Abstract. Podocytes are highly differentiated, postmitotic cells, whose function is largely based on their complex cytoarchitecture. The differentiation of podocytes coincides with progressive expression of maturity markers, including WT-1, CALLA, C3b receptor, GLEPP-1, podocalyxin, and synaptopodin. In collapsing forms of focal segmental glomerulosclerosis (FSGS), including idiopathic FSGS and HIV-associated nephropathy, podocytes undergo characteristic, irreversible ultrastructural changes. This study analyzes the expression pattern of the above differentiation markers and of the proliferation marker Ki-67 in collapsing idiopathic FSGS and HIV-associated nephropathy compared with minimal change disease, membranous glomerulopathy, as well as normal adult and fetal human kidney. In minimal change disease and membranous glomerulopathy, all mature podocyte markers were retained at normal levels despite severe proteinuria and foot process fusion; no cell proliferation was observed. In contrast, in collapsing idiopathic FSGS and HIV-associated nephropathy, there was disappearance of all markers from all collapsed glomeruli and of synaptopodin from 16% of noncollapsed glomeruli. This phenotypic dysregulation of podocytes was associated with cell proliferation in both diseases. It is concluded that the loss of specific podocyte markers defines a novel dysregulated podocyte phenotype and suggests a common pathomechanism in collapsing FSGS, whether idiopathic or HIV-associated.

Podocytes are highly specialized cells whose functions include support of the glomerular capillaries, synthesis of glomerular basement membrane, and regulation of glomerular permselectivity. These complex functions depend on a highly differentiated and unique cytoarchitecture. Based on their cytoarchitecture, podocytes may be divided into three structurally and functionally different segments: cell body, major processes, and foot processes. This segmentation of podocytes is also observed at the level of the cytoskeleton. Microtubules and intermediate filaments predominate in cell bodies and major processes. The foot processes contain an actin-based contractile apparatus, which is linked to the GBM at focal contacts by an α5β1-integrin complex (reviewed in reference (1)).

The expression of these specialized cellular features is developmentally regulated. As the developing glomerulus passes from the vesicle stage via the S-shaped body stage to the capillary loop stage and the mature glomerulus, podocytes acquire their characteristic complex cell architecture including foot processes and slit diaphragms (2,3). This maturation is associated with a loss of proliferative activity and progressive expression of distinctive podocyte markers, including the Wilms' tumor protein WT-1, CALLA (common acute lymphoblastic leukemia antigen), C3b receptor, GLEPP-1 (glomerular epithelial protein-1), podocalyxin, and synaptopodin.

WT-1 is a critical protein in nephrogenesis, as indicated by total failure of metanephric development in WT-1-deficient mice (4). Early in glomerular development, WT-1 is widely expressed in glomerular progenitor cells, but becomes restricted to podocytes as the glomerulus matures. WT-1 is a zinc finger transcription factor that downregulates proliferation (5), but may also be necessary to maintain the mature podocyte phenotype (6).

On their apical surface podocytes are equipped with a negatively charged glycocalyx which plays a dual role in permselectivity and maintenance of foot process cytoarchitecture. Podocalyxin, the major sialoglycoprotein of this glycocalyx, first appears in the S-shaped body stage of the developing glomerulus (7). In addition to podocytes, podocalyxin is also expressed by endothelial cells (8). GLEPP-1, a podocyte-specific protein tyrosine phosphatase, is another integral apical membrane protein that first appears in the S-shaped body stage. It has been proposed that signal transduction through GLEPP-1 is involved in regulation of foot process dynamics, cell differentiation, and contact inhibition (9).

A convergence has been demonstrated between antigenic determinants of B lymphocytes and leukemic cells on the one hand and podocytes of fetal and adult kidney on the other. Acquisition or loss of these hematopoietic antigens has been
correlated with the various stages of nephron differentiation and development (10). Some of these antigens, such as CD35, are expressed only transiently during the early stages of glomerulogenesis, whereas others such as CALLA and C3b receptor, whose expression first appears in the S-shaped body stage, are maintained in podocytes of the mature glomerulus (10,11).

Synaptopodin represents a novel proline-rich actin-associated protein of telencephalic dendrites and glomerular podocytes that may play a role in the actin-based shape and motility of podocyte foot processes (12). During rat kidney development, synaptopodin first appears at the capillary loop stage. Thus, the expression of synaptopodin coincides with the formation of foot processes (13). Because of its association with a highly developed cytoskeletal architecture, synaptopodin is an important marker of the mature podocyte phenotype.

In glomerular diseases with massive proteinuria, podocytes undergo dramatic structural alterations. In some conditions, such as minimal change disease, proteinuria and podocyte cellular alterations are reversible, whereas in other conditions, such as focal segmental glomerulosclerosis (FSGS), they are not. Among the many forms of FSGS (14), the glomerular degeneration in collapsing idiopathic glomerulosclerosis and HIV-associated nephropathy differs from that of other forms by its more severe clinical presentation, prognosis, and morphologic features. In these two diseases, the glomerular alterations are characterized by segmental or global tuft collapse, and most striking, the crowding of epithelial cells on the outer aspect of the tuft. It also differs in its lack of synechiae, at least in the early stages. It has been suggested that a primary podocyte injury plays a central role in the pathogenesis of certain forms of FSGS (15), although the nature of this injurious factor remains to be discovered.

The current study was designed to investigate to what extent the morphologic alterations seen in collapsing idiopathic glomerulosclerosis and HIV-associated nephropathy reflect an alteration of the mature podocyte phenotype. Based on the loss of all specific structural features as well as the loss of specific markers of differentiated podocytes, we propose a new pathogenetic mechanism of a dysregulated podocyte phenotype for both collapsing idiopathic glomerulosclerosis and HIV-associated nephropathy.

### Materials and Methods

We selected 28 cases from the archives of the Renal Pathology Laboratory at Columbia Presbyterian Medical Center, including 10 cases of collapsing idiopathic FSGS, eight cases of HIV-associated nephropathy, five cases of minimal change disease, and five cases of membranous glomerulopathy. Normal fetal and adult kidney were used as controls.

Three-micrometer-thick sections from each biopsy were stained with hematoxylin and eosin, periodic acid-Schiff, trichrome, and silver methenamine. Focal segmental and global glomerular sclerosis as well as glomerular collapse were evaluated and expressed as percentage of affected glomeruli per total number of glomeruli in each biopsy for each category. Sclerosis was defined as an expansile scar of the glomerular tuft characterized by increased matrix, with or without associated hyalinosis and adhesion to Bowman’s capsule. Collapse was defined as an implosive wrinkling and retraction of the glomerular basement membrane, with resulting narrowing or obliteration of capillary lumina and hypertrophy of overlying extracapillary cells (16).

### Immunohistochemistry

To characterize the expression profiles of podocyte marker proteins in glomerular development and in mature kidney, sections of normal fetal and adult human kidney were studied by immunohistochemistry using the full panel of podocyte-specific antibodies (Table 1). In addition, the proliferative activity of podocytes was analyzed using an antibody directed to the cell cycle protein Ki-67. Serial 3-μm-thick sections of formalin-fixed paraffin-embedded tissue were obtained from the cases listed above. The sections were rehydrated in a graded series of alcohol, blocked with 10% normal goat serum for polyclonal antibodies in 1% bovine serum albumin and 10% normal horse serum for monoclonal antibodies in 1% bovine serum albumin, and stained using avidin-biotin immunoperoxidase technique with monoclonal antibodies against GLEPP-1 (reference 9; courtesy of R.C. Wiggins, Ann Arbor, MI), podocalyxin (courtesy of R.C. Wiggins), synaptopodin (12,13), and Ki-67 (Clone MIB1, Dako), and a polyclonal anti-serum against WT-1 (C19, Santa Cruz Biotechnology). Sections were predigested by microwaving for 25 min and then incubated overnight with synaptopodin (1:20), GLEPP 1 (1:10), podocalyxin (1:10), Ki-67 (1:500), and WT-1 (1:200). Horse anti-mouse IgG and goat anti-rabbit IgG (both from Dako) were used as secondary antibodies (1:100) for 30 min. Sections were incubated for 30 min with avidin-biotin complex (1:10; Vector) and developed with diaminobenzidine as chromogen.

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<th>Table 1. “Expression of Ki-67 and podocyte maturity markers in glomerular diseases with nephrotic syndrome and in normal adult human kidney”*</th>
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*WT-1, Wilms’ tumor protein-1; GLEPP-1, glomerular epithelial protein-1; CALLA, common acute lymphoblastic leukemia antigen; HIV-AN, HIV-associated nephropathy; FSGS, focal segmental glomerulosclerosis; MCD, minimal change disease; MGN, membranous glomerulopathy; NAK, normal adult human kidney.
The percentage of glomeruli with one to five cells expressing Ki-67 was calculated in each category of disease. The percentage of non-sclerotic/noncollapsed glomeruli with decreased expression of three maturity markers (GLEPP-1, podocalyxin, synaptopodin) was also calculated in each category.

Frozen sections were obtained from fetal human kidney, adult normal kidney, and from 10 of the above cases, including membranous glomerulopathy (three cases), minimal change disease (one case), collapsing idiopathic FSGS (two cases), and HIV-associated nephropathy (four cases). Sections were fixed in acetone and incubated with monoclonal antibodies to CALLA (1:20; Innovex Biosciences) and C3b receptors (1:100; Accurate Chemical Scientific Corp.) for 1 h. The immunolabeling was visualized with the same immunoperoxidase technique described above.

Transmission Electron Microscopy

Specimens for transmission electron microscopy were fixed with 2.5% glutaraldehyde in phosphate-buffered saline (PBS), washed with PBS, and incubated with OsO4 for 60 min. The sections were counterstained with 1% tannic acid in PBS for 15 min, dehydrated in a graded series of ethanol (30, 50, 70, 95, and 100%), and finally embedded in Epon 812 according to standard procedures. Ultrathin sections were cut with a Reichert Jung Ultracut E ultramicrotome and studied under a Philips EM 301 electron microscope.

Results

Expression of the Cell Cycle Marker Ki-67 and of Mature Podocyte Marker Proteins during Renal Development and in Normal Adult Human Kidney

During ontogeny, podocytes develop from simple epithelial cells of the S-shaped body, where they divide avidly. Later, during the transition to the capillary loop stage, the cells stop dividing and transform into the mature mesenchymal-like cells with the characteristic pattern of primary and foot processes.

Expression of Ki-67 was found in up to 90% of cells comprising the glomerular vesicles, the earliest stage of glomerular development, with progressive reduction in the percentage of Ki-67-expressing cells in the course of glomerular maturation (Figure 1a). In the normal adult kidney, no Ki-67-expressing podocytes were observed. WT-1 was weakly expressed in all cells of renal vesicles, showed increased staining during the S-shaped body stage, and became restricted to podocytes in the capillary loop stage of development (Figure 1b) and in the adult kidney, where strong nuclear labeling of podocytes was found.

The expression of C3b receptor (data not shown), GLEPP-1 (Figure 1c), and podocalyxin (Figure 1d) commences at the early S-shaped body stage, increases thereafter, and persists at high levels in the adult podocytes. Podocalyxin was also strongly expressed in all vascular endothelia including glomerular endothelial cells. The expression of CALLA (data not shown) and synaptopodin (Figure 1e) was first seen at the transition from the late S-shaped body stage to the capillary loop stage. Synaptopodin staining was restricted to the basal portion of the podocytes, where foot processes form. In contrast, GLEPP-1 and podocalyxin were found in a polarized manner at the apical surface of the podocytes. C3b receptor and CALLA showed no preferential intracellular distribution (data not shown). Thus, the expression of podocyte markers in developing human kidney is consistent with previously described expression patterns of these proteins in developing rodent kidney (reviewed in reference (1)).

Dysregulation of Podocyte Phenotype in Collapsing Idiopathic FSGS and HIV-Associated Nephropathy Versus Minimal Change Disease and Membranous Glomerulopathy

We next analyzed the expression of the cell cycle marker Ki-67 and of the above podocyte markers in renal biopsies from collapsing idiopathic glomerulonephrosis and HIV-associated nephropathy (HIV-AN) compared with two other glomerular diseases associated with morphologic alterations of podocytes and nephrotic syndrome, i.e., minimal change disease (MCD) and membranous glomerulopathy (MGN).

Proteinuria was in the nephrotic range (>3.0 g/24 h) in 27 of 28 patients at the time of the biopsy. Proteinuria was more severe in patients with collapsing idiopathic glomerulonephrosis (mean 13.9 g/24 h) and MCD (mean 12.3 g/24 h) compared with HIV-AN (mean 7.0 g/24 h) and MGN (mean 9.8 g/24 h).

The biopsies from patients with MCD revealed no labeling of Ki-67, indicating the absence of cell cycle-engaged podocytes (Figure 2a). The staining profile of the podocyte marker proteins in MCD (Figure 3, c, e, and g) was virtually identical to that of normal adult kidney, with retention of all markers studied. This persistence of the mature podocyte phenotype was observed despite severe foot process effacement and high levels of proteinuria.

In MGN, another form of idiopathic nephrotic syndrome associated with severe morphologic alterations of podocytes including foot process fusion, the findings were similar to those observed in MCD. No proliferating podocytes were detected with Ki-67 immunostaining. The expression pattern of mature podocyte markers was comparable to that seen in normal adult kidney. The single exception was loss of mature podocyte markers overlying a lesion of segmental sclerosis in a single glomerulus from one of the five cases studied (data not shown).

The results in collapsing idiopathic FSGS and HIV-AN are virtually identical, both clinically and morphologically, and therefore will be described together. In agreement with previous studies (16–18), changes in podocytes appear to crucially underlie glomerular degeneration in these diseases. Podocytes lose many of their specific attributes including cellular architecture, cell cycle characteristics, and specific nuclear, cytoplasmic, and membrane proteins. In contrast to MCD and MGN, in both collapsing idiopathic glomerulonephrosis and HIV-AN, Ki-67-positive cells were seen in regions where the number of epithelial cells on the outer aspect of the tuft was obviously increased (Figure 2b). Eleven percent of glomeruli in collapsing idiopathic FSGS and 10% of glomeruli in HIV-AN showed from one to five podocytes with nuclear staining for Ki-67. We observed extensive loss of all podocyte markers (CALLA, C3b receptor, GLEPP-1, podocalyxin, synaptopodin, and WT-1) in all glomeruli with collapsing sclerosis (Figure 2D; Figure 3, d, f, and h). In segmentally affected
Figure 1. Expression of podocyte markers during normal human glomerulogenesis. (a) In the S-shaped bodies, most of the cells show nuclear positivity for the proliferation marker Ki-67. At the capillary loop stage, the absence of Ki-67 expression is consistent with entry of podocytes into G0 (resting phase) of the cell cycle. (b) WT-1 is weakly expressed in all cells in the early stages, but later becomes restricted to podocytes. (c) The tyrosine phosphatase GLEPP-1 is focally expressed in an apical localization during the S-shaped body stage and becomes restricted to podocytes in the capillary loop stage and in maturing glomeruli. (d) Podocalyxin has a similar distribution pattern as GLEPP-1, but in addition to podocytes is also found in endothelial cells. (e) The expression of synaptopodin commences at the transition from the S-shaped body to the capillary loop stage and coincides with podocyte foot process formation. S, S-shaped body stage; C, capillary loop stage; M, maturing glomerulus.
glomeruli (26% of glomeruli in collapsing idiopathic FSGS and 38% in HIV-AN), this loss was restricted to collapsed tuft areas, where the typical crowding of epithelial cells on the outer tuft surface was seen. In globally affected glomeruli (31% of glomeruli in collapsing idiopathic FSGS and 32% in HIV-AN), the loss was total. However, a reduction in synaptopodin expression (in contrast to the other markers) was also frequently observed in unaffected portions of the tuft of segmentally injured glomeruli, or even in glomerular profiles without any visible collapse or other histologic abnormalities (Figure 4, b and d). In collapsing idiopathic FSGS and HIV-AN, 16% of the histologically unaffected (i.e., nonsclerotic and noncollapsed) glomeruli showed marked reduction of synaptopodin expression versus only 3% in collapsing idiopathic FSGS and 4% in HIV-AN for GLEPP-1 and 0% in both categories for podocalyxin. With respect to the staining pattern of podocalyxin, an interesting observation was made. Glomerular profiles with global collapse were frequently found in which podocalyxin staining of podocytes had totally disappeared, whereas staining of endothelial cells was clearly maintained (Figure 3f). The results of the immunohistochemical studies are summarized in Table 1.

**Ultrastructural Alterations in Collapsing Forms of Glomerulosclerosis**

The loss of specific podocyte markers in these two diseases was accompanied by characteristic structural changes, among which the most prominent and characteristic were tuft collapse and the accumulation of epithelial cells on the outer aspect of the glomerular basement membrane (GBM) in the collapsed areas (Figure 5). In most of the affected glomeruli the tuft collapse was global, but some glomerular profiles were consistently encountered in which the injury was restricted to a portion of the tuft. The collapse was associated with a wrinkling and progressive thickening of the GBM and the disappearance of capillaries (Figure 5). Normally, the GBM is clearly separated from adjacent portions of the GBM either by the mesangium along its inner aspect or by podocytes along its...
Figure 3. Differential expression of mature podocyte markers in MCD and HIV-AN. (a, c, e, g) MCD. (b, d, f, h) HIV-AN. (a and b) Silver methenamine. In MCD, the normal pattern of the glomerular capillaries is well preserved (a), whereas in HIV-AN the glomerular tuft shows global collapse (b). The podocytes overlying the collapsed capillaries are hypertrophied. (c and d) GLEPP-1 is expressed in MCD at levels comparable to that of normal adult kidney (c), but has almost completely disappeared from the podocytes in the collapsed tuft (d). (e and f) In MCD, podocalyxin is expressed in podocytes and endothelial cells (e). In HIV-AN, podocalyxin disappears from podocytes, but is preserved in glomerular endothelial cells (f). (g and h) Synaptopodin is strongly positive in MCD (g) but is completely lost from podocytes in glomeruli with collapsing sclerosis (h). Magnification, × 260.
outer aspect. In the collapsed glomeruli, this separation is lost locally at both sites followed by a merging of folded segments of GBM, producing an irregular and “anastomosing” GBM network (Figure 5).

The second prominent structural change is the “crowding” (probably hyperplasia) of epithelial cells in Bowman’s space (Figure 5). In early stages of glomerular collapse, these cells can be identified as podocytes or podocyte-like cells by their attachment to the outer aspect of the GBM; the visceral and parietal cells are still separated by a urinary space. In later stages, such a space has disappeared rendering a distinction between parietal and visceral epithelial cells impossible. Areas with loss of cells leading to a peripheral exposure of naked GBM areas or tuft adhesions to Bowman’s capsule were not observed.

The cells that finally fill the entire Bowman’s space do not possess structural characteristics of the developed podocyte cell architecture. They are large, pale polygonal cells smoothly adhering to the GBM (Figure 6a) or to intervening newly formed extracellular matrix material (Figure 6b). Intermediates between a normal podocyte and the fully dysregulated phenotype point to a gradual loss of characteristic cellular features, which is different from what is usually seen when podocytes (e.g., in secondary forms of glomerular sclerosis) undergo simplification and degeneration (19,20). In addition to the loss of foot processes, these cells lose their ultrastructurally visible actin cytoskeleton (Figure 6c). In early stages of cell injury (i.e., partially injured cells with maintenance of some of their primary and foot processes), patches of the actin cytoskeleton are seen in the basal cytoplasm of the “fused” cell areas (Figure 6b). However, we never observed the rearrangement of the actin cytoskeleton into a continuous cytoskeletal mat (as occurs in MCD and MGN). In contrast, the cytoskeleton disappears leading eventually to the phenotype of pale, seemingly “empty” cells adhering smoothly to the GBM (Figure 6, b and c).

At some sites, these epithelial cells contain numerous electron-dense, membrane-bound droplets (generally interpreted as the result of extensive protein reabsorption at sites of protein

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**Figure 4.** Loss of synaptopodin in noncollapsed glomeruli. (a and c) Normal adult human kidney. (b and d) Noncollapsed glomeruli of collapsing idiopathic focal segmental glomerulosclerosis. Low-power view showing strong global distribution of synaptopodin immunoreactivity in all glomeruli (a). Synaptopodin is localized in podocyte foot processes along the glomerular basement membrane (c). (b) In the top left corner, there is a noncollapsed glomerulus with marked reduction of synaptopodin expression (arrow). Loss of synaptopodin staining is also seen overlying a lesion of segmental sclerosis in the bottom glomerulus (arrowheads) (d). Most of the podocyte foot processes have lost synaptopodin expression, although no wrinkling or collapse of the glomerular basement membrane is observed. These findings suggest that the dysregulation of the mature podocyte phenotype precedes the collapse of the tuft. Magnification: ×100 in a and b; ×1000 in c and d.
Figure 5. Transmission electron microscopy (TEM) morphology of collapsing glomerulopathy: HIV-AN. Overview of an entire glomerular profile (a). An accompanying drawing illustrates some details (b). The collapsed tuft area inside the glomerular basement membrane (GBM) is shown in dark gray, the area outside the GBM filled with cells is shown in pale gray, and remnants of a urinary space are displayed in white. A hatched line indicates the (greatly thickened) parietal basement membrane. Most of the “crowded” cells on the outer aspect of the GBM are of a pale undifferentiated type. A minor fraction of cells darker in appearance contains abundant lysosomal elements (absorption droplets; arrows). The pale cell type includes parietal (P) cells, one of which is undergoing mitosis (asterisk), and cells adhering to the GBM (stars). Cells in between appear to be simply piled up on top of others, thus being without any contact to a basement membrane. A distinction between these different cell types based on cytologic criteria is not possible. Note the dramatic wrinkling of the GBM resulting in an anastomosing pattern (arrowheads). TEM, ×1500.
leakage through the glomerular filter), consistent with hyperplasia of the endocytotic vesicular apparatus (Figure 5).

Discussion

The results of the present study provide direct support for the concept that collapsing forms of FSGS (both idiopathic and related to HIV infection) have a common pathomechanism leading to a dysregulated podocyte phenotype. This concept is based on three lines of evidence. (1) Both diseases showed identical changes in podocyte phenotype as reflected by the loss of all specific podocyte markers examined. (2) In both diseases, a comparable number of podocytes were recruited into the cell cycle. (3) The morphologic alterations of podocyte cytoarchitecture were indistinguishable between collapsing idiopathic glomerulosclerosis and HIV-AN. These findings are in sharp contrast to those obtained from the other two diseases studied, MCD and MGN, where severe podocyte damage also occurs. Our data are consistent with a report that in MCD and MGN expression of GLEPP-1 is preserved despite intracellular redistribution (21). Unlike in secondary forms of FSGS (19,20), in collapsing forms of FSGS the key event is not the loss of podocytes themselves but particular alterations of the podocyte phenotype that include loss of molecular markers and mature structural features. In collapsing forms, although podocyte detachment is common, we never observed segments of “naked” GBM in the periphery of the glomerular tuft. In contrast, the periphery of the collapsed GBM areas is completely covered by dysregulated epithelial cells. This lack of “naked” GBM areas may account for the absence of segmental synechiae typically seen in secondary forms of FSGS (19,20).

The dramatic structural changes as well as the complete loss of specific podocyte markers in collapsing idiopathic FSGS and HIV-AN raise the question whether these cells are indeed podocytes. Since these cells are located on the outer surface of the GBM, it is reasonable to assume that these cells originate from podocytes, but exhibit a severely dysregulated phenotype (22). Most convincing in this respect is the loss of WT-1 expression in collapsing forms of FSGS. In contrast, in glomerular diseases with reversible podocyte alterations but preserved phenotype, such as MCD and MGN, expression of WT-1 remains unchanged. The loss in collapsing sclerosis of WT-1, which is present during nephrogenesis from the very beginning of podocyte ontogeny, makes it very difficult to assign these cells the status of “dedifferentiated” podocytes, but lends strong support to the concept of a dysregulated phenotype. Because in the normal adult kidney, podocytes are the only WT-1-expressing cells (23), it has been suggested that WT-1 might act as a repressor of proliferation in mature podocytes (5,23,24). Therefore, the observed activation of cell proliferation in the dysregulated podocytes might be the consequence of the loss of WT-1. Alternatively, the loss of WT-1 may be responsible for the dysregulation of the podocyte phenotype in collapsing forms of FSGS, including the disappearance of podocyte processes and of synaptopodin, whose expression both in vivo and in vitro is associated with the formation and maintenance of processes (6,13). In recent in vitro studies of human and rodent podocytes, we were able to

Figure 6. Changes in podocyte cell architecture in HIV-AN. Changes in podocyte phenotype as seen in three different stages of injury: early (a), intermediate (b), and severe (c). Podocytes in areas with wrinkled GBM (asterisks) lose their foot processes, resulting in direct attachment of podocyte cell bodies to the GBM. Remnants of the actin-based cytoskeleton of podocyte foot processes are encountered in the basal cytoplasm of adhering portions of the podocyte cell body (arrows in a and b) associated with enlarging areas devoid of basal cytoskeleton (arrowheads in a and b). In the late stages (c), pale epithelial cells with few organelles smoothly adhere to the GBM; no cytoskeleton is detected. Podocyte surfaces facing the urinary space undergo “microvillous transformation” (star in a). TEM: ×3700 in a; ×5100 in b; ×4100 in c.
demonstrate that (like during development) WT-1 is expressed by both proliferating and nonproliferating cultured podocytes, and therefore seems more important for maintaining a certain degree or potential of podocyte differentiation rather than regulating podocyte proliferation per se (6,25).

There is an ongoing debate in the literature as to whether podocytes in the mature glomerulus are capable of undergoing cell division (19,26–28). In the collapsing forms of FSGS, the “crowding” of cells in Bowman’s space, together with the increased number of cycling cells clearly demonstrated in the present study, provides strong evidence that podocytes do indeed proliferate in association with an altered cellular phenotype. This disregulated phenotype may reflect a state of misdirected cell activation culminating in apoptosis (our unpublished observations) or may result in activation of pathways leading to sustained cell proliferation.

One hallmark of collapsing FSGS is the fact that the structural changes observed in the dysregulated podocyte phenotype are fundamentally different from the well established podocyte lesions seen in MCD and MGN. Although the foot processes disappear in all of these conditions, this is achieved by quite different means. In MCD and MGN, the effacement of foot processes is associated with a reorganization of the actin cytoskeleton, whereas the cell body and primary processes remain unaffected. In this reorganized actin cytoskeleton, we found undiminished levels of synaptopodin. These findings are corroborated by a study reporting the preservation of synaptopodin (previously termed pp44) expression in diseases with reversible foot process alterations (29). In contrast, in collapsing forms of FSGS, the disappearance of primary processes and foot processes is associated with a loss of cytoskeletal elements, including the disappearance of synaptopodin.

One central question in collapsing forms of FSGS is whether the podocyte damage is primary to the collapse of the tuft or a consequence of the collapse. Three lines of evidence support the hypothesis that a severe and direct injury of the podocyte represents the primary injury, which then leads to the collapse of the tuft. First, as shown in this study, synaptopodin is markedly reduced in noncollapsed glomeruli; podocalyxin, which is expressed by both podocytes and endothelial cells, is selectively lost from podocytes but is retained by the endothelial cells of the affected glomeruli. Second, in the glomerulus podocytes are the only cells that produce vascular endothelial growth factor (VEGF). Kitamoto and coworkers have recently shown that application of a neutralizing antibody to VEGF in newborn mice may effectively block the development of the glomerular capillary tuft (30). It will be interesting to investigate whether the dysregulation of the podocyte phenotype leads to a loss of VEGF production, which in turn causes tuft collapse. Third, strong support for the concept that the collapse is secondary to a podocyte damage comes from a transgenic mouse model of HIV-associated nephropathy carrying a construct lacking the gag and pol genes but retaining the other HIV regulatory elements (31). In this model, which shows HIV transgene expression in podocytes, we have observed a similar dysregulation of podocyte phenotype as in the human disease (32). It is intriguing to speculate that podocyte viral infection and resulting alterations of podocyte gene expression may underlie the observed phenotypic dysregulation in human HIV-AN as well.

At present, the pathogenesis of collapsing idiopathic glomerulosclerosis remains unknown, although possible viral mediators have been proposed (17). The striking morphologic and phenotypic similarities between HIV-AN and collapsing idiopathic FSGS raise the question whether the altered podocyte gene expression in collapsing idiopathic FSGS may also be due to a viral infection. This hypothesis is further supported by de novo occurrence of collapsing idiopathic FSGS in immunosuppressed renal transplantation patients (33) and by epidemiologic data. Similar to HIV-AN, collapsing idiopathic glomerulosclerosis first appeared as a recognizable entity in the early 1980s in our own institution, and its incidence progressively increased with a peak in the early 1990s, suggesting possible environmental factors (16). Moreover, the strong predominance in African-Americans points to a genetic predisposition for the development of collapsing forms of glomerulosclerosis (16,18).

In summary, we have demonstrated a novel, common pathomechanism for collapsing idiopathic glomerulosclerosis and HIV-associated nephropathy. A primary injury of the podocyte leading to dysregulation of the cellular phenotype appears to mediate the glomerular tuft collapse in both conditions. The availability of an HIV-transgenic mouse model (31) mimicking the human disease (32) should allow further avenues to identify factors responsible for altered podocyte gene expression in collapsing forms of glomerulosclerosis. It will be of particular interest to unravel the cellular signaling pathways that mediate podocyte dysregulation with the hope of designing therapeutic interventions to block their activation.

Acknowledgments

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References


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