Editorial Comments

What is the role of albumin in proteinuric glomerulopathies?

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Introduction

Albumin is a 69 kDa plasma protein (molecular radius \( \sim 36 \, \text{Å} \)) with a variety of functions, including maintenance of plasma oncotic pressure, buffering of plasma acid–base changes and functioning as a transport protein for hormones, fatty acids, phospholipids, ions and heavy metals [1]. Albumin was early identified as critical in regulating endothelial permeability, especially in studies on single perfused microvessels [2]. Later, plasma acidic glycoproteins, especially orosomucoid, were found to be (additional) crucial factors in maintaining normal microvascular permeability, particularly to negatively charged macromolecules (e.g. albumin) [3]. The critical role of albumin and orosomucoid in maintaining vascular permeability has also been demonstrated for glomerular capillaries [4,5]. Paradoxically, in Nagase analbuminaemic rats (NARs), a mutant of the Sprague–Dawley strain devoid of circulating albumin, the clearance of proteins (albumin and IgG) to the interstitium was, however, only moderately increased [6]. Furthermore, in a recent study, the glomerular permeability to macromolecules was only slightly altered in this rat strain [7].

Albumin and its ligands have also been implicated in the progression of proteinuric renal disease, by direct or indirect tubulotoxic effects [8]. This tubulotoxicity, during continuous protein overload, apparently results not only in injury to the tubular cells, but also in overall tubulointerstitial damage [8–10]. Although proteinuria is an established risk factor for the progression of glomerular disorders, the concept that albumin may be tubulotoxic is partly controversial. Also, the concept of albumin regulating microvascular (glomerular) permeability has been questioned [11]. Moreover, the classic concept of the glomerular barrier functioning as a highly size- and charge-selective filter has been put into serious doubt recently [12].

Determinants of normal glomerular permselectivity

According to the traditional view, the glomerular barrier selects molecules based on their size, shape and charge, and almost completely prevents large and negatively charged macromolecules from reaching Bowman’s space [13–15]. In the glomerular filter, the fenestrated endothelium with its glycocalyx, the glomerular basement membrane (GBM), and the podocyte slit diaphragm (PSD) in the epithelial filtration slits are arranged in series to produce a remarkable sieving barrier. Rodewald and Karnovsky [16] suggested a zipper-like arrangement of structures in the PSD to represent the major size-selective barrier in the glomerular filter. This view has gained renewed interest recently by the discovery by Tryggvason et al. that a defective expression of nephrin, a key molecule (among others) in the GBM, leads to massive proteinuria [17]. Ultrastructural data by Farquhar et al. [18] and Ryan and Karnovsky [19], however, have long indicated that the GBM, not the PSD, is the major sieving barrier of the glomerular filter. Thus the GBM effectively excludes large molecular weight dextrans during glomerular filtration and, furthermore, albumin is normally more or less totally excluded from the GBM [19]. Data further indicated that the GBM is the major site for the glomerular negative charge barrier [18]. However, recent evidence from investigations of isolated GBM indicates a similar selectivity for neutral and negatively charged Ficoll and for native (anionic) and neutral albumin across the GBM [13]. Hence, a number of authors have pointed to the possibility that the most effective charge-selective barrier may be located closer to the plasma compartment than in the GBM, possibly in the endothelial cell glycocalyx (ECG), whereas the most effective size-selective barrier may be located more ‘distally’ in the glomerular filter [20].
charged sieve plugs, the GBM and the podocytes/PSD, barriers in the glomerular membrane, the negatively barrier. It should be stressed, however, that all three determining the sieving properties of the glomerular be of less importance than presently conceived, in role than previously thought, and that the PSD may that the endothelial 'sieve plugs' are playing a greater the highest GFRs [22]. These evidence for concentration polarization occurring at function of GFR (1.5–4.5 ml/min). We found no neutralized and native albumin in intact rat kidneys as a intermediate size, neutral proteins, together with detail, but seems to consist of sulfated proteoglycans and sialoproteins, carrying negative charge. The ECG may thus represent a major charge barrier in the glomerular filter, and may also, to some extent, function as a size-selective barrier. Actually, if the major size- and charge-selective barrier were placed close to the plasma side, this would safeguard the glomerular filter from protein 'clogging' at high filtration rates. 'Clogging' implies that the concentration of proteins would increase in the GBM during high glomerular filtration rates (GFRs), because proteins would 'pile up' against the most impermeable portion (e.g. the PSD) under such conditions, a phenomenon called 'concentration polar- ization'. Our group has been able to assess glomerular sieving coefficients (θ, fractional clearances) for some intermediate size, neutral proteins, together with θ for neutralized and native albumin in intact rat kidneys as a function of GFR (1.5–4.5 ml/min). We found no evidence for concentration polarization occurring at the highest GFRs [22]. These in vivo data may indicate that the endothelial 'sieve plugs' are playing a greater role than previously thought, and that the PSD may be of less importance than presently conceived, in determining the sieving properties of the glomerular barrier. It should be stressed, however, that all three barriers in the glomerular membrane, the negatively charged sieve plugs, the GBM and the podocytes/PSD, seem to function as a unit. Damage to any portion of the membrane may lead to severe proteinuria [14].

The view of the glomerular filter as a highly size- and charge-selective barrier has been challenged recently [12]. Osicka et al. [23] worked with the isolated perfused kidney at 37°C and inhibited tubular protein reabsorp- tion (TPR) by various agents, including NH4Cl. The authors found that θ for albumin was nearly 100-fold higher than previously reported [24]. Furthermore, they found little evidence for charge selectivity of the glomerular filter. In contrast, Tojo and Endo [25], using a sophisticated tubular micropuncture technique, correcting for a number of the shortcomings of this technique, determined the θ value for albumin. It was found to be 6 × 10⁻⁴, which is similar to that obtained by our group [22] and by Haraldsson et al. [20]. However, if one assumes that the θ value for albumin to be as high as 0.07 [23], no less than ~600 g of albumin would pass the human glomerular filter every day! Comper et al. [12] have postulated a non-degradative 'retrieval pathway' to account for the reabsorption to plasma of almost all of the filtered albumin. Furthermore, a substantial fraction of urinary proteins was reported to be degraded and excreted in the final urine as protein fragments. It was proposed that a reduced protein ‘retrieval’ to plasma, or a reduced protein degradation, would be mainly responsible for the increased urinary protein excretion occurring in a number of proteinuric disorders.

Ohlson et al. [26] have specifically addressed the ‘retrieval pathway’ hypothesis by measuring θ for albumin and Ficoll in the isolated perfused kidney model at both 8 and 37°C. Using NH4Cl at 37°C to inhibit TPR, they indeed found a high θ value for albumin, approaching that for similarly sized (neutral) Ficoll. At 8°C, however, the θ value for albumin was only 0.001–0.002. They argued that at 37°C, the glomerular filter was clearly damaged (with loss of its negative charge), a damage that could be prevented by reducing the temperature to 8°C. At 8°C, the ratio of θ for 36 Å Ficoll vs native (negative) albumin was ~20–30 [20]. In the intact rat kidney in vivo, we determined the ratio of the θ values for neutral- ized albumin (35 Å) and for native albumin (radius 35.5 Å). The ratio was ~10 [22]. Other arguments against the ‘albumin retrieval hypothesis’ can be raised. (i) Practically all albumin transport through the prox- imal tubular cells occurs via a degradative pathway, after low-affinity binding to the recently described cubilin–megalin complex [27]. There is no evidence that labelled albumin crosses the tubule walls in between the cells [28], (ii) Ultrastructural studies using electron microscopy have shown that macromolecules such as large dextrans, or albumin, are normally not found in the GBM, suggesting that they are severely restricted at (or proximal to) the GBM [18,19,29]. (iii) Careful micropuncture studies [25], and studies in the isolated kidney perfused at 8°C [20,26], or studies using a tissue uptake technique in intact rat kidneys [22] come to the conclusion that the glomerular filter functions as a highly size- and charge-selective barrier.

![Fig. 1.](image-url) The four layers of the glomerular filtration barrier. Note the presence of 'sieve plugs' in the fenestrae [21]. Coat, endothelial surface coat; En, endothelial cell; GBM, glomerular basement membrane; Ep, epithelial cells/podocytes; US, urinary space.
The θ value for albumin was found to be only $\sim 6-10 \times 10^{-4}$. The θ value for neutralized albumin was found to be at least one order of magnitude higher [22].

Normal capillary permselectivity is dependent on interactions of the endothelium with plasma

It has been recognized for at least half a century that the normal function of the endothelial barrier is dependent on plasma proteins. In studies on isolated perfused microvessels or glomeruli, if one removes albumin almost completely from the perfusate (levels $< 0.1 \text{ g/l}$), large increases in microvascular hydraulic conductance ($L_pS$) are observed [2,5,30–32]. However, in whole organ studies, we found only a moderate increase in $L_pS$, as also noted by others [11,33]. Indeed, albumin is not the only plasma factor that is required for the maintenance of a normal capillary permeability. Haraldsson and Rippe [34] noted that, whilst plasma albumin can maintain $L_pS$ more or less intact in the perfused rat hindlimb, other plasma proteins are needed to prevent increases in the permeability of negatively charged proteins (e.g. albumin). We reported that plasma orosomucoid, even at levels as low as $\sim 0.1 \text{ g/l}$, apparently adds negative charge to the vessel wall, thereby reducing its permeability to anionic macromolecules [3]. Further detailed evidence supporting these findings was provided by Curry et al. [35]. Haraldsson et al. [4] were also able to demonstrate the ‘orosomucoid effect’ for rat glomerular capillaries. Furthermore, orosomucoid was shown to be able to bind specifically to microvascular endothelial cells in culture [36], and it was shown recently that orosomucoid can be produced by glomerular endothelial cells [37].

How albumin and orosomucoid interact with the endothelial cell surface, or actually the endothelial cell surface coat (glycocalyx), is not known in detail. From a strictly mechanistic point of view, the interaction of albumin with a gel structure (such as the ECG) would lead to an increase, not a reduction, in protein permeability [38]. The ECG is suggested to be constituted by a fibrous, three-dimensional network of glycoproteins, which is reinforced by plasma proteins, such as albumin and orosomucoid [2,3]. It has been suggested that albumin may reduce capillary permeability by transforming the lattice of fibrous macromolecules at the endothelial cell surface from an irregular array to a regular array. Furthermore, albumin has emerged as a ‘survival factor’ for various cell systems in culture because it scavenges reactive oxygen species [39]. Could this contribute to the beneficial effects of albumin on capillary permeability in isolated microvessels or glomeruli?

Concerning the role of other plasma proteins, Adamson and Clough [40] demonstrated that the presence of plasma in the perfusate actually alters the configuration of the ECG so that the thickness of the endocapillary layer is increased. This would support the role of plasma proteins in the reinforcement of the glycocalyx. Orosomucoid may bind to the glycocalyx, add negative charges, and confer charge-selective properties to the glycocalyx. It has thus been hypothesized that the lack of certain components of the glycocalyx, most notably orosomucoid, will alter the selectivity of the capillary wall, especially its charge barrier properties [3,35]. In the glomerular capillary, being the most selective microvascular filter in the body, even very subtle changes in the glycocalyx may lead to microalbuminuria, or even albuminuria. This view is in line with the so-called Steno-hypothesis [41], which proposes a link between endothelial cell dysfunction and impaired production of glycoproteins in the negatively charged ECG. This defect is thought to cause microalbuminuria, which, in turn, is associated with vascular morbidity and cardiovascular mortality.

Why albumin-deficient rats show a near normal capillary permeability is puzzling. However, in NARs, the lack of albumin is compensated for by an increased concentration of globulins and (acidic) glycoproteins (e.g. orosomucoid) in plasma [6]. It seems that plasma components other than albumin, most notably orosomucoid, may take over the role of albumin in maintaining capillary selectivity close to normal in NAR. Thus, it can be argued that albumin is far from being critical in regulating microvascular permeability [11].

The role of albumin in the progression of glomerular disease

Increased urinary albumin excretion is a hallmark of glomerular disease. Persistent proteinuria it thought to promote renal disease as a result of the increased load of filtered proteins in the proximal tubules. Protein loading of the tubular cells induces an inflammatory phenotype and genotype [42] of tubular epithelial cells, leading to interstitial fibrosis and progressive tubular atrophy. The candidate proteins responsible for this toxicity have not been defined in detail. Toxic effects have been ascribed to fatty acid ligands to albumin, to albumin itself or to proteins moving concomitantly with albumin, such as transferrin or complement factors [8–10]. Indeed, several groups have demonstrated that in patients with glomerular disorders, the urinary excretion of preferentially larger proteins than albumin, so-called ‘unselective proteinuria’ (as opposed to ‘selective proteinuria’, i.e. albuminuria), correlates better with the severity of the histological lesion and may better predict outcome than albuminuria [15,43]. It should be noted that in minimal change glomerulopathy (MCG), which presents with a highly selective, massive proteinuria, the prognosis is generally good. On the other hand, Ghiggeri et al. [44] found that urinary albumin in MCG was markedly lower in fatty acid content, particularly in linoleic and oleic acid, than urinary albumin collected from patients without MCG. It was reported recently that linoleic acid was the most tubulotoxic and oleic acid the most fibrogenic fatty acid bound to albumin [45]. It can also be
objected that the duration of proteinuria in MCG, due to its sensitivity to steroid treatment, is usually relatively short. Therefore, in MCG, periods of elevated protein traffic are relatively limited in time. Taken together, however, there is good evidence that tubular protein overload by albumin itself may not be the primary factor responsible for the progression of renal disease [46]. Even though unselective proteinuria actually seems to reflect a more severe glomerular lesion, the fact that the urinary excretion of high molecular weight proteins is a far better prognostic marker in proteinuric glomerulopathies than albuminuria indicates that filtered proteins larger than albumin may be more detrimental to tubular function than albumin itself.

Conclusion

In summary, it is questionable whether the claimed concepts on the role of albumin in regulating the normally high selectivity of the glomerular barrier, and the role of albumin in progression of glomerular disease, are actually correct. In analbuminaemic rats, the microvascular permeability is deranged to only a minor degree, if at all. It is thus thought that glycoproteins and globulins, substitute for albumin to keep permeability normal. Furthermore, in MCG, the outcome is good despite the presence of massive albuminuria. This appears to contradict the claimed detrimental role of albumin itself in the progression of proteinuric disorders. Further experimental and clinical studies are, however, warranted to establish the precise role of albumin in regulating glomerular permeability and in mediating tubulotoxicity in proteinuric glomerulopathies.

Conflict of interest statement. None declared.

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Lipid management in the proteinuric patient: do not overlook
the importance of proteinuria reduction

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Introduction

The markedly elevated cardiovascular risk observed in
renal patients is increasingly recognized as an important
treatment target [1]. Among the renal populations,
proteinuric patients are at particularly high risk, as
apparent from the observation of an almost 6-fold
increased incidence of myocardial infarction in such
patients [2]. Moreover, proteinuria has been shown to
be an independent risk factor for cardiovascular mor-
bidity and mortality [3,4]. Most likely, proteinuria-
associated lipid abnormalities play a main role in the
high cardiovascular risk in proteinuric patients, and
thus provide an important treatment target.

Several studies have underlined the efficacy of
statins, not only to improve the lipid profile, but also
to reduce cardiovascular morbidity and mortality in
hyperlipidaemic and hypertensive populations [5,6],
and recent post-hoc data from the CARE study showed
that statin treatment reduces cardiovascular morbidity
in subjects with chronic renal insufficiency [7]. There-
fore, statins will most likely be a cornerstone in cardio-
vascular prevention for years to come in non-renal
and renal populations.

For overtly proteinuric patients, however, solid
data on cardiovascular risk management are still lack-
ing, in spite of the obvious need for aggressive risk
management in this high-risk population. Rational
principles for cardiovascular risk management in this
population can nevertheless be formulated, based on
the available evidence. In this respect, lipid man-
agement is an important target. Importantly, protein-
uria reduction exerts a clear-cut lipid lowering effect,
irrespective of the way (class of drug, dietary inter-
vention, or both) it is achieved. Here, we will briefly

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