The glomerular basement membrane charge-selectivity barrier: an oversimplified concept?

N. P. Goode, M. Shires and A. M. Davison

Concept and background

The precise nature and role of a charge-selectivity barrier, existing at the level of the glomerular capillary wall has been the subject of continuing controversy since it was first postulated over 30 years ago. Recent studies suggest that some earlier observations may have been overinterpreted and that the conclusions reached should be re-examined.

The concept of a charge-selectivity barrier at the level of the glomerular filtration membrane which influences the permeation of circulating plasma proteins, dependent upon their charge, became widely accepted during the 1970s and 1980s [1,2]. Evidence was derived from investigations in which anionic sites were detected histochemically using various cationic tracers, and the demonstration that anionic component removal by specific enzymes or their neutralization by polycations resulted in increased glomerular permeability. Despite the contribution of epithelial and endothelial cell surface sialoglycoproteins (podocalyxin) to the glomerular capillary wall anionic charge [3], and the demonstration of hyaluronan [4] and chondroitin sulphate proteoglycans [5] in the GBM and mesangial matrix, the principal charge barrier was conceived to comprise of a linear array of anionic sites associated with heparan sulphate proteoglycan (HS-PG) networks in the laminae rarae of the glomerular basement membrane (GBM).

Much of the evidence for the existence of an anionic charge barrier has been derived from in vivo studies in experimental animals. Key studies included the demonstration that the fractional clearance of circulating plasma proteins, dependent upon their charge, became widely accepted during the 1970s and 1980s [1,2]. Evidence was derived from investigations in which anionic sites were detected histochemically using various cationic tracers, and the demonstration that anionic component removal by specific enzymes or their neutralization by polycations resulted in increased glomerular permeability. Despite the contribution of epithelial and endothelial cell surface sialoglycoproteins (podocalyxin) to the glomerular capillary wall anionic charge [3], and the demonstration of hyaluronan [4] and chondroitin sulphate proteoglycans [5] in the GBM and mesangial matrix, the principal charge barrier was conceived to comprise of a linear array of anionic sites associated with heparan sulphate proteoglycan (HS-PG) networks in the laminae rarae of the glomerular basement membrane (GBM).

Much of the evidence for the existence of an anionic charge barrier has been derived from in vivo studies in experimental animals. Key studies included the demonstration that the fractional clearance of circulating plasma proteins, dependent upon their charge, became widely accepted during the 1970s and 1980s [1,2]. Evidence was derived from investigations in which anionic sites were detected histochemically using various cationic tracers, and the demonstration that anionic component removal by specific enzymes or their neutralization by polycations resulted in increased glomerular permeability. Despite the contribution of epithelial and endothelial cell surface sialoglycoproteins (podocalyxin) to the glomerular capillary wall anionic charge [3], and the demonstration of hyaluronan [4] and chondroitin sulphate proteoglycans [5] in the GBM and mesangial matrix, the principal charge barrier was conceived to comprise of a linear array of anionic sites associated with heparan sulphate proteoglycan (HS-PG) networks in the laminae rarae of the glomerular basement membrane (GBM).

Disruption of the proposed charge barrier: experimental studies

Possible alterations of the proposed GBM anionic charge barrier have been studied in experimental puromycin aminonucleoside (PAN) nephrosis, an animal model of minimal change nephropathy, using different in vivo techniques including affinity labelling of anionic components with a variety of cationic probes; their detection with specific antisera; or the incorporation of radioisotopic precursors (reviewed in [2]). The different approaches used in these studies have yielded inconsistent and inconclusive results [2]. A major difficulty in the interpretation of studies involving in vivo infusion or perfusion of polycationic molecules is the possible influence of the probe itself on glomerular structure and permeability. Indeed, different probes may induce varying degrees of proteinuria either through toxic effects on glomerular cell metabolism, reversal of anionic site charge expression, or secondary alteration of glomerular matrix ultrastructure following binding of polycations to anionic sites [15].

Hunsicker and Bertolatus demonstrated that a massive but transient proteinuria induced in rats by infusion of the polycation hexadimethrine (HDM) resulted in a secondary alteration of GBM porosity (reduced size selectivity), which was manifest by increased sieving coefficients of anionic and neutral albumin and larger IgG 131I tracer molecules compared with controls [16]. These authors further showed that binding of tritiated HDM and cationic ferritin to isolated dog GBM was not due to heparan sulphate proteoglycans, or sialoglycoproteins such as podocalyxin, but was dependent upon charged carboxyl groups of the GBM [17].

Disruption of the proposed charge barrier: human studies

Anionic site distribution has been examined in human primary and secondary glomerular pathologies, using various cationic markers in an attempt to identify possible charge barrier aberrations. Investigations have favoured those markers (polyethyleneimine; cuproline.
blue; high-iron diamine-thiocarbohydrazide-silver proteinate; cationic colloidal gold) which are applicable to quantitative studies. Although anionic constituents with apparently diverse functions have been demonstrated in the glomerular capillary wall matrix, reported charge abnormalities have generally been assumed to be due to loss or alteration of HS-PG constituents. In many studies, this assumption is not based on direct evidence and may be fundamentally flawed.

We have applied a novel cationic marker poly-L-lysine coated colloidal gold, to the study of anionic charge in renal tissue [18]. The technique was subsequently applied to renal biopsy tissue obtained for routine diagnosis, to investigate the possible relationship between GBM charge aberrations and altered membrane permeability in different glomerulopathies [19–22]. We have been unable to find any direct relationship between GBM anionic charge loss and proteinuria [10]. Aberrations of GBM charge appear instead, to be associated with alterations in GBM structure [21,22]. Disrupted patterns may even show an inverse relationship with membrane permeability as in the case of ultrathin membrane disease [21]. The confused relationship between membrane permeability and charge expression is not surprising if the variety of GBM components which express anionic charge under different staining conditions and using different cationic markers is considered.

Diversity of charged components demonstrated using cationic colloidal gold

Application of the cationic colloidal gold (CG) staining technique to post-embedded tissue enables charge expression to be studied under diverse staining conditions in serial sections of the same area of tissue (Figure 1). Our studies show that pH and electrolyte concentration of the CG staining solution has a profound effect on charge expression by different tissue anionic components, not only in the GBM [18,22], but also by other constituents of renal parenchyma (Figure 1). The ‘normal’ CG staining pattern obtained in GBM at pH 1.0 (unpublished observations) resembles that seen at pH 5.8 under critical electrolyte conditions, with staining of GBM interna and externa sites [22]. It is generally agreed that under these conditions, only highly acidic sulphated glycoconjugates are dissociated [23]. The distribution of stained sites at pH 7.0 appears similar, but with additional cytoplasmic and nuclear staining [18]. However, at this pH carboxyl, sulphate and phosphate groups of weakly acidic glycoconjugates may all contribute to anionic charge expression. These patterns contrast with the discrete linear array of anionic sites localized in the GBM externa, seen at pH 2.5, with additional staining around the glycoalyx of glomerular epithelial and endothelial cells [18,19]. At this pH, carboxyl groups of sialoglycoproteins and sulphate groups of weakly acidic sulphated glycoconjugates are dissociated, together with phosphate groups of phosphated acidic glycoconjugates [23].

Other charged components

In addition to HS-PG, the contribution of minor components bearing these groupings, to overall GBM anionic charge has generally been understated or disputed. Evidence of hyaluronic acid sites, localized predominantly along the external aspect of rat GBM has been shown in our studies using enzyme degradation prior to cationic gold staining [18], and by direct staining with enzyme-gold complexes [16]. Chondroitin sulphate proteoglycans (CS-PG) has previously not been regarded as a normal GBM constituent, following reports of its absence from normal adult rat GBM [24]. However, we have recently shown by immunoelectron microscopy, that CS-PG is localized along the external aspect of ‘normal’ GBM and may contribute to the overall charge pattern [22]. The influence of these components on the permeability
characteristics of the GBM to albumin was reported over a decade ago [4].

Conclusion

It would appear then, that both GBM and the renal parenchyma as a whole contain diverse charged components. Abnormalities of GBM anionic charge expression are more complex than at first conceived, reflecting often subtle alterations in membrane matrix charged structures which may have a minimal or profound, direct or indirect influence on membrane permeability. The original concept of a charge-selective barrier maintained solely by the integrity of HS-PG networks in the GBM must now be inadequate.

References

2. Kanwar YS. Biophysiology of glomerular filtration and proteinuria. Lab Invest 1984; 51: 7–21
4. Rosenzweig LJ, Kanwar YS. Removal of sulfated (heparin sulfate) or non-sulfated (hyaluronic acid) glycosaminoglycans results in increased permeability of the glomerular basement membrane to 125I-bovine serum albumin. Lab Invest 1982; 47: 177–184

Endothelin: what role in acute contrast nephropathy?

P. Gross and E. Büssemaker

Nephrology, Department of Medicine, Universitätsklinikum C. G. Carus, Dresden, Germany

Acute renal failure following exposure to radiocontrast agents has been a well known clinical entity since the 1950’s. Its pathogenesis is believed to include at least four aspects: (i) direct toxicity of the contrast agent to renal tubular epithelial cells, mostly of proximal tubular origin; (ii) intrarenal haemodynamic instability, including a brief period of vasodilatation (increased renal blood flow) followed by vasoconstriction (decreased renal blood flow); (iii) intratubular precipitation of Tamm–Horsfall protein possibly causing tubular obstruction; (iv) complement activation. Clinically much has been learned about important risk