Collapsing glomerulopathy (CG) is associated with disorders that markedly perturb the phenotype of podocytes. The *kd/kd* mouse has been studied for immune and genetic causes of microcytic tubulointerstitial nephritis with little attention to its glomerular lesion. Because histologic examination revealed classic morphologic features of CG, the question arises whether podocytes in *kd/kd* mice exhibit additional phenotypic criteria for CG. Utilizing Tg26 mice as a positive control, immunohistochemical profiling of the podocyte phenotype was conducted simultaneously on both models. Similar to Tg26 kidneys, podocytes in *kd/kd* kidneys showed de novo cyclin D1, Ki-67, and desmin expression with loss of synaptopodin and WT-1 expression. Electron micrographs showed collapsed capillaries, extensive foot process effacement, and dysmorphic mitochondria in podocytes. These results indicate that the *kd/kd* mouse is a model of CG and raise the possibility that human equivalents of the *kd* susceptibility gene may exist in patients with CG.


Materials and Methods

Mice

All studies on Tg26 and *B6 kd/kd* tissues complied with Institutional Animal Care and Use Committee regulations of the New York University School of Medicine and the University of Pennsylvania School of Medicine, respectively. Archival formalin-fixed, paraffin-embedded kidneys from six homozygous *B6 kd/kd* mice ranging in ages from 15 to 43 wk and from two 15-wk-old wild-type *B6* controls were studied. Archival formalin-fixed, paraffin-embedded kidneys from three 6-wk-old heterozygous Tg26 mice and from one 6-wk-old nontransgenic littermate were used as positive and negative controls, respectively, for murine CG (20,21).
Figure 1. Collapsing glomerulopathy in B6 kd/kd mice. (A) Normal glomerulus in a B6 wild-type mouse. (B) Normal glomerulus in a nontransgenic Tg26 mouse. (C) B6 kd/kd mouse with glomerular collapse and podocyte hypertrophy and hyperplasia; focal injury to the parietal epithelium is also noted. (D) Tg26 heterozygote with glomerular collapsing features and prominent podocyte hypertrophy and hyperplasia with pseudocrescent formation and bridging to parietal epithelial cells. (E) B6 kd/kd mouse showing glomerular collapse and pseudocrescent formation adjacent to severe tubulointerstitial damage with prominent, protein-filled microcysts. (F) Tg26 heterozygote showing glomerular collapse with pseudocrescent formation adjacent to severe tubulointerstitial damage with prominent microcysts. (G) Electron micrograph of a glomerular capillary in a B6 wild-type mouse showing glomerular basement membranes (GBM) that are normal in thickness and contour, as well as podocytes with well-preserved foot processes. (H) Electron micrograph of a glomerular capillary in a diseased B6 kd/kd mouse shows GBM that are wrinkled and folded (indicating collapse) with subocclusion of the capillary lumen. There is also extensive foot process effacement accompanied by condensation of the actin-based cytoskeleton and swelling of primary cisternae forming truncated cisternae and granular-appearing matrix (arrows). Magnification, ×400 in A through D, ×100 in E and F, ×25,000 in G through J. The sections in A through F are silver-stained.

Ultrastructural Analysis

Small samples of renal cortex from B6 kd/kd mice were fixed in 2.5% glutaraldehyde and 2.0% paraformaldehyde in 0.1 M sodium cacodylate buffer, pH 7.4, overnight at 4°C. Samples were postfixed with 2.0% osmium tetroxide in 0.1 M cacodylate buffer for 1 hr at 4°C. After additional washing in 0.1 M cacodylate buffer and distilled H2O, sections were stained with 2% aqueous uranyl acetate for 30 min at room temperature. Samples were then rinsed in distilled H2O, dehydrated,
Results and Discussion

The glomerular lesion of collapsing glomerulopathy is defined morphologically by the presence of hyperplastic and hypertrophic podocytes overlying collapsed capillary loops in either a segmental or global distribution within the glomerular tuft (1,2). These diseased podocytes undergo a marked perturbation in their mature, quiescent phenotype, characterized by proliferation and dedifferentiation, which is not observed in other proteinuric lesions (8–13). Concurrent examination and quantitation of the morphologic injury within glomeruli of B6 *kd/kd* and Tg26 mice, coupled with quantitative profiling of the podocyte phenotype by immunohistochemical markers, demonstrate that the renal disease in B6 *kd/kd* mice fulfills the criteria for CG (Figures 1 and 2; Table 1). Similar to CG in Tg26 mice (20,21), diseased glomeruli in B6 *kd/kd* mice show segmental and global sclerosis and collapse of capillary loops with folding and wrinkling of the glomerular basement membrane, extensive foot process effacement with marked condensation of the actin cytoskeleton and focal loss of primary processes of podocytes, and hyperplastic and hypertrophic podocytes with de novo cyclin D1, Ki-67, and desmin expression and reduced synaptopodin and WT-1 expression. In addition to these significant alterations to podocytes, focal injury to the parietal epithelium lining Bowman’s capsule is evident in B6 *kd/kd* mice. Despite the variable, age-dependent penetrance of CG in B6 *kd/kd* mice, there appears to be a positive correlation suggesting causality between the extent of glomerular injury and the downstream tubulointerstitial disease in each animal. Together, these data indicate that the B6 *kd/kd* mouse is a previously unrecognized model of CG. Moreover, similar to prior observations of changes to the morphology of mitochondria in the tubular epithelium of B6 *kd/kd* mice (19), abnormal mitochondria are also found in diseased podocytes.

The exact pathogenic steps whereby mutant PLMP causes CG in B6 *kd/kd* mice are not known. Antisera to PLMP localize to dysmorphic mitochondria in renal epithelium of B6 *kd/kd* mice (19). This suggests that mutant PLMP might directly alter mitochondrial function in podocytes, lowering the threshold to injury from energetic stress. This is an attractive hypothesis as CG and focal segmental glomerulosclerosis can develop in patients with genetically-acquired mitochondrial cytopathies (22,23). Furthermore, CG is associated with a growing list of disease stresses (1). If this is indeed correct, B6 *kd/kd* mice would represent the first model of CG due to a mitochondrial disorder, providing a ready system to investigate how environmental factors may influence the manifestation of this abnormality within podocytes. Interestingly, bisphosphonate drugs, small molecules linked to podocyte injury and CG in humans (24,25), can perturb mitochondrial function (26), and human mitochondrial transprenyltransferases sharing homology with PLMP contain specificity determining residues for bisphosphonate binding (*i.e.* the amino acid sequence DDXXD). However, the extent to which bisphosphonates interact with and inhibit human transprenyltransferases is still unclear (Eric Oldfield, University of Illinois at Urbana-Champaign, personal communication).

Alternatively but not mutually exclusively, an aberrant autoimmune-like response to renal parenchymal damage specific to B6 mice may dictate the development of CG in this model, as suggested by prior studies on B6 *kd/kd* mice (18). Although the phenotypic manifestation of renal disease after transfer of the *kd* susceptibility gene to B6 mice, a strain biased toward T-
### Table 1.

| Mouse Age (wk) | Podocyte Hyperplasia/Fibrosis | Podocyte Hypertrophy | Tubular Atrophy | Interstitial Fibrosis | Interstitial Inflammation | Interstitial Microcysts/Acute Tubular Injury | Glomerulosclerosis | Segmental Sclerosis | Global Sclerosis | Segmental Collapse | Global Collapse |
|---------------|-------------------------------|----------------------|-----------------|----------------------|--------------------------|---------------------------------|-------------------|----------------|----------------|----------------|----------------|----------------|
| B6 wild-type  | 0                             | 0                    | 0               | 0                    | 0                        | 0                               | 0                 | 0              | 0              | 0              | 0              | 0              |
| B6 kd/kg     | 0                             | 0                    | 0               | 0                    | 0                        | 0                               | 0                 | 0              | 0              | 0              | 0              | 0              |
| Tg26 nontransgenic | 0                          | 0                    | 0               | 0                    | 0                        | 0                               | 0                 | 0              | 0              | 0              | 0              | 0              |
| Tg26 heterozygote | 0                          | 0                    | 0               | 0                    | 0                        | 0                               | 0                 | 0              | 0              | 0              | 0              | 0              |

**References**

7. Matussaka T, Xin J, Niwa S, Kobayashi K, Akatsu A, Hashizume H, Wang QC, Pastan I, Fogo AB, Ichikawa I: helper type 1 immunity (27), appears to be identical to that of the founder strain, we do not know if the degenerate glomeruli, glomerulosclerosis, and albuminuria noted in the original report on CBA/CaH kd/kd mice (14) is a product of the same podocytopathy reported here (i.e., CBA/CaH kd/kd tissues are no longer available). Indeed, although specific genes that may modify the nephropathy in B6 kd/kg have not been identified, the CG in B6 kd/kg mice may ultimately be attributable to background genetic differences between strains of mice. For example, using a mouse genetics approach with Tg26 mice to investigate the racial predilection of HIV-induced CG, Gharavi et al. identified susceptibility loci and strain-specific modifications to specific features of the renal disease in this model (28), including amelioration by BALB/C mice, a strain biased toward T-helper type 2 immunity (27). These intriguing observations regarding what is likely to be a polygenic disease raise the possibility that human equivalents of the kd susceptibility gene may exist and predispose some patients to develop CG. Further studies on B6 kd/kg mice, the first model of CG caused by a spontaneously occurring mutation identified through forward genetics, not a product of reverse genetics (3,4,6,7,20), and of patients with CG will help answer these questions.

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