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Minimal Change Disease: More Than a Podocytopathy?

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Minimal change disease (MCD) is the main cause of the nephrotic syndrome in children and accounts for 10% to 15% of adults presenting with the nephrotic syndrome. The term minimal change refers to the fact that glomeruli appear normal on light microscopy. The only histopathologic abnormality, visualized on electron microscopy, is diffuse podocyte foot process effacement. MCD is therefore considered a primary podocyte disease. Complete proteinuria remission in response to corticosteroid treatment is a hallmark of MCD, and progressive renal failure is rare.[1] Nevertheless, MCD causes severe morbidity. Disease-related complications observed mostly in adults include venous thrombosis and severe acute kidney injury requiring temporary dialysis.[2] In addition, as MCD is characterized by a chronic, relapsing course, prolonged immunosuppressive treatment is often necessary to maintain proteinuria remission. Chronic immunosuppressive treatment increases the risk of severe infections and carries the long-term risk of malignancies.

The underlying pathogenesis of MCD remains poorly understood. A longstanding hypothesis states that a circulating permeability factor produced by cells of the immune system triggers the disease.[3] Because a similar pathogenesis is considered for primary focal segmental glomerular sclerosis, it has been hypothesized that MCD and focal segmental glomerular sclerosis are part of the same disease spectrum.[4] Especially T cells were initially suspected as a source of circulating permeability factors, based on the association between MCD and non-Hodgkin lymphoma, remission induced by measles infection, and prolonged remissions after cyclophosphamide treatment. However, the T cell origin has been challenged by therapeutic efficacy of rituximab and other specific B cell-eliminating drugs.[5] Of note, also direct effects of both corticosteroids and rituximab on the podocyte have been suggested to have therapeutic effects.[6,7] Interestingly, a recent study identified autoantibodies targeting the podocyte slit diaphragm protein nephrin in a subgroup of patients with MCD, thereby providing potential links between podocyte injury, autoimmunity, and proteinuria response to anti-B cell treatment.[8]

Although observed podocyte injury is the main classical feature of MCD, disease mechanisms could also involve the glomerular endothelium. Glomerular endothelial cells (GEnCs) are covered by an endothelial glycocalyx with associated proteins. Together, GEnCs and the glycocalyx compose the glomerular endothelial surface layer (ESL), which forms a barrier to plasma proteins.[S1] The ESL is in direct contact with the blood, and thus with presumed circulating permeability factors. GEnCs contain large fenestrations that would, only based on size, permit the passage of most plasma proteins. The glycocalyx is a negatively charged network of proteoglycans, including glycosaminoglycans, which dictate ESL permeability properties. The main glycosaminoglycan in the glomerular endothelial glycocalyx is heparan sulfate (HS), which, among others, is bound to core proteins agrin and syndecan-1.[S2] *In vitro* studies have shown that enzymatic cleavage of HS by heparanase causes increased albumin permeability across a monolayer of GEnCs in culture.[S3] Damage of the glomerular endothelial glycocalyx has been demonstrated in many proteinuric kidney diseases, but limited data are available for MCD.[S4] Holt *et al.*[S5] have reported that plasma heparanase activity was decreased in children with steroid-sensitive nephrotic syndrome (consistent with MCD), whereas urinary heparanase activity was increased. Although not peer reviewed, increased glomerular heparanase and concomitant decreased HS expression has also been shown in kidney biopsy tissue of patients with MCD.[S6] Experimental evidence for a role of the endothelial glycocalyx in MCD pathogenesis is derived from studies of the serine protease and

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putative permeability factor hemopexin. *In vitro*, activated hemopexin caused podocyte injury, decreased lectin binding to the endothelial glycocalyx, and albumin leakage across the GEnC monolayers.[S7] The authors hypothesized that hemopexin initially causes increased ESL permeability by endothelial glycocalyx damage, allowing passage and subsequent injury to the podocytes to occur. Indeed, recent research has shown that there is extensive crosstalk between the GEnCs and podocytes in the glomerular filtration barrier. Endothelial cell viability depends on podocyte production of vascular endothelial growth factor (VEGF).[S8] The VEGF stimulates endothelial nitric oxide synthase-mediated nitric oxide production in the GEnCs. Nitric oxide negatively regulates the response of the GEnCs to the VEGF to prevent proliferation.[S9] Furthermore, endothelial nitric oxide synthase deficiency has been shown to exacerbate glomerular injury, possibly by inducing heparanase, and endothelial nitric oxide synthase gene delivery prevented proteinuria in experimental models of glomerular injury.[S10,S11] In addition, absence of the VEGF stimulates production of vasoconstrictor hormone endothelin-1 by the GEnCs.[S12] Notably, endothelin-1 was also shown to activate podocyte heparanase synthesis, resulting in a decrease of the glycocalyx.[S13] Interestingly, podocyte VEGF expression was decreased in MCD glomeruli.[S14] This observation suggests that ESL abnormalities may be involved in MCD and that ESL injury might be sustaining or even initiating the characteristic podocyte injury in MCD.

In this issue of the *KI reports*, Bauer *et al.*[9] present data suggesting the presence of systemic and glomerular endothelial injuries in MCD. Both pediatric ($n = 44$) and adult ($n = 21$) patients with MCD were included in the study. Blood samples were obtained both in the nephrotic phase (either newly diagnosed or relapse) in all patients and at the time of complete remission in the pediatric cohort. Serum creatinine concentrations were within the normal reference range for their age. In blood samples, the investigators measured markers for glycocalyx degradation (syndecan-1 and HS fragments), enzymes that cleave glycocalyx components (heparanase and matrix metalloprotease-2), and markers of endothelial activation (von Willebrand factor and thrombomodulin). All markers were strongly increased during the nephrotic phase and decreased during proteinuria remission, although levels remained elevated in comparison with healthy controls. Because of significant correlations between circulating syndecan-1 and HS with von Willebrand factor and thrombomodulin concentrations, the authors concluded that the systemic endothelium was the most probable source of endothelial glycocalyx products syndecan-1 and HS, respectively. Further experiments were performed to identify whether there could be specific abnormalities of the glomerular ESL in MCD. Data from Nephroseq, an open-source database of glomerular transcriptome, showed that endothelial activation markers thrombomodulin and nitric oxide synthase 3 were increased in MCD glomeruli. *In vitro* studies confirmed increased thrombomodulin expression in cultured GEnCs after incubation with sera of patients with MCD. Finally, immunofluorescence studies on MCD kidney biopsy material showed increased expression of endothelial activation marker caveolin-1.

The study has several limitations. The cohorts were relatively small, and follow-up of patients was limited. Only caveolin-1 and thrombomodulin were studied as markers of endothelial cell activation in cell culture and glomerular immunofluorescence experiments, and no evidence of direct glomerular endothelial glycocalyx damage was shown. Glomerular disease controls were not included, and it may therefore be questioned whether the observed abnormalities are disease specific. Increased caveolin-1 expression has indeed been demonstrated in the glomeruli in biopsy specimens from several other glomerular diseases, and significantly correlated with albuminuria.[S15] As discussed previously, endothelial and ESL injury has been shown to be involved in other glomerular diseases and experimental glomerular disease models as well.

Despite these limitations, the study is among the first to investigate a role of the endothelium in MCD. The results have important implications for further research. HS fragments, syndecan-1, heparanase activity, matrix metalloproteinase-2, thrombomodulin, and von Willebrand factor were increased in serum/plasma of patients with MCD, and elevated levels persisted during complete proteinuria remission. As mentioned previously, a previous study reported a decreased plasma heparanase activity in pediatric MCD.[S5] Validation studies of plasma heparanase activity in MCD and proteinuric/nephrotic disease controls are therefore needed. Ongoing endothelial activation and glycocalyx damage may have prognostic significance for development of future relapses or potential long-term cardiovascular complications. Prognostic studies of a larger patient

cohort with repeated measurement are needed. In addition, the study suggested that MCD sera can activate GEnCs, opening the possibility that GEnC-podocyte interactions play a central role in MCD pathophysiology. Therefore, this study stimulates further investigation into the relevance of endothelial injury in MCD and possibly also focal segmental glomerular sclerosis, and in particular disturbance in glomerular crosstalk, which could ultimately lead to the identification of the ESL as a novel therapeutic target.

DISCLOSURE

All the authors declared no competing interests.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary References.

REFERENCES

1. Vivarelli M, Massella L, Ruggiero B, Emma F. Minimal change disease. *Clin J Am Soc Nephrol*. 2017;12:332–345. https://doi.org/10.2215/CJN.05000516
2. Maas RJ, Deegens JK, Beukhof JR, et al. The clinical course of minimal change nephrotic syndrome with onset in adulthood or late adolescence: a case series. *Am J Kidney Dis*. 2017;69:637–646. https://doi.org/10.1053/j.ajkd.2016.10.032
3. Shalhoub RJ. Pathogenesis of lipoid nephrosis: a disorder of T-cell function. *Lancet*. 1974;2:556–560. https://doi.org/10.1016/s0140-6736(74)91880-7
4. Maas RJ, Deegens JK, Smeets B, et al. Minimal change disease and idiopathic FSGS: manifestations of the same disease. *Nat Rev Nephrol*. 2016;12:768–776. https://doi.org/10.1038/nrneph.2016.147
5. Gauckler P, Shin JI, Alberici F, et al. Rituximab in adult minimal change disease and focal segmental glomerulosclerosis - what is known and what is still unknown? *Autoimmun Rev*. 2020;19:102671. https://doi.org/10.1016/j.autrev.2020.102671
6. Kuppe C, van Roeyen C, Leuchtle K, et al. Investigations of glucocorticoid action in GN. *J Am Soc Nephrol*. 2017;28:1408–1420. https://doi.org/10.1681/ASN.2016010060
7. Fornoni A, Sageshima J, Wei C, et al. Rituximab targets podocytes in recurrent focal segmental glomerulosclerosis. *Sci Transl Med*. 2011;3:85ra46. https://doi.org/10.1126/scitranslmed.3002231
8. Watts AJB, Keller KH, Lerner G, et al. Discovery of autoantibodies targeting nephrin in minimal change disease supports a novel autoimmune etiology. *J Am Soc Nephrol*. 2022;33:238–252. https://doi.org/10.1681/ASN.2021060794
9. Bauer C, Piani F, Banks M, et al. Minimal change disease is associated with endothelial glycocalyx degradation and endothelial activation. *Kidney Int Rep*. 2022;7:797–809.