Why Do We Not All Have Proteinuria? An Update of Our Current Understanding of the Glomerular Barrier

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The key question is not why some patients have proteinuria but rather why not all people have it. In the present review, we will present an update on the glomerular barrier after the recent breakthroughs in podocyte biology. In particular, we will discuss the role of the endothelium, which seems to be a neglected part of the glomerular membrane.

We all know that proteinuria is a hallmark of renal disease. However, with a daily production of 180 l of primary urine from blood containing 50–70 g/l of protein, it is perhaps more relevant to ask why we do not all have massive proteinuria. Naturally, the design of the glomerular “filter” and the precise control of the filtration rate are absolutely crucial for everyday homeostasis and hence for our survival. The intricate properties of the glomerular barrier are, however, still well-hidden secrets, and even less is known of the mechanisms causing glomerular disorders.

In recent years there has been a renewed interest in the field of glomerular permeability, mainly due to the discovery of nephrin (9). Our understanding of podocyte cell biology has in a short time advanced immensely (11). Several new proteins besides nephrin are now known to be vital for an intact glomerular barrier, such as for example podocin, FAT, NEPH1, zonula occludens-1, CD2-associated protein, P-cadherin, α-actinin-4, dystroglycan, integrins, and laminin (11). Thus several hereditary renal disorders with hitherto unknown causes have now been characterized in detail, and mouse knockouts have shown that various podocyte component defects can cause proteinuria (11). Undoubtedly, the podocytes are important for an intact glomerular barrier. There is, however, a tendency to focus entirely on the role of the podocytes and to belittle the impact of the glomerular basement membrane (GBM), the endothelial cells, and in particular the endothelial surface layer, sometimes called the glycocalyx.

Currently, there are two rather extreme views of glomerular permselectivity in the literature. On the one hand, the classical concept of glomerular size and charge selectivity has been challenged in several publications from one laboratory (14) suggesting that the glomerular capillaries are quite leaky. On the other hand, recent findings on the podocyte slit membrane suggest that it totally restricts the passage of albumin (20). In the present review, we will try to reconcile these contradictory hypotheses and discuss the “true” properties of the glomerular barrier based on recent functional studies. Interestingly, there is growing evidence that the heavily fenestrated endothelial cells are important elements of the highly permselective glomerular barrier.

Structure of the glomerular barrier

The blood-to-urine barrier is highly organized and consists of four different layers that solutes and fluid must pass to reach the urinary space. A drawing of the glomerular barrier is shown in Fig. 1. First, there is an endothelial cell surface layer (sometimes called the glycocalyx) composed of proteoglycans, glycosaminoglycans (GAGs), and plasma proteins (see Fig. 2). Second, the endothelium contains numerous open fenestrae. Third, the GBM, which previously was considered to have three different laminae, is probably rather homogenous unless subjected to chemical fixatives. Finally, there is a slit membrane between the podocyte foot processes.

Exactly which part of the barrier is most important has been debated for decades. Currently, the focus has shifted from the GBM to the podocytes (11, 20). So far, the endothelial cell surface layer has received little attention, which may be unjustified as will be discussed below. Moreover, isolated GBMs show some size selectivity but lack charge selectivity in vivo (2), which has been taken as evidence of its limited role in the glomerular barrier. However, the isolation procedure may change the composition of the GBMs, and their permeabilities seem to depend on the hydrostatic pressures applied. In any case, removal of the principal proteoglycan of the GBM, perlecan, does not produce proteinuria (12), suggesting a limited role for the GBM in the glomerular barrier.

The story of leaky glomeruli and tubular reuptake of intact albumin

The classical view of a highly size- and charge-selective glomerular barrier (3) has been challenged by an alternative albumin retrieval hypothesis (14). Based on studies of isolated rat kidneys perfused with erythrocyte-free solutions at 37°C and using cytotoxic agents to inhibit tubular uptake, the authors suggested that the glomerular sieving coefficient of albumin is close to 8% rather than the more generally accepted values of 0.01–0.1% (14). The authors suggest that the absence of overt proteinuria is due to tubular reuptake of intact albumin and/or due to urinary degradation of protein (14). Albeit a new and fascinating concept, there are certain
facts that speak against the albumin retrieval hypothesis. First, a sieving coefficient of 8% would imply a daily filtration and reuptake of albumin of >700 g in humans (0.08 × 180 l/day × 50 g/l = 720 g/day). Second, the toxins used to inhibit tubular cell activity seem to damage the glomerular barrier per se (10), whereas cooling (the gold standard for inhibition of cellular activity) results in albumin-sieving coefficients closer to 0.1% (10). The latter value is slightly higher than the 0.06% obtained by Tojo and Endou (19) in a micropuncture study in which care was taken to minimize the effect of tubular activity. Thus the tubular uptake of albumin seems to be 9 g/day or less (0.001 × 180 l/day × 50 g/l = 9 g/day). Third, the mechanism behind tubular uptake of albumin is now rather well understood (4) (see Fig. 3). It involves the megalin-cubulin complex and an obligatory incorporation into lysosomes, degradation, and return to the circulation in the form of amino acids (4). Thus there is no evidence of reuptake of intact albumin (4).

Further studies of the mechanisms underlying the loss of glomerular charge selectivity (14) under certain experimental conditions will undoubtedly reveal more details of the design of the intricate charge barrier.

Do the podocytes prevent all proteins from reaching the urine?

As stated above, there is no doubt that the podocyte is a key component of the glomerular barrier. The question is, however, if it is the only important element for restriction of fluid and protein transport. The slit membrane has been suggested to have porous structures of 40 × 140 Å (20), e.g., with a slit half-width of 20 Å. Since albumin has a Stokes-Einstein radius of 35.5 Å, it would not be expected to reach the urinary space at all, except through a few “large pores” or “shunt pathways.” However, the slit membrane dimensions described above are incompatible with glomerular sieving of other solutes as well. Thus neutral solutes with Stokes-Einstein radii of 20 Å do in fact have sieving coefficients close to unity, suggesting almost free passage (3, 10), whereas a slit half-width of 20 Å would imply complete restriction. To illustrate the impact of “pore” dimensions, the podocyte slit membranes would (according to irreversible thermodynamics) exert 100% of the glomerular size selectivity if their functional pore radius were 47 Å instead of 20 Å. On the other hand, a further doubling of the pore radius to 100 Å would imply minimal restriction for molecules the size of albumin (with sieving coefficients >0.6). We therefore conclude that the attempts to calculate pore dimensions from electron microscopy so far have been incorrect (perhaps due to fixation artifacts) and that the role of the podocytes may be unduly emphasized (20).

FIGURE 1. Schematic drawing of the glomerular barrier and its 4 major layers, namely the podocyte slit membrane, the glomerular basement membrane, the fenestrated capillary endothelium, and the endothelial cell surface layer, often called the glycocalyx.

FIGURE 2. Schematic drawing of the fenestrated glomerular endothelium. The entire endothelial cell (EC) is surrounded by a stagnant cell surface layer, the composition and turnover rate of which remains to be elucidated. It is, however, conceivable that proteoglycans (pink, green) and glycosaminoglycans (yellow, blue) produced by the endothelial cells interact with certain plasma proteins (red) to form a gel compartment with size- and charge-selective properties.
Current understanding of glomerular selectivity

The classical view of a highly size- and charge-selective glomerular barrier (3) is still qualitatively true. The use of neutral and sulfated dextran did, however, lead to errors in the estimates of functional pore radii and of the charge density (3, 10). In vivo studies of the glomerular sieving of proteins are important but have serious limitations due to the complexity of the system. Therefore, most information has been obtained by using tracers such as Ficoll, a glucose polymer that behaves as a neutral hydrated sphere. Recent studies using neutral Ficoll have demonstrated that the glomerular barrier has numerous functional small pores \( \approx 50 \text{ Å} \) in radius and a few larger pores with radii between 80 and 100 Å. The large pores account for >1% of the total hydraulic conductance, and there is a huge area available for diffusion (10). These data were obtained in isolated kidneys perfused at low temperature (cIPK) to reduce tubular cell and urinary protease activities, but similar size-selective properties have been found in vivo in rats (7) and even in humans (1). Moreover, in the cIPK the sieving of neutral horseradish peroxidase has been found to be identical to that of neutral Ficoll of similar Stokes-Einstein radius (17, 18), strengthening the value of the experimental model. Our knowledge of the glomerular charge selectivity has increased substantially lately, and the data suggest a charge density close to 50 meq/l (7, 8, 10, 17, 18). Moreover, the estimated charge density is affected by changes in ionic strength (17, 18), which suggests a role for the endothelial glyocalyx (see below). Thus the glomerular barrier discriminates solutes on the basis of their size and charge in qualitative agreement with the classical concepts (3). The shape of the molecule also affects its glomerular passage, and elongated solutes have higher clearances than spherical ones of similar hydrodynamic size and charge.

On the basis of the biological data, it is possible to construct theoretical models that predict solute transport across glomerular capillary walls. The models use irreversible thermodynamics and nonlinear flux equations to describe the transport through functional pores, slits, or a fiber matrix (gel) (5). The functional pores can be uniform (homoporous) or have a lognormal pore size distribution, or consist of two distinct pore sizes (5). Furthermore, there needs to be some degree of heteroporosity with large pores or shunt pathways to fit the experimental data (1, 5, 10). Most models are, however, only applicable to neutral spherical solutes. Efforts have been made to combine glomerular size and charge selectivity in one model (10). The most advanced theoretical model is based on charged fibers interacting with neutral or charged spherical solutes (8). Such models are extremely complex but seem to describe the biological data with high precision as recently presented for mouse kidneys (8). On the basis of the charged fiber model, it is possible to construct the degree of restriction of solutes depending on their size and charge, as illustrated in Fig. 3.

The endothelial cell surface coat

The cell surface coat, sometimes called the glyocalyx, covers the endothelial cells. It consists of highly negatively charged proteoglycans and GAGs reinforced with plasma proteins such as orosomucoid. The latter protein, which has been shown to be of vital importance for capillary permeability, is in fact produced by the endothelial cells themselves (16). It is not possible to detect the surface coat by using electron
microscopy due to dehydration and destruction of the layer during sample preparation. However, with special fixation protocols the layer appears as a rather thick structure close to 300 nm (13). The presence of a cell surface coat has also been functionally tested in cremaster microvessels by Henry et al. (6), who described an exclusion zone for molecules up to ~0.5 μm in the periendothelial cell coat zone. Recently, it was shown that the synthesis of proteoglycans and GAGs was downregulated when glomerular endothelial cells were exposed to puromycin aminonucleoside (an agent used to induce nephrotic syndrome in rats) (15). Indeed, there is a growing interest in the participation of the endothelial cells in the glomerular barrier. More information, however, is required concerning the composition, signaling, and turnover rate of the endothelial cell surface coat under normal and pathophysiological conditions.

Summary

The glomerular barrier discriminates solute transport depending on size, charge, and shape. Undoubtedly, the podocyte slit membrane is a vital part of the barrier, and its structural components have been carefully characterized in recent pioneering work. Through this work, the background of several hereditary disorders has now been clarified. The role of the GBM is, however, less clear, with data suggesting minimal size and charge selectivity. Moreover, the endothelial cell surface layer may contribute substantially to the restriction of solutes. The research activity is currently high in the field, which hopefully will give us more information on different glomerular disorders and how to treat our patients.

This study was supported by Swedish Medical Research Council Grants 9898 and 14764, the Knut and Alice Wallenberg Research Foundation, the Ingabritt and Arne Lundberg Research Foundation, the National Association for Kidney Diseases, The Swedish Society for Medicine, and Sahlgrenska University Hospital Grant LUA-S71133.

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