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Genetic and Iatrogenic Defects in Peripheral Tolerance Associated with Anti-Nephrin Antibody-Associated Minimal Change Disease

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Minimal change disease · Nephrotic syndrome · Podocyte · Pathology · Anti-nephrin antibodies

Abstract
Introduction: Minimal change disease (MCD) is a common cause of nephrotic syndrome in children and adults. Immune dysregulation is a contributor, but the relative roles of individual components of the immune system in MCD pathogenesis remain unclear. ***Case Presentation:*** Here, we present 2 patients with defects in immune tolerance mechanisms that developed MCD associated with anti-nephrin antibodies. The first patient had a pathogenic deletion in *FOXP3*, leading to reduced regulatory T cells. Serum could not be obtained from this patient during the active phase of MCD to directly establish the presence of anti-nephrin antibodies. However, this patient demonstrated IgG dusting over podocyte cell bodies by immunofluorescence microscopy, as well as colocalization of IgG with nephrin in confocal microscopy. The second patient developed MCD in the context of immune checkpoint inhibitor treatment for metastatic carcinoma. Anti-nephrin antibodies were detected in this patient during active disease. The patient's kidney biopsy also showed evidence of binding of anti-nephrin antibodies within the glomeruli. ***Conclusion:*** These cases demonstrate that genetic and iatrogenic mechanisms of breakdown in peripheral tolerance can lead to MCD.

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Introduction

Minimal change disease (MCD) is the most common cause of nephrotic syndrome in children and one of the most common causes in adults [1, 2]. MCD is named for the minimal histologic changes seen in the kidney biopsy of these patients by light and immunofluorescence microscopy. The key diagnostic finding is extensive effacement of the foot processes of the specialized glomerular epithelial cell (podocyte) that are best visualized by electron microscopy, along with loss of filtration slit diaphragms. It has long been appreciated that immune dysregulation plays a role in MCD, though the exact immune system components responsible for disease pathogenesis have been a matter of debate [3–5]. Shalhoub proposed that MCD represented a scenario of T-cell dysfunction since it can be triggered by activated immune states such as viral infections, and remission can be induced with immunosuppression using steroids and cyclophosphamide [6]. Circulating factors such as cytokines produced by immune cells or podocyte products themselves have also been proposed as etiologic factors in animal models and human MCD [7–11]. B cells through their production of antibodies against CD40, ubiquitin carboxyl-terminal hydrolase L1 (UCHL1), and nephrin also seem to play a role in some cases of human MCD [12–14]. The success of B-cell-depleting agents, such as rituximab, also implicates a pathogenic role for B cells and their products (e.g., cytokines and antibodies) in MCD pathogenesis [15, 16]. Since B cells cannot produce high-affinity, class-switched antibodies without T-cell help, productive engagement between the cellular and humoral arms of the immune system is likely required to precipitate MCD.

Though most cases are idiopathic, known triggers for MCD include viral infections, lymphomas, and immunizations. Breakdown in immune tolerance mechanisms in these conditions may predispose to the development of MCD. A seminal study demonstrated the presence of autoantibodies targeting nephrin, a key podocyte slit diaphragm protein, in a large fraction of patients with MCD [12]. Since MCD is the most common cause of idiopathic nephrotic syndrome in children, a follow-up study confirmed the presence of these autoantibodies, which were identified in 70% of pediatric MCD patients with MCD and in 90% of those with treatment-naïve idiopathic nephrotic syndrome presenting with heavy proteinuria [17]. Both studies demonstrated that the titer of these autoantibodies correlated with disease activity. In the second study, the authors recapitulated key features of human MCD in an animal model by immunizing mice with recombinant nephrin extracellular domain. The immunized animals produced anti-nephrin antibodies and developed proteinuria and podocyte foot process effacement. The anti-nephrin monoclonal antibody 5-1-6 also causes severe proteinuria when injected into rats, demonstrating the nephrotic potential of antibodies targeting the nephrin extracellular domain [18]. In the mouse immunization model, the presence of anti-nephrin antibodies could be detected by changes in nephrin localization, resulting in a fine dusting pattern over podocyte cell bodies by immunofluorescence microscopy [17]. This phenocopies the 5-1-6 antibody-mediated redistribution of nephrin in rats, which alters its signaling properties and impairs podocyte cytoskeletal function, leading to proteinuria [19]. This nephrin redistribution has also been described in human kidney biopsy tissues and appears to correlate with the presence of anti-nephrin antibodies [12]. Together, these studies establish an autoimmune basis for MCD and demonstrate that a break in tolerance induced by immunization can generate pathogenic autoantibodies in a murine model.

Here, we present 2 patients whose disease courses further support the autoimmune basis of MCD. The first patient has an X-linked monogenic immune dysregulatory disorder, IPEX syndrome (immune dysregulation, polyendocrinopathy, enteropathy, X-linked), and developed membranous nephropathy followed by MCD. Patients with IPEX syndrome have reduced or absent expression of FOXP3 transcription factor, which is required for regulatory T cell (Treg) development and function [20–22]. Tregs are important for suppressing autoreactive T cells; therefore, IPEX patients are prone to develop multiple autoimmune diseases. The second patient developed MCD shortly after immune checkpoint inhibitor (ICI) treatment for metastatic carcinoma. ICIs are used to break peripheral tolerance and enhance antitumor immunity. Unfortunately, the presence of anti-nephrin antibodies could not be established through serum testing during the active phase of the disease for the patient with IPEX syndrome. Anti-nephrin antibodies were detected in the serum of the patient with ICI-associated MCD during active disease. However, concomitant with disease activity, both patients demonstrated anti-nephrin autoantibodies in their tissue as characterized by a dusting pattern for IgG in clinical immunofluorescence microscopy, as well as colocalization with nephrin in dual-color confocal microscopy. These cases demonstrate that both genetic and iatrogenic breaks in peripheral tolerance can lead to the development of autoantibodies targeting nephrin, resulting in MCD.

Patient 1 – IPEX Syndrome

Presentation and Medical History

A portion of the patient 1's history and diagnosis has been presented briefly by Baxter et al. [23]. To provide additional context here, the patient initially presented with diarrhea during infancy and was diagnosed (age 18 months) with Crohn's disease/enteropathy. He was subsequently diagnosed with type 1 diabetes (age 4 years) and juvenile arthritis (age 7 years). Other features consistent with his subsequent diagnosis of IPEX include chronic nonbacterial osteomyelitis (diagnosed age 14 years), dermatitis/psoriasis (diagnosed age 10 years), and uveitis. Over many years, his enteropathy and arthritis symptoms were variably controlled with monthly infliximab, cyclosporine, and weekly methotrexate. Given the subject's multiple autoimmune diseases, there was high clinical suspicion for IPEX, but an initial sequencing panel, that did not include noncoding regions, was inconclusive. At age 20, additional genomic analysis using a 462 gene oligonucleotide panel, which included coding and noncoding exons of *FOXP3*, identified a 4,466 bp deletion encompassing exon 1 of *FOXP3* (chrX:49,121,094-49,125,559, hg19 coordinates) [23]. This deletion removed an open chromatin region in Tregs corresponding to the conserved promoter of the *FOXP3* gene as well as the entirety of the nearby locus for the long noncoding RNA *FLICR* (FOXP3 regulating long intergenic noncoding RNA). As described in Baxter et al. [23], the deletion produced a nearly complete absence of FOXP3+ Tregs in the peripheral blood. Following the confirmation of the IPEX diagnosis, he was considered for peripheral blood stem cell transplantation, but the patient declined. He continues on infliximab, methotrexate, and cyclosporine maintenance therapy.

1st Kidney Biopsy during Episode of Nephrotic Syndrome (Age 12 Years)

At age 12, the patient presented with nephrotic range proteinuria without hematuria and a preserved serum creatinine of 0.7 mg/dL. A kidney biopsy was performed processed using standard clinical workflows for light, immunofluorescence, and electron microscopy. The biopsy demonstrated predominantly renal cortex with 69–83 glomeruli per level section, of which only 2–4 glomeruli per level were globally sclerosed. Segmental sclerosis was not seen. While epimembranous spikes were not readily seen in Jones-stained sections, some tangential sections of the glomerular basement membranes showed punched-out areas of lucency. A Masson

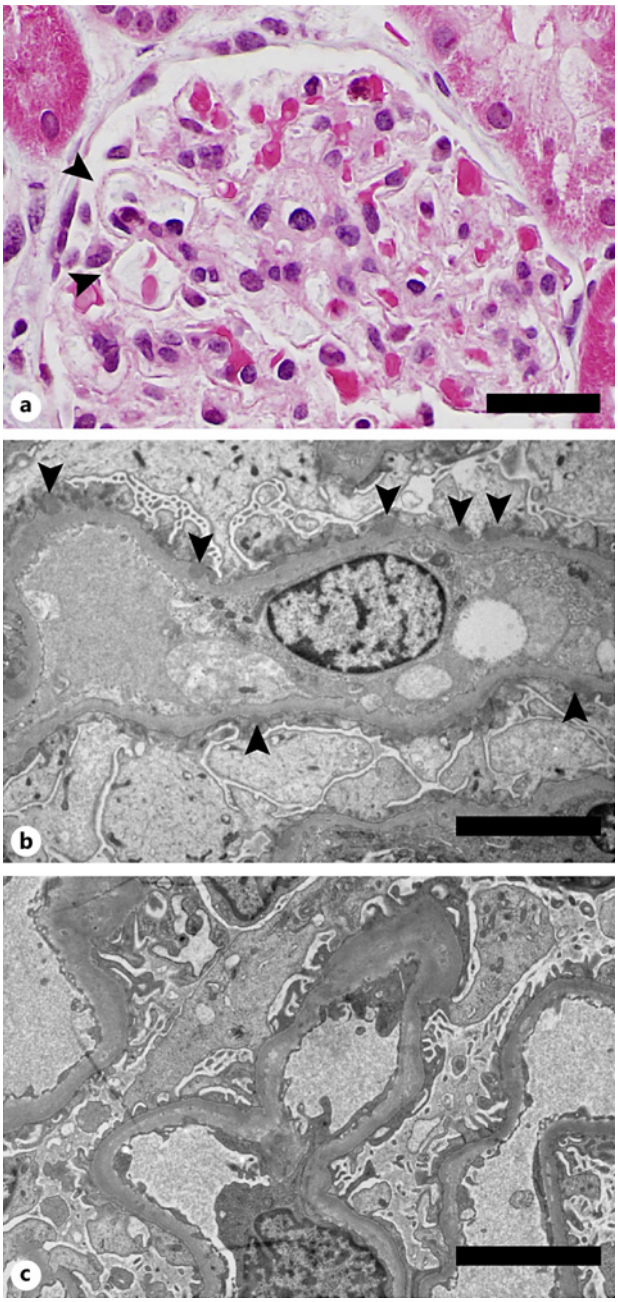

Fig. 1. Kidney pathology from patient 1's presentation with nephrotic syndrome at age 12 and acute kidney injury at age 16. **a** Masson trichrome stain from age 12 biopsy demonstrating fine fuchsinophilic granules along the outer aspect of capillary basement membranes (arrowheads). Scale bar = 20 μm. **b** Electron micrograph of patient 1's age 12 biopsy demonstrating electron-dense subepithelial deposits (arrowheads) along the glomerular capillary walls, which is associated with podocyte foot process effacement. Scale bar = 5 μm. **c** Electron micrograph from patient 1's age 16 biopsy demonstrating disappearance of subepithelial deposits (compared to **b**) with occasional intramembranous lucencies in the capillary basement membranes. The podocyte foot processes are largely intact in this field. Scale bar = 5 μm.

trichrome stained section showed possible capillary wall immune deposits (Fig. 1a). There was minimal chronic tubulointerstitial injury, and the sampled vessels were unremarkable. The immunofluorescence study showed finely granular peripheral capillary wall staining for IgG (3+) and C3 (3+). Electron microscopy showed scattered subepithelial electron-dense deposits with occasional glomerular basement membrane reaction and diffuse effacement of podocyte foot processes (Fig. 1b). The patient was diagnosed with membranous nephropathy and treated with cyclosporine and angiotensin receptor blockers which resulted in reduction of proteinuria and remission of nephrotic syndrome.

2nd Kidney Biopsy for Acute Kidney Injury (Age 16 Years)

During follow-up, the patient's serum creatinine was found to be elevated at 1.2 mg/dL without significant proteinuria. A 2nd kidney biopsy was performed and light microscopy contained renal cortex with 13–18 glomeruli, of which only 1 was globally sclerosed. Glomerular capillary wall thickening or epimembranous spike formation was not seen. In contrast to the prior biopsy, the immunofluorescence study showed no significant immune complex deposition within the glomeruli. Ultrastructural examination of the glomerular capillary basement membranes showed intramembranous lucencies and rare subepithelial electron-dense deposits consistent with treated membranous nephropathy (Fig. 1c). This was associated with only segmental (10–20%) podocyte foot process effacement. These biopsy findings correlated with clinical remission of his previously diagnosed membranous nephropathy. Acute kidney injury was thought to be due to cyclosporine, which was slowly tapered to 100 mg bid.

2nd Episode of Nephrotic Syndrome (Age 18 Years)

At age 18 years, the patient again presented with severe nephrotic syndrome (13.7 g/g proteinuria, serum albumin 2.1 g/dL, serum creatinine 1.2 mg/dL). This occurred in the setting of weaning of his maintenance immunosuppression (cyclosporine reduced from 75 mg to 50 mg twice daily). A kidney biopsy was not performed as this was felt to be a relapse of his membranous nephropathy. His cyclosporine was increased to 150 mg twice daily, prednisone 10 mg daily was added, and he had a rapid improvement. Within 4 weeks, his proteinuria had improved to 0.95 g/g and by 8 weeks, his proteinuria had resolved, and serum albumin (3.8 g/dL) and creatinine (0.9 mg/dL) had normalized.

3rd Episode of Nephrotic Syndrome and Kidney Biopsy (Age 24 Years)

Ten days prior to presentation, the patient had had a wisdom tooth extracted under general anesthesia complicated by facial swelling that was thought to be secondary to the surgery. However, 5 days later, the patient reported peripheral edema and an 11 kg weight gain. Ten days post-surgery, the patient was found to have acute kidney injury (serum creatinine 3.2 mg/dL) with nephrotic syndrome (proteinuria 6.6 g/g, serum albumin 2.5 g/dL) and was admitted to hospital. He reported that he had self-discontinued cyclosporine several months previously but had continued his maintenance infliximab and methotrexate. The clinical concern was for recurrence of the patient's membranous nephropathy with progression of chronic kidney injury or possible focal segmental glomerulosclerosis (FSGS).

A 3rd kidney biopsy was performed to evaluate these possibilities. By light microscopy, there were 12–15 glomeruli per level section, of which 2–4 per level section were completely sclerosed. FSGS was not seen. The remaining glomeruli showed variable but mild mesangial expansion due to accumulation of matrix and increased cellularity, changes consistent with early diabetic nephropathy (Fig. 2a). Epimembranous spikes were not seen in the capillary walls. There was also histologic evidence of acute tubular injury and chronic changes associated with calcineurin inhibitor toxicity. The immunofluorescence study did not show capillary wall immune deposits as would be seen for membranous nephropathy. Instead, there was only faint "dusting"-type staining over podocyte cell bodies for IgG, but not albumin (Fig. 2b). The electron microscopy study showed no evidence of immune deposits in the glomerular capillary walls. The glomerular basement membranes were intact and mildly thickened without evidence of electron lucencies or remodeling changes. The glomerular podocytes exhibited diffuse effacement of their foot processes with frequent microvillous transformation (Fig. 2c). A diagnosis of MCD was rendered.

The presence of IgG "dusting" by immunofluorescence microscopy has been reported for MCD patients with circulating anti-nephrin antibodies [12, 17] and in rats administered with the 5-1-6 anti-nephrin antibody [19]. Unfortunately, an attempt to collect plasma from the patient at the time of presentation with active disease to verify the presence of circulating anti-nephrin antibodies was unsuccessful. However, the frozen tissue block was costained for human IgG and nephrin and imaged using dual-color confocal microscopy as described previously

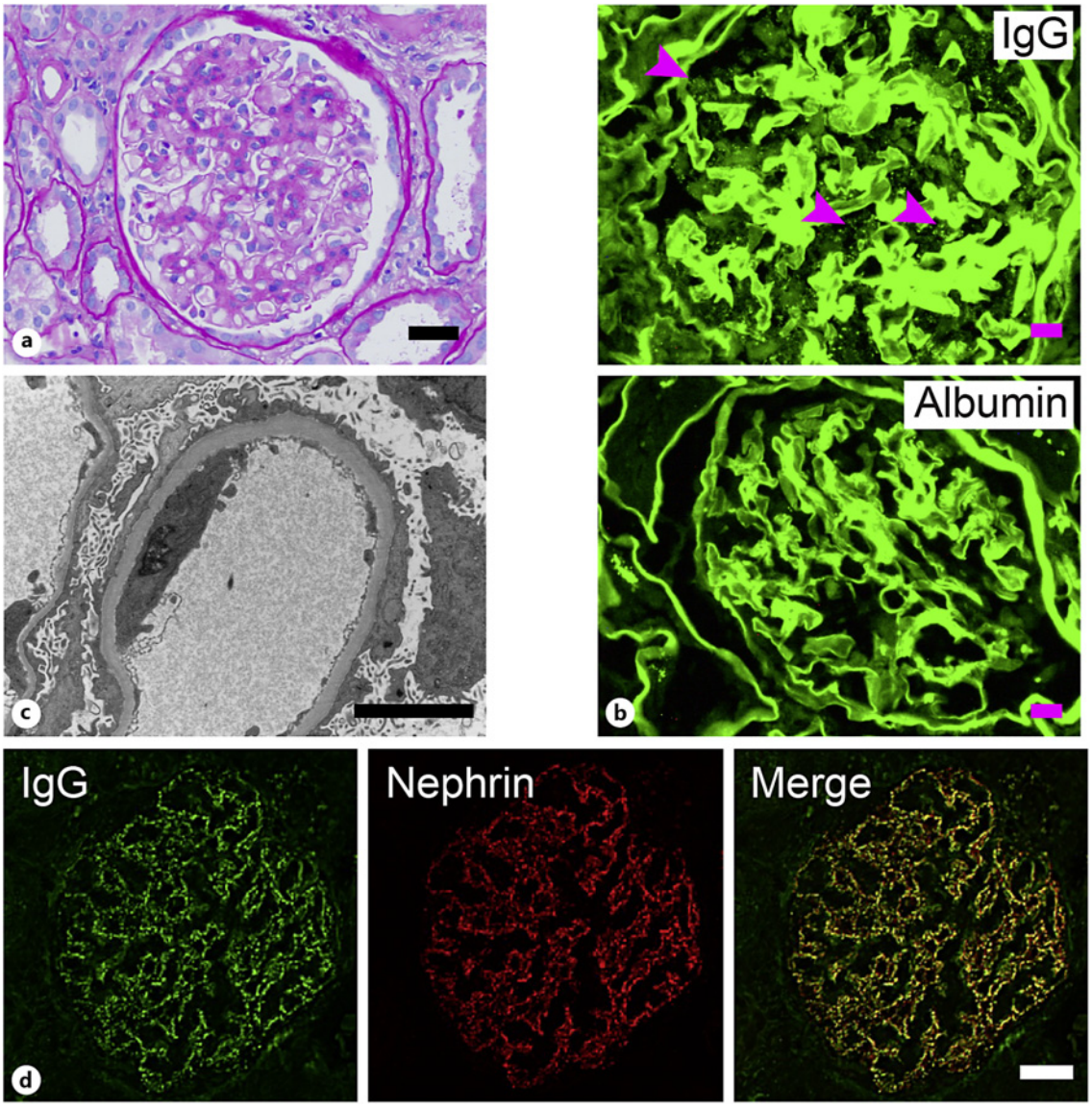

Fig. 2. Kidney pathology from patient 1's presentation with nephrotic syndrome at age 24. **a** Periodic acid-Schiff stain demonstrating mesangial expansion. Scale bar = 20 μm. **b** Immunofluorescence microscopy performed on a frozen tissue section from the patient's biopsy. Sections were stained for IgG (top) and albumin (bottom) and imaged using the same settings. Fine dusting is seen over podocyte cell bodies for IgG (arrowheads), but not for albumin. Long exposure (~800 ms) was required to visualize dusting, which increased autofluorescence signal from basement membranes. Scale bar = 20 μm. **c** Electron micrograph from the same biopsy demonstrating diffuse podocyte foot process effacement without subepithelial immune deposits. Scale bar = 5 μm. **d** Confocal immunofluorescence microscopy performed on frozen tissue sections demonstrates colocalization of IgG and nephrin signals. Scale bar = 20 μm.

[12]. This confirmed mislocalization of nephrin over the podocyte cell body instead of at the filtration slit diaphragm (Fig. 2d). This nephrin protein colocalized with human IgG confirming the "dusting" pattern seen by immunofluorescence microscopy. This pattern has been previously described in patients with MCD with circulating anti-nephrin antibodies [12].

His acute kidney injury began to recover prior to immunosuppression (3.2 mg/dL to 2.2 mg/dL within 3 days). Given his prior response to calcineurin inhibitors, it was decided to restart his cyclosporine at his prior induction treatment dose (150 mg twice daily, aiming trough level 150–200 ng/mL), rather than oral glucocorticoids. Within 4 weeks, he had a marked improvement in proteinuria

(1.3 g/g, serum albumin 3.8 g/dL), and by 3 months, he had a complete remission. Serum from this patient at 3 and 7 weeks post-biopsy, when he was receiving treatment and was undergoing remission, showed no detectable anti-nephrin antibodies. As of 2 years after the diagnosis with MCD, the patient has remained in remission.

Patient 2 – ICI-Associated MCD

Presentation and Medical History

The 64-year-old patient had past medical history significant only for hypertension and recent bilateral hip replacement. Imaging follow-up for persistent lower back pain after the hip replacement procedures identified an 8.1 cm renal mass as well as pulmonary nodules and numerous osseous metastases involving the skull, ribs, spine, pelvis, and knee. A CT-guided biopsy of the right renal mass and osseous metastasis revealed high-grade renal cell carcinoma, WHO/ISUP grade 4. Subsequent imaging also revealed metastases to the liver, adrenal glands, and regional lymph nodes. Despite undergoing radiation therapy to both hips and shoulder, the patient experienced persistent pain secondary to these metastases. He then started on systemic therapy with a regimen of nivolumab (anti-PD1 monoclonal antibody) and cabozantinib (VEGFR tyrosine kinase inhibitor). Cabozantinib was stopped about 1 month after initiation due to increasing but sub-nephrotic range proteinuria (~600 mg/24 h) and concern for thrombotic microangiopathy, a known complication of VEGFR inhibition [24]. The nivolumab was continued as a single agent. However, despite holding cabozantinib, the patient continued to complain of progressive edema, starting with the legs and worsening to generalized body edema. Within 3 months, he had developed nephrotic syndrome, with >12 g/g proteinuria (spot urine protein/creatinine ratio), hypoalbuminemia of 2.1 g/dL, and a preserved serum creatinine of 0.65 mg/dL. At this point, a kidney biopsy was obtained with a clinical differential diagnosis of cabozantinib-induced thrombotic microangiopathy vs. membranous nephropathy secondary to metastatic carcinoma. The patient received his last dose of nivolumab 2 weeks prior to the kidney biopsy.

Kidney Biopsy

By light microscopy, there were 10–13 glomeruli per level section, of which 1–2 per level section were completely sclerosed. Segmental glomerulosclerosis was not seen. The remaining glomeruli were histologically unremarkable, and there was only patchy mild tubular atrophy and interstitial fibrosis (Fig. 3a). Acute tubular injury and patchy active appearing mononuclear tubulointerstitial nephritis were also present. There was also mild-to-moderate chronic vascular disease. The immunofluorescence study showed faint "dusting"-type staining over podocyte cell bodies for IgG, but not albumin (Fig. 3b); granular capillary wall immune deposits were not seen. Electron microscopy confirmed the absence of immune deposits. The ultrastructural examination also showed diffuse effacement of podocyte foot processes (Fig. 3c). Costaining for nephrin and human IgG and confocal imaging showed colocalization of signal as was seen for patient 1 (Fig. 3d). The patient was diagnosed with MCD.

Clinical Follow-Up and Testing

The observed acute tubular injury and patchy tubulointerstitial nephritis raised concern that the biopsy had detected imminent kidney injury. The patient was recommended for a follow-up monitoring of kidney function. Three days after biopsy, the patient's serum creatinine had risen to 1.19 mg/dL. Quantification of patient serum for anti-nephrin antibodies was performed as described previously, with addition of a pre-adsorption step on sheep erythrocytes to reduce background from natural anti-α-Gal antibodies [12]. Serum from day 3 after the kidney biopsy was positive for anti-nephrin antibodies (191 U/mL, normal <100 U/mL). The patient was started on a steroid pulse and taper. Serum creatinine had dropped to 1.09 mg/dL 2 weeks after the biopsy had detected tubulointerstitial nephritis. Unfortunately, posttreatment serum was not available for anti-nephrin antibody testing. The patient's carcinoma was progressive, and he soon transferred to hospice care and expired 5 months after the diagnosis of MCD was rendered.

Discussion

The correlation of anti-nephrin autoantibody titers with MCD disease activity, and the recent demonstration of the pathogenicity of these antibodies in an animal model support, but do not yet completely prove, a causal role for these antibodies in human MCD [12, 17]. These two cases provide further evidence, from human patient experiences, that genetic or iatrogenic breaks in peripheral tolerance can lead to anti-nephrin autoantibody generation that is tightly correlated with onset of MCD and nephrotic syndrome.

Both MCD and membranous nephropathy have been reported in patients with IPEX. In a report on two brothers with IPEX, one developed MCD, while the other developed membranous nephropathy [25]. It is interesting that case 1 developed both these glomerular diseases at different times. A large series of IPEX patients

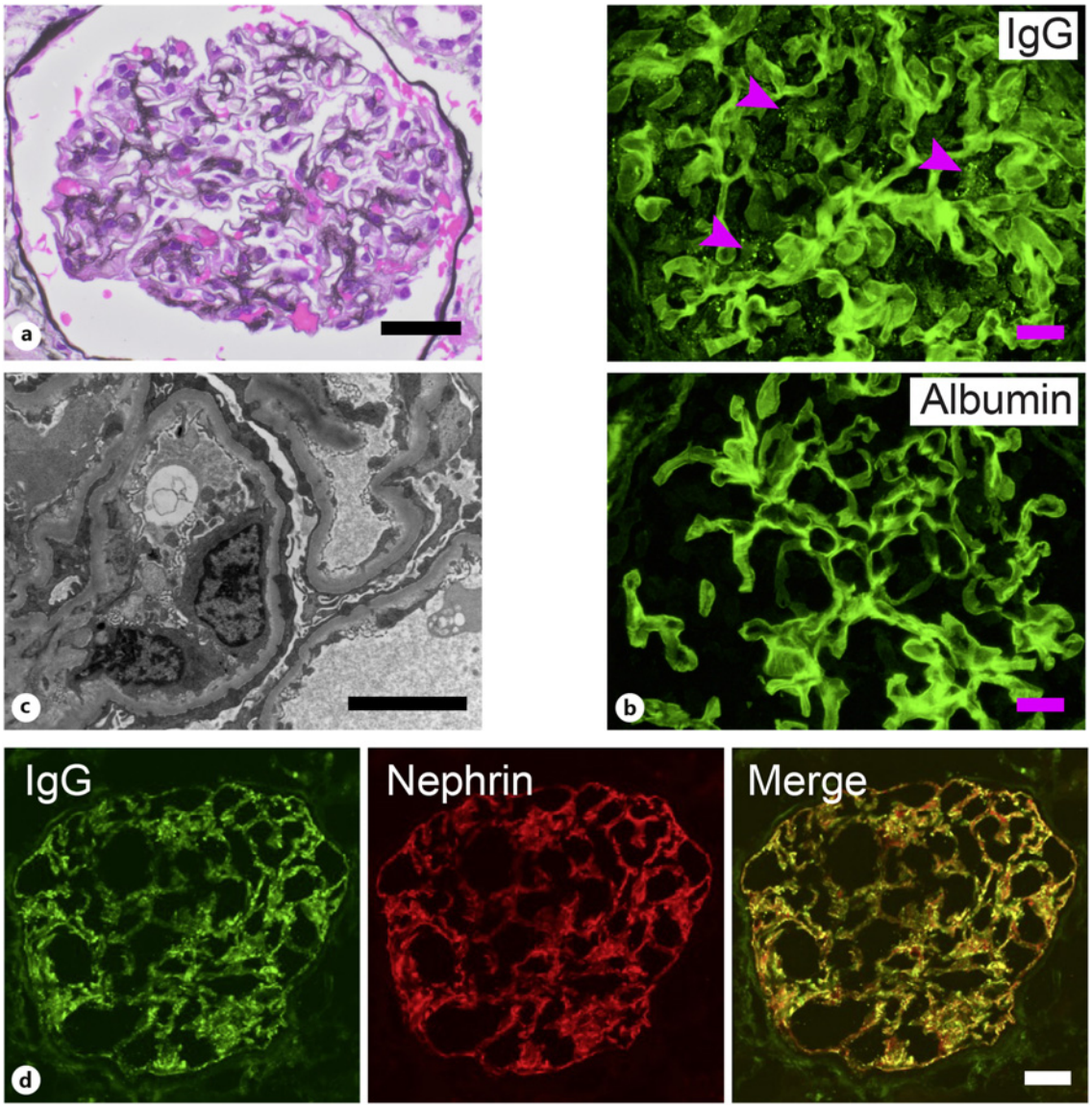

Fig. 3. Kidney pathology from patient 2's presentation with nephrotic syndrome at age 64. **a** Jones methenamine silver stain shows unremarkable glomerular histology. Scale bar = 20 μm. **b** Immunofluorescence microscopy performed on a frozen tissue section from the patient's biopsy. Sections were stained for IgG (top) and albumin (bottom) and imaged using the same settings. Fine dusting is seen over podocyte cell bodies for IgG (arrowheads), but not for albumin. Scale bar = 20 μm. **c** Electron micrograph demonstrating diffuse podocyte foot process effacement. Scale bar = 5 μm. **d** Confocal immunofluorescence microscopy performed on frozen tissue sections demonstrates colocalization of IgG and nephrin signals. Scale bar = 20 μm.

reported that nephropathies were present in 25% of patients [26]. Membranous nephropathy was the most common kidney pathology encountered in children with IPEX, followed by tubulointerstitial nephritis, MCD, and membranoproliferative glomerulonephritis. This series emphasized the variability in patient phenotypes and recommended sequencing in suspected cases. With that in mind, the hemizygous deletion of *FOXP3* in our IPEX patient also eliminated the X chromosome locus encoding for the long noncoding RNA *FLICR*, which negatively regulates *FOXP3* expression [27]. IPEX patients can present with multiple severe autoimmune diseases early in life, but we speculate that the deletion of this negative regulator of FOXP3 may have modulated the IPEX phenotype in patient 1. It has been reported that Treg function is impaired in sporadic cases of idiopathic MCD

[28–31]. Some animal models also support a protective role for Tregs in proteinuric disease. In the adriamycin-induced mouse model of FSGS, infusion of IL-2/anti-IL-2 antibodies, which cause Treg activation and proliferation, resulted in increased numbers of Foxp3+ Tregs and reduction of proteinuria [32]. Direct transduction of Foxp3 into T cells also resulted in proteinuria suppression in the same model [33]. Infusion of Foxp3+ Tregs into the buffalo/Mna spontaneous nephrotic syndrome rat model also resulted in improvement of proteinuria [34]. However, Tregs appear to be less protective in the LPS-induced proteinuric mouse model [35], suggesting that different pathogenic pathways may lead to proteinuria in humans and animal models of MCD.

Tubulointerstitial nephritis is the most common histologic presentation of kidney injury in patients receiving ICI [36, 37]. However, diverse glomerulopathies have also been reported in this patient population, including membranous nephropathy and MCD [38–44]. Some immune complex-mediated glomerulopathies may reflect exacerbation of sub-clinical disease in the context of ICI treatment. By contrast, membranous nephropathy and MCD may represent *de novo* breaks in tolerance with production of pathogenic antibodies that are detectable in the circulation and in the kidney biopsy. It is interesting that the autoantibodies in MCD do not form immune complexes readily detectable by electron microscopy. Even by immunofluorescence microscopy, they may appear as inconspicuous "dusting" over podocyte cell bodies. In this regard, anti-neutrophil cytoplasmic antibodies represent another precedent for pathogenic antibodies that can cause kidney injury by injuring endothelial cells, but without formation of detectable immune complexes. By contrast, in membranous nephropathy, autoantibodies against podocyte antigens such as PLA2R, NELL1, THSD7A, and others form readily detectable immune complexes that deposit beneath the podocyte within the glomerular basement membrane. This difference between membranous nephropathy and MCD autoantibody characteristics may be due to differences in biophysical properties, abundance and localization of podocyte antigens, as well as autoantibody abundance and affinity. Membranous nephropathy can also be triggered by altered immune states such as cancer, pregnancy, and autoimmune disease. Therefore, both MCD and membranous nephropathy can be viewed as autoantibody-induced podocytopathies that are triggered by breaks in peripheral tolerance mechanisms.

In summary, we present two cases which demonstrate the pathogenic potential of autoantibodies targeting nephrin in MCD. Due to a genetic defect resulting in Treg deficiency or due to treatment with ICI, both patients experienced breaks in peripheral tolerance that correlated with tissue-based and/or serological evidence of anti-nephrin antibodies. Our findings further support the idea that MCD is an autoantibody-mediated autoimmune disease, emphasize the importance of Tregs in maintaining peripheral tolerance to podocyte antigens, and justify screening of patients with nephrotic syndrome for anti-nephrin autoantibodies.

Statement of Ethics

For this study, clinical information was collected from the electronic health record and discarded serum no longer needed for clinical purposes was evaluated for anti-nephrin antibodies. This study protocol was reviewed and approved by the University of Washington's (UW) Institutional Review Board (IRB), Approval Nos. 19713 and 2591. Written informed consent was obtained from both patients for publication of this case report and the accompanying images.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

J.A.J., K.C., S.H., A.B.M., and S.S.T. were responsible for the clinical management of the patients and provided relevant information regarding clinical course and follow-up. Y.H., K.D.S., R.C.R., and S.A. interpreted the patient's kidney biopsies and provided pathology information, including images. A.W. and K.H.K. performed testing for circulating anti-nephrin antibodies in patient serum and confocal staining on patient biopsy tissue. A.W. and S.A. discussed findings, assembled figures, and wrote the initial draft of the manuscript. All authors reviewed and edited the manuscript and figures.

Data Availability Statement

The data that support the findings of this study are not publicly available due to their containing information that could compromise the privacy of research participants but are available from the corresponding author (S.A.) upon reasonable request.

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