Renal Pathology in Fabry Disease

JOSEPH ALROY,* SHARDA SABNIS,† and JEFFREY B. KOPP‡

*Department of Pathology, Tufts University Schools of Veterinary Medicine and Medicine, and New England Medical Center, Boston, Massachusetts; †Division of Nephrology, Armed Forces Institute of Pathology, Washington, DC; and ‡Kidney Disease Section, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland.

Fabry disease is an X-linked recessive lysosomal storage disease that is caused by deficient activity of the lysosomal enzyme α-galactosidase A (α-Gal A), an enzyme that cleaves terminal α-galactosyl residues. This deficiency results in progressive lysosomal accumulation of glycosphingolipid with terminal α-galactosyl residues, particularly globotriaosylceramide (Gb3). Gb3 accumulates in many cells, particularly in renal epithelial cells, endothelial cells, pericytes, vascular smooth muscle cells, cardiomyocytes, and neurons of the autonomic nervous system (1).

The genetic defect occurs in all cell types, but involvement differs greatly among different organs and cell types. This heterogeneity likely reflects different rates of sphingolipid metabolism. Thus the minimum threshold requirement for α-Gal A activity to prevent Gb3 accumulation varies across cell types due to the type and amount of substrates that are recycled by the different cells (2). Renal lesions are found in both hemizygous (male) and heterozygous (female) patients. Renal symptoms in the latter are typically milder and delayed by 2 to 3 decades, but there is considerable variability (3). The variability is likely the result of the random nature of X inactivation, resulting in considerable variability in α-Gal A activity among carriers and (at least theoretically) within one carrier individual among various tissues or regions of a single tissue.

Light Microscopy

The renal pathology of Fabry disease was first described just over 50 years ago and has been the subject of numerous case reports and series since then (4). Glomeruli on light microscopy show hypertrophic glomerular visceral epithelial cells (podocytes) distended with foamy appearing vacuoles, mesangial widening, and varying degrees of glomerular obsolescence (Figure 1, A and B). Within the glomerulus, the largest amount of lipid material is seen in podocytes, followed by the parietal epithelial, mesangial, and glomerular endothelial cells. With disease progression, glomeruli exhibit mesangial widening, in some cases segmental glomerulosclerosis, and ultimately global glomerulosclerosis.

Vacuolation is also present in the capillary endothelium and distal tubular epithelial cells, including those of Henle’s loop and the collecting duct, particularly intercalated cells (Figure 1C), and less commonly in proximal tubular epithelial cells. Vascular involvement includes deposition in capillary, arterial, and arteriolar endothelial cells, pericytes, and smooth muscle cells (Figure 1D and Figure 2B). In severe disease, there is progressive tubular atrophy, interstitial fibrosis, and varying amounts of interstitial fibrosis.

In general, making the diagnosis of Fabry’s disease on a needle biopsy is not difficult, particularly if electron microscopy is performed. There is somewhat more difficulty in determining whether the disease has progressed or stabilized over a period of time, because damage is not uniform in all glomeruli or all areas of the tubulointerstitium. Therefore, clinical research protocols that rely on histologic scores to determine outcome will require careful attention to the method of scoring renal pathology, and an adequate amount of tissue must be available.

Various special staining procedures may aid in making the diagnosis of Fabry disease on the basis of accumulation of sphingolipid. On tissue that has been subjected to routine processing, which removes some lipid components, hematoxylin and eosin or periodic acid Schiff (PAS) stains demonstrate only vacuolated cells. The storage material can be stained on routine sections with Luxol fast blue, which identifies polar lipids (5). On frozen sections, the storage material can be demonstrated with several approaches, including staining with PAS, Luxol fast blue, Oil red O (6), and Sudan black (6,7). Greater specificity can be obtained by using the lectins, Griffonia (Bandeiraea) simplicifolia-I and Ricinus communis-I, which identify α- and β-galactosyl residues (7). Furthermore, under polarized light, the storage material is birefringent and exhibits autofluorescence. Fabry deposits are best demonstrated using tissue that has been fixed in glutaraldehyde or Trump’s fixative, embedded in Epon, and stained with toluidine blue or methylene blue/azure II, yielding dark blue cytoplasmic inclusions (Figure 1, B through D).

Immunofluorescence is generally not contributory, although some patients may have focal mesangial deposits of C3 or IgM in a nonspecific fashion (3).

Electron Microscopy

Electron microscopic studies demonstrate enlarged secondary lysosomes (myeloid or Zebra bodies) packed with lamel-
lated membrane structures (Figure 2, A and B). These inclusions can vary in appearance, from granular to lamellated, the latter being more diagnostic. The periodicity of the lamellated membrane structures when measured using routine plastic thin sections is estimated to be 4 to 5 nm (3,8), but the periodicity of their structures is 14 to 15 nm when studied by freeze fracture electron microscopy, due to better tissue preservation (8).

With progression of the disease, there is fusion of podocyte foot processes in association with advancing proteinuria, occasionally focal glomerular and tubular epithelial necrosis, and thickening of glomerular and tubular basement membranes (3).

In our studies, focal duplication of glomerular basement membrane was noted (Alroy J, unpublished findings).

The presence of other, coexisting renal diseases may complicate the pathologic picture. Indeed, 2 of 21 Fabry patients who had renal tissue studied by electron microscopy in the context of a prospective therapeutic trial had membranoproliferative glomerulonephritis with subendothelial immune deposits, in one case associated with hepatitis C infection (Alroy J, unpublished findings).

Figure 1. Renal pathology in Fabry disease. (A) Glomerulus showing extensive inclusion bodies of glycolipid in podocytes (arrowhead), and mild mesangial widening (PAS stain; magnification, ×80). (B) Plastic embedded tissue showing in-site deposition of glycolipid in glomerular podocytes (arrowhead; toluidine blue stain; magnification, ×80). (C) Plastic embedded renal tissue demonstrating glycolipid inclusion bodies in distal tubules (asterisk), with relative sparing of proximal tubules, and interstitial fibrosis (toluidine blue stain; magnification, ×80). (D) Deposition of glycolipid in endothelial cells of peritubular capillaries (asterisk; toluidine blue stain; magnification, ×200). (E) Urine showing vacuolated urinary epithelial cells (oval fat bodies) in a Fabry patient (Papanicolaou stain; magnification, ×160). Figure modified with permission from Branton et al. (14).
Urine cytology presents a noninvasive and underutilized approach to making the diagnosis of Fabry disease or demonstrating renal involvement with Fabry disease, although the sensitivity of this approach, particularly early in the course of renal disease, is unknown. Most cells (76%) present in the urine of Fabry patients are tubular epithelial cells (9). Fabry inclusions can be identified with the PAS or Papanicolau stains.

Figure 2. Electron microscopy in Fabry disease. (A) Electron microscopy of a renal biopsy obtained from a 26-yr-old male Fabry patient demonstrates accumulation of storage material within the secondary lysosomes of podocytes and glomerular parietal epithelial cells. There is focal foot process effacement. There is free glycolipid in the urinary space and a thickened Bowman’s capsule (magnification, ×3760). (Inset) Higher magnification demonstrates enlarged lysosomes within a podocyte and a mixture of lamellated membrane structures and small vesicles in the glomerular parietal epithelium (magnification, ×11,200). (B) Electron microscopy of renal biopsy obtained from a male Fabry patient shows a small artery, with lysosomal storage located within endothelial cells (E) and smooth muscle cells (S) (magnification, ×3800). (Inset) Higher magnification of the endothelial cell cytoplasm demonstrates enlarged secondary lysosomes packed with storage material, which has a somewhat heterogeneous appearance (magnification, ×11,200).

Urine Cytology

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(Figure 1E), which demonstrate vacuolated renal epithelial cells. Electron microscopy demonstrates the electron-dense, multilamellar inclusions within lysosomes of tubular epithelial cells and macrophages. Similarly, Gb3 can be measured in urinary sediment.

Kidney in Renal Transplantation

Renal tissue of well-functioning allografts examined 6 and 8 mo after renal transplant has shown Fabry inclusions in the vascular endothelium, demonstrable only by electron microscopy (7,10). This has been proposed to represent colonization of the allograft vasculature by host endothelial cells, a process that is believed to affect all renal allografts (11). Appearance of Fabry inclusions in glomerular or tubular cells appears more rarely and after a longer duration; presumably, these cells are relatively protected from elevated circulating levels of sphingolipids by normal cellular expression of α-Gal A, as these cells are of donor origin (12,13).

Mechanisms of Renal Injury

We have recently reported that renal Gb3 content, renal pathology, and renal function correlate with residual α-Gal A activity in leukocytes (14). If renal α-Gal A activity correlates with leukocyte α-Gal A activity (a reasonable but untested assumption), this suggests that residual enzyme activity in renal parenchymal cells retards progression of renal disease. One case report has suggested that renal α-Gal A activity was reduced compared with liver α-Gal A activity when each was expressed as a fraction of normal α-Gal A activity in that organ; the mechanism for such a finding is unclear (15). Similarly, we found that Fabry patients with conservative missense mutations (defined as those that do not change the amino acid residue class) have delayed appearance of renal disease compared with patients with nonconservative missense mutations or other mutations (those resulting in deletions, insertions, or premature stop codons) (14).

We propose three mechanisms that might explain the segmental and global glomerulosclerosis that characterizes Fabry disease: microvascular disease, podocyte injury, and tubulo-interstitial injury.

Gubler et al. (3) made the perspicacious observation that in older Fabry patients, those 25 to 50 yr old, the progressive renal pathologic changes in the glomeruli and tubulointerstitium may be related to ischemic change. These changes include glomerulosclerosis, often with wrinkled and partially collapsed glomerular basement membrane, tubular atrophy, interstitial fibrosis, and vascular thickening. These changes were generally absent or mild in patients under 25 yr of age. In particular, these investigators noted that the earliest and most consistent degenerative alteration was arterial “fibrinoid” deposits and suggested that these were due to necrosis of smooth muscle cells fatally overloaded with Gb3 deposits. Hypertension is not a common feature of Fabry disease, although it may occur with progressive renal dysfunction (14). Therefore, following Gubler and colleagues, we hypothesize that one mechanism of renal injury in Fabry disease is accumulation of Gb3 within the arterial vessel wall and subsequent vascular compromise. In this regard, the renal vasculature is similar to the coronary and cerebral vessels, in which large vessel deposition of Gb3 is associated with premature vascular disease that is responsible for premature death in many patients.

Toxic accumulation of Gb3 within the podocyte may constitute a second important mechanism of glomerular injury. Podocytes are highly differentiated cells; their foot processes and slit-diaphragms constitute a critical portion of the glomerular filtration barrier that retards the entry large molecules into the urinary space. These cells are postmitotic and fail to undergo proliferation under most pathologic circumstances (an exception being the collapsing variant of focal segmental glomerulosclerosis), which means that they generally are not replaced when they are lost due to lethal injury. Kriz and Lemley (16) have proposed that when podocytes are lost, the denuded glomerular basement contacts the parietal epithelial cells and forms a synechia. Within the synechia, there is activation and proliferation of cells, especially mesangial cells, the entry of immune cells, including macrophages, and the accumulation extracellular matrix protein. This repair response may be driven in part by the leakage from the circulation into the synechia of macromolecules, including cytokines, chemokines, and growth factors, via the impaired glomerular filtration barrier. Matrix expansion and subsequent collapse of the capillary loop appears as the focus of solidification. This constitutes the lesion of segmental glomerulosclerosis, which progresses to global glomerulosclerosis. We propose as a working hypothesis that Gb3 induces podocyte injury, resulting in focal and ultimately global glomerulosclerosis.

Deposition of Gb3 within tubular epithelial cells may lead to focal tubular atrophy and interstitial fibrosis. As this process progresses, the glomeruli upstream of more severely affected tubules may function poorly or not at all. Other glomeruli may undergo hypertrophy to compensate, and hyperfiltration in these glomeruli may trigger a secondary form of focal segmental glomerulosclerosis. Evidence in support of this mechanism would include demonstration of glomerular enlargement, particularly in the early stages of glomerular and tubular injury in Fabry disease; these glomerular measurements have not been published to date.

What then are the most suitable pathologic measures to define the status of Fabry renal disease in a given patient and to predict whether therapeutic intervention will likely alter the future course of renal dysfunction in that patient? Eng et al. (17) selected the change in microvascular inclusions in interstitial endothelial cells of kidney, heart, and skin as a primary endpoint in their recent trial of α-Gal A replacement. We feel that the importance of this parameter as a potential surrogate marker for the progression of renal dysfunction (impaired GFR) and other renal pathology (glomerulosclerosis, interstitial fibrosis) is uncertain and needs to be tested in a longitudinal study. We have used additional pathologic markers, including mesangial expansion, glomerulosclerosis, and interstitial fibrosis in another recent trial of α-Gal A replacement (18). Arguably, these pathologic markers are also untested as suitable markers for progressive renal functional decline in Fabry disease. Nevertheless, similar composite in-
dices have been used to measure disease progression, such as lupus nephritis (19). Mesangial expansion correlates with impaired GFR in diabetic nephropathy (20). Interstitial fibrosis has long been recognized as correlating with GFR in most renal diseases (21). The extent of glomerulosclerosis does not correlate as well, perhaps due to the complete reabsorption of globally obsolescent glomeruli, leading to a milder pathology score than would otherwise be the case.

Conclusion
In Fabry disease, the kidney is affected in all male hemizygotes and in some female heterozygotes. Histologically, renal involvement is characterized by glycolipid deposits in glomerular cells (particularly in podocytes and also in mesangial cells and endothelial cells), tubular epithelial cells (particularly of the distal nephron), and vascular cells (endothelial cells of capillaries, veins, and arteries, and vascular smooth muscle cells). Progression of kidney disease is characterized by segmental and global glomerulosclerosis, tubular atrophy, and interstitial fibrosis. In patients with Fabry disease, renal involvement may be suspected by the appearance of proteinuria and/or renal insufficiency. The urine sediment contains cells with characteristic inclusions demonstrated by light microscopy and electron microscopy. Renal biopsy is occasionally helpful in making the diagnosis of Fabry disease when this diagnosis was not previously entertained, but in general other approaches (measurement of plasma or leukocyte α-Gal A activity, skin biopsy, examination of the urine sediment, or sequencing of the α-gal A gene) offer less invasive ways to establish the diagnosis. On the other hand, renal biopsy may be helpful in excluding coexisting renal diseases, and quantitative analysis of renal biopsy material in future studies may be useful in evaluating the efficacy of new treatments for Fabry disease.

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References