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REVIEW

Role of the podocyte in proteinuria

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Abstract In recent years, the podocyte, with its elaborate cytoarchitecture and slit diaphragm, has been the focus of extensive research, yet its precise role in the glomerular filtration barrier is still debated. There are puzzling observations indicating that a comprehensive mechanistic model for glomerular filtration is still necessary. There is no doubt that podocytes are essential for glomerular filtration barrier integrity. However, most albumin never reaches the podocyte because it is prevented from entering the glomerular filter at the endothelium level. Another puzzling observation is that the glomerular filter never clogs despite its high load of several kilograms of plasma proteins per day. Recently, we proposed a novel model in which an electrical potential difference is generated across the glomerular filtration barrier by filtration. The model offers novel potential solutions to some of the riddles regarding the glomerular filter.

Keywords Electrokinetic · Permselectivity · Albumin · Endothelial cells · Filtration

Introduction

The glomerular filtration barrier is composed of at least five layers (Fig. 1) [1–3]. The endothelial cell layer is perforated by multiple pores (fenestrae) and is covered by the glycocalyx, a mixture of negatively charged proteoglycans. For the purposes of filtration, the glomerular basement membrane (GBM) is essentially composed of a dense gel-like meshwork of negatively charged glycoprotein polymers. The outside of the filter is covered by multiple interdigitating foot processes of the visceral epithelial cells (podocytes). The filtrate passes across the filtration slits, which are bridged by specialized intercellular junctions, termed the slit diaphragm. Slit diaphragms are composed of the major protein complexes nephrin/nephrin-related protein 1 (NEPH1) and cadherin FAT1, which signal to the podocyte cytoskeleton [4–7]. Finally, a functional role of the subpodocyte space covering parts of the filtering surface for retarding filtration in selected areas has been proposed [8].

Every day, about 180 l of plasma containing several kilograms of plasma proteins are filtered across a glomerular filtration area of 0.5–2 m^2 [9]. More than 99.9% of the plasma proteins are retained by the filter, yet—under physiological conditions—the filter never shows any signs of clogging. To this day, it remains a mystery how this extraordinary task is accomplished by the glomerular filter. In this review, we focus on the role of the podocyte in glomerular filtration and discuss a novel theory that reconciles many of the seemingly controversial and so far unexplained phenomena. For a complete review of glomerular filtration, we refer to Haraldsson et al. [9].

Podocytes are essential for the glomerular filtration barrier

There is no doubt that podocytes are an essential and integral part of the glomerular filter [10]. The most significant evidence is derived from the identification of mutations in genes exclusively expressed in podocytes

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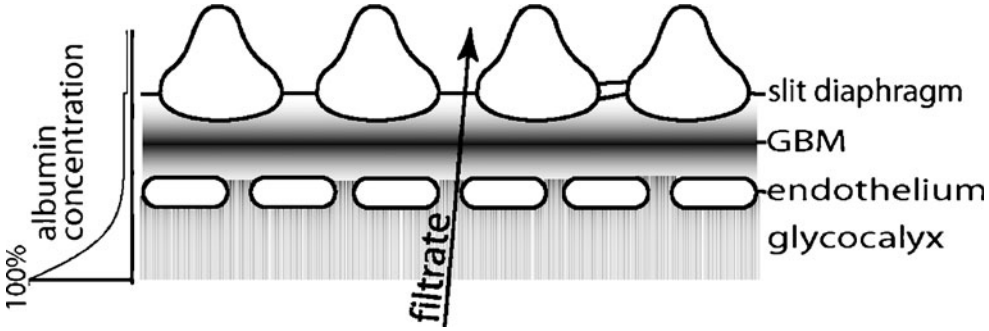

Fig. 1 Glomerular filtration barrier. The filtrate passes the layers of the filter as a laminar, nonturbulent flow along an extracellular route (*arrow*). Albumin is largely excluded from entering the filter, as indicated by the local albumin concentration on the *left*. *GBM* Glomerular basement membrane

within the kidney (e.g. podocin) [11]. Their mutation causes a breakdown of the podocyte cytoarchitecture (termed foot-process effacement) and of the integrity of the glomerular filter. As a rule, generalized foot-process effacement usually results in large-scale proteinuria, but—as discussed below—proteinuria can also occur with intact foot processes. In adult humans with nephrotic-range proteinuria, about 3–60 g of plasma protein per day are excreted, representing about 0.5% of the filter load. Interestingly, physiological foot-process effacement can be regularly observed along the nonfiltering part of the glomerular efferent arteriole [12], which is not associated with proteinuria.

Most plasma albumin never reaches the podocyte under physiological conditions

There are good indications that the bulk of the plasma proteins is excluded from the filtrate before it reaches the podocyte. When rat kidneys were fixed in vivo while filtration was ongoing, Ryan and Karnovsky showed that plasma albumin was retained within the capillary lumen and did not penetrate significantly into or across the filter [13]. Other groups, who used a more sophisticated immunoelectron microscopic technique, confirmed this finding [14, 15]. Theoretical considerations support the notion that the slit membrane cannot be the most selective layer of the filter. It is important to note that in a multilayered filter, the layers of the filter must be arranged with decreasing selectivity. This means that in a multilayered filter, the most selective layer must come first. If the slit membrane were a more selective filter layer than the GBM, retained plasma proteins would accumulate underneath the slit membrane (concentration polarization) and ultimately the filter would clog [9]. On the other hand, theoretical considerations do not necessitate the endothelial cell layer being the most selective part of the filter. It could also be possible that the endothelium contributes very little size selectivity to larger molecules and that the GBM is the first and most (size-) selective layer. However, based on these considerations, it seems very likely that the most selective layer of the filter cannot be the slit diaphragm of the podocytes.

Alternative filter systems without podocytes

There is at least one extrarenal filtration barrier, which lacks podocytes and which produces a primary filtrate that is also virtually free of plasma proteins: the choroid plexus. Cerebrospinal fluid contains about 5–40 mg/dl of protein, i.e. has a sieving coefficient of about 0.003–0.0008, which is similar to the sieving coefficient of the renal glomerulus. Interestingly, Kobessho et al. found in a small cohort study of diabetic patients that protein concentrations in cerebrospinal fluid increased with diabetes duration [16]. Podocytes are therefore not necessary for a highly selective biological filter.

Contribution of the glomerular endothelium to permselectivity

There is an accumulating body of evidence that endothelial dysfunction is a major determinant for the pathogenesis of (pre-) eclampsia. It occurs in up to 5% of pregnant women and is characterized by a reduced glomerular filtration area (−30% compared with normal pregnancy), glomerular capillary endotheliosis with hypertrophy of endothelial cells, loss of endothelial fenestrae, and subendothelial fibrin deposition [17]. Usually, there is only mild proteinuria (<3 g/day), but in severe cases, nephrotic-range proteinuria may occur. Podocyte foot processes are usually conserved. Recently, it was discovered that increased systemic levels of sFlt-1, which binds and inactivates vascular endothelial growth factor (VEGF) and placental growth factor (PLGF); and of endoglin, a transforming growth factor beta (TGF-β) antagonist; are released by the placenta of women affected by eclampsia [18, 19]. VEGF acts predominantly on endothelial cells. Specific inhibition of VEGF signaling in antiangiogenic therapies or in knockout mice also results in proteinuria and hypertension, similar to the effect in preeclampsia [20, 21].

A new model for the flux of proteins across the glomerular filtration barrier: not two but at least three different physical effects govern glomerular permeability to albumin

Until recently, the passage of albumin across the glomerular filtration barrier was believed to be driven by two effects [22]: diffusion, driven by a higher concentration of albumin within the capillary and a low concentration within the primary filtrate; and convection, driven by the drag of the flux of water across the filtration barrier. Recently, we proposed that electrical effects should be considered in addition to diffusion and convection when modeling the passage of albumin across the filtration barrier [23]. We describe this mechanism in two steps: First, a potential difference is generated across the filtration barrier by the passage of the water and ions within the plasma across the glomerular filter. Second, this potential influences the passage of negatively charged albumin across the filtration barrier.

Generation of a potential difference across the glomerular filtration barrier

At the 2005 annual meeting of the American Society of Nephrology, Dr. Somers proposed that electrokinetic effects are predicted to occur during the filtration process. Plasma is an ionic fluid consisting predominantly of the dipole water and the ions sodium (Na^+), chloride (Cl^-), bicarbonate (HCO_3^-), and potassium (K^+). When this ionic solution passes the glomerular filtration barrier, the ions interact with the charged filter walls (Fig. 2a,b). Multiple physical effects overlay each other in this process, which are beyond the scope of this review. We determined experimentally in *Necturus maculosus* (common mudpuppy, Fig.2c), that as the sum of these interactions, anions pass the glomerular filter slightly faster than cations, thereby generating an electrical potential across the filter, which is proportional to filtration pressures. The electrical field is quite weak (about 0.05 mV or more, i.e. 166 V/m assuming that the filter is

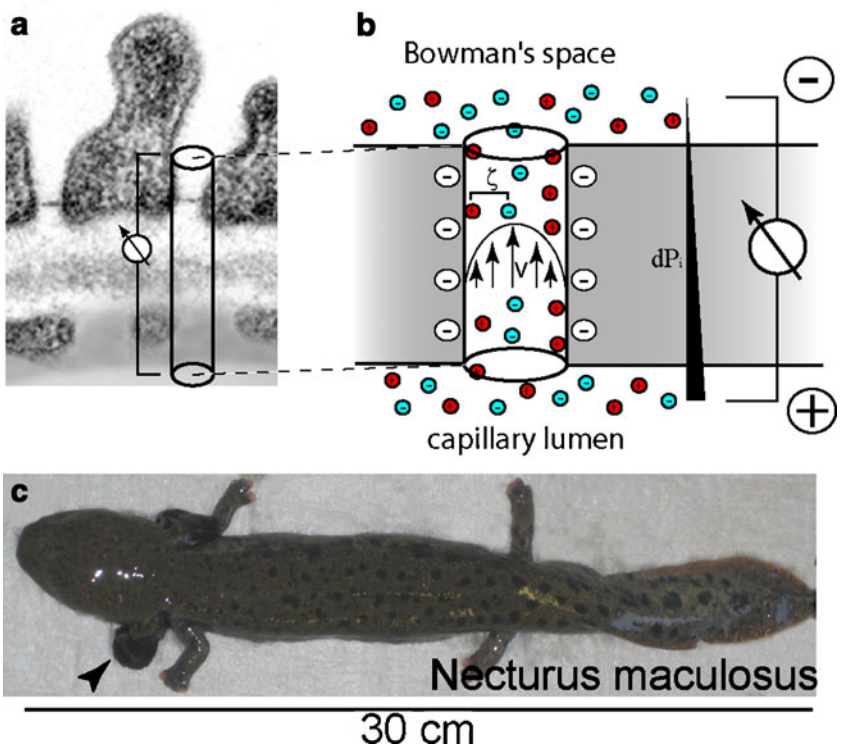

Fig. 2 Highly simplified model for the generation of streaming potentials across the glomerular filtration barrier. **a** The entire cross section of the glomerular filter can be modeled as a single pore. **b** Higher magnification of the pore. The ionic fluid [cations: sodium (Na^+), potassium (K^+); anions: chloride (Cl^-), bicarbonate (HCO_3^-)] passes through the pore driven by the effective filtration pressure dP_i. Within the pore, ions interact with the charged walls so that cations are displaced toward the periphery of the wall in a different fashion than are anions (determined by the local zeta potential, ζ). The convective flow with the velocity (v) is not constant across the diameter of the pore (*arrows*) so that cations and anions will move across the pore with different velocities, resulting in the generation of a filtration-dependent potential. As the physical interactions within the pore are too complex to be calculated, in the isolated perfused *Necturus* kidney, we determined that the sum of the effects generates a potential of about −0.045 mV per 10 cm H_2O within Bowman's space [23]. **c** *Necturus maculosus* (common mudpuppy). Note that the animal has external gills (*arrowhead*) as well as primitive lungs

300 nm wide) and is negative within Bowman's space. The energy for generating this field is derived from blood pressure.

Differences between a filtration-dependent potential and charge selectivity

Charge selectivity describes the fact that molecules the size of albumin or larger will pass the glomerular filter better when positively charged (i.e. cationic) and worse if negatively charged (i.e. anionic). As the glomerular filter bears fixed negative charge, this effect can be explained by electrostatic repulsion of the anionic macromolecules within the filter meshwork and should be independent of flow. However, discussions about this concept have reemerged in studies by several groups, following charge removal from the GBM using enzymes or homologous recombination in mice [24–26]. These groups demonstrated the surprising fact that removing charge from the GBM does not significantly influence albumin permeability. Interestingly, these experiments involved only the GBM, not the endothelial glycocalyx, which is predicted to be an important layer for filter selectivity (see above). Therefore, it could be argued that the GBM does not contribute significantly to any of the electrical effects (charge selectivity or filtration-dependent potential).

Consequences of the potential difference for passage of albumin

As albumin and almost all plasma proteins are negatively charged, the potential will induce an electrophoretic flux of albumin when it enters the glomerular filter (Fig. 3). This flux will be oriented toward the blood, i.e. in the opposite direction of diffusion and convection. In order to estimate the relevance of the electrophoretic flux, a mathematical model was created that indicated that electrical potential differences in the range of 0.02–0.04 mV are sufficient to induce an electrophoretic flux of albumin, which counterbalances the outward diffusive and convective fluxes [23]. This suggests that negatively charged macromolecules may be transported out of the filter back into the blood by electrophoresis. Cationic (i.e. positively charged) macromolecules, on the other hand, will be transported out of the filter into the urine by diffusion, convection, and electrophoresis. This is also true for larger molecules (i.e. immunoglobulins), as the electrophoretic flux component is predicted to increase relative to diffusion and convection with increasing macromolecule size. As virtually no macromolecule without electrostatic charge exists, this novel model may provide an elegant solution to why the glomerular does not clog under physiological conditions.

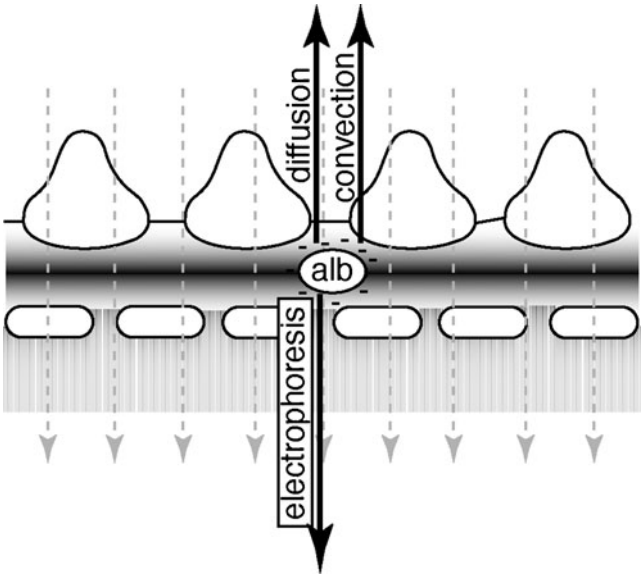

Fig. 3 Forces that influence the passage of albumin across the glomerular filtration barrier. Albumin (*alb*) is driven across the glomerular filter into the primary urine by convection (i.e. drag of the water) and diffusion (i.e. concentration difference between plasma and primary filtrate). A potential difference (*gray arrows*) is established across the filtration barrier by passage of the small ionic plasma components (i.e. dipole water, and small solutes sodium, chloride, etc.), which interact with the charged filter walls of the glomerular filtration barrier. As albumin and most plasma proteins are negatively charged, they are driven by electrophoresis back toward the blood

What are the functions of the podocyte?

Most researchers now agree that the glomerular filter cannot be regarded as individual layers but must be analyzed as a whole [27]. Nevertheless, several specific tasks can be attributed to the podocyte.

First, podocytes synthesize GBM. This has been demonstrated in elegant studies by Abrahamson et al. [28], who traced the origin of GBM components in equal amounts to endothelial cells and podocytes using a cell lineage tracing approach. Second, several groups showed in the 1960s that the podocyte is endocytically active [29–33]. As theoretically any macromolecule may pass the glomerular filtration barrier, it can be assumed that podocytes remove at least some of the retentate from the outer GBM by endocytosis. Akilesh et al. [34] detected immunoglobulin accumulation within the glomerular filter in FcRn knockout mice. FcRn retrieves albumin and immunoglobulin (Ig) from early endosomes to prevent lysosomal degradation. Third, we propose that the complex cytoarchitecture of podocytes is optimized to facilitate generation of a filtration-dependent potential. As the glomerular filtration barrier has a very low electrical resistance, charged particles (i.e. ions) must be continuously separated across the entire filtering surface to generate the potential difference. Even in lower vertebrates

with a closed circulatory system (e.g. frog, mudpuppy, freshwater fish, shark, lamprey), podocytes always form foot processes, and the podocyte cellular body is always detached from the capillary surface. The podocyte cell bodies and primary processes cover about one half to two thirds of the filtering surface [3], and it has been proposed that podocyte cell bodies float within the primary urine only to enlarge filtration surface. However, filtration surface could be increased much easier by increasing the number of glomeruli. So why does nature bother to detach podocyte cell bodies? We propose that this is to reconcile two conflicting situations: Podocytes are necessary to synthesize and clear the GBM but at the same time present a potential obstacle for filtration and thus for homogeneous generation of a potential difference. To solve this problem, the podocytes cover the capillaries exclusively with interdigitating foot processes. This allows filtration to occur homogenously across the entire filtering surface so that a potential difference can be established homogenously and thus proteinuria be prevented. This notion is also consistent with the pathogenesis of proteinuria in minimal changes nephropathy, where podocyte foot processes are effaced.

In summary, a model for glomerular filtration, which also considers electrical effects, provides a novel approach to our understanding of the function of the glomerular filter. Podocytes play an intricate and essential part in this highly efficient biological system. Advances in our understanding of the glomerular filter will provide the premises to design novel therapeutical concepts.

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References

1. Haraldsson B, Jeansson M (2009) Glomerular filtration barrier. Curr Opin Nephrol Hypertens 18:331–335
2. Jarad G, Miner JH (2009) Update on the glomerular filtration barrier. Curr Opin Nephrol Hypertens 18:226–232
3. Salmon AH, Neal CR, Harper SJ (2009) New aspects of glomerular filtration barrier structure and function: five layers (at least) not three. Curr Opin Nephrol Hypertens 18:197–205
4. Liu G, Kaw B, Kurfis J, Rahmanuddin S, Kanwar YS, Chugh SS (2003) Neph1 and nephrin interaction in the slit diaphragm is an important determinant of glomerular permeability. J Clin Invest 112:209–221
5. Garg P, Verma R, Nihalani D, Johnstone DB, Holzman LB (2007) Neph1 cooperates with nephrin to transduce a signal that induces actin polymerization. Mol Cell Biol 27:8698–8712
6. Moeller MJ, Soofi A, Braun GS, Li X, Watzl C, Kriz W, Holzman LB (2004) Protocadherin FAT1 binds Ena/VASP proteins and is necessary for actin dynamics and cell polarization. EMBO J 23:3769–3779
7. Huber TB, Benzing T (2005) The slit diaphragm: a signaling platform to regulate podocyte function. Curr Opin Nephrol Hypertens 14:211–216
8. Neal CR, Muston PR, Njegovan D, Verrill R, Harper SJ, Deen WM, Bates DO (2007) Glomerular filtration into the subpodocyte space is highly restricted under physiological perfusion conditions. Am J Physiol Renal Physiol 293:F1787–1798
9. Haraldsson B, Nystrom J, Deen WM (2008) Properties of the glomerular barrier and mechanisms of proteinuria. Physiol Rev 88:451–487
10. Patrakka J, Tryggvason K (2009) New insights into the role of podocytes in proteinuria. Nat Rev Nephrol 5:463–468
11. Boute N, Gribouval O, Roselli S, Benessy F, Lee H, Fuchshuber A, Dahan K, Gubler MC, Niaudet P, Antignac C (2000) NPHS2, encoding the glomerular protein podocin, is mutated in autosomal recessive steroid-resistant nephrotic syndrome. Nat Genet 24:349–354
12. Elger M, Sakai T, Winkler D, Kriz W (1991) Structure of the outflow segment of the efferent arteriole in rat superficial glomeruli. Contrib Nephrol 95:22–33
13. Ryan GB, Karnovsky MJ (1976) Distribution of endogenous albumin in the rat glomerulus: role of hemodynamic factors in glomerular barrier function. Kidney Int 9:36–45
14. Russo PA, Bendayan M (1990) Distribution of endogenous albumin in the glomerular wall of proteinuric patients. Am J Pathol 137:1481–1490
15. Fujigaki Y, Nagase M, Kobayasi S, Hidaka S, Shimomura M, Hishida A (1993) Intra-GBM site of the functional filtration barrier for endogenous proteins in rats. Kidney Int 43:567–574
16. Kobessho H, Oishi K, Hamaguchi H, Kanda F (2008) Elevation of cerebrospinal fluid protein in patients with diabetes mellitus is associated with duration of diabetes. Eur Neurol 60:132–136
17. Stillman IE, Karumanchi SA (2007) The glomerular injury of preeclampsia. J Am Soc Nephrol 18:2281–2284
18. Venkatesha S, Toporsian M, Lam C, Hanai J, Mammoto T, Kim YM, Bdolah Y, Lim KH, Yuan HT, Libermann TA, Stillman IE, Roberts D, D'Amore PA, Epstein FH, Sellke FW, Romero R, Sukhatme VP, Letarte M, Karumanchi SA (2006) Soluble endoglin contributes to the pathogenesis of preeclampsia. Nat Med 12:642–649
19. Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S, Libermann TA, Morgan JP, Sellke FW, Stillman IE, Epstein FH, Sukhatme VP, Karumanchi SA (2003) Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. J Clin Invest 111:649–658
20. Eremina V, Jefferson JA, Kowalewska J, Hochster H, Haas M, Weisstuch J, Richardson C, Kopp JB, Kabir MG, Backx PH, Gerber HP, Ferrara N, Barisoni L, Alpers CE, Quaggin SE (2008) VEGF inhibition and renal thrombotic microangiopathy. N Engl J Med 358:1129–1136
21. Zhu X, Wu S, Dahut WL, Parikh CR (2007) Risks of proteinuria and hypertension with bevacizumab, an antibody against vascular endothelial growth factor: systematic review and meta-analysis. Am J Kidney Dis 49:186–193

22. Dechadilok P, Deen WM (2006) Hindrance factors for diffusion and convection in pores. Ind Engin Chem Res 45:6953–6959
23. Hausmann R, Kuppe C, Egger H, Schweda F, Knecht V, Elger M, Menzel S, Somers D, Braun G, Fuss A, Uhlig S, Kriz W, Tanner G, Floege J, Moeller MJ (2010) Electrical forces determine glomerular permeability. J Am Soc Nephrol. doi:10.1681/ASN.2010030303
24. Wijnhoven TJ, Lensen JF, Wismans RG, Lefeber DJ, Rops AL, van der Vlag J, Berden JH, van den Heuvel LP, van Kuppevelt TH (2007) Removal of heparan sulfate from the glomerular basement membrane blocks protein passage. J Am Soc Nephrol 18:3119–3127
25. Harvey SJ, Jarad G, Cunningham J, Rops AL, van der Vlag J, Berden JH, Moeller MJ, Holzman LB, Burgess RW, Miner JH (2007) Disruption of glomerular basement membrane charge through podocyte-specific mutation of agrin does not alter glomerular permselectivity. Am J Pathol 171:139–152
26. Comper WD, Haraldsson B, Deen WM (2008) Resolved: normal glomeruli filter nephrotic levels of albumin. J Am Soc Nephrol 19:427–432
27. Ballermann BJ, Stan RV (2007) Resolved: capillary endothelium is a major contributor to the glomerular filtration barrier. J Am Soc Nephrol 18:2432–2438
28. Abrahamson DR, St John PL, Isom K, Robert B, Miner JH (2007) Partial rescue of glomerular laminin alpha5 mutations by wild-type endothelia produce hybrid glomeruli. J Am Soc Nephrol 18:2285–2293
29. Rennke HG, Venkatachalam MA (1977) Glomerular permeability: in vivo tracer studies with polyanionic and polycationic ferritins. Kidney Int 11:44–53
30. Farquhar MG, Wissig SL, Palade GE (1961) Glomerular permeability. I. Ferritin transfer across the normal glomerular capillary wall. J Exp Med 113:47–66
31. Fujigaki Y, Nagase M, Kojima K, Yamamoto T, Hishida A (1997) Glomerular handling of immune complex in the acute phase of active in situ immune complex glomerulonephritis employing cationized ferritin in rats. Ultrastructural localization of immune complex, complements and inflammatory cells. Virchows Arch 431:53–61
32. James JA, Ashworth CT (1961) Some features of glomerular filtration and permeability revealed by electron microscopy after intraperitoneal injection of dextran in rats. Am J Pathol 38:515–525
33. Lewis EJ, Schwartz MM, Pauli BU, Sharon Z (1978) Endocytosis: a property of the glomerular visceral epithelial cell. Nephron 22:91–96
34. Akilesh S, Huber TB, Wu H, Wang G, Hartleben B, Kopp JB, Miner JH, Roopenian DC, Unanue ER, Shaw AS (2008) Podocytes use FcRn to clear IgG from the glomerular basement membrane. Proc Natl Acad Sci USA 105:967–972