowing identification of injury related to each of these cell populations.

### **Glomerulosclerosis**

#### *Radiation Nephropathy*

Multiple models of glomerulosclerosis with progressive uremia exist. The radiation nephropathy model is well established in the rat, but the C57BL/6J mouse strain is resistant. This model mirrors chronic sclerosis that follows initial endothelial/thrombotic microangiopathy injury in humans, such as might occur not only after radiation, but also in chronic HUS. The model is non-hypertensive with gradual development of proteinuria. Morphologically, there is early endothelial injury with late sclerosis developing by week 12.

#### *Puromycin Aminonucleoside and Adriamycin Nephropathies*

Other models of progressive glomerulosclerosis have more in common with human idiopathic FSGS. These models are characterized by nephrotic range proteinuria and progression to ESRD. Most of these models have been developed in rats, with strain resistance in most mice to the selective epithelial cell toxins. Depending upon dosing and route of administration, e.g., i.v. or i.p., varying time courses of injury may be seen after injection with puromycin aminonucleoside (PAN). With single i.v. injection, there is an early abrupt onset of nephrotic syndrome that reaches its peak around day 8-10, with apparent resolution followed by long-term slow development of gradual proteinuria associated with FSGS lesions. Morphologically, there is early foot process effacement, which is maintained as sclerosis develops. Adriamycin causes a similar pattern of injury, but can only be administered

intravenously. Unfortunately, most mice strains are resistant to either of these models. Balb/c mice are, however, susceptible to adriamycin. Thus, the course and initial abrupt onset of nephrotic syndrome mirror well many aspects of human idiopathic FSGS. A major drawback is the lack of widespread susceptibility in most mice background strains.

#### *Remnant Kidney Model*

Other commonly used models of segmental sclerosis mirror well the secondary sclerosis that occurs in many human CKD conditions. These models have progressive, usually non-nephrotic, proteinuria with progression to ESRD. There is development of hypertension, and proteinuria with sclerosis over 8-12 weeks in the widely studied rat remnant kidney model. Several variations of induction of this model exist, including polectomy, cauterization and ligation. The most hypertensive and rapidly progressive injury occurs with ligation of branches of the left kidney, along with right uninephrectomy, to produce a total of 5/6 nephrectomy. Although most rat strains are susceptible to induction of sclerosis by this model, most mice strains are resistant, including C57BL/6J. The 129Sv mice strain is susceptible. Technically, induction of the model may be difficult in the mouse to achieve uniform ablation of renal tissue. We have therefore developed a combination approach to optimize development of hypertension and injury, with ligation associated with cautery of some of the left kidney mass to obtain more uniform ablation of renal tissue. In the susceptible 129Sv mouse early sclerosis is evident by 6-8 weeks, with well-developed and widespread sclerosis with associated tubulointerstitial fibrosis by week 12. Changes are quite similar to those seen in human disease, with accompanying proportional vascular lesions and tubulointerstitial lesions. Of note, in some rat strains, including the Munich-Wistar rat, there are surface glomeruli, which have been widely used for direct puncturing and measurement of pressures and hemodynamic factors that could be involved in glomerulosclerosis.

Although these remnant kidney models in mice and rats do mirror many aspects of human disease, it is of interest that effects on sclerosis in the rat in our experiments have not correlated directly to the effects on proteinuria. In contrast, in the mouse, effects on sclerosis did correlate with effects on proteinuria. Whether these observations mirror the relatively short period of follow-up after intervention, with longer time required to normalize proteinuria and restore podocyte foot processes, is under investigation.

#### **Podocyte-Specific Injury and Sclerosis**

Specific injuries related to the podocyte will be discussed in detail elsewhere in this symposium. I will here briefly discuss specific use of mice models where the injury is very specifically directed only to the podocyte. In studies led by Ichikawa, we created mice transgenic for a toxin receptor only on the podocyte. When mice were injected with exogenous toxin, only cells expressing this receptor, i.e., only the podocytes were injured. This model, depending upon dose of toxin used, results in rapid proteinuria and development of sclerosis with dedifferentiation of the podocytes. Sclerosis developed by five weeks after injection of toxin. A similar model developed by the group of Wiggins et al. in the rat has shown the importance of podocyte depletion in inducing progressive sclerosis. Of interest, this concept has also been used to create chimeric mice where only some podocytes express the hCD25 receptor. These chimeric mice developed equally severe injury as mice with all podocytes expressing the receptor, supporting that injury may spread from podocyte to podocyte within the glomerulus. As there subsequently also is injury to the mesangial cell and endothelial cells, this model also allows study of mechanism of transmission of injury from the initially injured cell to other glomerular cells.

## **HIV-Associated Nephropathy (HIVAN)**

Finally, excellent animal models exist for human HIVAN. The characteristic morphological findings of collapsing lesions of the glomerular tuft with overlying podocyte proliferation, due to dedifferentiated podocytes and foot process effacement with accompanying tubulointerstitial fibrosis, inflammation and tubular injury are well-mirrored in several models of HIV nephropathy where mice have been made transgenic for HIV structural genes, either in all cells, or specifically in the podocytes. Of interest, injury in response to HIV transgenic expression is also highly strain dependent, perhaps mirroring the difference in various human ethnic groups to HIVAN as a consequence of HIV infection. Specific mice transgenic for only single HIV structural genes have further shed light on which component of the HIV gene is necessary and sufficient to cause the HIVAN.

## **Summary**

In summary, although many animal models exist, a rodent model that faithfully reproduces key elements of the most frequent and compelling cause of CKD in humans, namely diabetic nephropathy, is still lacking. Excellent models exist for short-term study of mechanisms of tubulointerstitial fibrosis. The varying models of primary or secondary FSGS vary in their fidelity to capture properties of human disease. Importantly and increasingly recognized is the strain dependence of severity of injury, making appropriate key controls essential for interpretation of data and extrapolation to human conditions.

## **Selected reading**

### **Diabetic nephropathy models:**

Breyer MD, Bottinger E, Brosius FC, Coffman TM, Fogo A, Harris RC, Heilig CW, Sharma K: Diabetic nephropathy: Of mice and men. *Adv Chronic Kidney Dis* 12:128-145, 2005

Qi Z, Fujita H, Jin J, Davis LS, Wang Y, Fogo AB, Breyer MD: Characterization of susceptibility of inbred mouse strains to diabetic nephropathy. *Diabetes* 54:2628-2637, 2005

Zhao HJ, Wang S, Cheng H, Zhang MZ, Takahashi T, Fogo AB, Breyer MD, Harris RC: Endothelial nitric oxide synthase deficiency produces accelerated nephropathy in diabetic mice. *J Am Soc Nephrol* 17:2664-9, 2006

### **Tubulointerstitial fibrosis models (UUO):**

Klahr S, Morrissey J: Obstructive nephropathy and renal fibrosis. *Am J Physiol Renal Physiol* 283:F861-75, 2002

Ma LJ, Yang H, Gaspert A, Carlesso G, Barty MM, Davidson JM, Sheppard D, Fogo AB: Transforming growth factor-beta-dependent and -independent pathways of induction of tubulointerstitial fibrosis in beta6(-/-) mice. *Am J Pathol* 163:1261-1273, 2003

Nishida M, Fujinaka H, Matsusaka T, Price J, Kon V, Fogo AB, Davidson JM, Linton MF, Fazio S, Homma T, Yoshida H, Ichikawa I: Absence of angiotensin II type 1 receptor in bone marrow-derived cells is detrimental in the evolution of renal fibrosis. *J Clin Invest* 110:1859-1868, 2002

### **FSGS models:**

Fogo AB: Animal models of FSGS: Lessons for pathogenesis and treatment. *Semin Nephrol* 23:161-171, 2003

#### **Radiation nephropathy:**

Cohen EP, Robbins ME: Radiation nephropathy. *Semin Nephrol* 23:486-99, 2003

#### **Remnant kidney models:**

##### **Rats:**

Shimamura T, Morrison AB: A progressive glomerulosclerosis occurring in partial five-sixths nephrectomized rats. *Am J Pathol* 79:95-106, 1975

Anderson S, Rennke HG, Brenner BM: Therapeutic advantage of converting enzyme inhibitors in arresting progressive renal disease associated with systemic hypertension in the rat. *J Clin Invest* 77:1993-2000, 1986

Gretz N, Waldherr R, Strauch M: The remnant kidney model. In: *Experimental and Genetic Rat Models of Chronic Renal Failure*. Gretz N, Strauch M (eds), Basel, Karger, pp1-28, 1993 (variants of 5/6 Nx described)

Fogo A, Yoshida Y, Glick AD, Homma T, Ichikawa, I: Serial micropuncture analysis of glomerular function in two rat models of glomerular sclerosis. *J Clin Invest* 82:322-330, 1988

Adamczak M, Gross ML, Krttil J, Koch A, Tyralla K, Amann K, Ritz E: Reversal of glomerulosclerosis after high-dose enalapril treatment in subtotaly nephrectomized rats. *J Am Soc Nephrol* 14:2833-42, 2003 (model induced by polectomy)

Ma L-J, Nakamura S, Aldigier JC, Rossini M, Yang H, Liang X, Nakamura I, Marcantoni C, Fogo AB: Regression of glomerulosclerosis with high dose angiotensin inhibition is linked to decreased plasminogen activator inhibitor-1. *J Am Soc Nephrol* 16:966-976, 2005 (model induced by ligation)

**Mice:**

Ma LJ, Fogo AB: Model of robust induction of glomerulosclerosis in mice: importance of genetic background. *Kidney Int* 64:350-355, 2003

**PAN/ADM models:**

**Rats:**

Bertani T, Poggi A, Pozzoni R, Delaini F, Sacchi G, Thoua Y, Mecca G, Remuzzi G, Donati MB: Adriamycin-induced nephrotic syndrome in rats: sequence of pathologic events. *Lab Invest* 46:16-23, 1982

Grond J, Weening JJ, Elema JD: Glomerular sclerosis in nephrotic rats. Comparison of the long-term effects of adriamycin and aminonucleoside. *Lab Invest* 51:277-85, 1984

Ma LJ, Marcantoni C, Linton MF, Fazio S, Fogo AB: Peroxisome proliferator-activated receptor-gamma agonist troglitazone protects against nondiabetic glomerulosclerosis in rats. *Kidney Int* 59:1899-1910, 2001

Yang HC, Ma LJ, Ma J, Fogo AB: Peroxisome proliferator-activated receptor-gamma agonist is protective in podocyte injury-associated sclerosis. *Kidney Int* 69:1756-1764, 2006

**Mice:**

Chen X, Moeckel G, Morrow JD, Cosgrove D, Harris RC, Fogo AB, Zent R, Pozzi A: Lack of integrin alpha1beta1 leads to severe glomerulosclerosis after glomerular injury. *Am J Pathol* 165:617-30, 2004

Wang Y, Wang YP, Tay YC, Harris DC. Progressive adriamycin nephropathy in mice: sequence of histologic and immunohistochemical events. *Kidney Int* 58:1797-804, 2000

**Podocyte-specific injury models:**

Wharram BL, Goyal M, Wiggins JE, Sanden SK, Hussain S, Filipiak WE, Saunders TL, Dysko RC, Kohno K, Holzman LB, Wiggins RC: Podocyte depletion causes glomerulosclerosis: diphtheria toxin-induced podocyte depletion in rats expressing human diphtheria toxin receptor transgene. *J Am Soc Nephrol* 16:2941-52, 2005

Matsusaka T, Xin J, Niwa S, Kobayashi K, Akatsuka A, Hashizume H, Wang QC, Pastan I, Fogo AB, Ichikawa I: Genetic engineering of glomerular sclerosis in the mouse via control of onset and severity of podocyte-specific injury. *J Am Soc Nephrol* 16:1013-1023, 2005

Asano T, Niimura F, Pastan I, Fogo AB, Ichikawa I, Matsusaka T: Permanent genetic tagging of podocytes: fate of injured podocytes in a mouse model of glomerular sclerosis. *J Am Soc Nephrol* 16:2257-2262, 2005

**HIVAN models:**

Bruggeman LA, Dikman S, Meng C, Quaggin SE, Coffman TM, Klotman PE: Nephropathy in human immunodeficiency virus-1 transgenic mice is due to renal transgene expression. *J Clin Invest* 100:84-92, 1997

Zhong J, Zuo Y, Ma J, Fogo AB, Jolicœur P, Ichikawa I, Matsusaka T: Expression of HIV-1 genes in podocytes alone can lead to the full spectrum of HIV-1-associated nephropathy. *Kidney Int* 68:1048-1060, 2005

Zuo Y, Matsusaka T, Zhong J, Ma J, Ma LJ, Hanna Z, Jolicœur P, Fogo AB, Ichikawa I. HIV-1 genes vpr and nef synergistically damage podocytes, leading to glomerulosclerosis. *J Am Soc Nephrol* 17:2832-43, 2006

I will discuss the following models which I think are most useful in understanding human glomerular disease:

### **Nephrotoxic nephritis**

This model is induced by the injection of a heterologous antibody raised against a preparation of glomerular antigen. Classically there is acute injury produced by binding of the heterologous antibody to the glomerular basement membrane followed by a second phase of injury in which there is an autologous immune response to the heterologous antibody which then acts as a planted antigen. This second phase can be accelerated by pre-immunising the animals with heterologous immunoglobulin.

Typically the autologous phase is characterised by proliferative glomerulonephritis with crescent formation in susceptible animals. The heterologous phase closely resembles human anti-GBM disease but the autologous phase has more in common with an immune complex glomerulonephritis with the heterologous antibody acting as a planted antigen. Proliferative immune complex glomerulonephritis can also be produced by antigens planted by other mechanisms, for example, on account of their charge [3]

### **Heymann nephritis**

This is a model of membranous glomerulonephritis originally induced in rats by immunisation with a suspension of renal cortex and adjuvant. Subsequently a passive form of the model was induced by the injection of heterologous antibody raised against tubular brush border antigens. It is now known that the model depends on a cross reaction of these antibodies with antigens including megalin on podocyte foot processes.

### **Models of lupus nephritis**

There are several models of SLE in mice which are characterised by the generation of autoantibodies and the development of glomerulonephritis with immune complex deposition. In my opinion the model that most closely resembles human disease is that produced by crossing NZB and NZW mice but other models include the MRL/lpr mouse and the BXSB mouse. These models have been widely studied and have provided insights into genetic susceptibility to SLE, mechanisms by which tolerance is broken and have been used for elucidating the role of mediators of glomerular injury both by examining gene targeted animals and by administration of pharmacological agents.

### **Models of ANCA-mediated glomerulonephritis**

A major step forward in our ability to model human glomerular disease was the demonstration by Jennette and co-workers that transfer to normal mice of splenocytes or serum from myeloperoxidase (MPO) deficient mice immunized with MPO led to a pauci-immune necrotising glomerulonephritis [4]. This was the first demonstration that anti-MPO antibodies were pathogenic and has led to further insights into the mechanisms of injury in ANCA-mediated glomerular inflammation.

### **Dense deposit disease and thrombotic microangiopathy**

Pigs with a spontaneous deficiency of factor H and mice in which the factor H gene has been knocked out develop persistent activation of the alternative pathway of complement activation and a glomerulonephritis resembling human dense deposit disease [5]. If the Factor H deficient mice are then made transgenic for a truncated factor H protein they develop spontaneous thrombotic microangiopathy (Pickering et al submitted)

The following is a very selective discussion of what I consider the major insights that these models have given us into pathological mechanisms;

### **Neutrophils and macrophages**

It is clear that these cells play a major role in causing inflammatory glomerular damage leading to proteinuria and also to capillary wall rupture with crescent formation. Neutrophils are the key cell in heterologous nephrotoxic nephritis and macrophages are of central importance in the autologous phase. Macrophage depletion greatly attenuates injury in these models [6;7]. In anti-MPO induced glomerulonephritis in mice neutrophil depletion ameliorates disease [8] and the anti-MPO response has been shown to be directed against MPO on neutrophils and macrophages [9]. Macrophages in proliferative glomerulonephritis have an activated phenotype [3] and there is interesting work suggesting that they become programmed when they enter the glomerulus [10]. It is likely that the balance between different activation states in macrophages may determine whether there is progression or resolution of glomerular injury. Understanding the signals that attract and activate macrophages is of major importance.

### **Fc receptors**

Fc receptors on circulating leukocytes play a central role in the induction of glomerular injury in models of nephrotoxic nephritis [11;12] and lupus nephritis [13]. The class of immunoglobulin deposited in glomeruli affects which Fc receptors are activated and determines severity of inflammation [14] and interaction between activatory and inhibitory Fc receptors also influences the inflammatory response [15].

### **Complement**

Perhaps surprisingly, complement activation does not appear to play a major role in immune complex mediated crescentic nephritis nor in lupus nephritis [16] although there is evidence that this is in part because of the opposing effects of the classical pathway in facilitating removal of immune complexes and the alternative pathway in

promoting inflammation. Complement is of central importance in the Heymann nephritis model of membranous gn and the alternative pathway has been shown to be critical in a mouse model of anti-MPO glomerulonephritis [17]. Alternative pathway activation has also been shown to be central in mouse models of dense deposit disease [18] and HUS. Antibodies directed at C5 have led to amelioration in models of lupus nephritis and dense deposit disease.

### **T cells**

There is good evidence that as well as being important in controlling antibody synthesis T cells may have a direct effector role in glomerular injury in nephrotoxic nephritis in both mouse [19] and rat [20]. It is a challenge to define how important this mechanism may be in humans.

### **Genetics**

Animal models have provided insights into genetic susceptibility to glomerular disease in models of lupus nephritis in the mouse [21] and in a model of crescentic glomerulonephritis in the rat [22]. In the latter model susceptibility was associated with a variation in copy number of the *Fcgr3* gene and we then showed that there was also variation in copy number of the human *FCGR3B* gene that was associated with susceptibility to lupus nephritis.

This represents a very selective look at the insights that animal models have given into pathogenesis of glomerulonephritis and I believe that the examples I have chosen are likely to be of strong relevance to human disease. It is reasonable, therefore, to ask whether these insights into pathogenesis have led to advances in therapy for human glomerulonephritis. Disappointingly, I think there is very little in terms of treatment that has followed from work on these models. One exception is in the area of

complement inhibition. However, I think it is possible to speculate on where advances might come in future. I think promising areas for development of treatment include: inhibition of Fc receptor activation, modulation of macrophage activation, inhibition of complement, particularly the alternative pathway and C5 activation; inhibition of chemokines. It is also important to consider ways of targeting therapy specifically to the inflamed glomerulus and interesting strategies include the use of genetically modified macrophages [23] or of cytokines which are designed only to be activated at sites of inflammation [24]

It is inevitable that there will be differences between the way experimental animals respond to glomerular injury and the way humans do that may lead to misleading conclusions from animal experiments and, of course, this is one of the arguments that those opposed to animal experimentation rely on. However, there is one specific example of how animal experiments may be misleading that I would like to explore and this is not due to differences between species but to a failure to use appropriate controls. In 1999 we published data on a mouse in which serum amyloid P component (SAP) had been genetically deleted and which developed autoimmunity [25]. We concluded that SAP protected against autoimmunity and this has been widely quoted. However, the knock out had been created using embryonic stem cells derived from the 129 strain of mouse which were then transferred to C57BL/6 mice. This means that even when the knock out mice were subsequently back crossed to the C57BL/6 strain the genetic material around the SAP locus on chromosome 1 was of 129 origin and we subsequently showed that producing a congenic mouse with a 129 segment of chromosome 1 on a C57BL/6 background led to similar autoimmunity even when SAP was present [26]. This shows the need for tremendous caution when interpreting results with genetically manipulated animals.

In conclusion, there are a number of reproducible animal models of glomerulonephritis which, I believe, have led to insights into pathogenesis that are highly likely to be of relevance to human disease. However, it is disappointing that there is very little to show for all the work that has been done on these models in terms of rational development of new therapies.

#### Reference List

1. Lindemann W: Sur le mode d'action de certains poisons renaux. *Ann.Inst.Pasteur* 14:49-51, 1900
2. Kennedy D: Animal activism: out of control. *Science* 313:1541, 2006
3. Cook HT, Smith J, Salmon JA, Cattell V: Functional characteristics of macrophages in glomerulonephritis in the rat: O<sub>2</sub><sup>-</sup> generation, MHC class II expression and eicosanoid synthesis. *Am.J.Pathol.* 134:431-437, 1989
4. Xiao H, Heeringa P, Hu P, Liu Z, Zhao M, Aratani Y, Maeda N, Falk RJ, Jennette JC: Antineutrophil cytoplasmic autoantibodies specific for myeloperoxidase cause glomerulonephritis and vasculitis in mice. *J.Clin.Invest* 110:955-963, 2002
5. Pickering MC, Cook HT, Warren J, Bygrave AE, Moss J, Walport MJ, Botto M: Uncontrolled C3 activation causes membranoproliferative glomerulonephritis in mice deficient in complement factor H. *Nat.Genet.* 31:424-428, 2002
6. Iseme M, Fujinaka H, Adhikary LP, Kovalenko P, El Shemi AG, Yoshida Y, Yaoita E, Takeishi T, Takeya M, Naito M, Suzuki H, Yamamoto T: Important role for macrophages in induction of crescentic anti-GBM glomerulonephritis in WKY rats. *Nephrol.Dial.Transplant.* 19:2997-3004, 2004
7. Duffield JS, Tipping PG, Kipari T, Cailhier JF, Clay S, Lang R, Bonventre JV, Hughes J: Conditional ablation of macrophages halts progression of crescentic glomerulonephritis. *Am.J.Pathol.* 167:1207-1219, 2005
8. Xiao H, Heeringa P, Liu Z, Huugen D, Hu P, Maeda N, Falk RJ, Jennette JC: The role of neutrophils in the induction of glomerulonephritis by anti-myeloperoxidase antibodies. *Am.J.Pathol.* 167:39-45, 2005
9. Schreiber A, Xiao H, Falk RJ, Jennette JC: Bone marrow-derived cells are sufficient and necessary targets to mediate glomerulonephritis and vasculitis

- induced by anti-myeloperoxidase antibodies. *J.Am.Soc.Nephrol.* 17:3355-3364, 2006
10. Kluth DC, Erwig LP, Rees AJ: Multiple facets of macrophages in renal injury. *Kidney Int.* 66:542-557, 2004
  11. Tarzi RM, Davies KA, Robson MG, Fossati-Jimack L, Saito T, Walport MJ, Cook HT: Nephrotoxic nephritis is mediated by Fc $\gamma$  receptors on circulating leukocytes and not intrinsic renal cells. *Kidney Int.* 62:2087-2096, 2002
  12. Tarzi RM, Davies KA, Claassens JW, Verbeek JS, Walport MJ, Cook HT: Both Fc $\gamma$  receptor I and Fc $\gamma$  receptor III mediate disease in accelerated nephrotoxic nephritis. *Am.J.Pathol.* 162:1677-1683, 2003
  13. Bergtold A, Gavhane A, D'Agati V, Madaio M, Clynes R: FcR-bearing myeloid cells are responsible for triggering murine lupus nephritis. *J.Immunol.* 177:7287-7295, 2006
  14. Kaneko Y, Nimmerjahn F, Madaio MP, Ravetch JV: Pathology and protection in nephrotoxic nephritis is determined by selective engagement of specific Fc receptors. *J.Exp.Med.* 203:789-797, 2006
  15. Tarzi RM, Cook HT: Role of Fc $\gamma$  receptors in glomerulonephritis. *Nephron Exp.Nephrol.* 95:e7-12, 2003
  16. Turnberg D, Cook HT: Complement and glomerulonephritis: new insights. *Curr.Opin.Nephrol.Hypertens.* 14:223-228, 2005
  17. Xiao H, Schreiber A, Heeringa P, Falk RJ, Jennette JC: Alternative complement pathway in the pathogenesis of disease mediated by anti-neutrophil cytoplasmic autoantibodies. *Am.J.Pathol.* 170:52-64, 2007
  18. Pickering MC, Cook HT, Warren J, Bygrave AE, Moss J, Walport MJ, Botto M: Uncontrolled C3 activation causes membranoproliferative glomerulonephritis in mice deficient in complement factor H. *Nature Genet.* 4:424-428, 2002
  19. Tipping PG, Holdsworth SR: T cells in crescentic glomerulonephritis. *J.Am.Soc.Nephrol.* 17:1253-1263, 2006
  20. Robertson J, Wu J, Arends J, Zhou C, McMahon J, Torres L, Lou YH: Activation of glomerular basement membrane-specific B cells in the renal draining lymph node after T cell-mediated glomerular injury. *J.Am.Soc.Nephrol.* 16:3256-3263, 2005
  21. Fairhurst AM, Wandstrat AE, Wakeland EK: Systemic lupus erythematosus: multiple immunological phenotypes in a complex genetic disease. *Adv.Immunol.* 92:1-69, 2006
  22. Aitman TJ, Dong R, Vyse TJ, Norsworthy PJ, Johnson MD, Smith J, Mangion J, Robertson-Lowe C, Marshall AJ, Petretto E, Hodges MD, Bhangal G, Patel SG, Sheehan-Rooney K, Duda M, Cook PR, Evans DJ, Domin J, Flint J, Boyle JJ,

Pusey CD, Cook HT: Copy number polymorphism in *Fcgr3* predisposes to glomerulonephritis in rats and humans. *Nature* 439:851-855, 2006

23. Wilson HM, Stewart KN, Brown PA, Anegon I, Chettibi S, Rees AJ, Kluth DC: Bone-marrow-derived macrophages genetically modified to produce IL-10 reduce injury in experimental glomerulonephritis. *Mol. Ther.* 6:710-717, 2002
24. Adams G, Vessillier S, Dreja H, Chernajovsky Y: Targeting cytokines to inflammation sites. *Nat. Biotechnol.* 21:1314-1320, 2003
25. Bickerstaff MC, Botto M, Hutchinson WL, Herbert J, Tennent GA, Bybee A, Mitchell DA, Cook HT, Butler PJ, Walport MJ, Pepys MB: Serum amyloid P component controls chromatin degradation and prevents antinuclear autoimmunity. *Nat. Med.* 5:694-697, 1999
26. Bygrave AE, Rose KL, Cortes-Hernandez J, Warren J, Rigby RJ, Cook HT, Walport MJ, Vyse TJ, Botto M: Spontaneous autoimmunity in 129 and C57BL/6 mice-implications for autoimmunity described in gene-targeted mice. *PLoS. Biol.* 2:E243, 2004