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REVIEW

Minimal change nephropathy and focal segmental glomerulosclerosis

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Abstract The terms minimal change nephropathy and focal segmental glomerulosclerosis describe histopathological entities diagnosed by renal biopsy, typically in patients presenting with heavy proteinuria and its consequences including nephrotic syndrome. Numerous alterations in the immune response have been reported, but there is uncertainty about whether these play a causal role. In both conditions, there is evidence of injury to glomerular epithelial cells (podocytes), a cell type with limited potential for repair or replacement. The mechanisms of injury are poorly understood but may include immunologically mediated processes such as the effects of soluble mediators produced by lymphocytes. Empirical immunosuppressive therapy with corticosteroids, alkylating agents, and/or calcineurin antagonists is often effective, but the potential for toxicity of these drugs is enormous, and more specific forms of treatment are needed. The focus in recent years has been on the podocyte, and in particular the potential importance of mutations/polymorphisms in podocyte-specific genes as predisposing factors, mechanisms of podocyte injury including study of the role of podocytes as active participants in disease pathogenesis, indices of podocyte injury as markers of disease activity or possible diagnostic tools, and strategies for podocyte repair including the recognition that existing therapies may have effects (beneficial or adverse) on podocytes. Future improvements in the understanding of these diseases and in our ability to successfully treat them can be confidently expected as a result of rapid advances in the study of podocyte biology in health and disease.

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Introduction

Minimal change nephropathy (MCN) and focal segmental glomerulosclerosis (FSGS) may have very similar clinical presentations, and there has been much debate on the question of whether these two histopathological entities represent two subtypes of a single condition, opposite ends of a single spectrum of disease, or two entirely separate diseases. My own opinion is that they are two different diseases, but there is no doubt that they have in common the cardinal feature of injury to glomerular epithelial cells or podocytes, leading them to be classified, along with other forms of glomerular disease, as "podocytopathies" [1, 2].

In MCN, the podocyte injury is reversible, so that progressive loss of renal function is rare, whereas in FSGS, the podocyte injury is more severe and may be irreversible so that there is an appreciable risk of progressive nephron loss. In both conditions, empirical immunosuppressive therapies are widely used in the belief that the diseases are immunologically mediated: Corticosteroids, alkylating agents such as cyclophosphamide, or calcineurin antagonists such as cyclosporin or tacrolimus can be very effective (more so in MCN than in FSGS). We, thus, have the paradox that, in MCN and FSGS, our treatments are fairly effective despite our lack of understanding of the pathogenesis. The effectiveness of empirical therapy should not make us complacent: These treatments are nonspecific and potentially toxic. We use these approaches because they are effective rather than because we understand *why* they are effective. Treatment-related morbidity can be enormous,

especially in patients presenting in childhood and needing long-term therapy: There is a real risk that the treatments can be worse than the disease. The fact that therapies aimed at the immune system can alter the natural history of the conditions has contributed to the dominance of the hypothesis that both are the result of disordered immune responses. However, much of the literature on the immunological disturbances seen in MCN and FSGS does not allow a firm conclusion on which features (if any) are causal and which are merely consequences. I will consider MCN and FSGS separately, aiming to highlight those areas in which their clinical features, pathogenesis, and treatment overlap and where they differ. Then the remainder of this chapter will concentrate on podocytes as targets of injury in these diseases and the focus of therapeutic interventions.

Minimal change nephropathy

Clinical features

MCN typically presents with heavy proteinuria leading to nephrotic syndrome. The patient's main symptom is edema, affecting the face and limbs according to the influence of gravity so that periorbital swelling is typical on waking, and dependent edema of the legs develops in the upright posture during the day. Patients often also have profound lethargy and reduction in exercise tolerance. Excretory renal function is typically normal. Onset is often abrupt; there may have been a precipitating event such as an intercurrent infection or other illness, or a recent vaccination. All ages may be affected, but it is most common in childhood. There is an association with a personal and/or family history of atopy [3]. In rare cases, MCN is precipitated by a drug such as a nonsteroidal anti-inflammatory agent, and will resolve once the offending drug is withdrawn. There is also a well-described association with lymphoma, particularly Hodgkin's disease, in which treatment of the lymphoma leads to resolution of the nephrotic syndrome and relapse of proteinuria may signify recurrence of the underlying lymphoma [4]. However, in the majority of cases, no underlying cause can be identified. In adults, in whom MCN accounts for only around 15–20% of cases of idiopathic nephrotic syndrome and it is important to exclude other causes whose management may differ, the diagnosis is made by renal biopsy. By contrast, in children presenting with a first episode of nephrotic syndrome, MCN is the cause in the vast majority of cases so that renal biopsy is not considered essential before treatment is started. Initial treatment is usually with corticosteroids, and this explains one of the issues around the terminology on MCN. Much of the literature is based on pediatric practice and uses terms such as steroid-sensitive nephrotic syndrome rather than MCN because a histological diagnosis has not been obtained. Using the response to corticosteroids as a determinant of management of nephrotic syndrome is normal practice in pediatric nephrology: Children who do not respond are labeled as having steroid-resistant nephrotic syndrome, and this is usually taken as an indication for a renal biopsy. One of the likely histological diagnoses in this situation is FSGS.

In the renal biopsy appearances of MCN [5], the glomeruli are typically entirely normal by light microscopy. A mild degree of mesangial hypercellularity is considered by some authors to fall within the boundaries of MCN and may be an adverse prognostic factor. Typically, immunofluorescence microscopy is negative. Patients with isolated IgM deposition in otherwise normal or mildly hypercellular glomeruli are included in some series of MCN, but this author considers this indicative of IgM nephropathy (IgMN). Although there is considerable overlap between MCN and IgMN in clinical features, treatment, and prognosis, the fact that IgMN carries a small but definite risk of the development of renal failure [6] and may recur in renal transplants [7] indicate that it is a separate condition. Electron microscopy of the renal biopsy in MCN shows "effacement" or flattening of podocyte foot processes (Fig. 1) and an absence of electron-dense immune deposits in the glomeruli.

Patients with MCN may also present with complications of nephrotic syndrome [8] such as infection, a thrombotic tendency which may lead to arterial as well as venous thrombosis, and hyperlipidemia which may be severe. The predisposition to infection includes local factors such as the presence of edematous tissues in which cellulitis may occur and systemic abnormalities such as hypogammaglobulinemia (discussed further below). The reasons for the

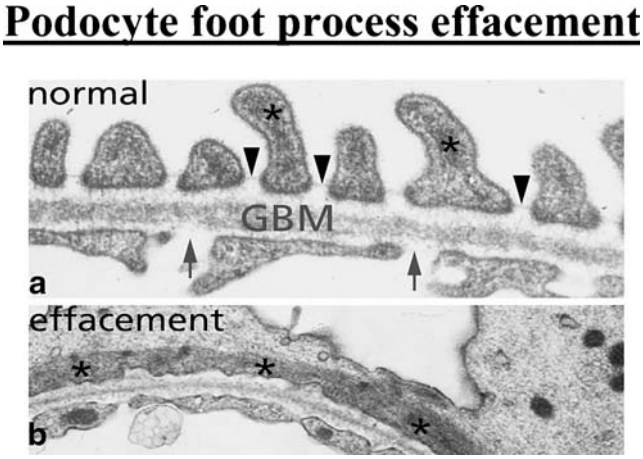

Fig. 1 Transmission electron micrographs of glomerular capillary wall. **a** normal (arrowheads indicate slit diaphragms between foot processes, arrows indicate fenestrations in glomerular endothelial cells) **b** effaced podocyte foot processes in minimal change nephropathy

thrombotic tendency are not well understood but may include preferential loss of anticoagulant factors and/or excessive synthesis of procoagulant factors. The hyperlipidemia is due to the combination of increased synthesis and reduced turnover of lipoproteins [9].

Is MCN caused by an abnormal immune response?

The suggestion that MCN is caused directly by an immunological disturbance has dominated the literature for decades. Shalhoub [10], in a seminal paper in 1974, presented the hypothesis that MCN results from a disorder of T cell function, and that the treatments such as corticosteroids that are effective in this condition are acting by modifying this. More recently, a key role for T cells was supported by a genetic screening approach [11]. However, the evidence for immune mechanisms is all indirect. There is no inflammation in the glomerulus, no infiltration with lymphocytes or other immune cells, and no deposition of immunoglobulin or complement in the kidney. Therefore, studies have concentrated on possible soluble mediators: The literature is conflicting, but there is a suggestion that a type 2 cytokine bias might be important (reviewed in [12]), and this has been linked to the "hygiene hypothesis" in a provocative review addressing the changing patterns of types of GN in the developed world [13]. Podocytes express receptors for interleukins (IL)-4, 10, and 13 [14, 15], and these cytokines have been shown in vitro to have direct effects on podocyte mediator production and barrier function [14–16]. Podocytes also express functional CCR and CXCR chemokine receptors and respond to exogenous chemokines [17]. Whether these known cytokines and/or chemokines are the elusive soluble mediator(s) in MCN remains unproven, but podocytes clearly have the potential to respond to various soluble products of the immune system.

An intriguing recent study [18] directly addressed the role of IL-13 by overexpressing this cytokine in rats and reported that an MCN-like nephropathy was induced, with changes in podocyte structure and gene expression similar to those seen in the human disease. This study provides the best evidence so far that a single cytokine such as IL-13 can induce nephrotic syndrome. Mice transgenically overexpressing IL-13 do not have proteinuria, but this may simply reflect the resistance that many mice strains show to the induction of glomerular disease. A mechanism whereby excess IL-13 might induce nephrotic syndrome is illustrated by changes in podocyte protein trafficking and proteolytic enzyme secretion seen when these cells are incubated with IL-4 or IL-13 in vitro [19].

Other evidence for an immune/autoimmune pathogenesis in MCN includes immunogenetic factors [20], especially associations with products of the major histocompatibility complex [21], and the response to drugs which have potent effects on the immune system, such as corticosteroids, cyclophosphamide, and cyclosporin. The relative specificity of cyclosporin's mode of action for T lymphocytes led to suggestions that its beneficial effects in MCN and FSGS strongly support the importance of T cells in the pathogenesis [22]. However, cyclosporin has numerous other effects including hemodynamic effects and actions on numerous other cell types, so that this interpretation may be overly simplistic. Of great interest is the recent observation that rituximab, a chimeric monoclonal antibody against the B cell marker CD20, can induce remission in nephrotic syndrome [23, 24]. In this case, the specificity of the therapeutic approach is such that its beneficial effects strongly imply a previously unsuspected role for B cells in these diseases.

Another agent whose effects in nephrotic syndrome may be instructive is levamisole. This drug's mode of action is unknown, but it is considered an immunostimulant rather than an immunosuppressive, having useful effects in helminth infections where it is believed to enhance the immune response to the parasite and favor parasite clearance. It has also shown benefit in certain forms of leprosy [25] and in cancer, especially colonic carcinoma [26]. In childhood MCN, levamisole treatment significantly reduces the rate of relapse [27], and my own (unpublished) experience in adult MCN suggests that this drug is also useful in maintenance of remission in a subset of patients. A plausible mechanism for levamisole's effects is suggested by our data in an experimental model in which there is a bias towards type 2 cytokines [28]. Levamisole leads to selective induction of gene transcription of IL-18 and is associated with augmentation of the type 1 response and reciprocal downregulation of the excessive type 2 response. These results support the hypothesis that MCN is associated with a biased type 2 cytokine response and suggest that levamisole acts by resetting the balance.

The older literature contains reports of a wide variety of immunological abnormalities in MCN, including observations in vivo and in vitro, affecting both humoral immunity and cell-mediated immunity. In considering these, it is important to ask whether the abnormalities are "cause" or "consequence" of the nephrotic state. Are they specific for MCN? Are they present only in the "active" phase of the disease, i.e., when the patient has nephrotic syndrome, or do they represent some more fundamental abnormality which is present even when the disease is in remission? Are the abnormalities explained by the effects of drug treatments? Are any similar abnormalities found in the families of affected individuals, implying an immunogenetic basis?

Hypogammaglobulinemia is a typical finding in patients with nephrotic syndrome [8]. In some forms of renal disease, there may be substantial urinary losses of IgG, but the proteinuria in MCN is typically "selective," and

urinary loss of IgG is slight or absent, so that the hypogammaglobulinemia must have other explanations. There is impaired IgG production in MCN [29, 30] as well as in other causes of nephrotic syndrome [30], and there is some evidence of increased IgG catabolism [8]. These abnormalities are substantially reversed by therapy, normalizing during remission [29, 30]. Importantly, the IgG subclasses are not equally affected: Circulating levels of IgG1 and IgG2 are profoundly depressed, and synthesis of these subclasses in vitro is impaired, but IgG3 and IgG4 are relatively spared [31]. This is further evidence that urinary loss cannot explain the hypogammaglobulinemia because the different IgG subclasses are proteins of similar size, and preferential loss of some subclasses is unlikely. Instead, these observations on the differential effects on IgG1 and 2 vs IgG3 and 4 may be very instructive in understanding the immune dysregulation in MCN. Other immunoglobulin isotypes are not depressed: Levels of IgM are normal or high [32], and levels of IgE are also markedly elevated [33, 34]. The elevation of IgE is not absolutely specific for MCN but is more frequent and greater in magnitude in this condition compared with other causes of nephrotic syndrome [35, 36]. The elevation of IgE is a feature during active disease but is also present during remission and when treatments have been stopped [33, 34], suggesting it is not simply a consequence of the disease or its treatments but instead reflects an underlying feature of the immune response in patients who develop MCN. These abnormalities of Ig isotype and IgG subclass may simply reflect cytokine dysregulation with a predominance of type 2 cytokines as discussed earlier, these being particularly concerned with class-switching of B cells to the production of IgG4 and IgE [37]. The literature on cytokine production in patients with MCN is complex and inconsistent [38], but does include reports of preferential production of type 2 cytokines, especially IL-4 [39, 40]. This is consistent with the association of MCN with a personal and/or family history of atopy [3], as atopy is itself typically associated with a type 2 cytokine bias [41]. The fact that atopy and allergy are more common in first-degree relatives as well as in the patients themselves suggests immunogenetic factors responsible for this type-2-biased immune response. There is a great deal of interest in genes which predispose to atopy, with genes encoding type 2 cytokines or their receptors being attractive candidate susceptibility genes [42]. We tested several of these candidate genes for any association with MCN and obtained negative results [43, 44]. However, analysis of candidate susceptibility gene loci in a complex disease such as MCN is inevitably speculative and many other genetic loci could be involved. It remains likely that there are immunogenetic factors underlying the tendency to produce a type 2-biased cytokine response. If it is correct that MCN arises in the context of a biased cytokine profile, identification of these genetic loci will be helpful in understanding the pathogenesis of MCN.

Other immunological phenomena reported in patients with MCN include various abnormalities of T cell subsets and of T cell responses in vitro; again, the literature is contradictory, and there are many reasons for believing that these abnormalities are secondary to the nephrotic state rather than primary. One striking example is the observation that the inhibitory effect of serum from nephrotic patients on lymphocyte proliferative responses in vitro could be reversed by removal of the lipoprotein fraction, implying that apparent defects in cellular immunity could be explained simply by the associated hyperlipidemia [45]. There is impairment of antibody-dependent cellular cytotoxicity [46], presumably consequent upon the hypogammaglobulinemia discussed above. Macrophage killing of *Candida* is impaired, although phagocytic function is normal [47]. Neutrophil and natural killer cell function seem to be normal [47].

Phenotypic analysis of lymphocyte subsets has given inconsistent results. Most of the described abnormalities are not specific for MCN, occurring in other causes of nephrotic syndrome; they are only present during "active" disease, i.e., associated with the nephrotic state itself; and they are only found in the patients themselves and not in their relatives. For these reasons, it is impossible to know whether these abnormalities are a cause or a consequence of MCN. The evidence suggests the latter so that these immunological abnormalities may not be instructive in understanding the pathogenesis of MCN. The elevation of IgE and the associations with atopy are possible exceptions, as discussed earlier.

Focal segmental glomerulosclerosis

Clinical features

The initial clinical presentation may be identical to that of MCN, except that hypertension and/or impairment of excretory renal function are more frequent in FSGS. As mentioned earlier, in children the diagnosis of FSGS may be suspected when the condition is steroid-resistant. In adults with unexplained nephrotic syndrome, FSGS is diagnosed by renal biopsy, and there is evidence that FSGS is becoming more common: In one series, between 1976 and 1979, 15% of cases were due to FSGS, and in a later cohort between 1995 and 1997, the proportion with FSGS had risen to 35% [48]. Other series have shown an even more dramatic increase which was greatest in African-Americans and Hispanics but also seen in whites [49] and confirmed that FSGS is increasing in importance as a cause of end-stage renal disease [50].

Variants of FSGS occur in association with severe obesity [51] and in human immunodeficiency virus (HIV) infection [52]: These will be mentioned further below. Some of the confusion in the literature about FSGS is explained by the fact that similar histological features can be seen when the glomerular injury is secondary to some other form of renal disease, either glomerular eg IgA nephropathy or nonglomerular eg reflux nephropathy. In these situations, the FSGS is a late phenomenon due to scarring, the level of proteinuria will be less, and there will be evidence of the other renal disease. Therefore, the entity of "idiopathic" or primary FSGS is best considered to be that with a presentation with heavy proteinuria and no evidence of preexisting renal disease.

The term FSGS merely describes the histological appearances: There is scarring (sclerosis) in some parts (segmental) of some glomeruli (focal). A number of histological subtypes have been described; it remains controversial whether these are predictive of outcome [53]. Immunochemistry is either negative or shows only IgM and/or C3 in sclerosed segments. Electron microscopy shows the sclerosis and also abnormalities of podocyte structure including fusion of foot processes which may overlie otherwise normal glomerular capillaries, focal detachments from the glomerular basement membrane (GBM), and lipid droplet accumulation. The podocyte as a target of injury in FSGS can therefore be inferred from the electron microscopic appearances, but it is from the study of rare inherited forms of the disease that the most significant recent advances have come, with the clear recognition that FSGS is the archetypal "podocytopathy" (see next section).

Differences between MCN and FSGS

Many of the immunological phenomena described above in MCN are also seen in FSGS and will not be repeated here. FSGS differs from MCN in four important ways: Empirical treatment using the same types of drug, namely, corticosteroids, alkylating agents, or calcineurin antagonists is less likely to be successful in FSGS than in MCN; there is a substantial risk of development of renal failure in FSGS; there is a high risk of recurrence of FSGS in renal transplants; and in FSGS, there is an abnormality of plasma composition which has led to a search for putative "permeability factors" in the circulation. Study of the latter two areas has been instructive in understanding FSGS and will be considered further.

The risk of recurrence of FSGS in a renal transplant is highest in pediatric patients, in white recipients of African-American kidneys, and in patients whose original disease was aggressive and rapidly progressive. Proteinuria may recur within minutes of the revascularization of the transplanted kidney, indicating that the cause lies in the recipient bloodstream. Various assays have been developed to analyze the putative permeability factor(s), either based on swelling of glomeruli in vitro when incubated in plasma from patients with FSGS (but not MCN) [54] or on in vivo infusion of plasma (or of supernatants from lymphocyte cultures) into rodents and the measurement of resultant proteinuria [55]. The precise identification of the permeability factor(s) has so far defeated investigators, and the utility of the approach of infusion of plasma fractions into rats has been seriously questioned [56]. The specificity for FSGS has also been questioned [57, 58]. A favored explanation for which there is now abundant evidence is that the abnormality is of something missing from nephrotic plasma compared to normal plasma, rather than of the presence of something abnormal. For example, the proteinuria-inducing effect of nephrotic plasma is lost when the plasma is preincubated with the urine of the same patient [59], the effect in the glomerular swelling assay is abrogated by mixing with normal plasma [60]; the effects of nephrotic plasma on human podocytes in vitro are reversed when the plasma is mixed 50:50 with normal plasma [61]. The evidence favors an imbalance of a protease—antiprotease pair, presumably due to loss of the inhibitor in the urine and/or the increased synthesis of the active protease but not its inhibitor [62]. The apparent success (albeit short-lived) of treatment of patients with recurrent FSGS using plasmapheresis may be explained by the removal of an abnormal substance and/or by the replacement of normal plasma. Immunoadsorption, which predominantly removes IgG, has also been reported to be successful, and here, no replacement plasma is given. There is evidence that the active component of the immunoadsorbed material is not IgG, also that the effect of immunoadsorption is not specific to FSGS [57].

One practical outcome of the recurrence of FSGS in renal transplants is the opportunity to study early events by biopsy of the transplanted kidney. The earliest histological features seen are changes in the podocytes' cellular phenotype including acquisition of macrophage markers and podocyte proliferation associated with changes in the cell cycle inhibitors which are believed normally to limit this cell types' proliferative capacity [63].

Treatment approaches in FSGS are similar to those used in MCN but less likely to succeed. This could simply reflect the greater severity of glomerular injury in FSGS or could be because the disease mechanisms are less amenable to these agents. It will be evident to the reader that, in both MCN and FSGS, there is a need for greater understanding of the pathogenesis leading to the design of more rational and specific forms of therapy. I am confident that study of podocytes will achieve these goals.

Podocytes

There have been dramatic advances in recent years in the understanding of the visceral glomerular epithelial cell, or podocyte. Podocytes lie on the extravascular (urinary) aspect of the glomerular capillary. Together with the glomerular endothelial cells which line the inner (luminal) aspect and the GBM lying between the two cell types, they form the tripartite glomerular filtration barrier. Podocytes have a complex structure with primary and secondary processes leading to foot processes which interdigitate with uniform slits between them, each bridged by a slit diaphragm (Figs. 1a and 2a). New impetus has been given to the study of this cell type by the identification over the last 10 years of a series of genes which when mutated in human or mouse give rise to early-onset nephrotic syndrome in which there is massive leakage of protein into the urine. Examples are the genes encoding nephrin, podocin, CD2AP, α-actinin IV, and TRPC6. In the normal glomerulus, these genes are only expressed in podocytes: The fact that podocyte-specific mutations lead to proteinuria has supported the conclusion that it is the podocyte that is the site of the selective permeability function of the healthy glomerular capillary. Thus, the focus has shifted from the GBM, which was previously thought to be the major site of regulation of protein permeability, onto the podocyte. In fact, study of MCN could already have led to this conclusion: The only morphological alteration seen in MCN is disruption of the structure of podocytes, seen in transmission electron microscopy of renal biopsies as flattening or "effacement" of foot processes (Fig. 1b) and shown more dramatically by scanning electron microscopy (Fig. 2b). However, it is worth noting the altered appearance of the GBM in Fig. 1b, making the point that alterations in one of the components of the glomerular filtration barrier may have consequences for the others. In my view, the glomerular permeability barrier is best considered as a composite of the three components, with disease processes primarily, but perhaps not solely, affecting one or more of these.

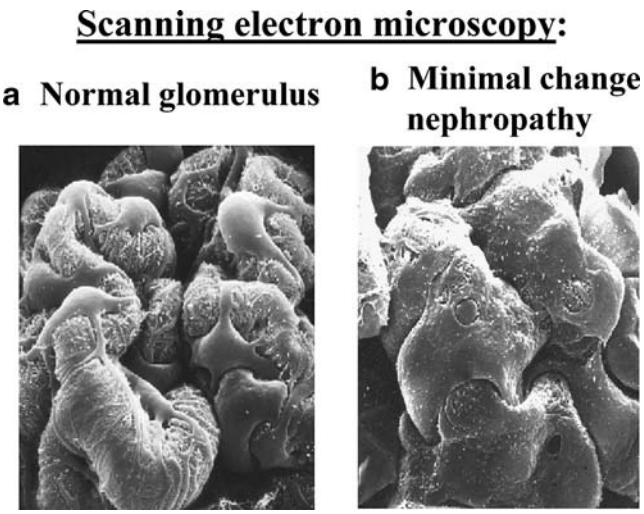

Fig. 2 Scanning electron micrographs of glomerular capillaries from urinary (external) aspect. **a** normal **b** minimal change nephropathy

Recent advances in podocyte biology

Congenital nephrotic syndromes are rare, but their study has been very instructive in improving our understanding of podocyte biology and the role of podocyte injury in FSGS. The breakthrough came with the identification by a positional cloning approach of the gene mutated in congenital nephrotic syndrome of the Finnish type, published in 1998 [64]. The protein encoded by this gene was termed nephrin and shown in the glomerulus to be podocyte-specific, localized to the slit diaphragm which bridges adjacent foot processes [65]. Soon afterwards, the importance of this gene was confirmed by the fact that mice lacking nephrin also develop severe congenital nephrotic syndrome [66]. During the last 10 years, several further single-gene defects have been identified in which mutation/deletion of genes which in the normal glomerulus are only expressed in podocytes leads to early onset nephrotic syndrome, the histological end-point in each case having the features of FSGS. As well as identification of novel proteins such as nephrin [64] and podocin [67], this work has highlighted new roles for known proteins such as CD2AP [68, 69], α-actinin IV [70], and TRPC6 [71, 72]. Subsequently, interest in these proteins and the podocyte in general has naturally extended to the more common acquired forms of glomerular disease. In relation to MCN and FSGS, noteworthy areas include study of mutations or polymorphisms in podocyte-specific genes as predisposing factors; identification of mechanisms of podocyte injury, including study of the role of podocytes as active participants in disease pathogenesis; indices of podocyte injury as markers of disease activity or possible diagnostic tools; and strategies for podocyte repair including the recognition that existing therapies may have effects (beneficial or adverse) on podocytes. These will be considered in turn in the next sections. Podocytes are terminally differentiated cells with limited potential for replication or repair, and it is believed that this fact underlies the importance of podocyte loss as a prognostic factor in a variety of renal diseases [73], most notably diabetic nephropathy [74]. The major difference between MCN and FSGS may turn out to be simply the irreversibility of podocyte injury in FSGS that gives it a more sinister prognosis. The identification of podocyte-specific genes has had the practical advantage of providing a means of specifically targeting podocytes in experimental models,

for example, by expressing a receptor specifically in podocytes than injecting an immunotoxin that selectively binds to that receptor and kills the cell bearing it. Using this approach, rodent experimental models have convincingly demonstrated that selective podocyte injury leads to FSGS [75, 76].

Podocyte-specific gene mutations/polymorphisms as predisposing factors

There are two aspects of the study of podocyte-specific genes in nonfamilial forms of the disease: First, the fact that some patients with apparently sporadic nephrotic syndrome may in fact have a genetic form of the disease without a family history. For example, in the autosomal recessive forms, the carriers are phenotypically normal so that the first affected individual will not have a family history; in addition, the FSGS associated with mutations in the gene encoding alpha-actinin IV has an autosomal dominant pattern of inheritance with variable expression so that previously affected individuals may simply not have been diagnosed. Patients whose nephrotic syndrome has a genetic basis are presumably less likely to respond to empirical treatment, so there has been interest in the question of whether genotyping at known disease-associated loci should be used in the study of steroid-resistant patients before use of more toxic agents, or even at diagnosis as a predictor of likely steroid responsiveness. Patients with a genetic defect leading to FSGS are also considered much less likely to have recurrence after renal transplantation, so genotyping could predict prognosis and affect management when transplantation is being considered.

The second aspect is in understanding pathogenesis: Could minor mutations or polymorphisms of podocyte-specific genes act as susceptibility factors for acquired FSGS? This has been best studied for the gene NPHS2 encoding podocin, for CD2-associated protein, CD2AP, and for the Wilm's tumor gene WT-1. Regarding podocin, a recent analysis suggested that, in European populations, heterozygosity for the most common variant of the gene, R229Q, conferred an increased risk of 20–40% of the development of FSGS [77]. Regarding CD2AP, one small study reported that heterozygous truncations can be identified in a small proportion of patients with sporadic FSGS [78]. As for WT-1, there is a report that polymorphisms of this complex gene were associated with idiopathic and HIV-associated FSGS [79]. Although at present, genetic testing of patients with nephrotic syndrome is not widely available, it is to be hoped that increasing knowledge of polymorphisms and mutations in podocyte-specific genes together with improvements in technology should allow greater access to this information in future. There is real optimism that this could allow clinicians to apply treatments in a more focused fashion and minimize unnecessary exposure to potentially toxic therapies if they are likely to be ineffective.

Mechanisms of podocyte injury

The identification of the genetic forms of FSGS mentioned earlier has allowed study of the consequences for podocyte structure and function: For example, the mutant form of alpha-actinin IV binds more avidly to actin and affects the mechanical properties of actin gels, so may also alter the mechanical properties of podocytes [80]; podocyte-specific transgenic expression of the mutated alpha-actinin IV gene leads to FSGS [81]; "knock-in" mice with induced expression of the mutant gene also develop FSGS [82] so that the relationship between the mutated gene and the disease is clear. A more recent example is the identification of mutations in TRPC6, a cation channel, in familial FSGS [71, 83]. One hypothesis is that mutated TRPC6 is constitutively active and allows unregulated calcium flux into the cell [72]. Intriguingly, when TRPC6 is overexpressed by podocytes in vivo, animals develop proteinuria [84]. Whether similar disease mechanisms operate in acquired forms of FSGS, by affecting alpha-actinin IV, TRPC6 or other podocyte proteins, remains unknown but it is of interest that TRPC6 seems to be overexpressed in a variety of forms of glomerular disease [84].

The ability to study podocytes in vitro has been helped enormously by the availability of temperature-sensitive transgene technology allowing the creation of cell lines which when differentiated show the phenotype of mature podocytes. This has been achieved for murine [85] and human [86] podocytes. Podocytes depend for their function on a complex cell shape which is maintained by a dynamic actin cytoskeleton and allows specific subcellular localization of proteins such as nephrin [87]. One of the most frequently cited animal models of nephrotic syndrome is that induced by puromycin aminonucleoside (PAN). PAN nephrosis is said to resemble MCN. In vitro, PAN injures podocytes and disrupts their actin cytoskeleton [87] at least partly explaining its effects in vivo. Another area of recent interest has been in the expression by podocytes of costimulatory molecules of the B7 family [88] suggesting that podocytes may be involved in antigen presentation. The functional significance of this remains to be established, especially because the podocyte lies in an extravascular site and is therefore normally inaccessible to cells of the immune system for direct cell–cell interactions. However, lipopolysaccharide (LPS) induces upregulation of B7.1 on podocytes in vitro and in vivo and B7.1-deficient mice were resistant to LPS-induced nephrotic syndrome

suggesting that this molecule is key to the ability of LPS to injure podocytes [88]. As discussed earlier, podocytes can also be treated in vitro with cytokines or chemokines and their effect on phenotype and function studied in a manner that is not possible in vivo: This approach has provided support for the importance of individual cytokines such as IL-13 [14, 15, 19].

Podocytes may themselves act as active participants in immune responses. As well as expressing cytokine and chemokine receptors and therefore being capable of responding to a variety of soluble mediators, podocytes are also capable of *producing* inflammatory mediators such as interleukins-1 [89] 6, and 8 [90] and may contribute to local injury in this way. Podocyte production of transforming growth factor β and fibroblast growth factor 2 is markedly enhanced in various experimental models of glomerulonephritis (GN), with potential deleterious effects on podocytes themselves and on other intrinsic glomerular cells [91]. The transcription factor nuclear factor-kappaB (NFκB) plays an important role in cytokine gene regulation in immune cells and is also important in podocytes, so could be a therapeutic target. In one experimental model of nephrotic syndrome, passive Heymann nephritis, there is evidence of gene activation via NFκB predominantly in podocytes at an early stage and blockade of this pathway reduces proteinuria [92]. Another transcription factor that has recently been targeted in podocytes is GATA [93].

The podocyte can also interact with the complement system. Podocytes produce complement proteins including C3 [94], again providing a potential mechanism for amplification of local inflammatory responses. However, podocytes also express the major cell surface complement regulator CR1 [95], which can bind and inactivate complement cleavage products and promote clearance of immune complexes, presumably acting as a podocyte defense mechanism against the consequences of complement-mediated attack. CR1 is lost from podocytes in severe forms of GN, and this may render the podocyte vulnerable to ongoing complement-mediated attack. Local complement activation, as in any site, can lead to lysis of target cells through generation of the membrane attack complex C5b-9. In the case of the podocyte, there is evidence that less profound complement activation also has important consequences: Sublytic levels of C5b-9 induce podocyte activation with release of proteases, oxidants, and other mediators, and also cause DNA damage which may itself further limit podocyte proliferation and repair [96].

The occurrence of FSGS in association with obesity or HIV was mentioned earlier. Obesity-related nephropathy has similarities with diabetic nephropathy, and the extent of (injury/proteinuria) correlates with the degree of insulin resistance shown by the patient [97]. Therefore, our recent work on the podocyte's ability to respond to insulin [98, 99] is relevant. We have shown that podocytes take up glucose in response to insulin, that this is dependent upon nephrin and on cortical reorganization of the actin cytoskeleton, leading to a radical change in cell shape and transient retraction of primary cell processes. This response is likely to explain the old observation that insulin infusion causes albuminuria [100, 101], and the loss of this dynamic ability is likely to have consequences for podocyte function. Therefore, in states of insulin deficiency (type 1 diabetes) or insulin resistance (type 2 diabetes or obesity), podocyte function is compromised: This ties in with the fact that the proteinuria seen in obesity is related to insulin resistance [97].

HIV is able to directly infect podocytes, and in vivo and in vitro leads to podocyte de-differentiation, loss of contact inhibition, and proliferation [102, 103]. It is likely that the same phenomenon explains the typical feature of HIV-nephropathy, the so-called collapsing variant of FSGS in which podocyte proliferation leads to collapse of the glomerular tuft [104]. The HIV gene nef is of key importance in induction of these podocyte changes, and its effects can be modulated by all-trans retinoic acid [105], suggesting a possible therapeutic avenue in future. Understanding the mechanisms of this could be very instructive: It would be therapeutically useful in other diseases including FSGS to be able to selectively induce and control podocyte proliferation, allowing podocyte renewal/replenishment.

Indices of podocyte injury as markers of disease activity or possible diagnostic tools

Study of podocyte-specific markers in renal biopsies of patients with MCN or FSGS is always susceptible to the problem that we do not know what the primary event is: Because we know that podocytes are injured in these diseases, how should we interpret changes in expression of podocyte-specific genes/proteins? For example, nephrin expression has been reported to be decreased in both diseases [106], but it is not certain whether this is just a nonspecific feature of podocyte injury. Furthermore, there is confusion in the literature: Other workers have reported that nephrin expression is normal in MCN and FSGS [107] or that there is no quantitative change in MCN, merely an altered distribution of nephrin expression [108]. At present, there is no clear consensus on the utility of tissue staining for podocyte proteins for assessment of diagnosis or prognosis in MCN and FSGS.

Because podocytes are situated in the urinary space, if they lose their ability to firmly adhere to the GBM, they will be shed into the primary urine. The identification of intact podocytes in urine has become possible with the new knowledge of podocyte-specific markers, and the extent of

"podocyturia" has been shown to correlate with prognosis in a variety of glomerular diseases including FSGS [109–111]. In diabetic nephropathy, podocyturia has been reported to correlate with proteinuria and to improve when patients are treated with drugs blocking the angiotensin cascade or insulin-sensitizers such as pioglitazone [112]. There is a note of caution about this work: Other groups have not found podocyturia to the same extent (reviewed in [113]) and it remains to be seen whether study of this phenomenon will have any widespread diagnostic or prognostic utility. Another possibility is to study soluble products of podocyte injury: This has the advantage that larger scale assays could be developed, for example, with enzyme-linked immunosorbent assays. Quantification of nephrin in the urine (so-called "nephrinuria") is one such possibility [114]. Another is measurement of multiple messenger RNAs in urine [115]. At present, these approaches remain research tools whose place in diagnosis and management remains to be established.

Strategies for podocyte repair/replacement

Because podocyte injury underlies MCN and FSGS, the obvious therapeutic aim is to identify ways to induce podocyte repair. The effectiveness of corticosteroids in MCN has prompted study of the effects of these drugs on podocytes. Our own work has shown complex effects of therapeutically relevant concentrations of dexamethasone on human podocytes [90] with upregulation of nephrin, down-regulation of vascular endothelial growth factor and promotion of podocyte survival. Another group [116] has shown protection by corticosteroids of murine podocytes against injury in vitro. These studies are in their early phases but serve to highlight the fact that podocytes are affected by drugs whose use in MCN and FSGS was predominantly motivated by the belief that the diseases are immune-mediated. Drugs which selectively achieve podocyte repair without the complex toxicities of these drugs would be very attractive forms of therapy for these diseases, and the best starting point for design of such agents is to study effects on podocytes of existing therapies. Other drugs widely used in patients with nephrotic syndrome may exert beneficial effects directly on podocytes: for example, statins, used as cholesterol-lowering agents, have other renal-protective actions [117] which may include specific effects on mediator expression by podocytes [118]. PPARgamma antagonists, used in diabetes mellitus as insulin-sensitizers, protect podocytes from injury in vitro [119]. Even darbepoietin alfa, used as an erythropoiesis-stimulating agent in patients with renal disease, protects podocytes from apoptosis [120]. Existing forms of therapy may also have deleterious effects on podocytes: for example, cyclosporine induces podocyte apoptosis [121]. Immunosuppressive agents that are used successfully in organ transplantation are not necessarily effective in primary renal disease: for example, sirolimus seems ineffective in nephrotic syndrome [122] and may even induce FSGS [123], presumably due to podocyte injury.

The prognostic importance of podocyte loss is believed to be explained by the limited capacity of this cell type to be replaced. There is great interest in stem cells as a source of cellular replacement: These could be intrinsic renal stem cells or bone marrow derived stem cells. There is some evidence that cells of bone marrow origin can become podocytes [124], and this is an active area of research that can be expected to yield new possibilities in the near future. Meanwhile, strategies to allow controlled proliferation of existing podocytes may be the most logical method of achieving podocyte replacement. As mentioned earlier, uncontrolled podocyte proliferation is deleterious (for example, in HIV nephropathy), but the fact that a cell type which normally has very limited proliferative potential can be induced to undergo active cell division highlights a potential therapeutic mechanism.

Conclusion

MCN and FSGS are important causes of proteinuria in children and adults, and share the defining characteristic of injury to podocytes. Whether this is mediated by an autoimmune response remains uncertain: Empirical immunosuppressive therapy is often effective, but this could be at least partly explained by direct effects of these agents on the injured podocytes. Potential toxicity of these treatments is sufficiently great to pose the risk of the treatment being worse than the disease. Evidence for immunologically mediated disease processes is indirect, but there is a suggestion of a role for soluble mediators (possibly IL-13) in MCN and a circulating abnormality (possibly an imbalance between proteases and antiproteases) in FSGS. Recent advances in the understanding of podocyte biology hold promise for novel therapeutic directions in the future so that we can be confident that the specificity of available treatments will improve. We can, therefore, hope for a concomitant reduction in the treatment-related morbidity that can currently dominate the clinical condition of patients "successfully" treated for these two diseases.

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