The \textit{kd/kd} Mouse Is a Model of Collapsing Glomerulopathy

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Collapsing glomerulopathy (CG) is associated with disorders that markedly perturb the phenotype of podocytes. The \textit{kd/kd} mouse has been studied for immune and genetic causes of microcystic tubulointerstitial nephritis with little attention to its glomerular lesion. Because histologic examination revealed classic morphologic features of CG, the question arises whether podocytes in \textit{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 \textit{kd/kd} kidneys showed \textit{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 \textit{kd/kd} mouse is a model of CG and raise the possibility that human equivalents of the \textit{kd} susceptibility gene may exist in patients with CG.

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 processes. (I) A healthy podocyte from a B6 wild-type mouse containing few normal mitochondria with regular matrix density (arrows). (J) A diseased podocyte from a B6 kd/kd mouse containing numerous abnormal mitochondria with compressed cristae 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.

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 with wrinkled foot processes, abnormal mitochondria, and intramembranous deposits. (I) A healthy podocyte from a B6 wild-type mouse showing numerous normal mitochondria with compressed cristae (arrows). (J) A diseased podocyte from a B6 kd/kd mouse showing numerous abnormal mitochondria with compressed cristae (arrows).
infiltrated, and embedded in Embed 812 (Electron Microscopy Science, Fort Washington, PA). Sections were examined in a JEOL100CX electron microscope. Digital images recorded on a Hamamatsu camera were analyzed for the presence of folding and wrinkling of the glomerular basement membrane and foot process effacement.

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. Morphologic injury and changes to the podocyte phenotype

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<th>Mouse Age (wk)</th>
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<th>Hypertrophy</th>
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References

7. Matussaka T, Xin J, Niwa S, Kobayashi K, Akasuka 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/kd* have not been identified, the CG in B6 *kd/kd* 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/kd* 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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