The podocyte’s response to stress: the enigma of foot process effacement

Wilhelm Kriz, Isao Shirato, Michio Nagata, Michel LeHir, and Kevin V. Lemley

1Centre for Biomedicine and Medical Technology Mannheim (CBTM), Anatomy and Developmental Biology, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany; 2Division of Nephrology, Department of Internal Medicine, Juntendo University, School of Medicine, Tokyo, Japan; 3Kidney and Vascular Pathology, Faculty of Medicine, University of Tsukuba, Tsukuba-City, Japan; 4Institute of Anatomy, University of Zurich, Zurich, Switzerland; and 5Division of Nephrology, Children’s Hospital Los Angeles, Los Angeles, California

Submitted 22 August 2012; accepted in final form 3 December 2012

Kriz W, Shirato I, Nagata M, LeHir M, Lemley KV. The podocyte’s response to stress: the enigma of foot process effacement. Am J Physiol Renal Physiol 304; F333–F347, 2013. First published December 12, 2012; doi:10.1152/ajprenal.00478.2012.—Progressive loss of podocytes is the most frequent cause accounting for end-stage renal failure. Podocytes are complex, terminally differentiated cells incapable of replicating. Thus lost podocytes cannot be replaced by proliferation of neighboring undamaged cells. Moreover, podocytes occupy a unique position as epithelial cells, adhering to the glomerular basement membrane (GBM) only by their processes, whereas their cell bodies float within the filtrate in Bowman’s space. This exposes podocytes to the danger of being lost by detachment as viable cells from the GBM. Indeed, podocytes are continually excreted as viable cells in the urine, and the rate of excretion dramatically increases in glomerular diseases. Given this situation, it is likely that evolution has developed particular mechanisms whereby podocytes resist cell detachment. Podocytes respond to stress and injury by undergoing tremendous changes in shape. Foot process effacement is the most prominent and, yet in some ways, the most enigmatic of those changes. This review summarizes the various structural responses of podocytes to injury, focusing on foot process effacement and detachment. We raise the hypothesis that foot process effacement represents a protective response of podocytes to escape detachment from the GBM.

foot process effacement; glomerular disease; podocyte apoptosis; podocyte loss; proteinuria

As the average age of the population rises, chronic kidney disease (CKD) is becoming an increasingly prevalent condition. Treatment of end-stage kidney disease requires regular dialysis or transplantation, leading to enormous burdens to health care budgets worldwide.

The overwhelming majority of kidney diseases that progress to CKD start in the glomerulus as a consequence of a very limited capacity of glomeruli for regeneration. This notable deficiency derives primarily from one cell type in the glomerulus, namely, the podocyte (42, 63). Podocytes are postmitotic cells. We are born with a certain number of podocytes, roughly 800/glomerulus in the 2 million nephrons of the two kidneys. So far no evidence has been found for the direct replacement of lost podocytes by replication of existing cells. The only way to immediately compensate for lost podocytes consists of cell hypertrophy to cover the glomerular tuft with a smaller number of podocytes. This mechanism, however, increases their vulnerability to any challenge.

We constantly lose podocytes; however, in absence of serious kidney disease, we maintain a sufficient number to last to an advanced age. Glomerular diseases dramatically accelerate the loss of podocytes. If in a given glomerulus the number of podocytes falls below a certain level (roughly 60% of normal), the glomerulus together with the entire nephron will be lost (49).

Currently, the replacement of lost podocytes from a niche population of precursors among the parietal epithelial cells is frequently discussed (46). However, unequivocal evidence for such a mechanism in the adult animal (that is, beyond a short postnatal period) (2) is lacking. Given the danger to podocytes of being lost by detachment from the glomerular basement membrane (GBM), as discussed in this paper, any migration of podocytes on the GBM over extended distances would be a hazardous maneuver.

Responses of Podocytes to Injury

Podocytes respond to injury and stress (such as exposure to toxins or increases in intracapillary pressures) in a unique way. In contrast to other cell types, podocytes maintain viability despite tremendous changes in shape. These changes follow stereotypical patterns (Figs. 1 and 2). They may be grouped into 1) those that develop as a consequence of excessive hypertrophy [cell body attenuation and the expansion of the subpodocyte space (59) into pseudocysts, when podocytes span extensive tuft areas (53)]; 2) those that indicate increased turnover of cell material [accumulation of lysosomal elements likely indicating increased au-
The phenomenon has never been undertaken. An in-depth study of this new site. An in-depth study of this phenomenon has been interpreted as showing that podocytes seek attachment at other sites (GBM or PBM) (8, 47) and may subsequently initiate migration of the entire cell to this new site. An in-depth study of this phenomenon has been undertaken.

**FPE**

The term FPE designates the loss of the usual interdigitating pattern of foot processes of neighboring podocytes, leading to relatively broad expanses of podocyte processes covering the GBM (Fig. 2). It is widely viewed as a pathological derangement that is associated with leakage of macromolecules such as albumin through the glomerular filtration barrier. Two stages may clearly be distinguished.

Within the first stage (Fig. 3A), foot processes undergo tremendous changes in shape, losing their regular interdigitating pattern and retracting into short irregularly shaped cell projections (clearly demonstrated in a recent study using scanning electron microscopy) (20). Slit diaphragms are lost or displaced from their usual position at the base and replaced by occludens-type junctions between the deformed, broadened foot processes. These changes are necessarily associated with considerable movements of these processes in relation to the GBM.

The fluid character of this initial phase is underlined by the observation that these changes occur within minutes after systemic infusion of protamine sulfate, which neutralizes the surface negative charge of the podocytes (79). After reestablishing of the surface charge by injection of heparin, an equally rapid reversal of these shape changes occurs. This is also the kind of FPE that is seen in the LPS model of nephrotic syndrome (71).

The second phase, the completed stage of FPE (Fig. 3, B and C), includes retraction of the foot processes into the primary podocyte cell processes, leading to broad flattened disc-like projections that cover the GBM, finally merging with the cell bodies. At this point, the cell bodies have lost their usual position “floating” above the GBM within the filtrate in Bowman’s space. Instead they broadly adhere directly to the GBM. The subpodocyte space beneath the podocyte cell body has largely disappeared in this stage.

During this final stage, a prominent rearrangement of the cytoskeleton is encountered, leading to the formation of a basal cytoskeletal “mat” closely apposed to the GBM (Fig. 4). This consists of a highly organized network of densely interwoven microfilaments regularly interconnected by α-actinin-positive densities, which appear to serve as cross-linkers for the microfilament bundles (84). This pattern is seen in both animal models and human glomerular diseases, including minimal change disease (MCD), membranous nephropathy, and IgA nephropathy (82). As shown in Masugi nephritis in the rat (84), these cytoskeletal rearrangements are accompanied by an increased expression of actin, α-actinin, and synaptopodin, with a clear localization of the α-actinin to interspersed dense areas. In puromycin aminonucleoside nephrosis, Smoyer and coworkers (85) have shown that the induction of α-actinin expression actually precedes FPE and proteinuria.

Of note, these prominent changes in structure are not accompanied by any obvious signs of decreased cell viability. They seem to be reversible although they may advance to more severe injuries, to cell death (e.g., by spillage of lysosomal enzymes into the cytoplasm followed by cell lysis) or to detachment from the GBM as viable cells. This review will deal with morphological, cell biological, and pathophysiological aspects of FPE and discuss its possible relevance as a mechanism opposing podocyte detachment.

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**Fig. 1. Podocyte injuries: schematic overview.** Schematic of a normal (top) and an injured podocyte (bottom). The podocytes are shown in blue, the glomerular basement membrane (GBM) in yellow, and the subpodocyte space in pink. Bottom: demonstration of the most characteristic structural changes in injured podocytes. In addition to cell hypertrophy, these include 1) cell body attenuation; 2) pseudocysts that develop out of the subpodocyte space (53) and may become associated with detaching areas of podocytes; 3) accumulation of lysosomal and or autophagic elements; 4) cytoplasm shedding; 5) microvillous transformation; 6) foot process effacement; and 7) detachments resulting in bare GBM areas.

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The mat of dense cytoskeletal filaments is closely apposed to the basal cell membrane, suggesting a reorganization of podocyte-GBM interactions. Intact foot processes are fixed to the GBM by various integrin dimers and by α- and β-dystroglycans. Integrin-α3β1 is the major podocyte integrin that binds to laminin, whereas integrin-α1β1 and α2β1 are considered to bind to type IV collagen (51). Deletion of any of the integrin components from podocytes has fatal consequences, most severe after deletion of α3- or of β1-integrin. Recently, it has been shown that deletion of Cd151, a member of the tetraspanin family, weakens the interaction of α3β1 with laminin in the GBM and leads to a progressive glomerulopathy (5, 75). Deletion of dystroglycans specifically from podocytes produces no obvious abnormalities (34). Very little is known about the changes in expression and distribution of integrins or dystroglycans in effaced foot processes compared with intact foot processes.

In vitro, in response to imposed mechanical stress, podocytes switch to a so-called “motile phenotype” that is generally considered to be the in vitro analog of FPE (77). This makes sense in a consideration of the earlier stages of FPE, which, taking into account the changes in length and width of foot processes, are certainly accompanied by increased motility in vivo as well. This analogy would seem less appropriate when one considers the later stage of FPE in vivo, in which, due to the large expanse of direct contact, the “effaced podocyte areas” appear to firmly stick to the GBM.

The change to the “motile phenotype” of cultured podocytes is accompanied by changes in the integrin subtypes expressed. α3β1-Integrins are decreased and αvβ3-integrins are upregulated, which appears to increase the ability of cultured podocytes to withstand mechanical stress (77). A downregulation of α3β1-integrins by mechanical stress was also observed by Dessapt and colleagues (12). In vivo, decreased expression of α3β1 integrins in podocytes has been described under diabetic conditions in animals and humans (9, 36, 70).

In summary, FPE is not a simple pathological derangement of normal podocyte architecture but seems likely to represent a regulated change in cell phenotype that may be considered as the reestablishment of a more typical epithelial cell structure with broad and firm adhesion of the cells to their underlying basement membrane. The fact that a knockout of part of the slit diaphragm-to-actin signaling system in podocytes prevents FPE also supports this interpretation (20). The associated pattern of stress fibers suggests that FPE is in part triggered by changes in the mechanical forces on the podocyte.

Functional relevance. So far, there has been only a single attempt to interpret FPE as an adaptive response, rather than simply as a result of cell injury. Taking into account an increase in the prominence of the actin bundles of the cyto-
Fig. 3. Stages of foot process effacement (FPE). A–C: the GBM is highlighted in yellow, capillary lumens in green. A: stage 1, the initial stage, consists of a deformation and thickening of foot processes, loss of slit diaphragms, and replacement by occludens-like junctions (arrows) that join the lateral surfaces of 2 processes. Note that these deformed foot processes do not attach smoothly to the GBM. B and C: stage 2, the completed stage, consists of retraction of foot processes into the primary processes, changing them to broad flattened cell portions that cover the GBM (B) finally fusing with the cell bodies (C). The basal cytoplasm of these fused cell portions contains a dense cytoskeletal mat exhibiting a regular pattern of more and less electron-dense areas (arrows). The endothelium in A and B is undergoing disconnection from the GBM. A: anti-GBM nephritis, mouse, day 10; bar = 10 μm; unpublished TEM from Ref. 47. B: Masugi nephritis, rat, day 3; bar = 2 μm; unpublished TEM from Ref. 83. C: growth stimulation by FGF-2 in rat, day 90; bar = 2 μm; unpublished TEM from Ref. 44.

Fig. 4. Cytoskeletal pattern associated with FPE. Cross section (A) and grazing section (B) through the basal cytoskeletal mat encountered in areas of FPE. It consists of a dense network of microfilament bundles that appear to be interconnected by regularly distributed densities (arrows). By immunohistochemistry, actin and α-actinin (restricted to the densities) were identified as the main components. The GBM is shown in yellow, a capillary lumen in green. A and B: Masugi nephritis, rat, day 7; bars = 2 μm (A) and 0.5 μm (B); unpublished TEMs from Ref. 83.
skeleton, we earlier interpreted FPE as representing an increase in the contractile capability of the podocyte, to counteract increased transmural pressure gradients in certain pathological circumstances (82, 84). A reevaluation of this interpretation (based on what has been reported in the literature and our own subsequent work) leads us to the conclusion that, although increased wall tension may in part trigger FPE, balancing increased pressure gradients is probably not the primary purpose of FPE.

First, FPE is generally a focal phenomenon, whereas increased intracapillary pressures are distributed throughout the entire capillary network of the glomerular tuft, affecting all capillaries to a similar degree. Second, in models of glomerular hypertension, including the Fawn-hooded hypertensive (FHH) rat (45) and DOCA-salt hypertension in rats (41) and mice (40), FPE is notably absent in early stages. In contrast, extensively dilated capillaries are regularly encountered that are covered with a normal pattern of interdigitating foot processes (Fig. 5). According to Laplace’s law, the wall tension of such dilated capillaries, i.e., the intramural restoring force necessary to balance the distending transmural pressure gradient, can be expected to be augmented by both the increased capillary pressures and the increased radius of the capillary. This argues strongly against a pressure-balancing rationale for FPE. Therefore, it seems necessary to consider alternative functional advantages for FPE.

Podocyte Loss

There is an ongoing discussion about why (and there may be many reasons) and how we lose podocytes in health and disease. The two major mechanisms in discussion for the latter question are apoptosis and detachment of viable cells.

Phylogenetically, podocytes are a very old cell type, with a structure largely preserved over hundreds of millions of years. Thus we may expect that their features have been extensively shaped by natural selection. For example, their complex structure and their unique position as cells floating in the primary filtrate, fixed to the outer aspect of the GBM only by their processes, presumably are somehow indispensable for their function. Moreover, these particular features most likely account for their inability to replicate in situ. This unusual structural arrangement makes it obvious that podocytes are constantly exposed to the risk of detachment. From this point of view, it is hard to understand why podocytes would be susceptible to programmed cell death, i.e., apoptosis.

In what follows, we will discuss the evidence for various possible mechanisms of podocyte loss, focusing on apoptosis and detachment as viable cells.

Podocyte loss by cell death before or in conjunction with detachment. Apoptosis is widely regarded as a major mechanism by which podocytes are lost in progressive glomerular diseases (48, 76, 87). The data in support of apoptotic podocyte loss come largely from in vitro experiments and from in vivo studies using terminal uridine deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) staining. The former are of course questionable, inasmuch as podocytes are not fully differentiated in vitro (even if they express podocyte-specific proteins) and they undergo significant cell division, something completely uncharacteristic of in situ podocytes. Thus in vitro studies are inherently unreliable from the point of view of establishing apoptosis as a common mechanism for podocyte loss in situ.

The data supportive of apoptosis occurring in podocytes in vivo are mostly based on TUNEL staining (74). This is a sensitive method for detecting apoptosis but is also known to be quite susceptible to producing false-positive results (52, 94). Podocyte apoptosis has certainly been discussed more than it has been reported. Those cases in which it has been reported in vivo (taking into consideration the chance of false-positive staining, as above) seem to be under very specific, unusual

Fig. 5. Lack of FPE in early stages of DOCA-salt hypertension. A and B: glomerular hypertension in the DOCA-salt model leads to prominent expansion of many capillary loops reflecting increased expansile forces. Nevertheless, a normal interdigitating pattern of foot processes (arrows) is generally encountered. In B, the GBM is highlighted in yellow. A and B: DOCA-salt hypertension, rat, after 6 wk of treatment; bars = 30 μm (A) and 5 μm (B); unpublished TEMs from (41).
conditions [e.g., transforming growth factor (TGF)-β overexpression] (7, 76).

The finding of apoptotic cells among the podocytes encountered in the urine does not necessarily prove that podocytes underwent apoptosis in situ; apoptosis may have occurred as a consequence of having lost a connection to a matrix substrate during their passage through the nephron (anoikis). Moreover, the fact that podocytes ever arrive as viable cells in the anoxic and often acidic bladder urine strongly speaks against a particular susceptibility of podocytes for undergoing apoptosis. Furthermore, mutations in genes for apoptotic factors have been found associated with renal cancer (35, 72) but not with focal segmental glomerulosclerosis (FSGS) or any other podocyte-related disease.

Our own observations. In advanced stages of glomerular diseases, isolated profiles of dead podocytes and areas of cell debris in Bowman’s space are occasionally found, suggesting that podocytes underwent cell death before or in conjunction with detachment. In most of these cases, signs of cell lysis are prominent, suggesting that the spillage of lysosomal enzymes into the podocyte cytoplasm may represent the proximate cause for an in situ cell death. This interpretation is supported by the frequent observation of neighboring podocytes with dense accumulations of lysosomal elements (Fig. 6). However, among all examples of dead podocytes ever encountered by us, we have seen no cases with a nuclear remnant of fragmented or condensed chromatin, suggesting an apoptotic cell death. In distinction to TUNEL staining, examination by transmission electron microscopy (TEM) provides essentially a gold standard to demonstrate apoptosis. In fact, in all our studies of progressive glomerular disease, comprising ~40,000 images, we have never encountered a single obvious instance of apoptosis in a podocyte. Even in heavily injured podocytes, with tremendous changes in overall cell structure, the chromatin of the nucleus is well preserved. These observations are in agreement with recent in vivo studies in a model of rapidly progressive glomerulonephritis (RPGN), in which one might expect apoptosis to occur frequently, but it was not found (6). Thus, if apoptosis ever occurs in podocytes (in these models), it must be a rare event indeed.

We may estimate the chances of missing any cases of apoptosis by TEM, if this mechanism were a significant source of loss of podocytes in situ. For this estimation, we need to know during what percentage of the apoptotic cell cycle the physical hallmarks (nuclear fragmentation, etc.) are easily visible and how long the process takes. As a rough estimation, consider that in very active diseases such as lupus nephritis or FSGS, one podocyte per glomerulus per day (on average) undergoes apoptosis. Let us presume that this process is recognizable for only 60 min (1/24 podocyte/glomerulus), and that of the 800 podocytes per glomerulus, the nuclei of 10–20 are caught in a single cross section: 1/24 × 1/40 to 1/24 × 1/80. This gives an a priori chance of seeing an apoptotic podocyte roughly once every 1,000–2,000 glomeruli in a biopsy. Under these assumptions, dozens or even hundreds of examples of apoptotic podocytes would be available in TEM images of renal biopsies each year, yet only a single case of an apoptotic podocyte has (to our knowledge) been reported (but not shown) in a human biopsy (37). If similar quantitative considerations apply to animal models, dozens of examples should have been apparent in our experience alone, yet we have not noticed any and we know of no pathologist who has.

Podocyte loss by detachment as viable cells. The unique situation of podocytes as floating cells, adhering to the GBM by their foot processes and being exposed to shear stress from the flow of filtrate, makes podocytes susceptible to detachment. The challenge of the adhesion of podocytes to the GBM is further increased by the evident fact of their migration along the GBM, which has been suggested for more than 50 years and has recently been shown experimentally (65). It is obvious...
that migration and attachment represent opposing challenges to
the podocyte.

**URINARY PODOCYTES.** More than 15 years ago, Hara, Naka-
mura, and coworkers (23, 27, 56–58) first identified podocytes
in the urinary sediments of patients with a variety of kidney
diseases. Vogelmann and colleagues (93) subsequently showed
that in inflammatory glomerular diseases, a majority of these
urinary podocytes are still viable, implying that they do not
detach from the GBM due to apoptosis or necrosis. These
authors also showed that a lesser number of podocytes are lost
into the urine even in healthy individuals. Since then, several
groups (1, 64, 96, 99) have presented convincing evidence that
shedding of podocytes is a common phenomenon in glomerular
diseases. Most importantly, the cumulative rate of podocyte
detachment and appearance in the urine correlates with path-
ological progression of disease in humans and experimental
animals (18, 26), suggesting that ongoing detachment underlies
podocytopenia and progression to glomerulosclerosis.

**IN SITU EVIDENCE.** Detaching and fully detached podocytes have
been found in all models of progressive glomerular disease that
we have investigated. These comprise (1) degenerative models
including uninephrectomy in young rats (53, 54), DOCA-salt
hypertension in the rat (41), and glomerular growth stimulation by
FGF2 in the rat (44); (2) genetic models including the FHH rat
(45), fa/fa rat (19), and overexpression of the AT1 receptor in rats
(30); and (3) inflammatory models including anti-GBM nephritis
in the rat (83, 84), anti-GBM nephritis in the mouse (3, 47), and
Thy-1-mediated nephritis in the rat (43).

Circumscribed detachment in various glomerular diseases
has been described in many previous papers and considered as
a mechanism of podocyte loss (8, 22, 36, 73, 79). Since
podocytes adhere to the GBM by multiple cell processes,
detachment of a podocyte must generally proceed through
stages, in which some processes lose their connections while
others still are affixed to the GBM. Such focal detachments do
not necessarily indicate that a podocyte as a whole has de-
tached or has entered an irreversible process of disconnection
from the GBM. Partial detachments are likely reversible to a
certain degree.

Podocytes in the process of detachment regularly show
remarkable shape changes (see above), with FPE being fre-
quently present. Of note, the overwhelming majority of these
cells appear to be viable; they have a well-formed nucleus with
a normal chromatin pattern and a cytoplasm with well-pre-
served cell organelles (Fig. 7).

Figure 7 presents two examples (from Masugi nephritis) (83)
of detaching podocytes leaving behind extensive areas of bare
GBM. Adjacent podocytes show FPE including effacement of
processes detaching from the GBM. Figure 8 presents two
examples of tuft areas (from rats after growth stimulation with
FGF-2) (44), in which several neighboring podocytes are
undergoing detachment. These podocytes establish contacts to
each other, forming an interconnected group. Note that among
them are binucleate cells, corroborating the observation that
urinary podocytes frequently have two cell nuclei (25, 93).
Figure 9 shows two more examples (from a fa/fa rat and
Thy-1-mediated glomerulonephritis rat) of groups of com-
pletely detached podocytes. It appears that simultaneous de-
tachment of several neighboring podocytes may represent a
common event under fulminant disease conditions.

![Fig. 7. Detachments of viable podocytes: examples of individual cells. A and B: podocytes in the process of detachment from the GBM. The GBM is shown in yellow, capillary lumens are in green, spaces between bare GBM and the detaching podocyte in pink. In A, 3 detaching podocytes (1–3) are seen. Podocytes 1 and 3 show extensive areas of FPE that are widely disconnected from the GBM even though the interposed space is still narrow. Profile of podocyte 2 does not show any connections to the GBM, although it appears to be connected to podocytes 1 and 3 by intercellular junctions (arrows); these latter cells are more closely attached to the GBM than the corresponding cells in A. Note that the cell nuclei of both detaching podocytes have a normal chromatin pattern (asterisks). A and B: Masugi nephritis; A: day 28; bar = 3 μm; B: day 7; bar = 5 μm; unpublished TEMs from Ref. 83.](image)
Detachment is mostly encountered in podocytes with effaced foot processes. In the inflammatory group of models [anti-GBM-GN in rats and mice (47, 83), also in the Kinjoh mouse (60)], in severely affected areas of a glomerulus, all podocytes show more or less complete effacement of their foot processes, including clearly detached areas, areas in intermediate stages of detachment, and others that are still adherent to the GBM (Figs. 10 and 11). Thus FPE frequently appears to precede detachment.

A special type of detachment affects podocytes that come to lie at the opening of Bowman’s capsule to the proximal tubule. Experimentally, this occurs regularly in disease models characterized by loss of mesangial support of the tuft (Thy-1-mediated glomerulonephritis) (16, 43), leading to protrusion of capillary loops into the urinary orifice. Such podocytes appear to be exposed to enhanced shear stress, as suggested by their elongated shapes as they protrude into the proximal tubule. The geometry of the tuft suggests that this is the region of the highest flow velocities of filtrate. The pictures (Fig. 12) strongly suggest that such podocytes are in extreme danger of being lost by detachment. Of note, in contrast to podocytes elsewhere in these glomeruli, only podocytes protruding into the urinary orifice have been found to exhibit FPE. Thus, also in these cases, FPE appears to be a reaction to (mechanical) stress and to precede detachment.

In summary, the majority of podocytes encountered in the process of detachment in TEM images of experimental models appear viable and display FPE. Thus FPE appears to precede detachment. The elaborate organization of the cytoskeleton associated with FPE contradicts the interpretation that FPE simply represents a form of cell injury, i.e., an injury stage preceding detachment. FPE is reversible, even when extensive, like in MCD, in which a complete reconstitution of the filtration barrier is clearly the rule. Thus FPE seems to represent a specific, reactive phenotype of the podocyte, which may enhance adhesion to the GBM and limit the risk of detachment, at least for some time. Consequently, our interpretation views FPE as a strategy for podocytes to escape detachment, providing the basis of the possibility for a reconstitution of the filtration barrier when local conditions have improved. FPE is clearly not always successful, as indicated by the widespread presence of urinary podocytes in many diseases.

**FPE: Response to Injury vs. Primary Injury**

So far, we have considered that podocytes are predominantly lost by detachment and that FPE likely represents a protective mechanism for podocytes to prevent detachment. This hypothesis has to be tested with respect to individual glomerular diseases. In the present context, two major groups of diseases can be distinguished: those that start focally in individual glomeruli (at least with respect to varying degrees of severity) with FPE considered as a protective response to the focal injury, and those in which all glomeruli are affected at the same time either as the consequence of a primary defect in the
Fig. 9. Detached podocytes in Bowman’s space and proximal tubule. A: group of 4 (1–4) detached podocytes with intact nuclei floating in Bowman’s space. A further podocyte (5), elongated in shape and with prominent microvilli (arrow), appears to be undergoing detachment from the GBM (GBM is highlighted in yellow, capillary lumens in green). B: group of detached podocytes in the lumen of the beginning segment of the proximal tubule. The cells appear viable with cell nuclei showing a normal chromatin pattern; one podocyte still contains a pseudocyst (asterisk). A: Fa/fa rat, 90 days; bar = 10 μm; unpublished TEM from Ref. 19. B: Thy-1-mediated glomerulonephritis, proliferating stage, rat; bar = 3 μm; unpublished TEM from Ref. 43.

Fig. 10. Podocytes detach from an “effaced” status. A and B: peripheral tuft areas with podocytes undergoing detachment starting from a status of almost complete FPE. The spaces between the GBM (yellow) and the detaching podocytes are highlighted in pink displaying the considerable extent of the detachment. Inside the GBM, the capillary endothelium has also detached from the GBM in many places. Within the spaces between capillaries and the GBM, or at sites where capillary and mesangial spaces have fused (left region in A), inflammatory cells (asterisks) are seen. Preserved capillary lumens are shown in green. Note that despite severe damage all podocytes have cell nuclei with a normal chromatin structure (stars). The holes in the podocyte cytoplasm in B are suggested to be pseudocysts. Arrowheads point to areas of cytoplasm shedding. PE, parietal epithelium. A: anti-GBM nephritis, mouse, day 10; bar = 5 μm; TEM reproduced from Ref. 47 with permission. B: Masugi nephritis, rat, day 7; bar = 5 μm; unpublished TEM from Ref. 83.
underlying network of proteins responsible for normal podocyte structure or an aberrant stimulation of components of this network.

**FPE in focal glomerular diseases.** FPE is seen in all glomerular diseases, in which the disease process is first noted focally in individual glomeruli. FPE is particularly prominent in inflammatory diseases (e.g., crescentic GN; anti-GBM-GN), and the idea that FPE is a protective response against the danger of detachment accords well with the risks to which the podocytes are exposed in these diseases. It is well known that connections of integrins to the actin cytoskeleton in podocytes are highly susceptible to disruption by complement activation products (89) and other harmful inflammatory mediators (4).

In diseases with subepithelial immune deposits (membranous and membranoproliferative GN, lupus nephritis), the fixation of podocytes to the GBM is disrupted at sites with interposed deposits. No information is available as to the strength of the attachment of podocytes to the deposited material, but due to the lack of specific integrin/dystroglycan receptors it is most likely weaker than to intact GBM. A weaker adhesion of podocytes to the GBM may also be expected to occur in diseases with changes in the matrix composition of the GBM (diabetic nephropathy; Alport and Pierson syndromes, among others).

In diseases associated with glomerular hypertension, podocytes are exposed to a particularly hazardous situation. Increased capillary hydrostatic pressures lead to increased distending forces within the GBM, and this may lead to an overtaxing of the cell-substrate interactions connecting the podocyte to the GBM. Substrate-level mechanical stress certainly leads to responsive changes in the podocyte actin cytoskeleton (15).

Podocytes are also constantly exposed to shear stresses resulting from the flow of filtrate within Bowman’s capsule. In cell culture experiments, they have been shown to be highly sensitive to shear stress (16, 86) just as they are to substrate-level stress (15), with attendant remodeling of the actin cytoskeleton. Exposure to increased shear stress probably represents a mechanical challenge that may play an important role in glomerular diseases associated with hyperfiltration (28) (e.g., diabetes mellitus, pregnancy, secondary FSGS) and may be exacerbated by locally increased filtrate flow due to preceding detachment of adjacent podocytes. Sheer stress due to filtrate flow may also be particularly relevant in diseases in which podocytes come to lie near or within the urinary orifice of the glomerulus (16, 43) (Fig. 12). In human diseases, such situations are regularly encountered in the so-called “tip lesion” (31) or tip variant of FSGS (11).

The most significant challenge to adhesion of podocytes to the GBM is likely associated with podocyte hypertrophy. Stimuli for cell hypertrophy in general tend to evolve into stimuli for cell proliferation. As shown previously (44), massive stimulation of podocyte growth forces podocytes toward cell division. Since they are unable to complete cytokinesis, the process may end in abortive mitosis (mitotic catastrophe) or may result in binucleate cells (Fig. 8). Binucleate cells in situ often show FPE (55) and they are frequently found among urinary podocytes (25, 93). The process of cell division necessitates an extensive reorganization of the cytoskeleton likely accompanied by an impairment of the adhesion of podocytes to the GBM.

**Fig. 11.** Localized areas with effaced foot processes appear to start the process of podocyte detachment. A and B: the GBM is shown in yellow, capillary lumens in green, spaces between bare GBM and detaching podocytes in pink. The podocyte in A forms bulging cytoplasmic domes (arrows) toward Bowman’s space, indicating the stepwise loss of connection to the GBM. Note that the GBM is markedly attenuated at the entire site of detachment (arrowheads). The detaching podocyte in B forms a single dome over a bare GBM area, suggesting unrestricted filtration as the cause for dome formation. Note that the cytoskeletal mat (arrows) is thinned as a result of the expansion. Also, the underlying endothelium is in the process of detachment (asterisk). A: Fawn-hooded hypertensive rat, 25 wk; bar = 10 µm; unpublished TEM from Ref. 45. B: Masugi nephritis, rat, day 3; bar = 3 µm; unpublished TEM from Ref. 83.
These examples illustrate the precarious situation that podocytes face in glomerular diseases, as floating cells attached to the GBM only by their processes. In all of these different diseases, FPE is a prominent feature, consistent with the interpretation of FPE as a protective response mitigating the risk of detachment. If this is correct, early impairment of podocyte-GBM connections (due to changes in the GBM itself or the connecting proteins), underlying impending detachment, should initiate FPE based on established signaling routes. Two mechanisms are likely to be involved.

The first is by integrin-mediated outside-in signaling resulting from changes in podocyte-GBM interactions under the influence of increased mechanical stress. This route involves the integrins (α3β1?, αvβ3?, α1β1?), the TPV (talin-paxillin-vinculin)-complex, the integrin-linked kinase (ILK), and the focal adhesion kinase (FAK). ILK over-expression in mice causes FPE followed by FSGS (4) and inhibition of FAK protects against proteinuria and FPE in experimental glomerular injury (50).

The second is via the slit diaphragm (SD) and its associated signaling proteins. The SD is generally thought to function as the key mechanosensor for the maintenance of the interdigitating foot process pattern and to play a role in the overall survival signaling of the podocyte (32). However, the specific cues for the initiation of survival signals have never been clearly defined. In the present case, any impending process detachment may first be structurally manifest in partial disconnections of individual foot processes. Such tiny changes (not easily recognized even by TEM) may mechanically affect the SD, changing its molecular architecture and leading to a
disturbed flow of filtrate through the slit. It is possible that these perturbations represent adequate mechanical cues for initiating danger signaling and consequent local FPE. A recent study (20), showing inhibition of FPE when nephrin-to-actin signaling is impaired, supports this hypothesis. Moreover, signaling routes via TRPC channels and the Rho family of small GTPases have recently come to the center of discussions of the regulation of the podocyte actin cytoskeleton (21). Such a regulatory scheme would fit perfectly into this concept; a detailed discussion of this issue is beyond the scope of this review.

**FPE as a result of a defect in the usual structure-preserving mechanisms.** The interpretation of FPE as a protective response of podocytes to a preceding stress does not apply to many genetic disorders or to acquired diseases with a ubiquitous distribution of lesions. In many genetic diseases, human and experimental, the mutation, gene deletion, or transgene responsible directly concerns proteins of the network of proteins responsible for the maintenance of the normal interdigitating foot process pattern. This may have a negative impact on responses to injury but may also by itself produce dysfunctional changes in the cytoskeleton leading to FPE (e.g., nephrin and podocalyxin knockout mice) (13, 66, 69). Most interesting in the present context is the CD2AP knockout mouse (90, 98). In this model, FPE emerges almost synchronously at the age of ∼2–3 wk and involves all podocytes in all glomeruli. Thus, in this case, FPE does not seem to represent a response to a preceding injury, but rather the genetic deficiency appears to directly initiate FPE (that this may have similar consequences will be discussed below).

Among acquired human diseases, MCD likely belongs in this group. In MCD, FPE is not focally distributed, indicating a response to local damage, but widespread and yet not accompanied by any other signs of podocyte injury. The longstanding hypothesis of a circulating factor being directly responsible for the cytoskeletal changes (10, 80) has been recently substantiated, suggesting that a circulating T cell factor in conjunction with some form of dysregulation of CD80 expression on podocytes is the culprit (33, 81). Thus, also in MCD, FPE is not a response to a preceding podocyte injury but due to directly induced cytoskeletal dysregulation. This could also explain why FPE in MCD is not associated with podocyte loss and thus progression.

**FPE and Protein Leakage**

The SD is generally considered to be the crucial barrier to the passage of macromolecules across the glomerular capillary wall. However, protein leakage of the glomerular filter may also be caused by damage to the upstream layers (the GBM and the endothelium), e.g., endotheliosis in preeclampsia (29, 88). Moreover, experimental models of proteinuria are known, in which the underlying failure of the podocyte does not produce any visible damage seen at the TEM level, e.g., models with removal of nephrin from the SD by inducing increased nephrin endocytosis (67, 68) or in models targeting nephrin-binding antibodies (mAb 5–1–6) to the SD (62). Of note, such situations do not progress, or progress only slowly, to more severe glomerular damage (38).

Generally, proteinuria is associated with structural changes in the podocyte, most commonly with FPE. This led early on to the idea that FPE causes proteinuria. However, data from the literature are discrepant in this regard. There are several studies (14, 78, 97) showing that FPE, i.e., foot process width, correlates with quantitative proteinuria, whereas others do not (91). It is not clear what could account for this discrepancy. Seen from a structural viewpoint, it is hard to imagine that foot processes “fused” to expansive sheets of continuous cytoplasm covering the outer aspect of the GBM should be “leaky” to proteins (even if we accept the proposition that some transcytosis of proteins through such areas may occur) (39). On the other hand, as discussed above, we need to distinguish different stages of FPE. The early stages appear to be accompanied by prominent motility of foot processes, with movements on the GBM likely being associated with transient localized detachment from the GBM, which would permit enhanced local passage of macromolecules. In an active glomerular disease, such transient denudations would be constantly created de novo at different sites, whereas at other sites the advancement to complete FPE would close the leaky patches.

Thus the present hypothesis is consistent with a causal association between FPE and proteinuria. Nevertheless, it argues that FPE is a response of podocytes to stress or injury aimed at preventing progression, including ongoing protein leakage. Here, we consider detachment of foot processes as a major cause of local protein leakage. This certainly is not a new idea. It has been raised in many previous studies (17, 61) and shown in tracer studies (92). New in the present hypothesis is the suggestion that FPE, by preventing further detachment, ultimately limits the amount of protein leakage. In the case of a severe, fulminant process, FPE appears to be able to prevent a worst-case scenario of widespread leakiness of the filtration barrier, admittedly at the cost of an acute (presumably transient) loss of filtration capacity.

FPE will never be able to completely prevent leakage of protein through the filtration barrier, since it) during its development, leaky (denuded) sites will inevitably arise (see above); and 2) at the borders of areas of complete effacement, abnormal junctional connections between adjacent podocytes will likely compromise the proper functioning of the SDs. Consequently, even in cases in which FPE does not develop in response to a preceding injury, but is directly triggered by systemic or genetic causes (as discussed above for MCD or the CD2AP knockout mouse), FPE will necessarily be accompanied by protein leakage.

In conclusion, FPE is considered as a protective strategy to prevent podocyte detachment and, in conjunction therewith, to limit long-term protein leakage. On the other hand, FPE will never be able to completely prevent proteinuria; it is necessarily associated with protein leakage and even a loss of glomerular filtration capacity. Its protective character is based on the premise that there is a reasonable chance for recovery of normal glomerular structure and that protein leakage of a limited magnitude and for a limited time does not result in irreversible harm to the organism.

**Concluding Remarks**

Podocytes “live and work” under precarious conditions: because of their position floating above the GBM attached only by their processes, they are at risk of detachment from the GBM as viable cells. Yet, they are unable to replicate when
some of them are lost from the tuft. It appears that nature has developed mechanisms to limit detachment under these challenging conditions. The stereotypical structural changes that podocytes display under stress do not appear to us to be simple pathological derangements but more likely represent adaptive responses to increase the chances for cell survival. In this review, we have raised the hypothesis that FPE constitutes a regulated change in podocyte phenotype, serving to protect podocytes against detachment from the GBM and loss into the urine. This may be accompanied by a loss of glomerular filtration capacity and the development of proteinuria, suggesting that filtration capacity and permselectivity are, to some extent, temporarily dispensable functions in the face of the risk of irreversible podocyte loss.

This hypothesis implies that research on aspects of podocyte biology relevant to their adhesion to the GBM might be particularly promising to uncover new strategies to help prevent podocyte loss and thus retard or prevent the progression of chronic renal failure. It also implies that proteinuria may not be the optimal marker of the effects of therapies in the acute stages of a glomerular disease; counting of urinary podocytes may more closely reflect the severity of the disease and its response to treatment.

ACKNOWLEDGMENTS
The authors thank Brunhilde Hähnel and Hiltraud Hosser for tireless technical and organizational help and Rolf Nonnenmacher for graphic assistance. The authors gratefully acknowledge the continuous support by the “Gotthard Schettler Gesellschaft fuer Herz- und Kreislaufforschung” and the “Professor Dr. Karl und Gerhard Schiller Stiftung.”

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

REFERENCES


