The cellular basis of albuminuria

Peter W. MATHIESON
Academic Renal Unit, Southmead Hospital, Bristol BS10 5NB, U.K.

ABSTRACT

The appearance of albumin in the urine has long been recognized as a cardinal feature of kidney disease and more recently has been shown to also be an independent cardiovascular risk factor associated with insulin resistance. Recent studies on rare human genetic variants, targeted gene disruption in mouse models and cultured glomerular cells in vitro have dramatically improved our understanding of the cellular and molecular basis of albuminuria. This review aims to summarize the current state of knowledge, to illustrate known mechanisms of proteinuria in disease states and to suggest a possible explanation for the link between albuminuria and insulin resistance.

ALBUMINURIA

Normal human urine contains only very small quantities of albumin, less than 30 mg of albumin being excreted by healthy adults in 24 h. The appearance of large amounts of albumin in the urine is a cardinal sign of kidney disease, especially glomerular disease, and is detectable by screening techniques using urinary dipsticks. Accurate quantification of the amount of albumin lost in the urine has important clinical connotations: excretion of amounts in excess of 300 mg in 24 h is termed ‘overt albuminuria’, excretion of lesser amounts of albumin, between 30 and 300 mg in 24 h, is termed ‘microalbuminuria’. In order to avoid the need for timed urine collections, which are notoriously inaccurately performed, many clinicians now rely on measurement of albumin/creatinine ratio (mg/mmol) on a random urine sample, which is much more convenient. The expression as a ratio is intended as a correction for urinary concentration. Creatinine excretion does vary between individuals according to muscle mass, but a good approximation which is useful in clinical practice is that the average creatinine excretion in an adult is of the order of 10 mmol/24 h, so that 10 times the albumin/creatinine ratio is approximately the number of milligrams of albumin excreted in 24 h. Thus an albumin/creatinine ratio of 31 approximates to 310 mg of albumin excretion in 24 h, i.e., overt albuminuria. Overt albuminuria is taken as a sign of established glomerular damage; in diabetic nephropathy, the single most important cause of renal failure in the developed world, the presence of overt albuminuria is a marker for a poor prognosis not only from renal disease itself, but also from associated cardiovascular disease [1–3]. In the early stages of diabetic nephropathy, microalbuminuria can be detected (and may be intermittent at first); later in the natural history of progressive diabetic nephropathy there is the development of overt albuminuria [4]. Intervention studies have increasingly been targeted at the early microalbuminuric stage in the belief that the pathological changes are modifiable and maybe even reversible at this stage [5,6]. It has also become apparent in recent years that microalbuminuria is a significant finding even in non-diabetic subjects: it is an independent cardiovascular risk factor [7,8] and is associated with insulin resistance [9,10]. The purpose of this review is to highlight recent advances in the understanding of the cellular basis for the appearance of albumin in the urine. Key observations have come from studies of the consequences of rare human gene mutations causing nephrotic syndrome (leakage of massive quantities of albumin into the urine), manipulation of single genes in mice and studies of cultured human cells. Together, these areas have provided important information about normal human renal physiology and indicated mechanisms of dysfunction in disease.

Key words: albuminuria, basement membrane, endothelial cell, glomerular filtration, nephrotic syndrome, podocyte, proteinuria.

Abbreviations: GBM, glomerular basement membrane; GEnC, glomerular endothelial cells; MCN, minimal-change nephropathy.

Correspondence: Professor Peter Mathieson (email p.mathieson@bris.ac.uk).

© 2004 The Biochemical Society
THE GLOMERULAR FILTRATION BARRIER

The glomerulus is the filtering unit of the mammalian kidney: it is a complex knot of capillaries and filtration takes place across the capillary wall into Bowman's space. Water and small molecules are freely filtered in large quantities: 180 litres of glomerular filtrate/day in a 70 kg adult. The detailed ultrastructure of the wall has been known for many years and comprises three layers: an inner layer of GEnC (glomerular endothelial cells), an outer (urinary side) layer of glomerular epithelial cells or podocytes and, lying between the two cellular layers, the GBM (glomerular basement membrane) (Figure 1). Each of these layers has specialized features distinguishing it from analogous components of capillary walls elsewhere in the body. GEnC have characteristic fenestrations filled with glycocalyx, podocytes have complex branching and inter-digitating processes called foot processes, GBM has unique constituents with typical isoforms of laminin, type IV collagen and proteoglycans, the latter imparting a strongly negative electrostatic charge. The composite structure of the glomerular capillary wall forms the basis of the selective sieving action that allows free passage of water and small molecules, but prevents the passage of albumin and larger molecules [11].

Glomerular epithelial cells (podocytes)

Until recently, it has been a matter of some controversy which component(s) of the glomerular capillary wall have primary responsibility for control of the selective sieve: recent advances have put the podocyte firmly at centre stage [12]. Filtration takes place through slits (Figures 2 and 3) formed between inter-digitating processes of the podocytes on the urinary side of the glomerulus. Each filtration slit has a membrane across it, apparently bridging the adjacent foot processes (Figure 2). In recent years, detailed knowledge of the molecular composition of the slit membrane spanning each slit has been obtained. The first step was the identification [13] in the late 1990s of the gene mutated in Finnish type congenital nephrotic syndrome, encoding a protein termed nephrin. In normal kidney, nephrin is expressed exclusively in the podocyte and is located at the slit membrane. Mutations in this gene result in severe congenital nephrotic syndrome which necessitates early nephrectomies as a life-saving procedure. Selective targeting of the nephrin gene in mice also leads to congenital nephrotic syndrome [14]. Since the description of nephrin, several other podocyte-specific genes have been identified which when mutated lead to severe nephrotic syndrome [15–19]. Table 1 provides further details of these. The fact that these gene mutations primarily affect the podocyte only and lead to massive proteinuria has led to the conclusion that podocytes are the cells responsible for the prevention of proteinuria in health [12]. In fact, disruption of podocyte structure had already been appreciated for many years as a feature of one of the commonest forms of proteinuric disease, MCN (minimal-change nephropathy). The condition is so-called because examination of glomeruli from affected patients by light microscopy is normal or nearly normal and higher resolution imaging by electron microscopy is required before any structural abnormality can be demonstrated. The characteristic feature of MCN in electron microscopic sections is effacement (flattening) of the podocyte foot processes (Figure 4). This is shown in another way by scanning electron microscopy in Figure 5: the complex architecture of podocyte processes and their intervening filtration slits has been obliterated. Since MCN is typically steroid-responsive, i.e., remissions can be induced in the majority of patients by treatment with corticosteroids, it is clear that the podocytes in this condition are not irreversibly injured. The efficacy of corticosteroids in MCN has traditionally been taken as...
Table 1  Podocyte genes, effects of mutations and putative functions

<table>
<thead>
<tr>
<th>Gene</th>
<th>Human mutation</th>
<th>Mouse knockout</th>
<th>Functions</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nephrin</td>
<td>Finnish-type congenital NS</td>
<td>Congenital NS</td>
<td>Located at slit diaphragm, Interacts with podocin and CD2AP, Intracellular signalling, Ligand unknown (forms homodimers with nephrin on adjacent podocytes?)</td>
<td>[12–14]</td>
</tr>
<tr>
<td>CD2AP</td>
<td>Unknown (susceptibility to sporadic NS?)</td>
<td>Congenital NS</td>
<td>Interacts with nephrin and podocin, Links with actin cytoskeleton</td>
<td>[12,15]</td>
</tr>
<tr>
<td>Podocin</td>
<td>Early onset NS</td>
<td>Congenital NS</td>
<td>Co-localises with nephrin, Interacts with nephrin and CD2AP, Intracellular signalling</td>
<td>[12,16]</td>
</tr>
<tr>
<td>α-Actinin IV</td>
<td>Familial FSGS (Dominant)</td>
<td>Early onset NS</td>
<td>Actin cross-linking</td>
<td>[12,17]</td>
</tr>
<tr>
<td>WT-1</td>
<td>Denys–Drash and Frasier syndromes (Dominant)</td>
<td>Failure of renal development</td>
<td>Transcription factor</td>
<td>[12,18,19]</td>
</tr>
</tbody>
</table>

Figure 3  Scanning electron micrograph of ‘external’ (urinary space) aspect of a normal human glomerular capillary

Pod, podocyte foot process. Arrows indicate filtration slits between inter-digiting processes of the podocyte.

Figure 4  Transmission electron micrograph of the glomerular capillary from a patient with MCN

Arrows indicate flattening (effacement) of podocyte foot processes (compare with the normal appearance of foot processes in Figure 1).

evidence of the involvement of immune mechanisms in the causation of the renal lesion [20]. We have been interested in an alternative possibility: that corticosteroids have direct effects on podocytes. Previously, human podocytes have been difficult to study in vitro, because available cell lines were not representative of the phenotype of the mature cells. We have addressed this by using temperature-sensitive transgene technology to develop human podocyte cell lines [21]. The resultant cell lines express markers of mature podocyte phenotype, including nephrin and podocin [21,22], and provide a useful resource for the analysis of podocyte structure and function in vitro.

Using these cells, we have shown that corticosteroids have profound effects on cell survival and phenotype, including up-regulation of nephrin and down-regulation of another key podocyte product, VEGF (vascular endothelial growth factor) [23,24]. The ability to study representative human cell lines in the laboratory represents another area of significant recent advance in our ability to analyse the glomerular filtration barrier.

It remains possible that podocytes exert some of their effects via the GBM (for whose synthesis podocytes are mainly responsible) and/or via interactions with GEnC.

Since podocytes are terminally differentiated cells with limited replication potential in vitro, the consequences of podocyte injury may be dire, and podocyte number is an important predictor of prognosis in diabetic and non-diabetic renal disease [25,26]. Whether the podocyte is the primary target of disease or merely the component
with the least capacity for repair [27] remains uncertain. Our own recent work with cultured human podocytes has shown that podocytes are insulin-responsive [28], taking up glucose in response to insulin with similar kinetics and magnitude to muscle cells, and changing their shape as a result of actin cytoskeleton re-organization after insulin treatment. We believe that these observations provide an explanation for the role of the podocyte as a target in diabetic nephropathy, when insulin responsiveness is lost due to insulin deficiency (Type I diabetes) or insulin resistance (Type II diabetes), and also may provide a unifying explanation for microalbuminuria in non-diabetic subjects, the link being due to insulin-resistance. If normal podocyte function depends upon glucose uptake and changes in cellular architecture in response to insulin, subtle podocyte dysfunction, manifest as microalbuminuria, could be the direct result of altered insulin responsiveness. Important questions remain. (i) Why should podocytes be insulin-responsive? Our hypothesis is that this reflects an aspect of the glomerular response to feeding: it is known that glomerular hyperfiltration follows calorie loading [29] and we assume that the podocyte must adapt to this. Insulin could act as a signal to the podocyte that feeding has occurred, and also as a means of providing rapid glucose uptake after a meal if podocyte energy requirements are increased after calorie loading. (ii) Are measures aimed at restoring insulin-responsiveness (e.g. treatment with glitazone drugs) successful in the reversal of microalbuminuria? There is some evidence that they are [30]. If so, is this due to direct effects on podocytes? (iii) What is the role of the other components of the glomerular capillary wall, GBM and GEnC?

GBM

The GBM is made up of unusual highly restricted isoforms of laminin and type IV collagen together with various proteoglycans, including heparan sulphate, agrin and perlecan [11,31]. The latter impart a highly negative electrostatic charge on the GBM, and much of the older literature on glomerular permeability focused on the charge characteristics of the GBM as a means of resisting the passage of albumin molecules, which are also negatively charged [32]. Mediators including hemopexin that are capable of modifying this charge have been shown to induce proteinuria when infused into animals [33]. The primacy of the podocyte in recent thinking should not detract from the possibility that GBM plays a role in glomerular permselectivity in health and disease: podocyte structure and function depend upon adhesion to GBM [34], so that abnormalities of GBM could directly influence podocytes by altering adhesion. There is growing interest in recovery of live podocytes from the urine in patients with glomerular disease [35]: why do they become detached and appear in urine? Also podocytes are responsible for at least part of GBM synthesis, and abnormal podocytes may not be able to synthesize and assemble GBM components. These questions can be studied in vitro using our wild-type and mutant podocyte human cell lines [21,36].

GEnC

Perhaps the least well-characterized of the components of the glomerular capillary wall are the GEnC, leading to the allegation that these cells have been neglected in the recent work on glomerular permeability [37]. This is partly explained by the previous difficulties in studying
these cells in vitro, since only small numbers of cells can be obtained by primary culture techniques and there is a generally accepted phenotypic marker of GEnC. In vitro, GEnC have distinctive morphological features, especially fenestrations, which are filled with glyocalyx, and there is evidence that the glyocalyx is important in permeability [38]. We have recently applied the same temperature-sensitive transgene approach described for the podocytes [21] to human GEnC, and successfully derived cell lines which show the phenotypic characteristics of mature GEnC [39]. The availability of these podocyte and GEnC human cell lines will allow us to study the two key glomerular cell types in vitro, in isolation and together, and to dissect communications between them [42].

CONCLUSIONS

As with all scientific advances, as old questions are answered, new questions appear. It is now generally accepted that the glomerular podocyte is the cell primarily responsible for the prevention of proteinuria in health, and that podocyte damage/dysfunction underlies proteinuria in disease. The next stages will be to devise measures to reverse podocyte dysfunction and/or enhance podocyte repair. The contribution from the other components of the glomerular filtration barrier, the GBM and the GEnC, remains poorly defined in health and especially in disease. Genetic manipulation in murine models [40,41] and study of human glomerular cells in vitro [21,39,42] will hopefully bring answers to these questions in the next few years. The grim significance of detection of protein in urine has been known since ancient times; we now know in great detail the cellular basis of this phenomenon. The next challenge is to bring this new understanding to fruition by developing novel more effective forms of therapy for our patients.

REFERENCES

4 Mogensen, C. E. (1997) How to protect the kidney in diabetic patients: with special reference to IDDM. Diabetes 46 (Suppl. 2), S104–S111
27 Kriz, W., Gretz, N. and Lemley, K. V. (1998) Progression of glomerular diseases: is the podocyte the culprit? Kidney Int. 54, 687–697
28 Coward, R. J., Welsh, G. I., Holman, G. D. et al. (2003) Human podocytes rapidly utilize glucose by both GLUT1 and GLUT4 in response to insulin; with significant differences in glucose transporter levels occurring in diabetic nephropathy. J. Am. Soc. Nephrol. 14, 388A

Received 10 June 2004/18 August 2004; accepted 27 August 2004
Published as Immediate Publication 27 August 2004, DOI 10.1042/CS20040168