The spectrum of podocytopathies: A unifying view of glomerular diseases

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Glomerular diseases encompass a broad array of clinicopathologically defined syndromes which together account for 90% of end-stage kidney disease costing $20 billion per annum to treat in the United States alone. Recent insights have defined the central role of the podocyte as both the regulator of glomerular development as well as the determinant of progression to glomerulosclerosis. We can now place all glomerular diseases within this spectrum of podocytopathies with predictable outcomes based on podocyte biology impacted by temporal, genetic, and environmental cues. This simplified construct is particularly useful to rationalize clinical effort toward podocyte preservation and prevention of progression as well as to focus basic research effort on understanding podocyte biology and for clinical research toward development of practical monitoring strategies for podocyte injury, dysfunction, and loss.

Kidney International (2007) **71,** 1205–1214; doi:10.1038/sj.ki.5002222; published online 4 April 2007

KEYWORDS: podocyte; glomerulus; glomerulonephritis; glomerulonephropathy; glomerulosclerosis; nephrotic syndrome

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Received 29 November 2006; revised 22 December 2006; accepted 16 January 2007; published online 4 April 2007

Glomerular diseases account for 90% of end-stage kidney disease (ESKD) at a cost of $20 billion per year in the US.[1] The traditional glomerular disease classification encompasses a bewildering array of descriptive pathologic entities and their clinical counterparts. And yet clinical trials and experience, guided by the hyperfiltration hypothesis of Brenner *et al.*,[2] tell us that effective blood pressure control, angiotensin II inhibition, and reduction in proteinuria are almost uniformly effective in reducing the rate of progression. This implies that there are common mechanisms driving disease progression.

Recent identification of mutations causing glomerular phenotypes, clinical biopsy reports, and experimental model systems reveal a simplified concept of glomerular diseases in which podocyte dysfunction, injury, or loss is a common and determining factor. Toxic, genetic, immune, infectious, oxidant, metabolic, hemodynamic, and other mechanisms can all target the podocyte. Depending on the stage of glomerular development and associated environmental factors, podocyte dysfunction, injury, or loss can result in a broad spectrum of clinical syndromes (Figure 1). Considering glomerular diseases as being related by these common podocyte-dependent mechanisms rather than as separate pathobiological entities facilitates progress towards prevention and treatment. Any national plan aimed at reducing the cost of health care will need to focus basic scientific effort on understanding podocyte biology and clinical research on learning how to prevent and monitor podocyte injury and depletion as major targets for intervention.

The development and maintenance of normal glomerular structure and function requires successful signaling and coordination between all glomerular cells including mesangial, endothelial, parietal epithelial, and visceral epithelial cells (podocytes). This point is emphasized by the critical requirement for vascular endothelial growth factor-A production by podocytes for normal endothelial and mesangial cell development and function.[3,4] However, the podocyte is the key organizer of glomerular development and maintenance.[5,6] This discussion will therefore be focused on the podocyte.

Figure 2 emphasizes, on the one hand, the key role of the podocyte in governing glomerular development culminating in the normal mature glomerulus. On the other, Figure 2 also depicts the impact of podocyte injury and depletion leading

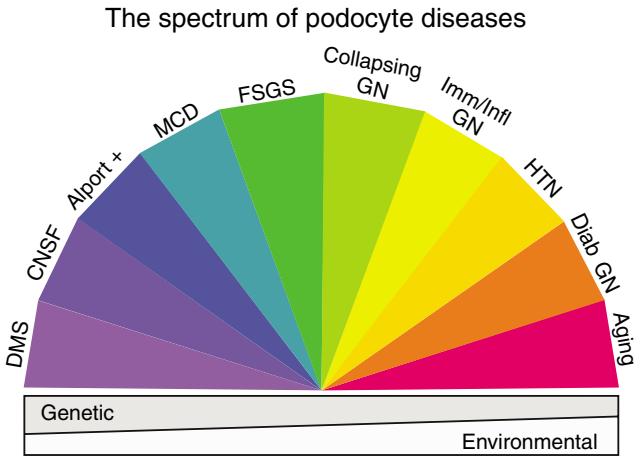

Figure 1 | The spectrum of podocyte diseases. Glomerular diseases can be considered as a consequence of podocyte dysfunction caused by genetic and/or environmental factors. Depending on when, during glomerular development and progression they occur and how significantly and rapidly they impact the glomerulus the clinical phenotype will vary. The clinical syndromes associated with podocyte dysfunction of various types include the following: diffuse mesangial sclerosis (DMS), congenital nephrotic syndrome of the Finnish type (CNSF), Alport's syndrome and variants (Alport +), MCD, FSGS, collapsing glomerulonephropathy (Collapsing GN), immune and inflammatory glomerulonephropathies (Imm/Inf GN), hypertensive nephropathy (HTN), diabetic glomerulonephropathy (Diab GN), and age-associated glomerulonephropathy (Aging). Together these conditions account for 90% of ESKD.

ultimately to global glomerulosclerosis. Variations in this basic pathway are specialized to particular clinical settings and result in a modified pathologic appearance of the glomerulus (e.g., diabetic glomerulosclerosis). Nevertheless, the major sequence of events can conveniently be considered as a continuum that plays out over time to a greater or lesser extent in an individual person depending on factors contributed by genetic and environmental milieus.

GLOMERULAR DEVELOPMENT

Podocytes become recognizable by their expression of key proteins in the S-shaped stage of nephron development (Figure 2).[5,6] They surround the in-growing blood vessels and mesenchyme to form a 'halo' or 'head-shaped' structure. By this time, podocalyxin is present on the apical surface of podocytes and tracks the intercellular junctions as they migrate down the lateral cell surface of the podocyte towards the developing glomerular basement membrane (GBM).[7]

During the capillary loop stage of glomerular development, there is a major expansion of the GBM requiring bulk protein synthesis of type IV collagens, laminins, and glycosaminoglycans.[8] At the same time, podocytes begin forming the interdigitating foot processes that markedly increases the space between podocytes necessary for efficient filtration. These foot processes connect the cell to the underlying GBM via an intergrin-linked adhesion mechanism.[9] The specialized intercellular junctions (slit diaphragms) that bridge between foot processes are made up of special proteins

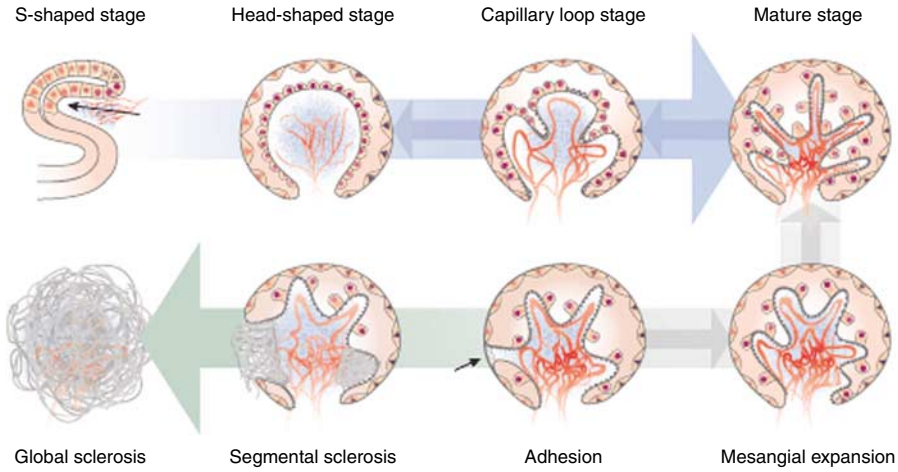

Figure 2 | Stages of glomerular development and progression with emphasis on the role of the podocyte. The S-shaped stage. Developing podocytes acquire podocyte markers during the S-shaped stage of nephron development at which time blood vessels and mesenchyme invade (arrow). The podocytes (round nuclei) separate from the parietal epithelial cells (triangular nuclei) forming what will become Bowman's space. The head-shaped stage: at this stage of glomerular development, the glomerulus consists of a ball of cell surrounded by developing podocytes. The capillary loop stage: this stage of glomerular development includes the infolding of the surface layer in order to enlarge the area available for filtration as well as the development of foot processes that interdigitate between podocytes and abut the underlying GBM which is being synthesized as a collaboration between the podocyte and underlying endothelial and mesangial cells. The mature glomerulus: the mature glomerulus has maximized filtration surface area by developing intertwining finger-like projections coated by fenestrated endothelial cells on the inside, a specialized strong thin GBM in the middle, and interdigitating podocyte foot processes connected by slit diaphragms on the outer surface. Mesangial expansion: loss of some podocytes (20%) is associated with mesangial expansion possibly as an attempt to reduce the filtration surface area. Adhesion formation: loss of podocytes resulting in appearance of bare areas of filtration surface results in adhesion of the bare surface to Bowman's capsule (synechia). Segmental sclerosis: loss of podocytes beyond a critical level results in a fibrotic glomerular response in that part of the glomerulus (segmental sclerosis). Global sclerosis: loss of podocytes beyond a critical level results in widespread scarring of that glomerulus (global sclerosis).

required for the filtration, sensing, and signaling functions of the podocyte. They include nephrin, podocin, CD2AP, neph1, fyn, yes, and others.[10–15] As the filtration surface of the mature glomerulus develops in association with an expanded GBM area, each podocyte has to communicate with and support its multiple and distant foot processes by outreach through major and intermediate cell processes that collectively give rise to the octopus-like shape of the mature podocyte. The GBM itself is constructed as a thin yet strong and efficient filtration surface through contributions by the podocyte, endothelial, and mesangial cells including the specialized α3, α4, and α5 type IV collagen chains.[16]

The key organizational signaling systems required to create and maintain the complex glomerular structure are not well understood. Master regulatory genes including the transcription factor *WT1* that control programs of downstream gene expression are necessary.[17–19] As noted above, coordination between glomerular cells also plays a key role as demonstrated by the critical requirement for vascular endothelial growth factor production by podocytes for normal endothelial and mesangial cell development.[3,4] Ultimately, the process of nephrogenesis results in the production of two kidneys, each containing approximately 1 million nephrons, which collectively filters 100 ml of blood plasma per minute with high efficiency.

REPLACEMENT OF PODOCYTES AND COMPENSATORY PODOCYTE HYPERTROPHY

The mature podocyte has limited capacity to divide *in situ*.[20,21] To what extent podocytes can be replaced at all during adult life, and if so, how and at what rate, has not yet been established. One potential mechanism for podocyte replacement is stem cell immigration from the bone marrow via blood as has been demonstrated in model systems.[22–24] A second possibility is the migration of cells from the parietal epithelial cell layer along Bowman's capsule onto the glomerular tuft together with a switch to the podocyte phenotype. In the first case, the stem cell has to traverse the GBM to home to its destination, whereas in the second case this is not necessary. A related question is the extent to which podocytes can hypertrophy to replace lost neighbors or to compensate for glomerular enlargement. Both human biopsy analysis as well as animal models show that podocytes can undergo significant hypertrophy but to a limited extent.[25–27] In a model system, as podocytes continue to enlarge they express different proteins reflecting an 'adaptation' stage and subsequently a 'decompensation' stage before becoming lost from glomeruli.[27] Whatever the mechanisms involved in podocyte hypertrophy and replacement it is possible that they occur slowly so that podocyte injury leading to podocytopenia can become a major threat to the glomerulus.

PROGRESSION TO GLOMERULOSCLEROSIS

The sequence of anatomic events resulting from podocyte injury has been comprehensively described in multiple animal model systems by Kriz.[20] This includes the appearance of denuded areas of GBM, adhesion to Bowman's capsule (synechia formation), focal segmental glomerulosclerosis, and global glomerulosclerosis associated with misdirected filtration into the interstitial compartment contributing to interstitial injury and fibrosis. The consequences of different degrees of podocyte loss is exemplified using rat models of regulated podocyte depletion.[28,29] In these models, loss $<$ 20% of podocytes causes mesangial expansion alone. Loss of more then 20% of podocytes leads to the appearance of denuded areas of GBM resulting in adhesion of the glomerular capillary loop to Bowman's capsule (synechia formation). With 20–40% podocyte loss, selected glomerular capillary loops become devoid of podocytes and result in a scarring response limited to that particular loop (segmental sclerosis). With more than 40% podocyte loss, the glomerulus becomes progressively more sclerotic until at $>$60% podocyte loss glomeruli become globally sclerotic and nonfiltering. These stages of podocyte depletion are accompanied by corresponding degrees of proteinuria and, as an increasing proportion of glomeruli become involved by measurable reduction in the clearance function of the kidney.

Glomerular enlargement is also associated with development of glomerulosclerosis.[30,31] This is true for reduction in renal mass (nephron number) as well as endocrine conditions such as acromegaly and obesity in man and in experimental models of overexpression of growth factors, partial nephrectomy, and high calorie intake. The sequence of events flowing from glomerular enlargement as outlined above is determined by the limited extent to which podocytes can hypertrophy to accommodate the increased GBM area to be served.[20,27] Exceeding this capacity appears to lead to glomerulosclerosis via similar mechanisms to those outlined for direct podocyte depletion.

THE PODOCYTE DEPLETION HYPOTHESIS

This hypothesis states that whatever the initial insult to the glomerulus (immune, toxic, infectious, ischemic, etc), the outcome depends on whether or not the complement of normal mature podocytes becomes depleted (Figure 3). On the one hand, if as a result of glomerular injury podocytes are not depleted, then the glomerulus has the capacity to remodel and recover essentially normal structure and function. On the other hand, if significant podocyte depletion occurs, then the glomerulus (or that part of the glomerulus) will not recover its normal structure and function. If progressive podocyte depletion is allowed to occur over time, then this will be associated with progressive glomerulosclerosis leading to progressive loss of renal function culminating in ESKD. Podocyte depletion can occur as a result of (a) direct podocyte loss because of necrosis, apoptosis, or detachment; (b) by glomerular enlargement leading to relative podocyte depletion; or (c) by a switch of the podocyte phenotype to one which cannot maintain normal glomerular structure and function.

As outlined above, many potential mechanisms may trigger podocyte injury and depletion. The loss of some

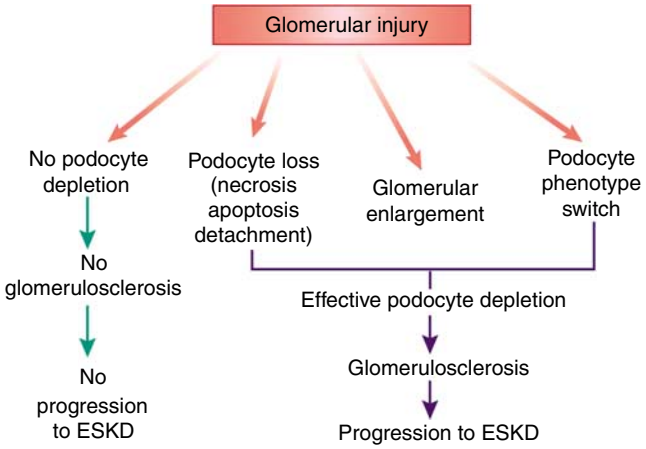

Figure 3 | The podocyte depletion hypothesis. This hypothesis states that the outcome for an individual glomerulus will depend on whether glomerular injury results in significant podocyte depletion or not. Podocyte 'depletion' is considered to be depletion of the complement of mature podocytes necessary for maintenance of the normal mature glomerulus. Podocyte 'depletion' can therefore occur by podocyte loss itself, by glomerular enlargement leading to expansion of GBM area to be served, or by alteration of the podocyte phenotype such that the podocyte-derived cell is no longer able to fulfill its normal mature functions, such as occurs in collapsing glomerulopathy and crescentic nephritis.

podocytes for any reason may trigger a vicious cycle leading to the loss of more podocytes.[32] Angiotensin II may itself directly amplify this effect,[33] thus providing a potential explanation for the well-known protective effect of angiotensin II inhibition in slowing and preventing progression in glomerular diseases.

THE SPECTRUM OF SYNDROMES ASSOCIATED WITH ALTERED PODOCYTE BIOLOGY

As indicated by Figure 1 and outlined in sections below, the different clinical syndromes of glomerular diseases can each be directly related to various aspects of altered podocyte biology. These include podocyte developmental arrest, defective podocyte products, phenotypic switch and podocyte loss, dedifferentiation, and proliferation.

Arrested glomerular development

Glomerular developmental arrest as a cause of diffuse mesangial sclerosis. Mutations that result in arrested glomerular developmental at the 'head-shaped' or early capillary loop stage of glomerular development (Figure 2) cause the clinical syndrome of diffuse mesangial sclerosis (DMS). Such arrested glomeruli can still filter to some extent but they leak protein into the filtrate (nephrotic syndrome) and rapidly progress to ESKD. The clinical syndrome of DMS includes proteinuria from birth (congenital nephrotic syndrome) and progression to ESKD within 6–12 months of age.[34,35] The pathologic appearance of DMS glomeruli includes a halo-shaped distribution of podocytes surrounding a matrix-containing glomerular center ('DMS'), absent foot processes, and lack of normal glomerular capillary loops.

The recent identification of phospholipase C epsilon (*PLCE1*) truncating mutations as a cause of DMS is helpful for understanding glomerular biology.[36] As PLCE1 is expressed by the podocyte in the developing glomerulus, the fact that mutations in *PLCE1* gene prevent normal glomerular development confirms the central role of the podocyte in this process. Similarly, WT1 is a major podocyte transcription factor present early in podocyte development, which regulates expression of key proteins by the podocyte including nephrin and podocalyxin.[37,38] Mutations in the *WT1* gene also cause DMS.[17–19,39] Furthermore, mutations in the β2laminin (*LAMB2*) gene cause DMS presumably as a result of defective GBM expansion and assembly required for normal glomerular development.[40,41] The fact that mutations in the above noted genes expressed by podocytes all cause a similar glomerular phenotype provides a glimpse of the part of this regulatory machinery necessary for early stages of glomerular development.

Glomerular developmental arrest as a cause of the congenital nephrotic syndrome of the Finnish type. Glomerular developmental arrest at the late capillary loop stage of glomerular development causes massive life-threatening proteinuria as congenital nephrotic syndrome of the Finnish type. Having constructed the enormous filtration surface area during the capillary loop stage of glomerular development, it becomes necessary that the filtration machine efficiently prevent loss of blood protein into the filtrate. This goal is achieved in part through the interdigitating podocyte foot processes and specialized intercellular junctions (slit diaphragms). By these mechanisms the podocyte provides physical support to the GBM to counteract the hydrostatic pressure of blood as well as supplies special molecules required by the GBM for its normal structure and function. The foot processes and slit diaphragms also serve as information mechanisms that signal outside-in to the podocyte about space between foot processes, pressure, and similar information required to maintain efficient filtration. The proteins that make up the slit diaphragm and its signaling system include nephrin, podocin, neph1, and their signaling partners fyn, yes, nck, CD2AP, and others.[10–15,32–46]

Failure to construct normal foot processes and intercellular junctions during development results in massive proteinuria before and immediately after birth, giving rise to the syndrome of congenital nephrotic syndrome of the Finnish type. The major causes of congenital nephrotic syndrome of the Finnish type in man are mutations in the *NPHS1* gene coding for the slit diaphragm protein nephrin and in the *NPHS2* gene coding for the foot process membrane protein podocin.[42,43] These mutations result in grossly normal capillary loop development with the key exception that foot processes and slit diaphragms fail to form normally. The result is a leaky glomerular filter and consequent life-threatening loss of protein into the urine.

Defective GBM construction and maintenance (Alport's syndrome and variants)

The requirement of the GBM to be strong and rugged yet thin and discriminating is achieved in part by special type IV

collagen trimers comprised an α3, α4, and α5 chain of type IV collagen.[16] These type IV collagens are produced by the podocyte and inserted into the GBM during development and adult life as required for GBM maintenance. Mutations in the α3 and α4 chains (*COL4A3* and *COL4A4*) can result in a thin GBM ('thin GBM disease'). Thin GBM is liable to rupture causing hematuria, but does not usually cause progressive loss of renal function or significant proteinuria.[47] Rarely, mutations in α3 and α4 type IV collagen genes (*COL4A3* and *COL4A4*) are associated with progressive loss of kidney function.[48] Mutations in α5 type IV collagen *COL4A5* cause Alport's syndrome resulting in defects in the glomerular GBM, the lens of the eye, and the cochlear membrane. The associated clinical syndrome includes renal failure, deafness, and visual defects.[49] Because the *COL4A5* gene is on the X chromosome, Alport's syndrome is a sex-linked condition primarily affecting males.

Podocyte phenotypic switch and minimal change disease

In minimal change disease (MCD) podocytes undergo a phenotypic switch from a cell with foot processes protruding from its basal surface to a cell without basal processes but which has acquired microvillus protrusions on its apical cell surface ('microvillus transformation') similar to those present in renal tubular cells. This remarkable alteration in podocyte structure is not associated with reduction in expression of key podocyte proteins such as nephrin or podocin, such as is seen in forms of focal segmental glomerulosclerosis (FSGS),[50,51] although podocyte α-dystroglycan, responsible in part for adherence of the podocyte to the underlying GBM is reduced in MCD.[52] MCD can therefore be considered as a podocyte phenotypic switch. It is typically triggered by allergic and immune events including lymphomas implying that circulating factors such as cytokines may trigger the switch, possibly in genetically susceptible individuals.[53] This concept is consistent with the ability of glucocorticoids to rapidly induce a switch reversal back to the normal podocyte phenotype, analogous to glucocorticoid being used to promote maturation (and surfactant production) by alveolar epithelial cells in the immature lung. The clinical consequence of the 'switched' podocyte in MCD is decreased efficiency of the filtration mechanism with loss of large amounts of albumin into the glomerular filtrate causing nephrotic syndrome. Podocytes are not lost into the urine in MCD with maintenance of the normal podocytes numbers compatible with the excellent long-term prognosis for renal function.[54]

Podocyte depletion from the glomerulus (podocytopenia) and FSGS

FSGS is a heterogeneous condition that has been recognized as being associated with podocyte injury for more than 30 years.[55,56] The sequence of events by which podocyte injury results in denudation of GBM, adhesions to Bowman's capsule, and progression through segmental to global sclerosis has been rigorously mapped by Kriz[20] and in numerous experimental reports. Direct quantitative demonstration of podocyte loss as a cause of FSGS has been demonstrated in rat models.[28,29] The concept that podocyte depletion causes FSGS in man is also supported by glomerular podocin mRNA being relatively depleted in FSGS compared with normal glomeruli[57] and finding that podocytes are lost into the urine in FSGS.[58] These data are compatible with the hypothesis that FSGS can be caused by podocyte depletion in man, but whether or not all FSGS in man is caused by podocyte depletion is not yet established.

For podocytes to serve their function, they must cover the filtration surface area with foot processes. Glomerular enlargement is known to be directly associated with development of FSGS, and is found in association with obesity, endocrine conditions such as acromegaly and diabetes, loss of kidney mass through nephrectomy, chronic glomerulosclerosis, and also in glomerulopenia resulting from premature birth and other causes.[30,31,59,60] Quantitative morphologic data show that FSGS is associated with increased GBM length per podocyte as further support for the hypothesis that relative podocyte depletion resulting from glomerular enlargement can contribute to FSGS.[25] Furthermore, in model systems of glomerular enlargement in rats the podocyte number did not increase as glomerular volume increased.[27] Rather, compensatory podocyte hypertrophy occurred up to a threshold point before FSGS supervened, as would be predicted if relative podocyte depletion was taking place in the enlarging glomerulus.

Mutations in genes coding for key podocyte proteins also cause FSGS. Autosomal recessive mutations such as *NPHS2* (podocin) and *PLCE1* (PLC epsilon non-truncating mutation) cause FSGS in children and young adults.[14,36] Autosomal dominant gain of function mutations in genes coding for α-actinin 4 and TRPC6 cause FSGS in adults.[61–65] We do not yet know whether these mutations cause FSGS through promoting podocyte loss in man or through some other mechanism. Similarly, experimental deletions of genes coding for proteins that play a role in podocyte structure and function causes FSGS in model systems under normal aging or stressed conditions (reviewed in Mundel *et al.*,[10] Pavenstadt *et al.*,[11] Asanuma and Mundel,[12] Ly *et al.*,[13] Antignac,[14] and Huber and Benzing[15]). Transforming growth factorβ overexpression is an example of a model where podocyte number has been shown to be reduced in association with development of FSGS.[66] Circulating factors,[67] toxic factors including drugs, oxidants, and deposition of glycosphingolipids in Fabry's disease[68] and crystallization of myeloma protein in podocytes[69] all cause podocyte injury leading to FSGS and account in part for the heterogeneity of this pathologic entity.[56]

Podocyte dedifferentiation causes 'collapsing glomerulopathy'

HIV-associated nephropathy and pamidronate toxicity are associated with a glomerular phenotype described as 'collapsing glomerulopathy' in which the glomerulus loses

its normal structure including its capillary loops.[56,70–73] In this condition, mature podocyte markers including WT1, nephrin, podocalyxin, GLEPP1, the cyclin-inhibitors p21, and p27 and foot processes are lost. In contrast, markers of immature dividing podocytes are acquired and the podocyte becomes capable of proliferating. Expression of the HIV protein *nef* specifically by podocytes in a transgenic model system is sufficient to cause this phenotype, thereby confirming that this is a podocyte disease.[70] In the presence of such dedifferentiated podocytes (absence of normal mature podocytes), the whole glomerulus can be viewed as itself dedifferentiating backwards towards a phenotype resembling the head- or halo-shaped early capillary loop stage of glomerular development where podocytes are arranged on the peripheral surface of the glomerular tuft before capillary loop formation and which is also present in DMS (Figure 2). Collapsing glomerulopathy is therefore mechanistically and structurally distinct from other forms of FSGS. Accumulating data suggest that eradication of the HIV infection allows podocytes to differentiate back to the mature phenotype with improvement in the filter characteristics and preservation of renal function.[74]

Podocyte injury, loss, and proliferation in immune and inflammatory glomerulopathies

The outcome in immune and inflammatory glomerulonephropathy is strikingly variable even within descriptive pathologic types. According to the podocyte depletion hypothesis, whatever the underlying form of immune injury, those individuals in whom immune injury causes podocyte loss would develop proteinuria, glomerulosclerosis, and progressive loss of kidney function, whereas those where similar injury did not result in podocyte loss would not develop glomerulosclerosis. For example, in immunoglobulin A nephropathy biopsy analysis shows a correlation between podocyte depletion and development of glomerulosclerosis.[75] People with active immunoglobulin A nephropathy, systemic lupus erithematosus, and membranoproliferative glomerulonephritis have increased numbers of podocytes in their urine,[76–79] which can be eliminated by effective treatment.[80] Membranous nephropathy is well known to be associated with podocyte injury owing to insertion of complement C5b–9 complex into podocyte plasma membranes.[81–83] To what extent the difference between progressive and non-progressive membranous nephropathy is determined by whether or not podocytes are lost from glomeruli is not yet known.

The special case of crescentic nephritis. In crescentic nephritis, dedifferentiated podocytes in the process of losing their mature markers can be identified as present in the glomerular crescent filling Bowman's space, possibly contributing to podocytopenia via this mechanism.[84,85] Quaggin and co-workers have demonstrated using a transgene model of Von Hippel–Lindau gene deletion from podocytes that podocytes themselves can drive the crescentic process in part via expression of the hypoxia-inducible factor target gene Cxcr4.[86] They also demonstrate that this signaling system is present in crescentic human glomeruli from patients with systemic vasculitis. Thus, under certain conditions podocytes can change their phenotype, proliferate to form at least a part of the glomerular crescent, and themselves drive the crescentic process towards a sclerotic outcome.

Hypertensive glomerulosclerosis and the podocyte

There is no doubt that systemic hypertension superimposed on glomerular disease of any kind leads to more rapid loss of renal function.[87] Blood pressure control and use of angiotensin-converting enzyme-inhibitors in glomerular diseases reduces loss of podocytes in the urine.[75–77] African Americans are particularly susceptible to developing ESKD in association with hypertension and serve as an important example of hypertension-related kidney failure.[88] Low glomerular number associated with low birth weight cannot account for this susceptibility, at least in African Americans.[89] Biopsy material from adult hypertensive African Americans shows that about 13% have typical FSGS lesions.[90] A large proportion of the remainder have a focal and global solidification of the glomerulus described as 'decompensated benign nephrosclerosis'.[91] Kimmelstiel and Wilson[92] and Helmchen and Wenzel[93] noted that the earliest glomerular changes in this lesion, which they called 'alterative glomerulitis', was 'swelling of the epithelial cells of the glomerular loops' and that 'early fibrinous adhesions to the parietal layer of Bowman's capsule may be present'. The recent observation that mutations in *WT1* are associated with FSGS in the African-American population fits in with the general concept that a podocyte abnormality could play a role.[94] African Americans, like the general hypertensive population, are protected from progression by angiotensin-converting enzyme inhibition and effective blood-pressure control.[95] To what extent progression to ESKD in different hypertensive groups can be attributed primarily or secondarily to podocyte dysfunction/depletion has not yet been determined.

Diabetic glomerulosclerosis as a consequence of glomerular enlargement and podocyte loss

The concept that podocyte injury is central to progression in diabetic nephropathy is now well established.[96–98] Pathologic biopsy reports in type I diabetic glomerulosclerosis and type II diabetic glomerulosclerosis directly link reduced glomerular podocyte number to increased proteinuria[99–102] and show that glomerular podocyte number is the best predictor of glomerular outcome in type II diabetes in Pima Indians.[103] This biopsy data are supported by urine podocyte number data in man where progressive diabetic glomerulopathy is associated with increased podocyturia and data showing that some diabetic treatment strategies reduce podocyturia.[104–107] Furthermore, there is extensive experimental data showing that podocytes injury is an early detectable event after onset of diabetes as well as reports linking progressive loss of podocytes to progression of glomerular disease in model systems.[108] Thus, the concept of podocyte loss as a driver of

the glomerulosclerotic process in diabetes is well documented. The underlying mechanisms are likely to be in part related to oxidant injury of the podocyte combined with glomerular enlargement resulting from insulin and other growth factors.

High calorie intake, obesity, and glomerulosclerosis

Several experimental models demonstrate that high-calorie diet results in glomerular enlargement and glomerulosclerosis.[109–111] This process may be podocyte-dependent as suggested by analysis of the mechanism for the protective effect of dietary restriction in rats.[27] Obese humans also develop proteinuria and FSGS,[26,56,112] and obesity is an independent risk factor for progression to ESKD.[113,114] The mechanism by which obesity and high-calorie intake target the podocyte is not known, but like diabetes is likely to include glomerular enlargement under the influence of growth factors and oxidant injury to podocytes.

Aging-associated glomerulopathy

Glomerulosclerosis is closely associated with aging, so that by over 40 years most individuals have sclerotic glomeruli and the proportion increases with further aging.[115–119] ESKD is also directly related to aging with a peak incidence of treated ESKD at 64 years.[1] The observed reduction in treated ESKD above 65 years of age is probably the result of a selection not to treat ESKD or a failure to recognize ESKD in old age, particularly in women with small muscle mass and therefore lower serum creatinine levels. Ninety percent of ESKD is because of glomerular disease.[1] In older age, most of this ESKD is currently assigned to the category of hypertension and/or diabetes as a causative mechanism although the validation of this assignment is not well established, leaving open the possibility that the aging process itself may contribute significantly age-associated ESKD by as yet unrecognized and potentially treatable mechanisms.

In rats, development of glomerulosclerosis occurs in relation to aging itself.[118,119] This process can be accelerated by high-calorie intake and retarded by calorie restriction in the absence of either hypertension or diabetes.[27] This tendency to age-related glomerulosclerosis in rats is strain-dependant, thereby demonstrating that genetic background also plays a role in age-associated glomerulopathy.

Glomerulosclerosis in the aging rat is a 'podocyte disease' as was first pointed out by Floege *et al.*[118] and supported by recent data.[27] The underlying mechanisms will possibly include alterations in DNA methylation and/or histone modifications (epigenetic mechanisms) that cause miss-expression of key genes in the podocyte as well oxidant injury to mitochondrial DNA and other macromolecules.[120,121] This would be analogous to other age-associated diseases that impact key cells which, like podocytes, are highly differentiated post-mitotic cells with limited capacity to divide. Examples include neurons in Alzheimer's and Parkinson's disease, pancreatic β cells in diabetes, photoreceptors in the eye, hair cells in the ear, and conducting cells in the heart. Age-associated glomerulopathy can therefore be seen as a senescent process, which sets up the podocyte, and thereby the whole glomerulus, for failure. This aging process can be accelerated by a combination of factors including genetic background, diet, and superimposed conditions such as hypertension and/or overt diabetes.

PREVENTION AND TREATMENT IMPLICATIONS OF THE PODOCYTE INJURY/DEPLETION HYPOTHESIS OF GLOMERULOSCLEROSIS AND PROGRESSION

Podocyte depletion (absolute or relative to glomerular enlargement) can be considered as the mechanistic explanation underlying the hyperfiltration hypothesis of Brenner *et al.*[2] that has proved so useful in preventing progression in the clinic. According to this hypothesis, the primary clinical focus should be on minimizing factors that tend to promote injury, podocyte detachment, and depletion. This includes control of systemic and intraglomerular hypertension and particularly the use of angiotensin II blockade as a central element of the prevention strategy. In this sense, reducing podocyte injury and depletion and reducing proteinuria are facets of the same strategy. Additional clinical strategies such as reducing glomerular enlargement and oxidant injury through calorie restriction, weight control, reduction in circulating insulin, and other growth factor levels will probably be confirmed to be clinically important by future studies. The permissive role of mutations and polymorphisms of genes that play a role in the propensity to lose podocytes in individual patients will need to be defined and targeted for therapeutic intervention. Measurement of podocyte products in the urine as a potential mechanism for monitoring accelerated podocyte loss non-invasively holds good potential for clinical application. Podocyte replacement by stem cells may prove to be a useful strategy.

CONCLUSION

A unifying concept, which puts podocytes at the center of the glomerular disease spectrum, is conceptually simple and makes intuitive sense to the practicing clinician. It also reinforces rational clinical and scientific approaches towards preventing progression.

ACKNOWLEDGMENTS

I am grateful for grant support from NIDDK at the National Institutes of Health (grants DK46073 and P50 DK39255) and for the support and council of our esteemed colleagues at the University of Michigan and worldwide.

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