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REVIEW

Pathogenesis of proteinuria in idiopathic minimal change disease: molecular mechanisms

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Abstract Minimal change disease (MCD) is the most common type of nephrotic syndrome in children and adolescents. The pathogenesis of proteinuria in this condition is currently being reassessed. Following the Shalhoub hypothesis, most efforts have been placed on identifying the putative circulating factor, but recent advancement in podocyte biology has focused attention on the molecular changes at the glomerular capillary wall, which could explain the mechanism of proteinuria in MCD. This report critically reviews current knowledge on the different postulated mechanisms at the glomerular capillary wall level for increased permeability to plasma proteins in MCD. The report helps describe the rationale behind novel therapies and suggests future targeted therapies for MCD.

Keywords Minimal change disease · Podocytopathy · CD80 · Nephrin phosphorylation · Angiopoietin-like 4

Introduction

Minimal change disease (MCD) defines a type of nephrotic syndrome characterized histopathologically by the absence of major structural glomerular changes and an absence of immune deposits, with features of podocyte injury and foot process (FP) fusion by electron microscopy [1]. The terms lipoid nephrosis, minimal lesion, and nil disease have been used as synonyms for MCD.

Molecular basis of proteinuria in idiopathic MCD

MCD is characterized by an increased glomerular permeability to plasma proteins, mostly albumin. Plasma proteins in MCD are transferred from the capillary lumen to the Bowman urinary space crossing the width of the glomerular capillary wall.

Glomerular capillary wall

An update on composition of the glomerular capillary wall is appropriate in order to understand the pathogenesis of proteinuria in this disease. The capillary wall consists of three structural layers (Fig. 1).

Endothelium

The glomerular capillaries are lined by a thin, fenestrated endothelium. Fenestrae represent tunnels of 70- to 100-nm diameter. The surface of the endothelial cells is covered by a carbohydrate-rich layer known as endothelial glycocalyx, which is connected to the endothelium trough "backbone" molecules, mainly proteoglycans and glycoproteins. Proteoglycans consists of glycosaminoglycan chains (heparan sulfate, chondroitin sulfate, dermatan sulfate, keratan sulfate, hyaluronan) linked to a core protein (syndecan, glypican, perlecan, versican, decorin, biglycan, mimecan). Glycoproteins comprise three families of cell adhesion molecules, such as selectin, integrin, and immunoglobulin superfamily [2].

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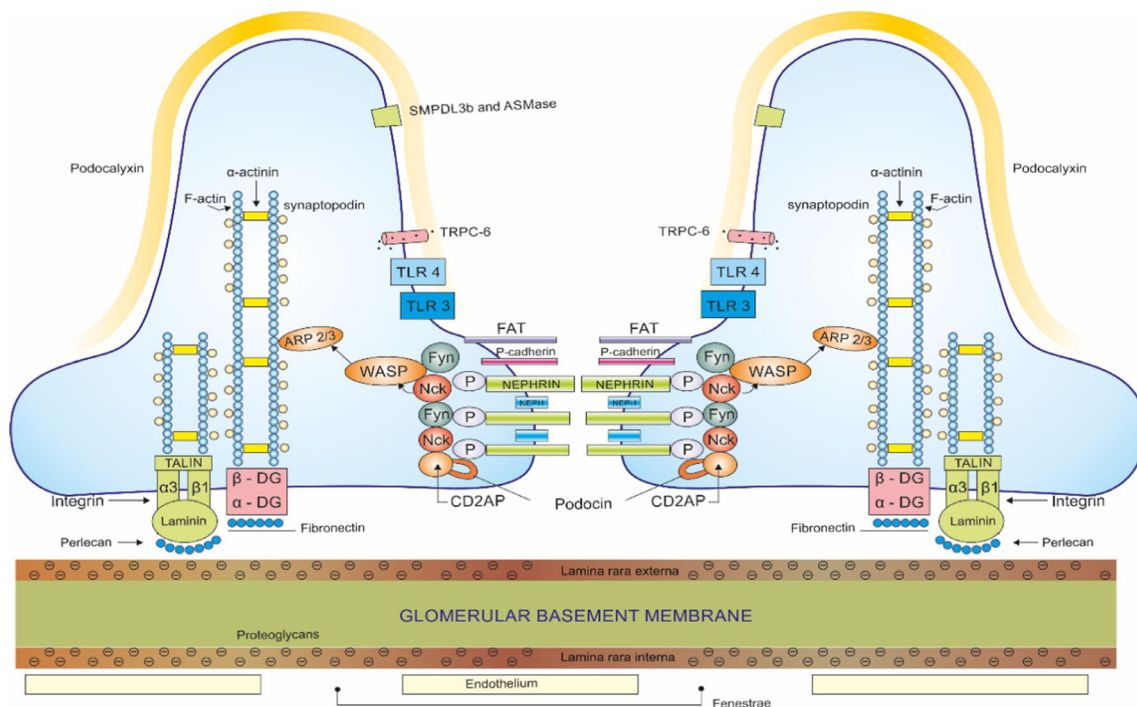
Fig. 1 Molecular anatomy of the glomerular filtration barrier in the healthy state. *SMPDL-3b* Sphingomyelin phosphodiesterase acid-like 3 b protein, *ASMase* acid-sphingomyelinase, *TLR* toll-like receptor, *ILs* interleukins, *ARP 2/3* actin-related protein 2/3 complex, *WASP* Wiskott-Aldrich syndrome protein, *CD2AP* CD2-associated protein, *P* phosphorylated site, *DG* dystroglycans

Glomerular basement membrane (GBM)

On electron microscopy, the GBM is composed of a central dense layer (lamina densa) and two thinner electrolucent layers (lamina rara interna in contact with the endothelial cell, and lamina rara externa in contact with the podocyte). The GBM has been considered to represent a charge-selective barrier. The anatomical base for this barrier is a lattice-like network of anionic sites distributed throughout the lamina rara. These anionic sites are composed mostly of heparan sulfate proteoglycans, including laminin, perlecan, and other related proteins [3].

Podocytes

Podocytes are highly differentiated visceral epithelial cells. The pedicels or FPs come into contact with the lamina rara externa of the GBM, affixing the podocyte to the membrane. The FPs of neighboring podocytes interdigitate, leaving gaps between them (filtration slits) of ~30- to 40-nm wide, which are bridged by a thin zipper-like-pattern membrane [slit diaphragm (SD)]. The apical or luminal membrane of the podocyte and the SD are covered by a surface coat rich in sialoglycoproteins, mainly podocalyxin [4].

Podocyte cytoskeleton

The podocyte cytoskeleton in the cell body and the FPs has microtubules and intermediate filaments, such as vimentin and desmin. In the FPs is a dense network of F-actin filaments connected to cell receptors located at the SD and apical and basement domains of FPs through several adaptor proteins (Nck, CD2AP, ZO-1), effector proteins (WASP, 2/3 Arp), and small guanosine triphosphatase (GTPases: RhoA, Rac1, and Cdc42), which in turn modulates actin polymerization [5]. F-actin forms insoluble filaments upon assembly of G-actin-soluble monomers. Two actin-associated proteins, α-actinin-4 and synaptopodin, are also necessary to maintain podocyte adhesion and shape by bundling actin filaments and forming actin stress fibers. Actin polymerization is a dynamic and tightly regulated process that provides podocytes with mechanical support to modulate FP shape and orchestrate cell motility and migration.

SD composition

This structure includes several proteins that imbricate, forming a zip-like configuration.

Nephrin A type 1 transmembrane protein consisting of a large extracellular portion with eight immunoglobulin (Ig)G-like domains, a single fibronectin type-3 motif, and an intracellular domain. Nephrin displays a dual role: it provides structural support to the SD by forming bridges between adjacent podocyte FPs, and it is the key regulator of signaling pathways linking SD and cytoskeleton proteins [5]. There is strong clinical and experimental evidence supporting the key

role of nephrin in SD formation and maintenance [6, 7]. The regulatory role of nephrin is determined by its level of phosphorylation at the tyrosine sites of its intracellular domain. Fyn, a tyrosine kinase of the Src family, directly binds and phosphorylates nephrin within lipid rafts, resulting in the recruitment of the protein adaptor Nck, which in turn enhances Fyn activation and promotes the downstream signaling cascade regulating actin polymerization [8, 9].

NEPH protein family   Comprises three proteins, NEPH 1, NEPH 2, and NEPH 3, which retain structural similarities to nephrin but lack the fibronectin domains and have shorter extracellular domains. Similarly to nephrin, NEPH proteins contain phosphorylates sites. The phosphorylated NEPH contributes to SD stability by forming heterodimers with nephrin and by recruiting scaffolding molecules, such as ZO-1 or the PAR-3/PAR-6 atypical protein kinase polarity complex [5].

Podocin   A 42-kDa hairpin-like raft-associated protein serves as anchorage of nephrin to the podocyte cell membrane. Furthermore, podocin recruits nephrin to lipid rafts, which is required for proper initiation of nephrin signaling [5].

Connections between cytoskeleton and SD

Phosphorylated nephrin functionally links SD to actin cytoskeleton through a growing number of adaptor proteins.

Nck1/Nck2   Adaptor proteins that localize to lipid rafts and bind to phosphorylated nephrin through their SH2 domain in vivo. Nck plays a dual role by enhancing Fyn activity and serving as a link, through its SH3 domain, between phosphorylated nephrin and the effector protein neuronal Wiskott–Aldrich syndrome protein (N-WASP), which is a potent inducer of actin-related protein 2/3 complex (Arp 2/3), thereby regulating actin polymerization. Similarly to nephrin, podocyte Nck and N-WASP expression are required for the maintenance of normal FPs [8, 9].

Crk family   Adaptor proteins that serve as links between nephrin and cytoskeleton. Crk proteins are thought to play a key role in transducing signals that regulate the cytoskeleton dynamics and mobility by forming a complex with two proteins—phosphorylated cellular apoptosis susceptibility (CAS) and focal adhesion kinase (FAK) [10].

CD2AP   An 80-kDa cytoplasmic adaptor protein that serves as a bridge between SD and actin by binding to nephrin and podocin and interacting with actin-binding proteins such as cortactin and synaptopodin, which regulate actin polymerization through Arp 2/3 and α-actinin-4 activation, respectively [5].

Synaptopodin   A proline-rich actin-associated protein expressed in neurons and differentiated podocyte FPs. Synaptopodin binds to podocin, providing mechanical support, and to α-actinin-4, regulating its bundling activity. In addition, synaptopodin promotes formation of actin stress fibers by preventing degradation of the GTPase RhoA. A cytosolic variant of cathepsin L (CatL), a member of the cathepsin family of cysteine proteases, promotes degradation of nonphosphorylated synaptopodin, resulting in proteinuria. Phosphorylation of synaptopodin protects it from cathepsin-L-mediated degradation and helps maintain integrity of the FP [11].

Connections between podocytes and GBM

Podocyte are attached to the GBM by transmembrane cell proteins including α3β integrins, tetraspanins, and α and β dystroglycans. Dystroglycan (DG) is a negatively charged glycoprotein that covers the basolateral and apical cell membranes of the podocyte [12]. Dystroglycans are known to bind laminin and agrin in the GBM, whereas in the cytoplasm, the dystroglycan complex is connected to the actin cytoskeleton of FPs via utrophin [13].

Podocyte receptors

A review of all cell receptors is beyond the scope of this manuscript; we focused on those that may play a role in proteinuria in MCD.

Transient receptor potential channel 6 (TRPC6)   This plays a role in the regulation of cytoskeletal dynamics by regulating cytoplasmic Ca^{2+} flux into the podocyte [5].

Toll-like receptors (TLR)   Human kidneys express TLR1-10 messenger RNA (mRNA).TLR-3 is the most predominant TLR expressed. CD80, a key molecule in proteinuria in MCD, is overexpressed by human podocytes when incubated with either TLR-3 ligand (Poly:IC) or TLR-4 ligand (LPS), suggesting a link between microbial products, podocyte CD80, and proteinuria [14].

Sphingomyelin phosphodiesterase acid-like 3b (SMPDL-3b) protein

SMPDL-3b is suggested to regulate β3 integrin activation in podocytes, thereby participating in the process of cytoskeleton reorganization and cell motility. SMPDL-3b mainly localizes to lipid rafts [15].

Glucocorticoid receptors (GRs)   All cell types in the glomerulus, as well as immortalized human podocytes, express GRs.

Effects of dexamethasone on in vitro human podocytes include enhanced and accelerated maturation of podocytes, downregulation of protein p21 and vascular endothelial growth factor (VEGF), and reduction in interleukin (IL)-6 podocyte expression [16]. In addition, dexamethasone induces nephrin phosphorylation in cultured human podocytes by activating the Src family kinases [17].

Mechanism of proteinuria in idiopathic MCD (Fig. 2)

Role of capillary wall electrical charges

The increased glomerular permeability to plasma proteins, mostly albumin, in MCD has been attributed to a defective glomerular charge-selective barrier caused by the loss of fixed negative charges in the capillary wall. In 1981, Myers found that the uptake of colloidal iron, a polycation, by glomeruli from MCD patients was diminished compared with controls, suggesting loss of glomerular polyanion [18].

Endothelial cells

Endothelial cells are not usually considered an impediment to the transfer of proteins into Bowman space because of the size of the fenestrae. However, lately, it has been thought that podocalyxin, which coats endothelial cells and is negatively charged, could reduce the diameter of fenestrae, significantly impairing movement of albumin through the endothelium. No studies have been performed to test this hypothesis, but early studies by Karnovsky et al. using catalase as a tracer showed that these molecules crossed the endothelial layer and were retained not at the level of the endothelium but at the GBM [19].

Glomerular basement membrane

In MCD, there is a decreased number of anionic sites in the lamina rara interna [20], with a segmental or total absence of heparan sulfate GBM staining demonstrated by using an antibody that recognizes heparan sulfate core protein and glycosaminoglycan chains [21]. In addition, an increase in the heparan sulfate/urinary creatinine ratio has been noticed in patients in relapse [22].The significance of these findings has been recently questioned by Goldberg et al. studying agrin and perlecan mutant (knockout) mice. Despite the absence of these proteoglycans and a significant reduction in GBM anionic charge, mice did not develop proteinuria, and the fractional clearance of an anionic tracer was unchanged. The
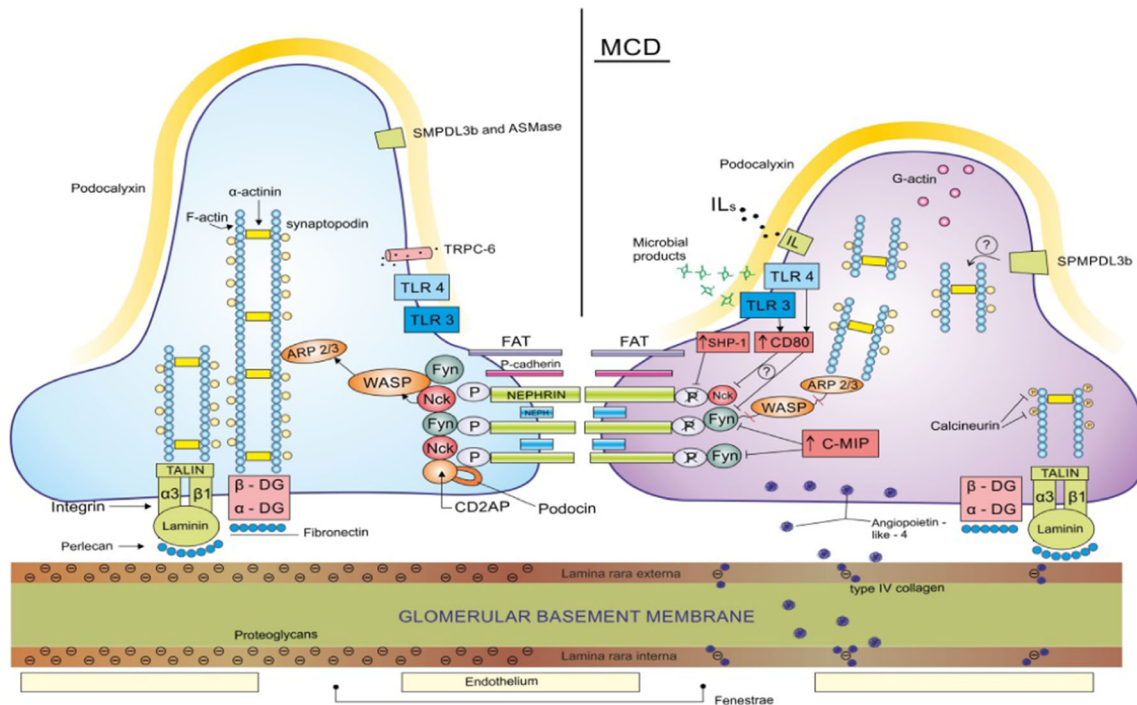

Fig. 2 Molecular anatomy of the glomerular filtration barrier in the healthy state (*left*) and in minimal change disease (MCD) during relapse (*right*). In MCD, microbial products and/or interleukins (ILs) bind to toll-like receptors (TLRs), leading to CD80 and C-mip overexpression, which interfere with Nck and/or Fyn proteins, resulting in nephrin dephosphorization. The latter cause dysregulation of the downstream pathway that links nephrin with cytoskeleton, resulting in rearrangement of the actin cytoskeleton. The same mechanism is postulated for hemopexin. Angiopoietin-like-4 and IL-8 are thought to induce proteinuria by reducing anionic sites at the glomerular basement membrane (GBM) level. *SMPDL-3b* sphingomyelin phosphodiesterase acid-like 3 b protein, *ASMase* acid-sphingomyelinase, *TLR* toll-like receptor, *ILs* interleukins, *ARP 2/3* actin-related protein 2/3 complex, *WASP* Wiskott–Aldrich syndrome protein, *CD2AP* CD2-associated protein, *P* phosphorylated site, *DG* dystroglycans

authors concluded that the loss of heparan sulfate proteoglycan in MCD may be a part of the phenotypic change that occurs in this disease, but it may not be responsible for the proteinuria per se [23]. In a similar study, Chen et al. knocked out *Ext1* gene expression in podocyte mice, halting polymerization of heparan sulfate glycosaminoglycans of the proteoglycan core proteins secreted by podocytes. The mice showed FP effacement, and immunohistochemical analysis demonstrated a significant decrease in heparan sulfate glycosaminoglycans confirmed by ultrastructural studies using polyethylenimine staining. Despite the fusion of FPs and loss of heparan sulfate glycosaminoglycans, knockout mice demonstrated only mild albuminuria [24]. Van den Hoven et al. used mice overexpressing heparanase to evaluate the expression of different heparan sulfate domains in the kidney with antiheparan sulfate antibodies [25]. Glycosaminoglycan-associated anionic sites were reduced about five fold in the GBM of transgenic mice, whereas glomerular ultrastructure and protein excretion remained normal.

Mediators of GBM loss of negative charges Three molecules, IL-8, angiopoietin-like-4 (angptl-4), and hemopexin have been implicated in the loss of GBM-negative charges in MCD.

Angiopoietin like-4 Human angptl-4, a 45- to 65-kDa glycoprotein, has been proposed as a mediator of proteinuria in MCD and has been found in plasma, urine, and glomeruli of MCD patients during relapse. Angptl-4 oligomers (220 kDa) with pI < 8 were detected in urine in four MCD patients. One of these patients also had urinary angptl-4 molecules (55–70 kDa) with a pI>8. Similarly, angptl-4 oligomers (100–160 kDa) with pI < 8 and angptl-4 molecules with pI > 8 were also detected in blood of some MCD patients [26]. Serum levels of angptl-4 were elevated in patients with active MCD, focal segmental glomerulosclerosis (FSGS), and membranous nephropathy compared with controls [27]. Immunofluorescence of kidney tissue revealed mild angptl-4 staining in MCD in relapse compared with controls; it also colocalized with podocyte, GBM, and endothelial cell markers. The proposed mechanism(s) of angptl-4 in MCD includes podocyte-secreting angptl-4 (mostly pI > 8), which migrates to the GBM and endothelium, resulting in reduced GBM anionic charges and, therefore, proteinuria [26]. In our cohort of MCD patients, urinary angptl-4 positively correlated with proteinuria. Thus, higher urinary angptl-4 levels were found in MCD during relapse than in controls [28]. This may be the result rather than the cause of the leaky glomerular filtration barrier. Indeed, urinary angptl-4 levels were also increased in patients with other glomerulopathies, such as FSGS and membranous nephropathy, when compared with controls. Furthermore, serum angptl-4 levels were decreased in MCD patients during relapse compared with in the same patients in remission and in controls. In addition, and in contrast with a previous study, no or minimal angptl-4 staining by immunofluorescence was observed in kidney tissue from our MCD patients in relapse. Most importantly, no increased angptl-4 expression was observed in human podocytes cultured with sera from MCD patients in relapse compared with those exposed to sera from MCD patients in remission. Finally, we found no evidence of urine cationic angptl-4 (pI > 8.0) [28]. In our MCD patients in relapse, we observed angptl-4 with a pI 5.4, thus challenging the hypothesis of a high pI angptl-4 as mediator of proteinuria in MCD. In summary, the clinical significance of angptl-4 in MCD remains to be determined given the contrasting findings between two groups.

IL-8 We and others have shown increased serum IL-8 levels in MCD patients during relapse compared with those observed during remission and in controls [29, 30]. We found that rats infused with IL-8, reaching serum IL-8 concentration similar to that observed in MCD patients, experienced mild proteinuria. Administration of anti-IL-8-neutralizing antibody prevented proteinuria, thereby suggesting a causal relationship between IL-8 and proteinuria [31, 32]. In addition, we observed in rats infused with IL-8 an increased GBM glycosaminoglycan catabolism. These data suggest some role of circulating IL-8 in proteinuria in MCD by reducing anionic charges at the GBM, although, as previously mentioned, the decrease in GBM heparin sulfate has been questioned.

Hemopexin Hemopexin is a β1 glycoprotein synthetized by the liver. Its receptors are expressed on hepatocytes, neurons, and macrophages. No such receptor has been described in kidney [33]. Bakker et al. isolated a fraction from normal human plasma called as 100KF fraction, containing several proteins, of an apparent molecular weight of 80–100 kDa [34]. When infused for 15 min into rat suprarenal artery, there was a decrease in anionic sites in the lamina rara interna and an increase in urinary protein excretion [35, 36]. The authors attributed these effects to hemopexin contained in the fraction having protease activity. However, there are problems attributing hemopexin as the circulating factor in MCD. First, the plasma of MCD patients in relapse contained less of this 100KF fraction than did plasma from healthy controls [37]. The authors tried to circumvent this issue by claiming the presence in nephrotic patients of a pure "active hemopexin" that they were unable to isolate. Second, there have been numerous unsuccessful attempts by this group to purify the fraction and

isolate the putative hemopexin. Initially, the authors found that most proteins isolated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) on the 100KF fraction were contaminants, not native human hemopexin [34]. Subsequently, Bakker et al. used different chromatographic methods to analyze the 100 KF fraction. However, the isolated hemopexin by these techniques [38] continued to be associated with contaminants (hyaluronidase or other proteins) or exhibited substantial cleavage [38, 39]. Third, Bakker et al. suggested that hemopexin has serine protease activity, since a variety of protease inhibitors blocked the biological effects of hemopexin observed in a selection of assays [40, 41]. However, these results have not always been consistent or reproducible. The validity of these findings is rather questionable, as the same authors later reported opposed findings using the same protease inhibitor [36, 42]. Recombinant hemopexin was also suggested to have protease activity measured by spectrophotometry. However, recombinant hemopexin was required at higher doses and for a longer reaction time than expected for proteases to cause absorbance changes [40]. Thus, it appears unlikely that the rapid onset of proteinuria observed in rats infused with recombinant hemopexin is due to its protease activity. Therefore, it has not been proven that the 100KF fraction actually has serine protease activity. Attributing this activity to plasma hemopexin or to a modification of hemopexin in nephrotic patients without more direct evidence remains presumptive. Fourth, there is no evidence that podocytes express hemopexin receptor, and no study has shown hemopexin deposited in the glomeruli of nephrotic patients [33]. Finally, the pattern of proteins excreted into urine by rats infused with hemopexin or plasma 100KF fraction is not consistent with MCD because half of the proteins excreted were immunoglobulins [43].

Podocyte

As previously mentioned, podocalyxin covers the podocyte apical membrane as well as the SD. Using a monoclonal primary antibody against podocalyxin, Kavoura et al. found a significant decreased expression of podocalyxin in MCD compared with healthy kidneys [44]. The authors could not determine whether the reduced expression of podocalyxin in MCD was due to effacement of FPs or to podocyte injury. Others, however, have not observed differences in podocalyxin expression between MCD and controls [45].

Role of podocytes in increased glomerular permeability in MCD

Changes in podocyte cytoskeleton and SD

MCD has been postulated to be a podocytopathy. The increased glomerular permeability to plasma proteins is thought to be due to changes in podocyte cytoskeleton, leading to a leaking SD and resulting in proteinuria.

Role of nephrin in the maintenance of podocyte cytoskeleton and integrity of SD

Nephrin phosphorylation is the key regulator of the signaling cascades between SD and cytoskeleton in MCD. Nephrin has been studied in MCD using a variety of techniques, with conflicting results. Several studies have shown that the glomerular nephrin expression in MCD is no different than that observed in normal controls [46, 47]. In contrast with previous studies, Furness et al. [48] found reduced amounts of nephrin mRNA in glomeruli isolated from three patients with MCD; Doublier et al. [49], using an immunofluorescence technique, reported loss of staining for nephrin in 30 patients with proteinuria (including some with MCD). These conflicting results could, at least in part, be explained by differences in methodology, but at this time, we can only conclude that there is no definite evidence of reduced nephrin in MCD.

Phosphorylated nephrin Recent studies have demonstrated a pathway through which nephrin regulates actin polymerization [8, 9]. Reduction in nephrin phosphorylation has been observed in animal models of proteinuria (puromycin aminoglycoside) and in glomeruli of MCD patients (nephrin phosphorylation at pY228) compared with that in normal glomeruli [50]. In agreement with these findings, we found reduced nephrin phosphorylation (pY1217) in kidney tissue from MCD patients during relapse compared with those in remission. Similarly, we found that nephrin phosphorylation was decreased in human podocytes when cultured to sera from MCD in relapse compared with those in remission [51]. Podocytes cultured with serum from MCD patients in relapse, in addition, showed morphological changes consistent with reorganization of actin cytoskeleton. Based on these findings, it has been postulated that podocyte cytoskeletal changes will disrupt the morphology of the SD, opening them to the passage of plasma proteins and, therefore, providing a mechanism for proteinuria in MCD. The mechanism (s) by which phosphorylation of nephrin is impaired in MCD remains unknown. Three proteins—CD80, c-mip, and SHP-1—may interfere with the process of nephrin phosphorylation.

CD80 CD80 (also termed B7-1) is a transmembrane protein expressed on podocytes. The link between podocyte CD80 and proteinuria was based on the observation that LPS-injected mice developed increased podocyte CD80 expression, FP effacement, and proteinuria. In contrast, such findings were not observed in CD80 knockout mice when injected with LPS. Furthermore, severe combined immunodeficient (SCID) mice still developed proteinuria in response to LPS, thus showing that the proteinuria can occur independently of T cells [52]. We have shown that urinary CD80 is increased in patients with MCD when compared with normal controls and patients with other glomerulopathies (FSGS, lupus nephritis) [53, 54]. The source of urinary CD80 appears to be the podocyte, since (a) lower serum level of s-CD80 (the soluble CD80 molecule of molecular weight 23 kDa) is observed in MCD patients in relapse compared with normal controls [53]. (b) The molecular weight of CD80 in urine was shown by Western blot to be 53 kDa, consistent with CD80 being the whole-cell-membrane-associated CD80 as opposed to the circulating 23-kDa soluble CD80, which is known to be secreted by circulating B cells [54]. (c) Finally, CD80 was present in glomeruli of seven of seven MCD patients in relapse but was minimal or absent in two of two with FSGS and in the one with MCD in remission. Of the seven MCD patients in relapse, none showed tubular staining for CD80 [54]. We also found that human podocytes incubated with sera from MCD patients in relapse display an increased CD80 expression along with diminished nephrin phosphorylation in comparison with podocytes incubated with sera from MCD patients in remission. CD80, therefore, may interfere with nephrin phosphorylation by binding and thus inhibiting Fyn and/or Nck proteins. Trigger stimulus for increased CD80 podocyte expression in the experimental animal have been Poly:IC, LPS model, and IL-13 [14, 52, 55]. These studies support the hypothesis that a circulating factor mediates proteinuria in these experimental models. The supposed role of circulating IL-13 in proteinuria in MCD is questionable because: (a) elevated serum IL-13 levels were not detected by others or by us in MCD patients during relapse [56]; (b) elevated serum IL-13 levels, compared with controls, have been reported in patients with nephrotic syndrome during remission [57]; (c) although serum IL-13 is increased in asthma patients, only a few present associated proteinuria. Cultured human podocytes constitutively express TLR 1–6 and -9 [58] and -10 [59]. Activation of TLR-3 and TLR-4 by TLR ligands such as Poly:IC and LPS (mimicking viral and bacterial products, respectively) promote podocyte CD80 expression. Viral infection in MCD patients triggers relapse [60]. Thus, it may be hypothesized that microbial products, rather than ILs, are the pathogenic circulating factor leading to proteinuria in MCD patients [56].

C-mip This is an 86-kDa protein containing a pleckstrin homology (PH) domain, often present in proteins recruited to lipid rafts. Balb/c and SCID mice exposed to LPS developed proteinuria and podocyte c-mip overexpression. When LPS was administered to mice pretreated with small interfering RNA targeting c-mip (c-mip siRNA), a significant reduction in proteinuria was observed, suggesting a link between c-mip and proteinuria [61]. Transgenic mice that overexpressed podocyte C-mip developed podocyte FP effacement, massive proteinuria along with reduced phosphorylated nephrin, and active Fyn. Glomerular c-mip was increased using in situ hybridization and immunohistochemical techniques in MCD patients during relapse compared with those in remission. C-mip is selectively expressed in Reed-Sternberg cells and podocytes of patients with Hodgkin's lymphoma who develop MCD, whereas no increased c-mip expression was found in Hodgkin's patients presenting without proteinuria [62]. Data from in vitro and animal studies suggest that podocyte c-mip binds to Fyn, blocking its interaction with nephrin and resulting in reduction of nephrin phosphorylation, thereby altering the downstream signaling cascade linking SD and cytoskeleton proteins [61]. The clinical relevance of podocyte c-mip in MCD, suggested by the above observations, is unknown since: (a) Fyn-deficient mice experienced proteinuria and upregulation of podocyte c-mip; thus, one may think that c-mip expression is the result rather than the cause of Fyn dysregulation, (b) we found no changes in Fyn expression in human podocytes cultured with sera from MCD patients in relapse compared with those cultured with sera from patients in remission (unpublished observations). This finding argues against the proposed mechanism by which c-mip inhibits Fyn in MCD. C-mip may reflect a nonspecific response of podocytes to a noxa rather than the molecule-driven proteinuria in MCD.

SHP-1 SHP-1, belonging to the family of protein tyrosine phosphatases (PTPs), is expressed in podocyte lipid rafts under certain circumstances. A tight regulation between protein tyrosine kinases (such as Fyn) and PTPs is required to maintain a steady level of phosphorylation of the target protein (for instances, nephrin), which in turn regulate downstream signaling pathways. Cultured human podocytes, when exposed to high glucose,

overexpressed SHP-1 resulting in nephrin dephosphorylation selectively at Tyr 1193 and Tyr 1217 residues. SHP-1 inhibition prevented nephrin dephosphorylation in cultured human podocytes, suggesting a link between SHP-1 and nephrin [63]. To date, there are no published data on SHP-1 in MCD.

Galectin-1 (Gal-1) This is a 135 amino acid protein encoded by the *LSGALS1* gene, with specific affinity for β-galactosides that serve as regulators of cell motility and adhesion and as an immunomodulator [64]. Gal-1 is expressed in endothelial and mesangial cells in normal human kidney tissue. In addition, it co-localizes with the nephrin ectodomain. Human podocytes cultured with recombinant Gal-1 exhibited an increase in nephrin phosphorylation, suggesting that Gal-1 may play a role as signaling mediator in the SD. In MCD patients in relapse, Shimizu et al. found decreased glomerular Gal-1 expression when compared with patients with "minor glomerular abnormalities" [65]. Finally, Ostalska et al. found no Gal-1 expression in glomeruli from MCD patients in relapse or in controls (nephrectomy due to Wilms tumor), whereas Gal-1 expression was detected in podocytes from patients with FSGS and those with diffuse mesangial proliferation [66].

Hemopexin Hemopexin has been postulated—based on in vitro experiments—to mediate proteinuria by inducing nephrin-dependent cytoskeleton changes [41]. Bakker et al. suggested that the cytoskeletal changes were the result of hemopexin serine protease activity. Thus, the authors found that low dosages of protease inhibitors [4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSF)] reduced reorganization of actin fibers in podocytes exposed to hemopexin. The significance of these findings remains unclear because: (a) the protease inhibitor used (SPI) is known to have independent effects on actin reorganization, and (b) preincubation of podocytes with 10 % normal human plasma completely blocked the effects of hemopexin on actin fiber reorganization [41].

Alterations of the linking between F-actin and SD proteins

Synaptopodin Immunohistochemistry for synaptopodin showed weaker intensity in MCD patients compared with controls in one study [67]. In contrast, no such difference was found by others [68, 69].

Podocin In children with nephrotic syndrome, Horionuchi et al. [69] found podocin to be decreased in MCD. The MCD group, however, included patients with steroid-resistant nephrotic syndrome, and in those cases, decreased expression of podocin may also be attributed to mutations in *NPHS2*. In contrast, in a study that included six MCD patients, immunohistochemical analysis indicated that the amount of podocin did not decrease in any MCD patient when compared with normal controls [70].

Alterations in adhesion of podocytes to GBM

Dystroglycans The expression of α-DG and sialidase were investigated by immunofluorescence in kidney biopsies of five patients with MCD and four normal human kidneys. The authors also studied urinary excretion of sialic acid using a newly developed tandem mass spectrometry method. No major changes in the expression of α-DG or urinary sialic acid excretion were observed in MCD patients compared with normal controls [71]. These findings differ from a previous report in which the density of α- and β-DG was studied using immunohistochemistry and quantitative immunoelectron microscopy [72] In MCD, the density of expression of α-DG was significantly reduced to 25 % of controls and that of β-DG to ∼50 %. According to the authors, because immunogold labeling for α-DG was more intense than that for β-DG, the values obtained for the former may have reflected the true pathophysiology more accurately. Interestingly, steroid treatment of MCD patients apparently restored DG expression, along with reformation of FPs. However, the presence of decreased DG does not allow us to conclude that the decreased concentration may play a role in proteinuria. Recently, mice showing no α-DG glycosylation in glomeruli had flattening of podocyte FPs but did not develop proteinuria [73].

Integrins The study of integrins in glomerulopathies, including MCD, using indirect immunofluorescence have been handicapped by the small number of cases involved and the subjective grading of fluorescence by observers. Nevertheless, Bains et al. found no differences in glomerular staining intensity for β1, α2, α3, and Vβ3 integrins between normal controls and MCD cases [74]. Baraldi et al. studied the expression of α2, α3, α5, and α6 subunits of the β1 integrin family in kidney specimens by immunofluorescence. Six normal kidney tissues were compared with ten samples from patients with various glomerulopathies (MCD, membranous nephropathy, and systemic lupus nephritis). In normal glomeruli, α3 was the dominant integrin, being mainly present on podocytes and showing a linear fluorescent pattern that codistributed with laminin. In MCD and lupus nephritis, the pattern of α3 staining was normal [75]. Finally, in the study by Regele et al., the expression of β1-integrin remained at normal levels in both MCD and FSGS [72]. Thus, there seems to be no decrease in adhesion proteins in MCD. Supporting this finding, Lahdenkari et al. observed that the podocytes were firmly attached to the GBM in kidneys of patients with MCD and Finnish-type congenital nephrotic syndrome with heavy proteinuria [76].

Significance of recent advances on the pathogenesis of proteinuria in MCD: therapeutic implications

The therapeutic benefits of drugs currently used in MCD have been based on the concept that a pathogenic cytokine is released in the circulation of these patients, with therapy aimed at controlling production of this cytokine at the T-cell level. However, with the recent understanding that MCD is a podocytopathy, it is increasingly recognized that our current therapies (steroids, cyclosporine) may actually be directly acting on the podocyte [16, 77] instead of T cells. As previously shown, progress has been made in the attempt to understand the pathogenesis of proteinuria in MCD. So far, CD80 and synaptopodin are indicated as possibly playing a role. However, the pathway(s) for CD80 leading to proteinuria has (have) not been identified, though its role in actin reorganization is tantalizing.

There is no well-defined MCD animal model, although the LPS and Poly:IC models fulfill many requisites for MCD [14, 52]. However, both conditions present only transient proteinuria, making drug evaluation difficult, since there is only a small therapeutic window.

The podocyte has the characteristic of engulfing particles that are retained in the extracellular matrix. Using this endocytotic function, compounds can be delivered that will activate the production of underexpressed proteins and/or mRNA involved in the pathogenesis of proteinuria. Thus, gene delivery to podocytes has been demonstrated in vivo through plasmid transfer [78]. Conversely, siRNA bound to anti-mouse podocyte antibodies reduce disease-causing overexpression of podocyte proteins. The bound antibody is internalized, with subsequent release of siRNA, and has been used to reduce nephrin and TRPC-6 in mice. Using similar techniques, purified proteins may be delivered and taken up by podocytes to replace degraded or dysfunctional native proteins.

The discovery that increased podocyte CD80 expression during relapse in MCD plays an important pathogenic role in proteinuria has multiple therapeutic implications. The increased expression of CD80 by podocytes in MCD and/or its effect could be attenuated at different levels: (a) blocking the effect of microbial products at TLR-3 receptor, cytokines, or other products at the TLR-4 receptor, (b) decreasing the production of CD80 by the administration of specific siRNA, and (c) increasing the concentration of CTLA-4, the natural inhibitor of CD80, via administration of CTLA-4 bound to a carrier or by stimulation of podocyte CTLA-4 production using complementary DNA (cDNA) coding fragments. We have shown that in MCD patients in relapse, urinary excretion of CTLA-4 is low in contrast with urinary excretion of CD80, which is increased [79]. Administration of CTLA4-IgG1 resulted in a transient reduction of urinary CD80 levels and resolution of proteinuria in a MCD patient [80].

Conclusions

The hypothesis that MCD is a podocytopathy and that CD80 or other still undefined molecules are the triggers of proteinuria in MCD is tantalizing. Recent and future findings should lead to new therapeutic approaches for this disease. Instead of broad suppression of the immune system, new drugs would target specific podocyte protein abnormality. The challenge at this time is to define the specific abnormality and then develop specific drug therapy.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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