glomerular disease

Revisiting the determinants of the glomerular filtration barrier: what goes round must come round

Detlef Schlöndorff[1], Christina M. Wyatt[1] and Kirk N. Campbell[1]

The glomerular filtration barrier (GFB) is characterized by a very high hydraulic permeability, combined with a marked permselectivity that excludes macromolecules such as albumin. Thus, the GFB retains most of the plasma proteins, with only 0.06% of albumin getting across the basement membrane. The GFB consists of 3 layers: fenestrated endothelial cells, the glomerular basement membrane, and podocytes. Injury to any of these components can result in the development of proteinuria. The contribution of the major components of the GFB has recently been reexamined and is discussed in the context of our past and present understanding.

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Glomerular filtration barrier (GFB) function and morphology were established with the application of micropuncture and electron microscopy techniques in the 1960s and early 1970s. The fenestrated glomerular endothelium, the multilayered glomerular basement membrane (GBM), and the podocyte, with its intricate interdigitating foot processes separated by the interposed slit diaphragm, were described by Farquhar *et al.* as well as Rodewald and Karnovsky.[1,2] Farquhar *et al.* performed a sophisticated functional analysis of the GFB with the use of size-selective proteins (albumin and dextrans) and charge-selective tracers (ferritin). They showed that the GBM represented the major permselective barrier for both molecular size and charge, excluding molecules with the size and negative charge of albumin from glomerular filtration under normal conditions. The slit diaphragm was identified as a structure connecting foot processes of adjacent podocytes, but its functional role in the GFB remained controversial.

The glomerular basement membrane as a component of the filtration barrier

Over the ensuing decades the meshwork of extracellular matrix proteins constituting the GBM was analyzed in detail. The major components of the GBM include type IV collagens with alpha 3, 4, and 5 chains, laminin β2, and negatively charged heparin-sulfate proteoglycans such as agrin and nidogen. These components generate the molecular scaffold essential for the adult GBM. The GBM provides not only structural support for the glomerular capillaries, but also contributes to cell-matrix and cell-cell cross-talk, which are essential for the maintenance of the functional unit of the glomerulus.

The contribution of individual components of the GBM to its permselectivity was elucidated in large part through genetic studies demonstrating that mutations in different components of the GBM lead to albuminuria and progressive renal failure.[3] For example, mutations in collagen IV α3 in Alport syndrome and in laminin β2 in Pierson syndrome both result in albuminuria, proof of the importance of the normal GBM as a major barrier for proteins. Laminins represent not only important links for the collagen meshwork of the GBM, but also for cell-cell and cell-matrix signaling. The podocyte-matrix signaling system depends on interactions with podocyte transmembrane integrins, specifically α3 β1 integrin, as well as with the dystroglycans.[3]

The changes in collagen or laminin composition resulting from mutations in collagen IV α3 and in laminin β2 were also

[1]*Department of Medicine, Division of Nephrology, Icahn School of Medicine at Mount Sinai, New York, NY, USA*
Correspondence: *Christina M. Wyatt, Mount Sinai School of Medicine, Division of Nephrology, Box 1243, One Gustave L. Levy Place, New York, New York 10029, USA. E-mail: christina.wyatt@mssm.edu*

visualized by disruption of the normal ultrastructural architecture of the GBM. Furthermore, in the GBM of patients with Alport syndrome and mutations in collagen IV α3, ectopic deposition of laminin α1 and increased levels of laminin α5, 6, and 7 have been noted.[3] These observations suggest that the laminin and collagen networks are intrinsically connected as major determinants of the GBM permselectivity barrier.

Based on these observations, a model for the permselectivity of the GBM was proposed, with the interwoven molecular architecture generating a fine-pore mesh filter with negative charge, which excluded molecules such as albumin based on size and to some extent also on charge. The Nobel laureate Oliver Smithies then proposed, based on his intricate knowledge of gel electrophoresis for size separation of proteins, that the GBM acts as a size-selective gel for diffusion of molecules, excluding macromolecules such as albumin from entering the GBM.

He stated: "My permeation diffusion hypothesis depends on 2 main assumptions: (i) that the GBM is a gel having size-selective properties determined by permeation and diffusion, not by filtration; and (ii) that the slit diaphragm is essential for normal glomerular structure but does not act as a molecular sieve, even though it introduces considerable resistance to hydrodynamic flow."[4] Nearly 15 years later, the concept of the GBM as a size-selective gel is widely accepted.

The endothelial glycocalyx as part of the filtration barrier

The glomerular endothelial layer was initially thought to only exclude cellular components of the blood from filtration as a result of the fenestrations. More recently, it has been recognized that a fine meshwork of glycosaminoglycans covers the entire luminal glomerular endothelial layer, even bridging the fenestrations.[5] Recognition of the endothelial glycocalyx required special fixation and staining techniques, including the use of specific lectins for visualizing the glycocalyx, that are not routinely performed on kidney biopsies. By using these tools, the contribution of the endothelial glycocalyx to the permeability properties of the GFB is now well established, as are alterations in its components as contributors to albuminuria in various glomerular diseases.[5] Thus, the endothelial glycocalyx is now considered the initial, coarse barrier for macromolecular exclusion from ultrafiltration.

Contributions of the podocyte and its slit diaphragm to the filtration barrier

During the last decades an impressive number of proteinuric glomerular diseases have been ascribed to mutations in podocyte-expressed genes.[6] This association has led to the proposal that podocytes and their slit diaphragm directly contribute to the GFB, especially as a number of mutated genes contributing to nephrotic syndrome, most notably nephrin, are integral components of the slit diaphragm. Other genes mutated in hereditary glomerular diseases include molecules involved in cell-cell and cell-matrix interaction and signaling, as well as cytoskeletal organization. Disruptions in any of these can be associated with proteinuric kidney diseases. Furthermore, podocyte injury occurs in a wide variety of acquired proteinuric disorders, such as diabetic nephropathy and immune-mediated glomerulonephritis. In all of these conditions the architecture of the slit diaphragm is disturbed, resulting in what renal pathologists refer to as "foot process effacement;" as a result, the conclusion that the slit diaphragm is an important component of the GFB appeared logical.

Questions about the role of the slit diaphragm as a direct contributor to the permselectivity of the GFB were raised as early as 1975 by Farquhar, and more recently by Smithies and colleagues[4,7] and Grahammer *et al.*[8] Nonetheless, even if the slit diaphragm itself does not directly contribute to glomerular permselectivity, it may indirectly contribute to the function of the GFB. This might be due to functions of the slit diaphragm in connecting foot processes from adjacent podocytes, and thereby in cell-cell signaling. The slit diaphragm also separates the podocyte cell membrane in the urinary space from that facing the basement membrane, which may be important in maintaining the GFB through cell-matrix/GBM interactions and integrin signaling.

What is the role of the special glycocalyx membrane domain between podocytes and the basement membrane in glomerular permselectivity?

The base of the podocyte foot processes directly interacting with the GBM is covered by a special and unique glycocalyx, which is different from that covering the luminal surface of podocytes.[9] It has been speculated that this glycocalyx between podocyte and GBM may be part of the charge-dependent GFB. If and how changes in this unique glycocalyx secondary to alterations in the slit diaphragm, podocyte injury, or inside-out signaling might alter the GFB remain to be examined.