Proteinuria with and without Renal Glomerular Podocyte Effacement

Raghu Kalluri
Division of Matrix Biology, Department of Medicine, Beth Israel Deaconess Medical Center, Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, and Harvard-MIT Division of Health Sciences and Technology, Boston, Massachusetts

Renal biopsies of patients with proteinuria and kidney disease most often are associated with podocyte foot process effacement. For several decades, nephrologists have wondered whether proteinuria is a result of podocyte foot process effacement or the cause of it. In the past few years, the author’s laboratory has addressed this issue using different mouse models of proteinuria. Although in most cases, podocyte effacement is associated with proteinuria and glomerular disease, in three different mouse models, it was demonstrated that proteinuria can be observed without podocyte foot process effacement. The first model is generated by injection of antibodies to vascular endothelial growth factor or soluble vascular endothelial growth factor receptor 1. The second model is a mouse with deletion of type IV collagen α3 chain in the glomerular basement membrane. The third model was generated by genetic deletion of a slit diaphragm protein known as nephrin. Collectively, these experiments and the supporting evidence from several human studies demonstrate that severe defects in either the glomerular basement membrane or the glomerular endothelium can lead to proteinuria without foot process effacement.

Published online ahead of print. Publication date available at www.jasn.org.

Address correspondence to: Dr. Raghu Kalluri, Division of Matrix Biology, Department of Medicine, Beth Israel Deaconess Medical Center, 330 Brookline Avenue, Boston, MA 02215. Phone: 617-667-0445; Fax: 617-975-5663; E-mail: rkalluri@bidmc.harvard.edu

Copyright © 2006 by the American Society of Nephrology

ISSN: 1046-6673/1709-2383
within the slit diaphragm, and they associate with each other and are connected directly or indirectly to the actin filaments of the podocytes and regulate its function (19,21). An understanding of the precise function of podocytes still is evolving, and much more work needs to be done.

Debates about which one of the three components of the glomerular filtration apparatus is the defining barrier that keeps albumin from escaping from the blood have been going on for many years (1,9). It now is generally believed that the charge barrier of the GBM may not be the most prominent filter, but the composite effect of the GBM charge and also slit diaphragm integrity are essential for the successful retention of albumin and other proteins that are $>$70 kD. Under normal physiologic conditions, how the trapped albumin in the GBM and the slit diaphragm returns back into the circulation still is not understood, but many theories exist. Tubular and glomerular reabsorption, cellular endocytosis, and an active glomerular reflow into the blood in the reverse direction from the slit diaphragm against flow and pressure are all a possibility. Collectively, evidence gathered from our laboratory in the past few years suggest that damage to any of the three components of glomerular filtration apparatus results in proteinuria without effacement of podocyte foot processes.

Vascular endothelial growth factor (VEGF) is a key endothelial survival factor and induces vascular permeability (22–24). We tested the hypothesis of whether circulating physiologic levels of VEGF can provide survival cues to the glomerular endothelial cells and help maintain the fenestrations. The motivation for these experiments also stems from observations in oncology clinics that in a significant percentage of patients, anti-VEGF antibody therapy leads to proteinuria and hypertension (25,26). In addition, between 1998 and 2002, several reports indicated that women with preeclampsia (who among other things exhibit proteinuria and hypertension) present with elevated levels of soluble VEGF receptor 1 (sFLT-1), detected as an increase in the amniotic fluid and cytotrophoblasts (27–29). To test this clinical observation experimentally, we neutralized circulating VEGF in mice using equimolar amounts of mouse anti-VEGF antibody or sFLT-1 (30). In these experiments, we observed that proteinuria can be induced by neutralizing circulating VEGF without altering the levels of endogenous kidney tissue associated VEGF (30). Interestingly, we demonstrate that proteinuria can be induced in these mice with anti-VEGF antibody and sFLT-1 without podocyte foot process effacement (30) (Figure 1). Predominant lesions observed in these mice are glomerular endothelial damage, endotheliosis with large vacuoles, and detachment from the GBM, resembling the histopathology that is observed in women with preeclampsia (30).

Predominant components of GBM are type IV collagen and laminin (15). The predominant type IV collagen constituents of GBM proper are α3, α4, and α5 chain (31). Therefore, deletion of α5 chain of type IV collagen (α5KO) in mice leads to severe GBM defects as a result of elimination of all three chains of type IV collagen due to obligatory assembly that is required among the type IV collagen chains (14,32). Electron microscopy (EM) pictures show significant defects in the GBM, early in the life of the α5KO mice on the 129/sv background (1) (Figure 2). By approximately 5 wk, these mice develop proteinuria, and careful EM examination of glomerular architecture reveals intact endothelial layer, significant GBM defects (splitting, thinning, basketweave pattern, and thickening), and intact podocyte foot processes (Figure 2). Continued proteinuria with time results in podocyte effacement in these mice (Figure 2). The α3KO mice are a model for autosomal recessive Alport syndrome, and our results suggest that early hematuria and albuminuria that are seen in these patients could occur without podocyte foot process effacement. Our results with the α3KO mice demonstrate for the first time that significant GBM structural and functional defects can lead to massive proteinuria in mice without podocyte foot process effacement.

Nephrin is a component of the podocyte slit diaphragm and also of other structures and cellular constituents of the body, including the nervous system (33,34). Mutations in nephrin have been identified in patients with nephrotic syndrome of the Finnish type (33). We generated mice that are deficient in nephrin, and these mice die at approximately 2 d after birth and are associated with massive proteinuria. We are not sure whether the phrase “nephrotic-range proteinuria” should be used in the context of mice; therefore, we use the term “massive proteinuria” here. This massive proteinuria in mice occurs without obvious podocyte foot process effacement (Figure 3). It is not clear yet whether the early death that is seen in these mice is due to the kidney phenotype or due to some other, unknown defects (1). Other investigators also have demonstrated that targeting nephrin can lead to slit diaphragm defects (35) and proteinuria without significant podocyte foot process effacement (36–38). Collectively, studies with nephrin-deficient mice demonstrate that massive proteinuria can be observed without any defects in the GBM, glomerular endothelium, or podocyte foot processes (Figure 3).

Our findings demonstrate that defects that are induced in any of the three components of the glomerular filtration apparatus can lead to initial proteinuria without podocyte foot process effacement. Sustained proteinuria, eventually in all three settings, is associated with podocyte foot process effacement, so what causes proteinuria without podocyte effacement? Our contention is that all three components of the glomerular filtration apparatus are in constant molecular and biochemical communication with each other, via GBM–cell interactions and possibly also via growth factor and other soluble ligand-receptor influences (Figure 4). Therefore, although gross morphologic changes in the podocyte foot process may not be observed during the early phases of the abnormal protein leak in the urine, subtle molecular alterations in the slit diaphragm composition, assembly, and signaling are possible at this stage without overt morphologic changes (Figure 4). Subsequently, sustained insult/injury potentially leads to an eventual overt morphologic defect, observed as podocyte foot process effacement. Foot process effacement is associated with an enhanced generalized, nonspecific adhesion that engages neighboring foot processes and a loss of well-organized adherens junctions (slit diaphragms). Such defects potentially lead to loss of specific signaling pathways and compromised functional adhesion to the GBM. Therefore, what causes proteinuria still is an...
open-ended question. Nevertheless, it is clear now that podocyte foot process effacement is not required for initiation of proteinuria. Defects that are induced in the glomerular endothelial cells, GBM, or the slit diaphragm can lead to proteinuria without podocyte foot process effacement. In this regard, several other studies in mice and rats have demonstrated that proteinuria can be observed without podocyte foot process effacement, supporting the studies documented here (36,39,40). Most interesting, male MWF rats develop spontaneous proteinuria with age but without podocyte foot process effacement (41).

In the 1950s, Farquhar et al. (42,43) first described patients with nephrosis, glomerulonephritis, and lupus erythematosus with extensive podocyte foot process effacement. Defects that are induced in the glomerular endothelial cells, GBM, or the slit diaphragm can lead to proteinuria without podocyte foot process effacement. In this regard, several other studies in mice and rats have demonstrated that proteinuria can be observed without podocyte foot process effacement, supporting the studies documented here (36,39,40). Most interesting, male MWF rats develop spontaneous proteinuria with age but without podocyte foot process effacement (41).

In the 1950s, Farquhar et al. (42,43) first described patients with nephrosis, glomerulonephritis, and lupus erythematosus with extensive podocyte foot process effacement. Since then, several other human studies advance the notion that proteinuria can occur without obvious podocyte foot process effacement (44–47). Van den Berg et al. (44) documented in elegant studies that podocyte foot process is not correlated with the level of proteinuria in several human glomerulopathies. Variants of minimal-change nephritic syndrome with proteinuria are not associated with podocyte foot process effacement (44). Branten et al. (45) report that a familial form of nephrotic syndrome occurs in the absence of podocyte foot process effacements. Additionally, several other anecdotal reports with human biopsies that support the notion that proteinuria can occur without podocyte foot process effacement exist. It is interesting that the most convincing of such reports have been around for a few decades now. These include EM studies of the kidney glomeruli of women with preeclampsia, a syndrome that is associated with proteinuria and hypertension and is seen in approximately 5% of pregnant women (48,49). Proteinuria, hypertension, and glomerular endotheliosis in these women are not associated with podocyte foot process effacement (Figure 5).

Proteinuria still represents a key biomarker for kidney dysfunction. What causes excessive protein leak and albuminuria
is not yet known. Therefore, experiments with a mechanistic focus on what causes proteinuria still might represent the best approach to identifying biomarkers for most renal diseases.

Figure 2. Transmission electron microscope analysis of the α3KO kidneys with and without proteinuria. (A) Control wild-type kidney at 4 wk of age. Illustrates normal glomerular basement membrane (GBM) architecture. (B) Nonproteinuric 4-wk-old α3KO mice with significant GBM defects, normal glomerular endothelial cells, and normal podocyte foot processes. (C) Proteinuric 5-wk-old α3KO mice with GBM defects, normal glomerular endothelial cells, and normal podocyte foot processes. (D) Proteinuric 8-wk-old α3KO mice with GBM defects, mild to moderate glomerular endothelial damage, and significant podocyte foot process effacement. These data were originally reported by Hamano et al. (1), except for panel C. Magnifications: ×35,000 in A and B; ×30,000 in C; ×12,250 in D.

Figure 3. Transmission electron microscope analysis of glomeruli of nephrin-deficient mice. (A) Wild-type mice at day 2 after birth. (B) Heterozygote mice at day 2 after birth. (C) Nephrin −/− mice at day 2 after birth with massive proteinuria but without podocyte foot process effacement. These data were originally presented in by Hamano et al. (1) Magnifications: ×180,000 in A and B; ×115,000 in C.

Acknowledgments

The work reported here is supported by the National Institutes of Health, 1998 ASN Carl Gottschalk Award, and 1998 Joseph Murray Award from National Kidney Foundation, the Emerald Foundation (New York, NY), and research funds of the Center for Matrix Biology and the Division of Matrix Biology at the Beth Israel Deaconess Medical Center.

I am extremely grateful to all the talented scientists and trainees who spent their precious time in our laboratory and launched a partnership with me to study the kidney in health and disease. I am honored that my colleagues found our scientific approach interesting and helped to shape it with their hard work, innovative thinking, and team spirit. Therefore, this award from the American Society of Nephrology is a true recognition of a partnership between all dedicated scientists in our group and their collective efforts toward a goal of unraveling the mysteries behind how kidney functions and its pathologies. My first exposure to the kidney and matrix came as a graduate student in the laboratory of Billy G. Hudson in 1988, and my special thanks to Parvin Todd, Sripad Gunwar, and Usha Ponnappan for helping me to become a scientist and providing me with valuable friendship during my graduate school days. My time with Eric Neilson at Penn was very
special, as he nurtured my career as a research associate and prepared me to take on the challenges of starting an independent laboratory. He has been a constant supporter of our laboratory, a good colleague, and, importantly, a caring friend. I thank Vikas Sukhatme and Robert Glickman for recruiting me to my first job as an assistant professor at Harvard Medical School and giving me the freedom to pursue any of my scientific interests. The infectious enthusiasm of Vikas Sukhatme was critical in my pursuit of innovative ideas. Robert Moellering, Jr., as my chairman for the middle 7 yr at the BIDMC was critical in helping us set up the Center for Matrix Biology and protecting us from various distractions and providing us all of the support that we needed to pursue our science. Judah Folkman rekindled my passion for patient care and continues to be a valuable teacher and a mentor on many fronts on a daily basis. The continuing mentorship of James Watson has been pivotal for my sustained enthusiasm for biology and medicine. The constant support at all levels from our current chairman Dr. Mark Zeidel has been critical in being focused on our mission of performing innovative biomedical research. Dominic Cosgrove has been our collaborator for the past 8 yr, and I thank him for help with studies related to the α3KO mice. I thank Michael Zeisberg for the help in preparing this manuscript, and I am grateful to Dr. Issac Stillman in the Department of Pathology at the Beth Israel Deaconess Medical Center for providing the EM picture in Figure 5.

References


