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Update on the glomerular filtration barrier

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Abstract

Purpose of the review—The nephrology community lacks a unified view of protein sieving through the glomerular capillary wall (GCW). The GCW consists of three distinct but closely interacting layers: the fenestrated endothelium, with its glycocalyx; the podocytes, with their interdigitated foot processes and slit diaphragms; and the intervening glomerular basement membrane (GBM). Proteinuria is associated with abnormalities in any one layer, suggesting that each contributes to the glomerular filtration barrier (GFB). Proteinuria can also be induced in the context of a normal GCW. Here we review some classic studies as well as some newer concepts and present competing hypotheses about the GFB.

Recent findings—Two almost forgotten concepts have recently emerged. One group has challenged the exquisite selectivity of the GFB to albumin and suggested that proteinuria is the result of abnormal tubular uptake. There has also been a reemphasis on diffusion through the GBM as the driving force behind macromolecular filtration. New evidence suggests that the endothelial glycocalyx is an important charge-selective barrier.

Summary—We suggest viewing the GFB as a dynamic rather than as a rigid barrier, requiring three healthy layers and a hemodynamic steady state. Multiple challenges to studying the endothelium, the tubular handling of albumin, and the role of hemodynamic forces will require new tools, new hypotheses, and open minds.

Keywords

Proteinuria; Glomerular filtration barrier; Permselectivity; Gel permeation; Podocytes

Introduction

The terms glomerular capillary wall (GCW) and glomerular filtration barrier (GFB) were coined during the 1950s to describe the novel structure observed by electron microscopy (EM). The GCW consists of three distinct but interacting layers: fenestrated endothelial cells, podocytes with their foot processes (FPs) and slit diaphragms (SDs), and a shared intervening extracellular matrix called the glomerular basement membrane (GBM) [1]. We now have a better understanding of the complex nature of these three layers and the interactions among them (Figure 1). Either genetic or acquired abnormalities in each of the three layers of the GCW can lead to a defective GFB, proteinuria, and kidney disease. However, it is unclear whether the pathway leading to increased protein concentration in the glomerular filtrate is similar in all cases, or if it differs considerably depending on the primary defect. Importantly, if proteinurias can be segregated based on their causes, can we better predict outcomes or employ better treatments?

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We would like to direct readers to multiple excellent reviews that discuss in more detail than possible here the many aspects of glomerular filtration and physiology [2–4••,5–7••].

Historical prospective

Karl Ludwig in the 1800s was the first to propose that the glomerulus works as a protein sieve. Multiple studies in the first half of the twentieth century supported his ideas [8]. With the advent of EM and the discovery of the intricate nature of the GCW [1], investigators attempted to localize the GFB within the GCW using different tracers, with variable results [9]. With the arrival of the genomic age, we have gained a much better understanding of the molecular structure of the GCW, but our understanding of the functional aspects of the GFB has not advanced at the same rate. In fact, many of the current controversies surrounding the GFB (size vs. charge selectivity; SD vs. GBM as predominant albumin barrier; glomerular barrier vs. tubular reabsorption as the major "anti-albuminuria" mechanism; determination of sieving coefficients) are not new, as some go back more than 50 years [10,11].

Glomerular sieving coefficient (GSC)

The local sieving coefficient of a molecule is the ratio of its concentration in filtrate to that in plasma at a particular point along a capillary [2], whereas the GSC is the ratio of its concentration in Bowman's space to that in plasma. Because albumin is the most abundant plasma protein, and considering that albuminuria is viewed by most as one of the most important signs of glomerular disease, determining albumin's GSC is central to understanding the GFB.

How tight is the GFB?

The glomerulus works as a macromolecular sieve, retarding the passage of plasma proteins and certain exogenous tracers, while allowing relatively free flow of water and small solutes. Early micropuncture studies showed albumin's concentration in the proximal tubules of normal rats to be 0.7–1 mg/100ml [12], which increased after the induction of proteinuria. Using differential micropuncture techniques to eliminate interstitial contamination, Tojo and Endou determined the albumin concentration in rat glomerular filtrate to be 22.9 μg/ml, with a calculated GSC of 0.00062 [13]. Recently, much higher albumin GSCs of ~.03, about 50 times the long accepted estimates, have been reported by one group of investigators using different techniques [14–21••]. Yet even using the highest GSC estimates, only ~3% of plasma albumin passes through the GFB. Nevertheless, the difference between 3% and the more widely accepted 0.06% is staggering, and it suggests a fundamental flaw in one or perhaps both measurements.

Role of size vs. charge selectivity

The existence of size selectivity is universally accepted. Studies with inert tracers such as Ficoll and dextran indicate that the GSC is inversely related to molecular weight and radius [22–24]. However, the location of the size barrier is ill-defined. The contributions of podocyte SDs, the GBM, and the endothelium are not fully understood.

Charge selectivity was established from classic studies using charged dextrans [25,26]. Negatively charged dextran was more restricted than neutral dextran, which was more restricted than positively charged dextran of similar size. There is a detectable charge selectivity defect associated with proteinuria in animal models and human subjects. Charge selectivity was also suggested to explain the difference between the GSCs of native (anionic) proteins and uncharged Ficoll of similar size [27,28]. However, charge selectivity is not universally accepted. The concept was formulated through the use of charged polysaccharides (dextran

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and Ficoll) that do not behave like the more rigid spherical proteins [29–31]. Charge selectivity has been challenged by Comper and coworkers to explain the higher than anticipated GSC they have reported.

The location of the charge barrier, if it exists, has not been fully defined. Despite earlier reports, reducing fixed anionic charge sites in the GBM by more than 50% via removal of agrin has no consequences on urinary albumin concentration [32,33]. Cellular glycocalyces (of podocytes and endothelial cells), however, may provide sufficient charge to form an effective barrier (see below) [34], but this is underappreciated, understudied, and requires further morphological analysis.

Where is the barrier?

Data generated over decades suggest that normal macromolecular filtration requires the contribution of all three layers of the GCW: endothelium, GBM, and podocytes [3,7••].

Endothelium

The fenestrated glomerular endothelium may play a direct role in determining protein sieving [35,36]. Although the fenestrae are too large to form any meaningful barrier and lack a diaphragm, endothelial cells have an elaborate luminal surface glycocalyx forming a highly negatively charged coat that covers the fenestrae with plugs (Figure 2), which could be central for charge selectivity [35]. However, the glycocalyx is highly sensitive to the hypoxia encountered during tissue fixation, making it difficult to visualize and contributing to underestimations of its importance [36]. Visualizing the glycocalyx requires fixation under near normoxic conditions or perfusion with positively charged tracers [37•,38]. The extent of the glycocalyx's interactions with plasma proteins, considering that such interactions may change its restrictive properties, is poorly understood [39].

Pre-eclampsia/eclampsia is the classic proteinuric disease associated with endothelial dysfunction [40] and is now known to be caused by blockade of trophic factors, such as vascular endothelial growth factor (VEGF), that are required for endothelial cell health [41]. In animal models, manipulating VEGF signaling results in an endothelial injury similar to pre-eclampsia, with proteinuria and kidney failure even in the absence of detectable podocyte and GBM injury [42••,43,44]. Diabetic nephropathy is also associated with endothelial dysfunction [45]. Despite advances in our understanding of the mechanisms of glomerular endothelial cell injury, our knowledge of the full extent of the glomerular endothelium's contribution to permselectivity is limited.

GBM

Classic studies localizing different tracers by electron microscopy indicated an important role for the GBM in glomerular permselectivity [46–48]. The pattern and distribution of ferritin and high molecular weight dextran (62–125 kD) in normal rats was restricted to the subendothelial aspect of the GBM and changed dramatically after puromycin aminonucleoside-induced proteinuria, with deeper penetration of the tracers into the GBM and the appearance of tracer-laden endocytic vesicles within podocytes [47,49]. Studies using endogenous albumin and IgG showed similar distributions, suggesting that the GBM is central to determining GSC [48]. However, many of these studies showed the GBM to be leaky at the site of endothelial cell or podocyte detachment in puromycin aminonucleoside (PAN)-induced proteinuria or after clamping the renal artery and vein [50–52].

It is well known that GBM abnormalities can result in proteinuria. The best example is the absence of laminin β2, which results in congenital nephrotic syndrome in both mice [53,54]

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and humans [55,56•]. Laminin β2 is normally the only laminin β chain in the GBM, so its absence would be expected to change the properties of the GBM. *Lamb2*−/−mice are born with mild proteinuria, initially in the absence of significant endothelial or podocyte changes [57], but eventually urinary albumin levels increase greatly, foot processes become effaced, and the mice die at about 1 month of age [54]. The mutant GBM shows increase permeability to ferritin [57]. Pierson syndrome is the corresponding human disease, and the observed pathology includes diffuse mesangial sclerosis [55]. Alport syndrome, a collagen IV disease, is also associated with a disrupted GBM and but initially only mild proteinuria both in humans and in mouse models [58], with increased ferritin permeability observed in mice [59•].

Podocytes and their SDs

The nature of the SD's molecular structure [60–64] and the link between primary SD abnormalities and development of congenital nephrotic syndrome in humans and in animal models indicates a tight link between SD function and the GFB. Furthermore, podocyte FP effacement and SD abnormalities are the hallmark of proteinuria in nearly all settings.

Rodewald and Karnovsky proposed the current model of the SD as a ladder-like structure with a thick central strand [65]. Studies showed that horseradish peroxidase (40kD) and catalase (210kD) concentrations drop sharply at the level of the SD, suggesting it has barrier properties [52]. The structure was further investigated by electron tomography and shown to consist of multiple layers of nephrin strands connecting adjacent FPs. Pores within this meshwork form elongated channels with a maximum width equals to that of albumin [66]. In the setting of *Nphs1* (nephrin) mutation, the slit pores become narrower with only shorter, thinner, less organized strands bridging adjacent FPs and forming relatively larger pores and channels.

However, because the SD is located most distally in the GCW, it is unlikely to function as its most restrictive barrier, even if its pores are smaller than albumin. Proteinuria has been reported in the absence of FP and SD changes [57,67]. Conversely, the degree of such changes in other cases does not correlate with the level of proteinuria [68], and foot process effacement and SD changes are not always associated with proteinuria. Furthermore, albumin and other serum proteins can be podocyte-toxic [69••,70•,71••,72], suggesting that podocytes under normal conditions must be sheltered from high concentrations of plasma proteins.

Mechanisms of protein filtration

Convection refers to drag by fluid flow across the barrier, with the GFB acting as a physical sieve for macromolecules. Convection, however, cannot be the only force driving macromolecular filtration, because changing GFR results in an opposite change in the macromolecular filtration fraction [24,73]. Diffusion has been suggested to explain the lower filtration fraction of macromolecules with increasing GFR. Diffusion also explains the sudden appearance of albumin and IgG in the urinary space after clamping the renal artery, followed by their disappearance after releasing the clamp [48]. Most recently, Oliver Smithies reintroduced this concept in his "Gel Permeation/Diffusion Hypothesis" [6]. According to this hypothesis, diffusion through the GBM, which acts as a modified gel, is the predominant force governing macromolecular movement through the GFB. Diffusion is independent of fluid flow (i.e., GFR), but dependent on the gel's properties. According to this hypothesis, increased protein concentration in the glomerular filtrate can occur by two different pathways. The first is by an increase in the rate of passage of protein by changes in the gel's properties (i.e., by alterations to the GBM and perhaps also to the endothelial glycocalyx). The second is by a reduction in the available surface area for filtration, as occurs either with FP effacement or reduced endothelial fenestration. This reduction results in increased hydraulic resistance and reduced single nephron GFR [2,40], while diffusion of plasma proteins remains constant; i.e., approximately the same amount of protein is diluted in a smaller volume. (A normal, properly

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functioning gel requires stable interactions with the adjacent cells under stable hydrodynamic pressures, which can explain some of the results from studies of isolated GBM [74,75].) The increased protein concentration in the filtrate then overwhelms the tubular resorption pathway, resulting in albuminuria. However, as elegantly simple as Dr. Smithies' hypothesis appears, there are issues to be resolved. Data in the literature show that the filtered load of albumin is more dependent on GFR [7••] than predicted by the hypothesis, and the hypothesis makes no allowance for the effects of tubule flow rate or residence time [76]. As yet there is no direct experimental evidence published in its support.

In overload proteinuria [69••], the GFB and GSC are normal, but both plasma and glomerular filtrate albumin concentrations are higher, with the latter eventually exceeding the tubular threshold for filtered protein resorption. Furthermore, we postulate that in the appropriate setting, podocyte FP effacement can occur without significant proteinuria as long as the GBM or glycocalyx becomes more restrictive, a situation in which reduced albumin diffusion would compensate for reduced GFR [77••].

Role of tubular absorption

Even considering the most restrictive GSC proposed for albumin in humans (~0.0001), a significant amount of albumin will cross the GFB in the normal kidney. And with the much higher proposed GSC estimates, the amount of filtered albumin would easily exceed the nephrotic range if present in the final urine. Thus, in any scenario there must be significant post-glomerular processing of albumin by tubules.

Tojo and Endou, using a fractional micropuncture technique, found that 94% of filtered albumin was absorbed by proximal tubules [13]. Park and Maack studied the fate of albumin in isolated rabbit proximal tubules [78]. They failed to show any transcellular or paracellular transport of intact albumin. Proximal tubule albumin absorption capacity was estimated to be 3.7 ng/min per mm tubule length, and the rate of albumin removal was proportional to fluid absorption. Most of the absorbed albumin was degraded and transported through the basal surface into the bathing solution.

An earlier micropuncture study comparing glomerular filtrate and final urinary albumin concentrations in normal and nephrotic PAN-treated rats showed that urinary albumin increased by a factor of 43, while glomerular filtrate albumin increased by a factor of only 4.5, suggesting abnormal tubular handling of the filtered albumin [12]. More recently, Comper and colleagues have published multiple papers suggesting that the GFB is more leaky to albumin than generally accepted, by ~50 fold. They hypothesize and have provided evidence that filtered albumin is reclaimed in the S1 segment of proximal tubules, with most of it returned intact to the circulation [15,17–21••,79]. They conclude that proteinuria is a tubular rather than a glomerular disorder. If true, we must change the way we view the GFB and think of even selective proteinuria in the context of tubular abnormalities. However, this concept is not accepted by most in the nephrology community. A new report utilizing a specially labeled albumin that can be visualized by fluorescence only after hydrolysis shows that albumin endocytosed by proximal tubular cells is degraded quickly [80••]. However, there was no indication of the ratio of degraded to intact albumin at the basal pole of the cells.

Concluding remarks

We suggest viewing the GFB as a functional unit consisting of three different elements [3]. We also suggest viewing it as a dynamic sieve, rather than as physically inert. Normal GFB function requires not only three intact GCW layers, but also a hemodynamic steady state in the glomerular capillary and the urinary spaces. A change in any factor (GBM, cell, glycocalyx, local GFR, or plasma albumin concentration) will alter albumin's concentration in the primary

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filtrate. When the level of albumin in the filtrate exceeds the tubular absorption threshold, albuminuria will ensue.

We suggest the following approaches for the future:

1. Analyze *in vivo* the growing list of proteinuric animal models of different etiologies to better delineate the pathophysiology of proteinuria.
2. Define the endothelium's contribution to the GFB, in part by developing the tools necessary to manipulate glomerular endothelial cell gene expression.
3. Investigate the endothelial glycocalyx with better and reproducible morphological and physiological assessments.
4. Devise a better "mousetrap" to understand tubular handling of albumin, especially the fate of resorbed albumin: degradation, return to the circulation, or both.

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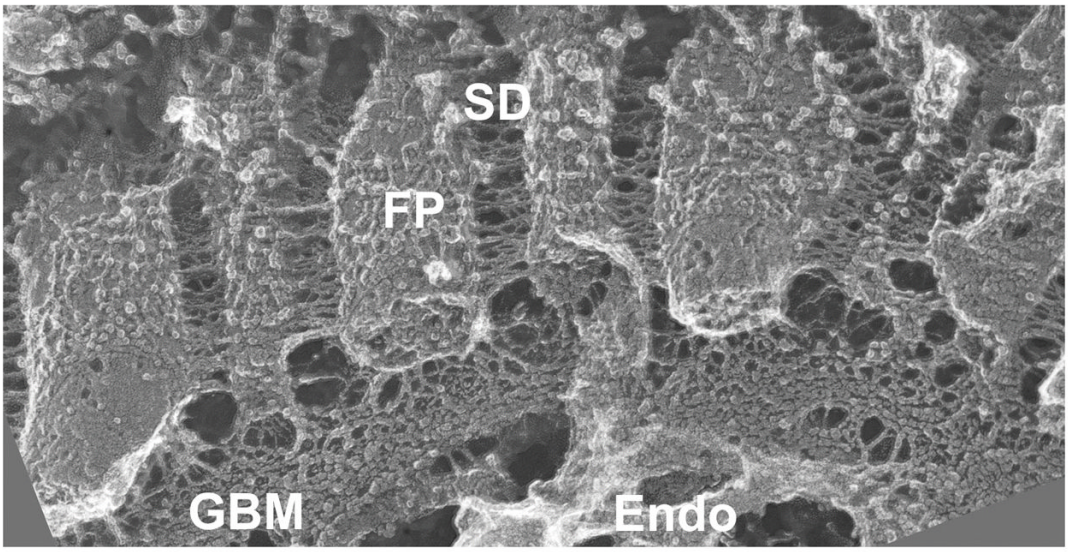

Figure 1. View of the glomerular capillary wall by freeze fracture deep-etch scanning electron microscopy

The GCW consists of the diaphragm-less fenestrated endothelium (Endo), the GBM with its thick central layer (corresponding to the lamina densa by transmission EM), and podocyte FPs with bridging SDs. Note the thin strands connecting podocytes and endothelial cells to the GBM. Image provided by Dr. John Heuser, Washington University School of Medicine.

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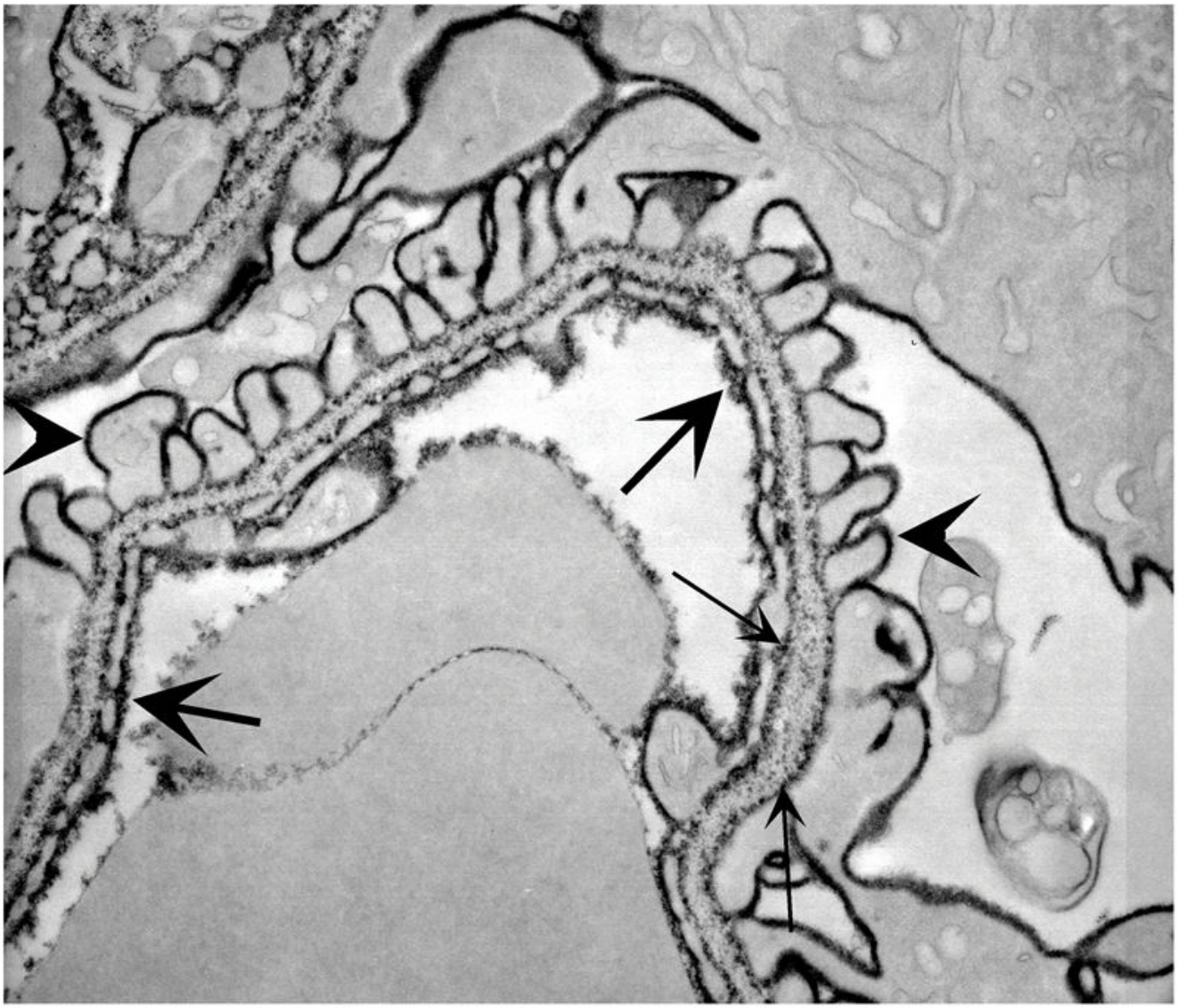

Figure 2. GCW charges

Kidneys were perfused with a high concentration of polyethylenimine (PEI) and post-fixed in phosphotungstic acid and osmium. The extent of the endothelial glycocalyx and fenestral plug is clear (large arrows). Note also the podocyte glycocalyx (arrowheads) and GBM charges (small arrows), which are strongest at the laminae rarae internae and externae.