Inducible rodent models of acquired podocyte diseases

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Pippin JW, Brinkkoetter PT, Cormack-Aboud FC, Durvasula RV, Hauser PV, Kowalewska J, Krofft RD, Logar CM, Marshall CB, Ohse T, Shankland SJ. Inducible rodent models of acquired podocyte diseases. Am J Physiol Renal Physiol 296: F213–F229, 2009. First published September 10, 2008; doi:10.1152/ajprenal.90421.2008.—Glomerular diseases remain the leading cause of chronic and end-stage kidney disease. Significant advances in our understanding of human glomerular diseases have been enabled by the development and better characterization of animal models. Diseases of the glomerular epithelial cells (podocytes) account for the majority of proteinuric diseases. Rodents have been extensively used experimentally to better define mechanisms of disease induction and progression, as well as to identify potential targets and therapies. The development of podocyte-specific genetically modified mice has energized the research field to better understand which animal models are appropriate to study acquired podocyte diseases. In this review we discuss inducible experimental models of acquired nondiabetic podocyte diseases in rodents, namely, passive Heymann nephritis, puromycin aminonucleoside nephrosis, adriamycin nephrosis, liopolysaccharide, crescentic glomerulonephritis, and protein overload nephropathy models. Details are given on the model backgrounds, how to induce each model, the interpretations of the data, and the benefits and shortcomings of each. Genetic rodent models of podocyte injury are excluded.

glomerulus; animal models

HISTOLOGICAL ANALYSIS is a cornerstone for studying mechanisms of glomerular disease. However, analysis in human disease is limited by a relative paucity of tissue availability. Renal biopsies are only pursued if a presumptive diagnosis cannot be established on clinical grounds. Tissue sampling is typically restricted to the time of disease presentation and is rarely performed in follow-up, providing a mere snapshot of disease course.

Animal models have significantly advanced our understanding of the pathogenesis of glomerular disease by overcoming these hurdles. Serial assessment of renal tissue in experimental models affords the opportunity to study development and progression of disease over time. Furthermore, the host response to injury may be deliberately modified, for example, through pharmacological intervention, selective disruption (knockout strategies), or overexpression (transgenic strategies) of a particular gene, allowing for mechanistic evaluations. A variety of animal species have been employed in the study of glomerular disease, but rodent models are preferred due to lower cost, maintenance requirements, and short gestational periods. Although both rats and mice are utilized, there are some important advantages and disadvantages for each (Table 1). The development of transgenic technology has proven an invaluable tool in elucidating the function of individual genes in health and disease. However, they cannot replace experimental models in furthering our understanding of the mechanisms of glomerular disease.

Research into mechanisms of glomerular disease in recent years has sparked a renewed interest in the biology of podocytes. Terminally differentiated epithelial cells lining the outer aspect of the glomerular basement membrane (GBM), podocytes are indispensable to preserving glomerular architecture and function. Dating back to the isoporous zipperlike model proposed by Karnovsky and colleagues (78, 149) almost 40 years ago, the slit diaphragm bridging the space between neighboring foot processes is widely considered the anatomic basis of the size selectivity of the glomerular filtration barrier. However, beyond a mere structural role as a macromolecular sieve, mounting evidence has established the slit diaphragm as a dynamic signal transduction complex mediating various downstream cellular responses including regulation of prosurvival pathways, cell polarity, actin remodeling, endocytosis, and transcriptional activation (54, 73, 97, 103).

Podocytes are vulnerable to many forms of injury. Immune-mediated processes include immune complex deposition with subsequent complement activation, as seen in membranous nephropathy (125), as well as cellular mechanisms characteristic of minimal change disease (MCD) or focal segmental glomerulosclerosis (FSGS) (109). Nonimmune injury includes hemodynamic insults (systemic hypertension, states of reduced nephron mass) and metabolic...
(diabetes mellitus), infectious (human immunodeficiency virus), toxin-mediated (puromycin, adriamycin), and genetic mutations essential to slit diaphragm assembly (nephrin, CD2AP, α-actinin 4, TRPC6, PLC-1, podocin). Despite this diverse range of inciting triggers, the cellular responses typically include rearrangement of the complex actin cytoskeleton, subsequent spreading of the foot processes along the GBM (this shape change is called podocyte effacement), loss of filtration slits, and apical redistribution of slit diaphragm proteins. These changes may be reversible. In some instances, podocytes undergo apoptosis (programmed cell death), whereas in others, they detach from the underlying GBM. Because of its limited proliferative capacity in vivo, a progressive reduction in podocyte number from apoptosis and/or detachment fuels the development of glomerulosclerosis, the pathological basis of chronic renal failure (155).

The hope that a greater understanding of podocyte biology in health and disease may provide novel therapeutics to treat glomerular disease clinically has fueled interest in podocyte research. Studies of conditionally immortalized podocyte lines in culture, which mimic the quiescent phenotype of podocytes in vivo, have proved invaluable (154). Ultimately, however, the biologic relevance of findings generated from cell culture studies must be verified in vivo, through the use of experimental models of glomerular disease.

Accordingly, the purpose of this review is to highlight rodent models where podocytes are the primary cell undergoing injury such as occurs in lupus nephritis, antineutrophilic cytoplasmic antibody (ANCA)-associated glomerulonephritis, and IgA nephropathy. In these diseases, glomerular injury is typically “primarily” to endothelial cells, parietal epithelial cells, GBM, and mesangial cells, and podocytes then respond to that injury by undergoing changes such as effacement. We fully recognize that diseases of the GBM and glomerular endothelial cells also may lead to proteinuria and glomerulosclerosis, but these are beyond the scope of this review.

Defining Terminology Related to Animal Models

Before reviewing rodent models of podocyte injury, it is important to define the terminology used. Similar to human diseases, the initial/primary injury to podocytes in experimental models occurs during the disease induction phase. This can be immune mediated, which occurs when the primary injury is caused by immune complex formation, usually initiated by an antibody to renal antigens rather than by an agent or drug. Non-immune-mediated models involve injury that is initiated typically by a toxin or drug.

An active immune model utilizes the production of antibodies to renal antigen(s) by the animal’s own immune system by sensitizing the animal to renal antigen(s) to elicit immune complex formation within the glomerulus. To stimulate a potent and prolonged immune response, the antigen is usually combined with an adjuvant. Freund’s adjuvant is most typically used as a nonspecific immune system stimulant. There are two types of Freund’s adjuvant, complete and incomplete, defined by the respective presence or absence of killed Mycobacterium tuberculosis. Although complete Freund’s invokes a

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Table 1. Advantages and disadvantages of rats and mice for inducible, nondiabetic models of podocyte disease

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
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<tbody>
<tr>
<td>Larger kidneys available for histology, mRNA, and protein isolation.</td>
<td>Limited genetic manipulation available.</td>
</tr>
<tr>
<td>Podocyte-specific cre-mice available.</td>
<td>Many models are strain dependent.</td>
</tr>
<tr>
<td>Genetic manipulation technology well developed and commonly used (knockouts, transgenics).</td>
<td>Larger quantity of agents required to induce and/or treat disease.</td>
</tr>
<tr>
<td>Microarray platforms readily available.</td>
<td>Many models are strain dependent.</td>
</tr>
<tr>
<td>Podocyte-specific cre-mice available (podocin- or nephrin-driven cre recombinase).</td>
<td>Limited glomerular complement activation.</td>
</tr>
<tr>
<td>Use of larger animals means surgical procedures are easier.</td>
<td>Current number of models of podocyte injury is limited.</td>
</tr>
<tr>
<td>Short reproduction period.</td>
<td>Glomerular isolation free from tubular fragments requires perfusion with magnetic beads, and protein/RNA yields are lower.</td>
</tr>
<tr>
<td>Cheaper to purchase and maintain.</td>
<td>Use of mouse monoclonal antibodies increases background immunostaining.</td>
</tr>
<tr>
<td>Many different strains and genetically modified mice commercially available.</td>
<td>Requires more expertise for surgical interventions and injections due to small kidney size.</td>
</tr>
<tr>
<td></td>
<td>Requires expertise for intravenous injections due to smaller veins.</td>
</tr>
<tr>
<td></td>
<td>Urine collection volumes smaller and more evaporation occurs during collection.</td>
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more robust immune response, it leads to formation of granulomas, so it is only used for the primary immunization, whereas incomplete Freund’s is used for subsequent immunizations.

A passive model of immunization utilizes antiserum to an antigen(s) generated in another animal (often another species, including rabbits, goats, or sheep), which are then injected into a rat or mouse to elicit immune complex formation. The initial 5- to 7-day period of a passive model is referred to as the heterologous, phase, because it relies on an immune response to the antibody injected from a foreign or heterologous source. Subsequently, the rat or mouse mounts its own immune response to the deposited heterologous antibody and thereby enters the autologous phase of disease.

After disease induction, the course of podocyte injury can be 1) repair with complete healing and no disease progression, 2) lack of repair and continued podocyte injury and loss, fueling chronic proteinuria and/or glomerulosclerosis, or 3) secondary podocyte injury with eventual repair and healing, leading to disease resolution. Thus, when determining which podocyte model to use from an experimental standpoint, one must first establish what is the specific hypothesis being tested so that the appropriate animal model can be utilized.

The rodent models discussed in this review are either directly analogous to specific human disease entities or have no direct analogy to a particular human disease but exhibit specific responses of the podocyte to injury (Table 2). We have organized this review around these distinctions.

**Rodent Models of Membranous Nephropathy**

Membranous nephropathy (MN) is a leading cause of nephrotic syndrome in adults. The major cause is “idiopathic,” although efforts are underway to delineate this better. One also has to consider secondary causes in adults, which is usually due to a preformed antigen-antibody complex such as occurs in certain solid tumors. The models described below are more germane to human idiopathic MN.

**Rat Model of Passive Heymann Nephritis**

Background of the rat model of passive Heymann nephritis. The Heymann model of MN is one of the most widely studied models of podocyte injury. Heymann et al. (70) first described the injection of kidney extracts with Freund’s adjuvant to induce nephritic syndrome in 1959. In subsequent studies, an insoluble subfraction from rat proximal tubule brush borders termed fraction 1A (Fx1A) was isolated and utilized to produce antibodies for a passive heterologous model termed passive Heymann nephritis (PHN) (48, 47, 55). Although both active and passive models exist, the active model is not as widely utilized, because it is more difficult to induce, is more variable, and has a longer disease course.

The antigenic targets of Fx1A antibody in rats were identified to be a 600-kDa membrane-bound glycoprotein called megalin (81, 84, 105) and a 45-kDa protein called receptor-associated protein (RAP) (53). Cunningham et al. (32) demonstrated that when monoclonal antibody to megalin alone was injected, it was not capable of inducing proteinuria without inhibiting complement regulatory proteins CR1-related gene y protein product (Cry) and protectin (CD59). Although antibodies to megalin alone and RAP alone do not initially induce PHN, megalin antibodies can eventually cause proteinuria, whereas RAP antibodies do not (106). The antigen(s) causing MN in humans has proved elusive. Some clues have emerged from recent studies demonstrating neutral endopeptidase as an antigen in neonatal cases of MN (39, 143). Meanwhile, studies by Beck et al. (10) to identify candidate antigens in human MN are ongoing. Since its establishment, the Heymann model has been used to study mechanisms of cellular injury as well as the podocyte’s responses to injury (125).

**Disease induction of the rat model of PHN.** Because PHN has been so widely studied over the years, a variety of methods for inducing disease have been utilized. Various strains such as Sprague-Dawley, Wistar, Munich Wistar, Lewis, and Piebald Viral Glaxo have been utilized. Generally, male rats are used, but female rats also have been used, including pregnant females (50). Male (200–300 g) and female (100–200 g) rats typically receive a single dose of heterologous Fx1A antibody produced in rabbits or sheep. Uninephrectomy (11, 12), as well as multiple injections followed by sensitization with rabbit IgG (119), have been utilized to produce accelerated models of PHN. Antibody is administered either intravenously through the tail vein or intraperitoneally. Doses range from 2–7 ml/kg when serum is administered to 20–240 mg/kg when isolated

### Table 2. Acquired rodent models of nondiabetic podocyte injury classified by the relevant human disease entity

<table>
<thead>
<tr>
<th>Human Disease</th>
<th>Rat Models</th>
<th>Mouse Models</th>
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</thead>
<tbody>
<tr>
<td>FSGS:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Classic variant</td>
<td>Puromycin aminonucleoside nephrosis (high or repeated doses)</td>
<td>Adriamycin nephrosis</td>
</tr>
<tr>
<td></td>
<td>Adriamycin nephrosis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Remnant kidney</td>
<td></td>
</tr>
<tr>
<td>FSGS:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collapsing variant</td>
<td>Not available</td>
<td>HIV-associated nephropathy</td>
</tr>
<tr>
<td>Membranous nephropathy</td>
<td>Passive Heymann nephritis</td>
<td>Cationic BSA</td>
</tr>
<tr>
<td>Minimal change disease</td>
<td>Active Heymann nephritis</td>
<td></td>
</tr>
<tr>
<td>Crescentsc glomerulonephritis</td>
<td>Puromycin aminonucleoside nephrosis (low dose)</td>
<td>Not available</td>
</tr>
<tr>
<td>No specific human disease, but rather a generalized response of podocytes to injury in clinical disease:</td>
<td>Not available</td>
<td>Anti-GBM nephritis</td>
</tr>
<tr>
<td>Foot process effacement</td>
<td>LPS</td>
<td></td>
</tr>
<tr>
<td>Slit diaphragm protein abnormalities</td>
<td>Protein overload Protamine sulfate</td>
<td>LPS Protamine sulfate</td>
</tr>
</tbody>
</table>

FSGS, focal segmental glomerulosclerosis; HIV, human immunodeficiency virus.
IgG is administered and are determined empirically for each batch of antibody (24–26, 28–38). Controls consist of saline or PBS, preimmune serum, or preimmune IgG from sheep or rabbits before Fx1A immunizations, as well as serum or IgG from nonimmunized rabbits or sheep (summarized in Table 3). However, an important control is to coadminister cobra venom factor (CVF), which depletes C3 and C5 fragments, thereby decreasing terminal membrane attack complex formation.

Expected outcomes of the rat model of PHN. PHN is a progressive model of glomerular injury. After injection of anti-Fx1A antibody, circulating gp330 antibodies bind to megalin expressed in the clathrin-coated pits along the sides and bases of podocyte foot processes. The antibody is capped and shed into the subepithelial space, where it deposits in the lamina rara externa of the GBM. Immune deposits continue to accumulate in this fashion until they obscure the slit diaphragm (53). Sublethal complement activation occurs on affected podocytes, causing damage and intracellular signaling activation (162, 35). It has been well documented that deposition of the terminal components of complement (c5b-9) occurs not only in PHN (3, 33, 82, 147) but also in MN in humans (30, 49, 91, 133). Moreover, c5b-9 is required in some models of PHN (9, 34, 124, 135). However, it should be noted that in some experimental models of PHN utilizing the C6-deficient Piebold Viral Glaxo strain of rats, disease formation and progression occur in the absence of c5b-9 (160). Four to seven days after injection, the onset of proteinuria occurs, but this varies depending on initiating antibody (24–26, 28–38). Proteinuria persists throughout the autologous phase of the model. Although the only histological changes are GBM thickening at the light microscopy level, foot process effacement can be observed by electron microscopy (EM). Recently it was demonstrated that changes in nephrin at the slit diaphragm can be detected before the onset of proteinuria (122, 148). Eventually glomerular and tubulointerstitial sclerosis occurs as a result of ongoing proteinuria and podocyte injury (11, 125, 136, 137, 141, 144, 155).

Relevance of the rat model of PHN. Several features of PHN support the notion that this is the experimental model of choice to study MN. First, it is relatively easy to both produce the heterologous antibody and induce disease in rats. Second, the time course for the onset and progression of disease is relatively short (days to weeks). Finally, PHN closely follows the pathogenesis of human MN. Healthy podocyte foot processes and slit diaphragms are shown by transmission EM in Fig. 1, A (human) and B (rat), whereas C (human) and D (rat) demonstrate foot process fusion, loss of slit diaphragms, and subepithelial deposits in MN in humans and rats with PHN, respectively. So, although induction utilizes heterologous antibody and the target antigens are different, the PHN model has proved to be very relevant to human disease.

Establishing the rat model of PHN in your laboratory. To establish the rat model of PHN, one must first produce the Fx1A antigen. Rat cortices are pressed through a 150-μm mesh and resuspended in saline. Glomerular and tubular fragments are removed by low-speed centrifugation, and the supernatant containing the renal tubular epithelial fraction (Fx1) is sedimented by centrifugation. The pelletted fraction (Fx1A),

### Table 3. Details of disease induction of acquired rodent models of nondiabetic podocyte injury

<table>
<thead>
<tr>
<th>Animal Model</th>
<th>Species (Strains)</th>
<th>Dosing</th>
<th>Renal Phenotype</th>
<th>Foot process effacement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Passive Heymann nephritis</td>
<td>Rat (Sprague-Dawley, Wistar, Munich Wistar, Lewis, Piebold Viral Glaxo)</td>
<td>IP, 0.4–1.4 ml/200 g BW serum, IP, 4–48 mg/200 g BW IgG</td>
<td>Progressive Proteinuria onset, 4–6 days</td>
<td>Foot process effacement</td>
</tr>
<tr>
<td>Glomerular antibody model (anti-Glom)</td>
<td>Mouse (Balb-C, C57BL6)</td>
<td>IP, 0.5 ml/20 g BW, 2 consecutive days IV, 10–20 mg/20 BW ×1</td>
<td>Progressive Proteinuria onset, 7 Days</td>
<td>Apoptosis</td>
</tr>
<tr>
<td>Puromycin aminonucleoside nephrosis</td>
<td>Rat (Lewis, Wistar, Sprague-Dawley)</td>
<td>SC, 1.5–166 mg/100 g BW daily, ≤13 days IV, 1.5–15 mg/100 g BW ×1</td>
<td>Transient Proteinuria onset, 5–8 days Multiple injections, progressive proteinuria ≤123 days</td>
<td>Reversible foot process effacement</td>
</tr>
<tr>
<td>Adriamycin nephrosis</td>
<td>Rat (Most rat strains show complete susceptibility) Mouse (BALB/c, 129/SvJ)</td>
<td>IV/IP, 2–10 mg/kg BW</td>
<td>Progressive Proteinuria onset, 5–7 days</td>
<td>Segmental glomerulosclerosis</td>
</tr>
<tr>
<td>Protein overload</td>
<td>Rat (Wistar, Dark Agouti, Piebold Viral Glaxo; negative: Sprague-Dawley)</td>
<td>IV/IP, 1–15 mg/day−1 g BW−1 injected 3–5 days up to 3–4 wk</td>
<td>Transient Proteinuria onset, 6–24 h</td>
<td>Tubulointerstitial fibrosis</td>
</tr>
<tr>
<td>Protamine sulfate model</td>
<td>Rats (Wistar, Sprague-Dawley)</td>
<td>AI, 50–500 μg/ml LPS, 30 min AI, 10–100 μg/ml heparin, 10 min</td>
<td>Transient Proteinuria onset, 40–70 min</td>
<td>Reversible foot process effacement</td>
</tr>
<tr>
<td>Lipopolysaccharide model</td>
<td>Mouse (Balb-C 129SV, C57BL6, SCID)</td>
<td>IP, 200 μg LPS/mouse at 1 mg/ml PBS</td>
<td>Transient Proteinuria onset, 24 h, up to 72 h</td>
<td>Reversible foot process effacement</td>
</tr>
</tbody>
</table>

IP, intraperitoneal; IV, intravenous; SC, subcutaneous; AI, aortic infusion.

AJP-Renal Physiol • Vol. 296 • FEBRUARY 2009 • www.ajprenal.org
containing organelles and cell membranes, is washed in water and used for the production of heterologous antibody (47). The Fx1A antigen is combined with Freund’s and injected into either rabbits or sheep. In addition, gp330, one of the pathogenic antigens in Fx1A, also has been used to produce heterologous antibodies that can induce PHN (106, 187). The heterologous antibody against Fx1A should be injected into rats as serum, plasma, or isolated IgG. A dose response/time course should be performed to establish the optimal dose and onset of proteinuria. Finally, it is important to measure proteinuria in all rats, because in our experience, only 80% of injected animals actually get proteinuric, and thus the nonproteinuric animals should be excluded. Moreover, all animals need to be tested for antibody deposition in the glomerulus to ensure that there was appropriate binding of the Fx1A antibody.

Mouse Models of MN

Although we and others have made attempts, there are relatively few published reports of a robust mouse model of MN. In one published study (8), a single injection of rabbit antiserum against homologous, pronase-digested renal tubular antigens was given to C57.B10 mice. Although subepithelial deposits of rabbit IgG were described, only transient albuminuria occurred, and there was no spike formation, podocyte vacuolization, or foot process fusion. Assmann et al. (8) speculated that because the subclass of rabbit anti-Fx1A was IgG1, little complement fixation occurred and the model lacked many of the hallmarks of MN. Using unpublished data, we have tried a similar approach utilizing both rabbit and sheep antibodies to mouse pronase-digested renal tubular antigens in C57BL6 mice. Virtually identical results were obtained to the

Fig. 1. Examples of human vs. rodent podocyte pathology. Transmission electron microscopic images show normal glomerular basement membrane and preserved podocyte foot processes and slit diaphragms (arrows) in a human (A) and a rat (B). In contrast, subepithelial immune-type electron dense deposits associated with effacement of foot processes and loss of slit diaphragms (arrows) are shown in a human with membranous nephropathy (C) and a rat with passive Heymann nephritis (D). Likewise, podocyte flattening and extensive effacement of foot processes (arrows) in a human with minimal change disease (E) and a rat 10 days following puromycin aminonucleoside (PA) administration (F). Immunogold labeling of sheep antibody to rabbit glomeruli, used to induce crescentic glomerulonephritis (GN) in mice, shows binding predominantly to the glomerular endothelium (G, arrow). Light microscopic images show a cellular crescent in a human with crescentic GN (H, arrow) and in a mouse 14 days after administration of glomerular antibody induced crescentic GN (I, arrow). A typical focal segmental glomerulosclerosis lesion (circled) characterized by segmental obliteration of capillary lumina by expanded matrix and accumulation of hyaline and the presence of fibrous adhesion of the glomerular tuft to the adjacent Bowman’s capsule is shown in a human glomerulus (J) and in a rat administered cumulative doses of PA (K).
Assmann study. More recently, a mouse model of MN was reported (28). In this study, three different strains of mice (ICR, BALB/c, and C57BL6) were preimmunized with cationic bovine serum albumin (cBSA) every other day for a week. Two weeks later, mice were reimmunized with cBSA in Freund’s adjuvant. Both ICN and BALB/c mice developed disease; however, BALB/c mice required a higher dose. In this study, mice developed features of MN, including severe proteinuria, diffuse thickening of the GBM, subepithelial deposits, and GBM spikes.

Rodent Models of Crescentic Glomerulonephritis

Background of the mouse model of crescentic glomerulonephritis. Crescentic glomerulonephritis (GN) is the histological description of a glomerular disease characterized by an extra-capillary crescent-shaped proliferation of cells within the urinary space (31). There are several causes, which can be classified into ANCA-positive and ANCA-negative diseases. The majority are part of systemic disease processes such as vasculitis, although in certain instances the disease may be limited to the kidney. Although earlier studies identified parietal epithelial cells as the main kidney cell type proliferating in crescents, recent studies have shown that podocytes also constitute a proportion of crescents (117, 165). This might explain in part why many patients with crescentic GN develop nephrotic range proteinuria. This review focuses on models where the podocyte is the primary target of disease. We do not discuss disease models that are characterized by an initial endocapillary inflammation that later leads to podocyte disease and crescent formation, such as the anti-GBM models in rats and mice or autoimmune/ANCA-associated models of crescentic GN.

Disease induction of nephrotoxic mouse model of crescentic GN. The passive nephrotoxic nephritis model of crescentic GN is induced by intraperitoneal injection of heterologous antibody to heterologous whole glomeruli (132) and sheep anti-rabbit glomeruli antibody, either serum (0.5 ml·day⁻¹·20 g body wt⁻¹ for 2 consecutive days) or isolated IgG (10–20 mg/20 g body wt). Male C57BL/6 and BALB/c mouse strains have been used (Table 3). The heterologous antibody binds predominantly to glomerular endothelium as demonstrated by immunogold staining (Fig. 1G), thereby passively injuring the podocyte. Mice are usually killed 7, 14, and 28 days after disease induction. Nephrectomy, 24-h urine collection, and blood collection are performed at each time point to determine renal function and histology. Control animals receive vehicle only.

Expected outcomes of mouse model of crescentic GN. After injection of heterologous antibody to heterologous glomeruli, mice develop severe glomerular lesions with crescents (132). By day 7, a 20-fold increase in urine protein has been documented, along with segmental and circumferential cellular crescents in 20–34% of glomeruli. Crescents were associated with vacuolization of podocytes and segmental parietal glomerular epithelial cell and mesangial proliferation. Formation of synechia or adhesions of the glomerular capillary tufts to Bowman’s capsule have been noted. At day 7, Bowman’s capsule was still intact and occasional infiltrating neutrophils and macrophages were present, although they were observed outside Bowman’s capsule. Segmental sclerosis was present in 15% of glomeruli. By days 14 and 28, the percentage of crescentic glomeruli remained unchanged (27 and 29%). However, sclerosis and matrix expansion increased significantly. By day 28, many glomeruli were obsolete and crescents became fibrocellular. Proteinuria persisted throughout.

Relevance of mouse model of crescentic GN. Experimental crescentic nephritis is typically induced by antibodies to whole glomeruli (“nephrotoxic nephritis”) or to isolated GBM (“anti-GBM disease”). These animal models, as well as studies in humans (104), suggest that crescents are composed of both parietal and visceral glomerular epithelial cells as well as infiltrating monocytes, macrophages, lymphocytes, and fibroblast-like cells (111). However, Moeller et al. (117) recently showed convincingly that part of the cellular origin of glomerular crescents lies with the podocyte. Therefore, examination of the passive nephrotoxic nephritis model, where Bowman’s capsule remains intact and no infiltrating cells are necessary to initiate the glomerular injury, is a superior model for studying the role of the podocyte in crescent formation (132). Shown in Fig. 1H is an example of a typical cellular crescent found in a human glomerulus, and Fig. 1I shows a crescent found in a mouse with crescentic GN. Unfortunately, to our knowledge this model is only available in mice.

Establishing the mouse model of crescentic GN model in your laboratory. Sheep anti-rabbit glomeruli antibody can be produced by immunizing sheep at least three to four times at 2-wk intervals with lyophilized whole rabbit glomeruli emulsified in complete Freund’s adjuvant for the first immunization and in incomplete Freund’s adjuvant for subsequent immunizations. To produce antigen, rabbit glomeruli are isolated by differential sieving and should contain fewer than 5 tubular fragments per 100 glomeruli to maintain glomerular specificity. Importantly, not all sheep produce antibodies capable of inducing crescentic GN. In our hands, only one in four attempts have been successful. Antiserum should be heat inactivated at 56°C for 30 min to eliminate complement and reduce toxicity to mice. To further improve tolerance, IgG should be isolated from the serum.

Animal Models of Minimal Change Disease and Focal Segmental Glomerulosclerosis

Minimal change disease (MCD) and focal segmental glomerulosclerosis (FSGS) are the leading primary causes of nephrotic syndrome in children and adults, respectively. Although likely distinct disease processes, some have suggested that they form a continuum where MCD precedes and then develops into FSGS. Although we do not necessarily share this viewpoint and believe these are indeed distinct diseases with different etiologies, animal models such as puromycin aminonucleoside nephrosis (see below) have been used experimentally to study both disease processes and are therefore “grouped” together in this section. As stated earlier, there are several well-characterized genetic mouse models of FSGS that will not be reviewed.

Rat Model of Puromycin Aminonucleoside Nephrosis

Background of the rat model of puromycin aminonucleo-side nephrosis. With hallmarks of podocyte flattening and nephrotic-range proteinuria, puromycin aminonucleoside nephrosis (PAN) has become the experimental prototype of human MCD and FSGS (24). In the early 1950s, Silvestre Frenk discovered the renal pathology serendipitously while...
developing an animal model to study protein-energy malnutrition states (60, 61). Administration of puromycin aminonucleoside (PA) has reinforced observations in humans that MCD and FSGS are two pathological processes on a continuum of podocyte disease and that FSGS is a histological progression of MCD. Subsequent studies revealed that podocytes are the primary target of PA-induced injury, and thus the PAN rat model has become a powerful tool for investigating podocyte pathophysiology.

**Disease induction of the rat model of PAN.** Typically, PA studies utilize male Sprague-Dawley rats weighing between 90 and 200 g. Despite slight differences in quantity and onset, proteinuria and ultrastructural changes are reproducible among many different rat strains. Although there are a few exceptions, historically mice have been resistant to PAN (29, 41, 120).

Subcutaneous (19, 22–25, 52, 57, 67, 71, 83, 168–170, 180), intravenous (43, 72, 79, 80, 89, 93, 108, 130, 145, 177, 178, 180), and intraperitoneal (65, 75, 102, 157, 180) administration routes have all been used. In most studies, control rats receive distilled water or normal saline (Table 3). Factors influencing the onset of proteinuria and extent of injury include rat strain, PA dose, and route of administration; however, cumulative PA exposure is the most critical factor determining whether rats develop histological features of MCD or FSGS (43). Many investigators assessed ultrastructural changes at early time points; thus it is difficult to draw conclusions about the progression of podocyte lesions over time. However, the available data suggest that progression of glomerular lesions from podocyte effacement to glomerular scarring largely depends on cumulative PA dose. Olson et al. (130) administered a single intravenous dose of 15 mg/100 g body wt to male Wistar-Furth rats. At day 9, proteinuria peaked and EM showed changes characteristic of human MCD with podocyte foot process effacement and detachment from the GBM; however, proteinuria normalized by day 28 (130). Similarly, Shiki et al. (156) showed that rats given puromycin subcutaneously for 5 days developed lesions consistent with MCD that eventually regressed. However, 5 additional days of PA led to the development of irreversible sclerotic lesions. In another elegant study, progressive podocyte depletion induced by increasing doses of PA correlated directly with degree of glomerulosclerosis at 90 days (87). On balance, induction of FSGS with sclerosis in 7–10% of glomeruli requires a higher cumulative PA dose over a period of 11–12 wk (63, 64).

**Expected outcomes of the rat model of PAN.** The main focuses of study with the PAN model have been 1) deciphering the mechanism by which PA injures podocytes, 2) characterizing ultrastructural podocyte changes after PA exposure, and 3) correlating the time course and extent of podocyte injury with onset of proteinuria. With respect to the mechanism of injury, PAN causes direct DNA damage via the production of reactive oxygen species (42, 108, 153). Proof of principle was shown when rats pretreated with oxygen radical scavengers before receiving PA had less proteinuria and podocyte injury (42, 164).

Ultrastructural changes seen in PAN parallel those described in human nephrosis (51). Between 2 and 4 days following PA, podocyte foot processes disorganize into broad expanses of epithelial cytoplasm (22, 24, 52, 113, 145, 168, 169). As mentioned earlier, Fig. 1A (human) and B (rat), shows healthy podocytes by transmission EM, whereas Fig. 1E shows the typical flattening and loss of foot processes observed in MCD in humans. Similarities in the ultrastructural changes seen in a rat 10 days following PAN are shown in Fig. 1F. After this preproteinuric phase, podocyte detachment and proteinuria occur simultaneously (24, 56, 67, 170). Subsequent apoptosis of podocytes is associated with the development of FSGS (156).

Changes in cytoskeletal and slit diaphragm proteins underlie the transformation of podocytes from stawlcart cells to shapeless cytoplasmic masses. Altered mRNA levels and protein levels, mislocation, and function of podocalyxin, actin, podocin, and nephrin have been reported in rats with PA nephrosis (25, 65, 75, 79, 80, 83, 157, 178). In one study, an antibody directed against a membrane glycoprotein led to rapid reversible podocyte flattening. This protein, subsequently named podoplanin, is significantly downregulated in PA-treated rats (19, 110).

The most perplexing question is how cytoskeletal changes in podocytes relate to proteinuria. One theory is that loss of slit diaphragm integrity and orientation leads to loss of size and charge selectivity. Another theory is that podocyte detachment leaves denuded areas of GBM, thereby allowing protein leakage. Whiteside et al. (177, 178) showed that podocyte detachment from the GBM closely correlated with onset of massive proteinuria. Actin cytoskeletal changes occurred 3 days after PA and was followed by podocyte detachment and massive proteinuria.

**Relevance of the rat model of PAN nephrosis.** The PAN rat model offers insights into how podocyte lesions evolve in the pathological processes of MCD and FSGS. Studies suggest there may be an injury threshold beyond which podocytes are irreversibly damaged and detach from the GBM, leading to proteinuria and scarring. Detachment of podocytes from the GBM seems to be the critical event underlying formation of the sclerotic lesions seen in FSGS. In this model, cumulative PA dose determines the degree of podocyte injury and whether MCD or subsequent FSGS develops. For example, Fig. 1J shows a typical FSGS lesion in a human glomerulus characterized by segmental obliteration of capillary lumens by expanded matrix and accumulation of hyaline and the presence of fibrous adhesion of the glomerular tuft to the adjacent Bowman’s capsule. A similar lesion is shown in Fig. 1K in a rat with cumulative PA dosing. The PA rat model affords the opportunity to evaluate serial changes in the podocytes. With current advances in molecular biology and other technologies, this model continues to provide critical information for effective diagnosis and treatment of these disease entities.

**Establishing the rat model of PAN nephrosis in your laboratory.** These recommendations are based on an extensive review of the literature and our experience with this rat model. Although disease has been successfully induced in at least four rat strains, most investigators use male Sprague-Dawley rats weighing between 100 and 150 g. We recommend a single tail vein injection of 15 mg/100 g body wt PA (Sigma-Aldrich, St. Louis, MO; InvivoGen, San Diego, CA; or EMD Chemicals, Gibbstown, NJ). At this dose, proteinuria develops between days 4 and 6, peaks around day 8, and normalizes at 4 wk. Glomerular changes at onset of proteinuria resemble those in human MCD with pronounced podocyte effacement, foot pro-
cess fusion, and structural and molecular reorganization of slit diaphragm proteins. Resolution of these changes parallels normalization of proteinuria (Table 3).

Rat Model of Adriamycin Nephrosis

Background of the rat model of adriamycin nephrosis. First described in 1970 (161), anthracycline-mediated nephropathy has been utilized in hundreds of animal studies as a model of progressive glomerulosclerosis associated with nephrotic syndrome. The anthracycline antibiotics, produced by the fungus Streptomyces peucetius, include daunorubicin and its 14-hydroxy derivative doxorubicin (Adriamycin, or ADR). ADR-induced nephropathy has become the prototypical experimental model of human primary FSGS. In both experimental models and affected humans, the podocyte is the site of the initial injury in the development of FSGS. A unique advantage of using ADR to induce a model of FSGS is that it allows the precise timing of the onset of injury to the podocyte. In fact, ADR-induced nephropathy has proved to be a very reproducible model of FSGS, and its application has provided tremendous insight into the mechanisms that initiate FSGS and development of chronic kidney disease. This model also has been useful in testing the efficacy of various therapeutic strategies to prevent or retard progressive FSGS (185).

Disease induction of the rat model of ADR nephrosis. Although most rat strains show complete susceptibility to Adriamycin’s nephrotoxicity, the most commonly utilized strain is the Sprague-Dawley rat. There are, however, sex differences in disease susceptibility. Sakemi et al. (146) found that male rats were more likely to develop massive proteinuria than female rats and that castration significantly reduced proteinuria to the levels seen in female rats. In addition to worse proteinuria, male rats also demonstrated more severe hypoalbuminemia, a greater impairment in renal function, and a higher degree of glomerulosclerosis (146).

A single intravenous injection of an anthracycline, most commonly ADR, leads to proteinuria and progressive renal disease, with the onset of proteinuria occurring within 5–7 days of exposure. One of the first reports of ADR-induced nephrotic syndrome in rats utilized a dose of 7.5 mg/kg body wt via tail vein injection (13). Since then, various dosing regimens have been reported, ranging from 2 to 10 mg/kg body wt, typically utilizing 0.9% NaCl as vehicle, administered intravenously (118) or intraperitoneally (119) in a single dose or, more rarely, in divided doses (Table 3). Control rats receive vehicle only. Our preferred route of administration is intravenous injection, which predictably induces disease. Intraperitoneal injection has the advantage of ease of administration, whereas intravenous injection provides more direct delivery and eliminates the dependence of absorption across the peritoneal membrane. Although some studies have utilized multiple doses, including some reported protocols of weekly intravenous injections of 1 mg/kg for up to 7 wk (96), multiple studies have established that even in the absence of continued ADR exposure, ADR induces self-perpetuating nephropathy.

Expected outcomes of the rat model of ADR nephrosis. Rats treated with a single dose of ADR develop nephrotic syndrome, characterized by heavy proteinuria, hypoalbuminemia, and hypercholesterolemia. This is accompanied by a progressive reduction in glomerular filtration rate, as measured by increased serum creatinine and/or blood urea nitrogen. Biochemical abnormalities appear within 5–7 days following the injection of ADR and reach maximum levels by 4–5 wk (13).

After ADR administration, rats exhibit early podocyte foot process fusion, segmental glomerulosclerosis by week 4, and progressive global sclerosis and interstitial fibrosis by week 6 (13). In studies using lower doses given twice separated by a 3-wk interval, there is an early phase of proteinuria with focal foot process effacement progressing over the first 8 week, followed by segmental sclerosis at week 16, which progresses to widespread glomerulosclerosis with tubulointerstitial fibrosis by week 24. Some rats die of uremia by week 28 (59, 129).

In rats, clipping of the renal artery during ADR infusion prevents the development of nephropathy, suggesting that sensitivity to the agent is independent of extrarenal metabolism and that direct exposure of the kidney to anthracycline is a requirement for the development of podocyte injury (14, 38). The exact pathophysiological mechanisms underlying the initial cytotoxicity and the delayed progression remain to be determined. Studies have shown that several potential pathways of injury may be operational (62). Acute cytotoxicity is thought to be secondary to DNA intercalation, cross-linking, or binding, inhibition of topoisomerase II, free radical generation with consequent induction of DNA damage or lipid peroxidation, direct cell membrane effects, cell death via necrosis or apoptosis (62, 134), and/or promotion of a senescence-like growth arrest (139).

Delayed progression may be secondary to generation of reactive oxygen species (116, 182), leading to damage of mitochondrial DNA with subsequent respiratory chain dysfunction (96) and increased glomerular heparanase expression with associated reduction in heparan sulfate in the GBR (88); chemokine release by injured resident kidney cells, including monocyte chemoattractant protein-1 (MCP-1) and regulated on activation normal T-cell expressed and secreted (RANTES), with subsequent renal infiltration by macrophages and T cells (181); and/or apoptosis secondary to reactive free radical formation or activation of Fas/Fas ligand signaling pathway (158).

Relevance of the rat model of ADR nephrosis. Over the last 30 years, ADR has been administered in rats to yield a chronic proteinuric renal disease similar to human FSGS. Although the mechanisms that mediate progressive renal dysfunction and glomerular scarring are not yet fully elucidated, experimental ADR nephropathy has allowed the ability to probe mediators that may modulate or accelerate disease progression, including free radical generation (158), the renin-angiotensin-aldosterone system (77, 88, 100), and hyperlipidemia (158).

Establishing the rat model of ADR nephrosis in your laboratory. There is variability in the potency of ADR depending on the manufacturer of the drug. In addition, animal-to-animal variability in response to ADR exists. Finally, the skill of the operator will determine whether an adequate dose is delivered to the kidneys via intraperitoneal or intravenous routes. Given the narrow therapeutic range of ADR, a pilot study should be performed to determine the optimal dose whenever a new manufacturer or a new batch of the reagent is used, using doses reported in the literature to serve as rough guides for initial trials. The onset of proteinuria within 5–7 days of ADR administration serves as a good measure of adequate disease induction. Further validation can be obtained following anal-
ysis of other biochemical parameters and histopathology for the characteristic abnormalities of FSGS.

Mouse Models of PA and ADR Nephrosis

**Background of the mouse model of ADR nephrosis.** Attempts to apply the well-established models of nephropathy induced by injection of PA or ADR to mice were initially quite disappointing. Although most rat strains show complete susceptibility to both agents, most mouse strains do not. In the mid-1990s, Chen et al. (27) determined that BALB/c mice proved susceptible to ADR administration, with selective injury to podocytes resulting in severe proteinuria and progressive renal failure. This was described as the first experimental model of FSGS in mice (26, 27).

The reason for the susceptibility of BALB/c was determined in 2005 by Zheng et al. (185), who showed that ADR susceptibility segregates as a single gene defect with recessive inheritance by mapping the trait locus to chromosome 16A1-B1 (DOXNP H locus). Although 15 genes were found to have significant interaction between strain and exposure to ADR, none localized to the DOXNP H linkage intervals. One gene, protein arginine methyltransferase 7 (Prtm7), localized to the region of the DOXNP H locus following ADR exposure (185). Although arginine methylation of proteins is involved in many cellular processes such as protein-protein interactions, signal transduction, transcription, and RNA processing, methylation of ADR has been reported to decrease its cytotoxicity potential, raising the possibility that direct methylation of ADR by Prtm7 may mediate protection from kidney injury (185). In the study by Zheng et al. (185), BALB/cJ and 129/SvJ mice demonstrated severe susceptibility to ADR, with the onset of proteinuria 5 days after injection and progression to significant glomerulosclerosis (Table 3). In contrast, C57BL/6J, FVB/NJ, and CAST/EiJ mice were completely resistant to ADR with renal histology and proteinuria indistinguishable from saline-treated controls (185). In subsequent studies, 10 additional strains have been tested for susceptibility to ADR nephropathy. AKR/J, C3H/HeJ, CBA/J, C57BL/10J, LP/J, SWR/J, SJL/J, and 129S6/SvEvTac mice were found to be resistant to ADR nephropathy, whereas 129S1/SvImJ and BALB/cByJ mice were susceptible (184).

**Disease induction of the mouse model of ADR nephrosis.** Similar to what has been observed in rats, male mice demonstrate a tendency toward an increased likelihood to develop more severe disease. There also have been reports of age-related differences in susceptibility to ADR. Hahn et al. (66) showed that following exposure to ADR, 12-wk-old mice developed significantly greater proteinuria, transforming growth factor (TGF)-β excretion in urine, and interstitial fibrosis compared with mice that were only 5 wk old.

The initial studies by Chen et al. (27) utilized two intravenous injections of ADR at a dose of 10 mg/kg body wt. The animals that received two doses developed heavy proteinuria on day 2, became anuric on day 5, and died on days 6 and 7. Subsequent studies reduced the dose to a single injection of 10 mg/kg body wt. In these preliminary studies, 18–25% of their mice died of severe dehydration and cachexia by days 19 and 20. Therefore, the mice were killed on day 18 (26). Wang et al. (172) later extended these studies to 6 wk to more firmly establish and characterize a stable and reproducible murine model of chronic progressive nephropathy with significant and persistent proteinuria by administering ADR as a single intravenous injection of 10–11 mg/kg body wt. Since then, most studies have utilized doses ranging from 10 to 12 mg/kg body wt as a single intravenous injection, although doses have varied from 5 to 20 mg/kg body wt. In addition, several studies have employed the use of a second dose administered 2–4 wk following the initial dose without an increase in animal death rates (Table 3). In these studies, administration of vehicle alone (typically 0.9% NaCl) has been used as the control.

**Expected outcomes of the mouse model of ADR nephrosis.** In the study by Wang et al. (172), mice developed nephropathy characterized by proteinuria, hypoalbuminemia, increased serum creatinine, and progressive renal injury. Overt proteinuria appeared at day 5 (Table 3). By week 2, histology revealed glomerular hyper trophy, hyaline deposits, reabsorption droplets, and intratubular casts. By week 4, glomeruli were reduced in size with glomerular vacuolization, tuft collapse, and mesangial expansion. By week 6, extensive FSGS and severe interstitial fibrosis and inflammation were present. In addition, global sclerosis was observed in many glomeruli by week 6 (172). EM demonstrated segmental podocyte foot process fusion at week 1 that was widespread by week 6 (172).

Mechanisms for the initial cytotoxic effect similar to those detailed in Rat Model of Adriamycin Nephrosis have been suggested in the murine model (171, 40). However, emerging data from mouse knockout experiments have provided insight into potential mediators that may be important in the delayed progression of disease following the initial cytotoxicity. By using knockout animals for various complement cascade components, including C3, C1q, factor D, factor B, or the complement regulatory protein CD59a, studies have identified the alternative pathway of the complement cascade as important in mediating the early podocyte injury and the later onset glomerulosclerosis, tubulointerstitial injury, and renal dysfunction (99, 166). Our group has shown that cell cycle regulatory proteins may be important mediators in ADR-induced podocyte apoptosis. The cyclin-dependent kinase inhibitor p21 was shown to be a critical regulator of podocyte survival, with p21 knockout mice demonstrating increased blood urea nitrogen, albuminuria, and apoptosis by terminal deoxynucleotidyl transferase dUTP-mediated nick-end label staining, correlating with decreased podocyte number and more severe glomerulosclerosis (unpublished data). Others have shown that lymphocytes are critical players in the delayed progression of ADR nephropathy. In mice with established ADR nephropathy, depletion of CD4+ T lymphocytes worsened glomerular and interstitial injury, whereas depletion of CD8+ T lymphocytes ameliorated disease (173, 174).

**Relevance of the mouse model of ADR nephrosis.** In mice, the ADR nephropathy model has proved to be a robust experimental model of human FSGS. Because FSGS is the final common pathway for loss of functioning glomeruli, and because podocyte loss is a critical event in the initiation of FSGS, the murine ADR model is ideal to elucidate the underlying mechanisms that govern the response of the podocyte to injury. Establishing ADR nephropathy in mice carries the special advantages of ease of handling, economy, and the potential for application of genetic and monoclonal antibody manipulation to study pathogenesis (181).
Since the establishment of a reproducible murine model of FSGS, understanding of the pathogenesis of progressive kidney disease has been strengthened. Recent and future studies hopefully will provide a basis for the development of novel strategies that may protect podocytes from injury, prevent subsequent glomerular damage, and/or promote tissue repair.

Establishing the mouse model of ADR nephrosis in your laboratory. Recommendations for the murine model of ADR nephropathy are similar to those already discussed in Rat Model of ADR Nephrosis. However, the appropriate dose is much more critical. Given a smaller body mass, too high a dose will lead to multiple organ failure in mice. Therefore, establishing an optimal dose via a dose-response study is a necessary initial step. Similar biochemical and histopathology parameters can be utilized to validate that the model is working. A significant limitation of the use of the murine model of ADR nephropathy is the restricted strain susceptibility. Many genetically altered mice currently in use are on the C57BL6 background, a strain that is known to be completely resistant to ADR nephrotoxicity. To employ the ADR-induced nephropathy model, these mice would need to be backcrossed to a genetically susceptible strain carrying the genetic mutation of interest, a process that may consume a great deal of time, effort, and financial resources.

Disease Models of Podocyte Injury That Cannot Yet Be Classified As a Specific Human Disease

The rodent models discussed above are used experimentally to study specific nondiabetic human diseases of primary podocyte injury including MN, MCD, FSGS, and crescentic GN. Several other rodent models of podocyte injury have emerged that have significantly contributed to our understanding of podocyte biology in disease and also have served as useful models to study the responses of podocytes to injury, rather than representing a specific human disease entity. These disease models are discussed below.

Protein Overload Nephropathy

Background of the rat model of protein overload nephropathy. Scientific investigations into the use of plasma substitutes for battle casualties during World War II led to the observation that have been used), as well as the sex and strain of rats (2, 4, 7, 14, 20, 36, 37, 95, 98, 101, 142). The lack of a

Expected outcomes of the rat model of protein overload nephropathy. The onset of proteinuria typically occurs as early as 6–24 h after the initial protein injection, accompanied by increased podocyte pinocytosis and lysosomal activity, which leads to formation of vacuoles and reabsorption droplets (4, 7, 36, 95, 142). Podocytes swell and foot processes become partially obliterated along with partial lifting from the GBM. Ultimately, podocytes detach from the basement membrane, leaving bare segments of GBM (155). Importantly, most of these alterations are reversible. Thus urine protein excretion and glomerular lesions tend to regress and normalize. Although urinalysis of BSA disappears within days, elevated levels of rat albumin remain for several weeks after the last BSA injection (107). This is due to persistent glomerular lesions including mesangial proliferation, glomerular sclerosis, and tubular dilation.

In general, the amount of BSA administered to rats correlates with the severity of the glomerular injury and the magnitude of proteinuria (36). As expected, these effects are even more pronounced in uninephrectomized animals (46, 175). Most endogenous and exogenous proteins used induce similar glomerular changes and increases in proteinuria (up to 400-fold), which consists of equivalent amounts of both endogenous (rat albumin, hemoglobin, γ-globulins) and exogenous proteins (injected BSA, human serum albumin) (36, 101, 175) as a result of a small but significant defect in size selectivity (98, 175). In contrast, investigators using egg albumin have reported different results. Egg albumin has a smaller molecular mass (48 kDa) compared with human or bovine albumin (69 kDa) and induces selective proteinuria of injected egg albumin with no glomerular changes and no increased excretion of endogenous rod proteins (20, 36, 37, 98, 101).

Relevance of the rat model of protein overload nephropathy. Clinical observations in children (112) receiving repeated plasma transfusions to treat inherited coagulation disorders have reported massive proteinuria and described a phenotype similar to that reported in the rat model. The protein overload nephropathy model also has been recognized as a useful model to study the relationship between glomerular dysfunction and the consequences of proteinuria on the tubulointerstitium, as well as the mechanisms leading to scarring, apoptosis, and atrophy (98, 175). Altogether, this model best serves as a model of general injury to podocytes for the study of specific molecular and cellular events.

Establishing the rat protein overload model in your laboratory. Although numerous protocols have been published, on the basis of our experience we would recommend an intraperitoneal injection of 5 mg·day⁻¹·g body wt⁻¹ BSA dissolved in 2.5 ml of 0.9% NaCl twice daily for 5 days in male Wistar rats (Table 3). Control animals should receive saline injections only. Marked proteinuria, which will consist of both BSA and
rath albumin, can be observed 24 h after the first injection. Glomerular lesions become evident within 24–48 h. Days after the last BSA injection, urine protein concentrations and glomerular lesions regress and normalize.

Mouse Model of Protein Overload Nephropathy

Background of the mouse model of protein overload nephropathy. The protein overload model also has been reported in mice. In contrast to the rat model, however, the glomerular lesions are typically mild. Similar to rats, the severity of disease strongly depends on the mouse strain utilized. Ishola et al. (76) showed more severe proteinuria, tubulointerstitial infiltration, and fibrosis in 129S2/Sv mice compared with C57BL/6 mice. However, there were no differences with respect to glomerular injury between the two strains or between treated and vehicle-only control animals.

Among murine models of protein overload nephropathy, the albumin dosages vary (44, 45, 68, 121, 179, 186) between 10 and 20 mg·day⁻¹·g body wt⁻¹ administered daily for 3–5 days (Table 3). Glomerular changes range from no change to first week, beginning with 2 mg·day⁻¹·g body wt⁻¹ and increasing the BSA dosage during the first week, beginning with 2 mg·day⁻¹·g body wt⁻¹ initially.

Establishing the mouse model of protein overload model in your laboratory. Several protocols have been published describing various protocols to set up the protein overload model in mice. On the basis of our experience, we would recommend gradually increasing the BSA dosage during the first week, beginning with 2 mg·day⁻¹·g body wt⁻¹ initially and increasing the daily dose by 2 mg·day⁻¹·g body wt⁻¹ until the final concentration of 10 mg·day⁻¹·g body wt⁻¹ has been reached. This final dose should be administered for 3–5 days. Proteinuria will increase throughout the induction period and should be observed as early as 24 h after the first injection (Table 3).

Reversible Models of Podocyte Foot Process Effacement

Two models have been used to evaluate rapid but reversible foot process effacement and proteinuria, the protamine sulfate (PS) model in the rat and mouse and the lipopolysaccharide (LPS) model in the mouse.

Rat Model of PS-Induced Foot Process Effacement

Background of the rat model of PS-induced foot process effacement. The GBM and podocyte foot processes are both negatively charged (118, 128, 138). In the 1970s, several studies reported a change in glomerular anion charge, related to a change in surface sialoproteins, which altered the barrier to negatively charge plasma proteins in minimal change disease in humans and in the rat PAN model (15, 16, 115, 151). Infusions of different cations, hexadimethrine (74), polylysine, polyarginine, and protamine sulfate (PS) to induce the change in epithelial surface charge have been reported; however, polylysine and polyarginine are cytotoxic, so PS has proved the most useful (150). Based on these findings, the PS model was developed to alter the anionic charge in the glomerulus resulting in foot process fusion (115). Disease induction of the rat model of PS-induced foot process effacement. Original studies were conducted in female Sprague-Dawley rats, but later studies have been performed in male animals (90) as well as Wistar rats (159). Initial descriptions of the model described bilateral renal perfusion in situ with an aortic catheter. Kidneys were perfused for 1 min with a Ringer-bicarbonate buffer, followed by a 10-min perfusion with various concentrations of PS. Control rats were infused with either cationic or neutral substances in equivalent concentrations. PS infusions (50–500 µg/ml) alone resulted in foot process fusion, and this process was reversible with infusion of heparin (10–100 U/ml for 10 min) (Table 3) (151).

Time-course studies showed that both PS dose and infusion time were important for inducing foot process effacement. Andrews et al. (5) showed that an infusion of 50 µg/ml PS resulted in focal (<50%) flattening of podocyte pedicels and major processes, but infusion of 500 µg/ml resulted in more generalized effacement after 10 min that was even more extensive after 30 min of PS infusion. Similar studies performed by Sonnenburg-Hatzopoulos et al. (159) confirmed the dose (range 110–330 µg/ml) and time (40–70 min) effects on foot process effacement and proteinuria.

Finally, studies to evaluate systemic vs. direct renal artery infusion showed that systemic infusion of PS at 25–75 µg/g body wt induced proteinuria that was primarily albuminuria. However, single kidney infusions proved that the resultant proteinuria and foot process effacement was specific to the PS infusion and not a generalized or a systemic side effect.

Expected outcomes of the rat model of PS-induced foot process effacement. High doses of PS can decrease glomerular filtration rate and renal plasma flow by inducing hemodynamic changes, but these changes do not account for the increased proteinuria (167). Although there were reports of glomerular thrombosis (92) or capillary congestion with platelets and fibrin, as well as vacuolization of the glomerular epithelium and microthrombi formation (114), the most prominent histological feature consistently reported in this model has been foot process effacement. The exact cellular mechanisms by which PS infusion results in foot process effacement have not been elucidated. Studies in cultured mouse podocytes have shown similar structural changes in podocytes exposed to PS in vivo. A change in intracellular calcium occurs following incubation of cultured podocytes with PS, and this correlates with studies by Kerjaschki (85), who showed that a decrease in extracellular calcium resulted in less foot process effacement in the PS model.

Relevance of the rat model of PS-induced foot process effacement. The use of cationic infusions linked experimental and clinical forms of the nephrotic syndrome and allowed for the definitive demonstration that nephrotic syndrome was associated with foot process fusion. More recent studies have used this model to evaluate slit diaphragm structure and function, specifically looking at the subcellular position and quantity of specific proteins, such as ZO-1 (90). Both the acute and reversible features of this model make it a potentially useful tool for evaluation of the malleability of the podocyte actin cytoskeleton and the reorganization of slit diaphragm proteins that result in foot process effacement and proteinuria.
Mouse Model of PS-Induced Foot Process Effacement

The PS model has not been fully described or developed in the mouse. Asanuma et al. (6) described in situ bilateral renal perfusion with PS at 2 mg/ml in HBSS for 15 min, followed by perfusion with heparin at 800 µg/ml in HBSS in mice. Histological examination documented reversible foot process effacement in wild-type and synaptopodin null mice. No proteinuria data were reported (6). Although there have been no other studies to confirm the reproducibility of this model in mice, if verified, this model might be useful to study the mechanisms involved in actin cytoskeleton rearrangement in podocytes and the effects on proteinuria.

Mouse Model of LPS-Induced Proteinuria

Background of the mouse model of LPS-induced proteinuria. LPS is the major glycolipid component of the outer membrane of gram-negative bacteria. It binds to Toll-like receptor 4 (TLR-4), which interacts with other extracellular proteins, LPS binding protein, CD-14, and MD-2. These interactions trigger an intracellular cascade that results in activation of NF-kB and the release of proinflammatory cytokines. TLR-4, which is found on all white blood cells, also has been identified on many other nonhematopoietic cells, such as mesangial cells and podocytes (18, 140).

Although microalbuminuria occurs early and transiently after multiple systemic insults such as ischemia, pancreatitis, and trauma, it also is an early feature of sepsis and correlates with organ dysfunction (1). Although studied extensively as a model for sepsis, LPS was recently described by Reiser et al. (140) as a reversible model of proteinuria.

Disease induction and expected outcomes of the mouse model of LPS-induced proteinuria. Intraperitoneal injection of 200 µg of LPS diluted in PBS at a concentration of 1 mg/ml was used to induce disease in three different mouse strains, BALB/c, WT 129, and C57BL/6. Proteinuria, measured by dipstick, and foot process effacement, as shown by EM, were identified within 24 h. Proteinuria was transient, returning to baseline by 72 h (Table 3). Because the focus of that article (140) was to show that B7-1, a transmembrane protein on B cells and antigen presenting cells, was essential for podocyte cytoskeletal rearrangement and subsequent proteinuria, these experiments also were repeated in SCID mice. The mechanisms by which LPS interacts with podocyte TLR-4 receptors to induce proteinuria is unknown, but the studies in SCID mice show that the mechanism is independent of T and B cell involvement (140). Because of the risk of septic shock, care should be taken when inducing and interpreting the LPS-induced model, because injury to the podocyte is not the only mechanism involved.

Relevance of the mouse model of LPS-induced proteinuria. Although the clinical relevance of this model is limited, it has potential as a complementary model to the PS model to study reversible foot process effacement. Further studies are needed to define the podocyte-specific pattern and mechanisms of injury.

Summary

Our current understanding of podocyte biology has advanced rapidly over the past decade. Although new podocyte-specific genes are being identified each year, and the incredibly ingenious and powerful tools of inducible and podocyte-specific genetic mice have substantially delineated the roles of podocyte-specific genes, the function of these genes often remains unknown in disease. Understanding the molecular and cellular events of podocyte injury in human disease ultimately requires induction of experimental disease models in rodents that reflect those in humans. Likewise, identifying and testing new therapeutic targets in podocyte disease also requires induction of an experimental model of disease that resembles changes in humans. Thus, although we have made major strides in defining the molecular and cellular events underlying nondiabetic acquired diseases of podocytes, we still have a way to go before we can replicate all disease events in humans. The experimental models described in this review are intended to guide researchers to the animal models that are available and their advantages and disadvantages. We have focused on common nondiabetic models where the podocyte is the primary glomerular cell injured. We recognize that other diseases such as lupus nephritis, ANCA-associated glomerulonephritis, IgA nephropathy, and the 5/6 nephrectomy (remnant kidney) model of progressive glomerulosclerosis also may be associated with podocyte abnormalities. However, in these diseases, glomerular injury is typically primarily to glomerular endothelial cells, parietal epithelial cells, GBM, or mesangial cells, and the changes seen in podocytes are regarded as “secondary” to the primary events. As such, they are not as ideal for determining the function(s) and biological role of podocyte-specific proteins or to identify podocyte targets for treatment and were therefore not discussed in this review.

One may notice that in contrast to the rat, there is a relative paucity of models of acquired podocyte diseases in mice. This is a shame given that mice are typically used for deletion or overexpression of specific genes in podocytes. In the absence of a developmental phenotype in a genetically altered mouse, a logical next step would be to determine the biological role of specific genes in disease. Therefore, if more reliable mouse models were available that mimic those in humans, they could be induced in these genetically altered mice. One might ask, why are mouse models less available? Similar to the difficulties experienced producing murine models of diabetic kidney disease, we and others have found that what works well in rats does not always translate to mice. Antigen and receptor expression differ in mice, making the currently available antibody-mediated models more challenging. As with PAN and ADR, susceptibility to toxin-induced injury differs in mice. Intraglomerular pressure might simply not be high enough to augment the progression of glomerular injury. Complement activation differs in mice compared with rats. Our group and many others have put a great deal of time and energy into establishing reliable models in mice. We successfully developed the nephrotoxic nephritis model of crescentic GN reviewed above (132). An important feature of this model is that unlike models such as the anti-GBM models in rats and mice or the autoimmune/ANCA-associated models of crescentic GN, where initial endocapillary inflammation later leads to podocyte disease and crescent formation, the primary injury is to podocytes.

On a more positive note, excellent models of acquired podocyte diseases exist in the rat. In our opinion, the best model to study membranous nephropathy is the rat passive...
Hemyn nephritis model; likewise, puromycin nephrosis and adriamycin nephrosis are well suited to study FSGS. The protein overload model provides a good tool for studying the pathophysiology of the podocyte’s response to excess protein and its response to this form of injury. The PS and LPS model are less specific to a specific human disease and are perhaps best used to study the podocyte’s response to injury. With so many more models available for study in the rat, it makes one speculate whether the renal community should place more effort into producing transgenic rats for renal research. With the successful production of a renal-specific transgenic rat by Wharram et al. (176) in 2005 and advances being made in producing transgenic rats (58), it is clear that this may be a worthy direction for the future.

Investigators need to be very clear on the hypotheses they are testing to determine which animal model(s) of podocyte disease should be used and what time points are best to study the prespecified end points. Strain, age, and sex all need to be carefully considered, because they affect disease severity and outcomes. The appropriate controls should always be used, and sufficient numbers of animals are essential for valid statistical analysis. Administering a reagent to rodents does not guarantee disease induction, and therefore validation of disease induction should be checked whenever possible.

In conclusion, because podocyte disease underlies most forms of proteinuric kidney diseases, it is essential that we employ a combined basic, clinical, and translational science research approach to ultimately improve the care of our patients. Animal models of experimental podocyte disease therefore serve an essential and vital approach to addressing these challenges by complementing studies in humans.

REFERENCES
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