Membranous nephropathy (MN) is one of the more common causes of nephrotic syndrome in the adult population, accounting for about 20% of cases. It can be idiopathic, without identified cause (70%-80%), or secondary to various clinical conditions, including infections (hepatitis B, syphilis), systemic lupus erythematosus, cancers, and drug intoxications.1

MN is an immunologically mediated disease defined by immune complex deposition in the subepithelial space that causes a membrane-like thickening. The immune deposits consist of IgG, antigens that have long eluded identification, and the membrane attack complex of complement C5b-9. IgG4 is the most prominent deposited subclass in idiopathic MN, although variable amounts of IgG1 are usually associated; in secondary MN, IgG1, IgG2, and IgG3 exceed IgG4.2,3 The formation of subepithelial immune deposits and complement activation are presumably responsible for functional impairment of the glomerular capillary wall, causing proteinuria. Evidence now suggests that MN is triggered by antibodies directed against podocyte proteins. Two major antigens, both membrane glycoproteins, have been identified. The first is neutral endopeptidase, the alloantigen involved in rare neonatal cases of MN that occur in newborns from neutral endopeptidase–deficient mothers.4 The disease could be transferred to rabbits injected with immunoglobulin purified from the infant’s mother’s serum but not from the father’s serum.5 The second antigen is the M-type phospholipase A2 receptor (PLA2R), the first antigen identified in idiopathic MN in adults, which is considered an autoimmune disease.6

Although anti-PLA2R antibodies are found in about 70% of patients with idiopathic MN6–9 and seem to correlate with disease activity and proteinuria,6,10,11 there is no definitive proof that these

ABSTRACT
Up to 80% of patients with idiopathic membranous nephropathy have non–complement-fixing IgG4 autoantibodies to the phospholipase A2 receptor (PLA2R). Membranous nephropathy recurs in approximately 40% of patients after kidney transplantation, but the mechanism is unknown. Here, we describe a patient with recurrent membranous nephropathy 13 days after kidney transplantation whose graft biopsy specimen showed granular staining for C3, C5b-9, C1q, and IgG3; electron microscopy revealed subepithelial nonorganized deposits. A search for hematologic disorders was negative. Retrospective evaluation of a biopsy sample from the native kidney revealed a similar pattern: monotypic IgG3x deposits together with C3, C1q, and C5b-9. Glomerular deposits contained PLA2R in both the graft and the native kidney, suggesting that the recurrence was the result of circulating anti-PLA2R antibodies binding to PLA2R antigen expressed on donor podocytes. Confocal analysis of anti-PLA2R and anti-human IgG3 showed colocalization, and the patient had IgG3x-restricted circulating anti-PLA2R antibodies. Treatment with rituximab stabilized both proteinuria and serum creatinine, and circulating anti-PLA2R became undetectable. In summary, this case of recurrent membranous nephropathy in a graft suggests that circulating monoclonal anti-PLA2R IgG3x caused the disease and activated complement by the classic pathway.

antibodies are pathogenic. First, PLA2R-related MN could not be induced by transfer of patients’ serum or IgG to mouse, rat, or rabbit because these species do not express PLA2R antigen in glomeruli. Second, as yet there is no animal model of PLA2R-related MN that could phenocopy Heymann nephritis, a reliable form of MN in the rat in which the target antigen, megalin, is also located at the podocyte surface.12,13 Third, anti-PLA2R antibodies can occasionally be found in patients with idiopathic MN but without PLA2R antigen in subepithelial immune deposits, a finding suggesting that at least some anti-PLA2R antibodies might not be pathogenic.14 Fourth, although PLA2R-related MN can recur in the kidney graft, sometimes after only a few days,15–17 some patients with high-titer anti-PLA2R antibodies at the time of transplantation will not have clinical or histologic recurrence.16 In those cases, however, differences between donor and recipient PLA2R sequence variants might account for the lack of recurrence.

Here we report an exceptional case of recurrent PLA2R-related MN with monotypic IgG3k deposits and circulating anti-PLA2R antibodies restricted to IgG3k, which provides an argument favoring the pathogenicity of anti-PLA2R antibodies, at least in this particular situation.

A kidney allograft biopsy was performed 13 days after transplantation because of delayed graft function (plasma creatinine, 2.82 mg/dl) and proteinuria (1.85 g/d) in a 52-year-old man in whom MN had been diagnosed 13 years earlier and who has been receiving hemodialysis for the last 6 years. Pretransplantation assessment of the glomerulopathy failed to identify a cause, thereby suggesting idiopathic MN. The biopsy revealed early recurrence of MN, characterized by abundant granular deposits of IgG on the outer aspect of the glomerular basement membrane (Figure 1A). These deposits did not show any organization by electron microscopy (Figure 1B). We performed a subclass and light-chain isotype analysis of deposited IgG, which exclusively stained for IgG3k (Figure 1C). Biopsy specimen also contained C3, C1q, and 

![Figure 1. Characterization of immune deposits in kidney biopsy specimens from grafted (A–D) and native (E) kidneys. (A) Immunofluorescence study showing early recurrence of the MN (day 13) characterized by granular deposits of IgG. (B) Representative segment of the capillary wall analyzed by electron microscopy. Electron-dense deposits seen on the outer aspect of the glomerular basement membrane do not show any organization. (C) Immunostaining for IgG subclasses and light-chain isotypes showing the presence of monotypic IgG3k. (D) Complement components, including C3, C1q and C5b-9, detected in the absence of MBL. (E) Kidney biopsy specimen from native kidney stained for IgG3 and light-chain isotype. The specimens shown in E are paraffin sections, whereas those shown in A, C, and D are cryostat sections. Original magnification for A, C, D, and E x400; for B x40,000.](1950_JASN_Figure1.png)
C5b-9 in deposits but no mannose-binding lectin (MBL) (Figure 1D). The positive control for MBL staining is shown in Supplemental Figure 1.

The finding of monotypic IgG3k deposits prompted an extensive search for a hematologic disorder. Electrophoresis and immunofixation of serum proteins did not disclose any qualitative anomaly. Serum κ and λ free light-chain levels and immunoglobulin classes were normal, except for IgG, which was moderately decreased. Blood lymphocyte immunophenotyping was unremarkable. Positron emission tomography did not reveal hyperfixation, and the bone marrow biopsy specimen was normal, revealing rare, polyclonal plasma cells.

We then asked whether deposits in the native kidney biopsy specimen were also monotypic. The pathologic report described granular deposits of IgG, C3, and C1q, but there was no information on light-chain isotype. Because of the lack of frozen kidney biopsy specimen, we developed a new technique to perform subclass and isotype analysis in paraffin-embedded sections. The deposits stained for IgG3 and κ but did not stain for the λ isotype (Figure 1E).

PLA2R antigen was detected in a granular pattern typical of subepithelial immune deposits in the native and kidney graft biopsy specimens (Figure 2, A and B). Co-localization with IgG3 was established by confocal microscopy (Figure 2, C–F).

Because both the native and kidney graft biopsy specimens featured monotypic IgG3κ deposits in the absence of IgG4, we reasoned that the circulating anti-PLA2R antibodies could also be monotypic. Using the indirect immunofluorescence test (Euroimmun AG, Lübeck, Germany), we first showed that in patient’s serum anti-PLA2R antibodies were present at the time of transplantation (Figure 3). Next, using subclass- and light-chain isotype–specific revealing antibodies, we found that anti-PLA2R antibodies were restricted to IgG3κ, which is the immunoglobulin isotype detected in glomerular deposits (Figure 2).

On the basis of these findings, the patient received four injections of rituximab (375 mg/m²) at 2-week intervals. Six months later, proteinuria had decreased dramatically (from 5.1 g/d before rituximab to 0.4 g/d), kidney graft function had stabilized (serum creatinine, 2.72 mg/dl before rituximab versus 2.56 mg/dl), and anti-PLA2R antibodies had disappeared (Figure 3).

**DISCUSSION**

To our knowledge, this is the first report of monotypic anti-PLA2R IgG3κ associated with MN, activation of the classic complement pathway, and very early recurrence of the disease on the kidney graft. The finding of IgG3κ being co-localized with PLA2R in both the native and the kidney graft biopsy specimens, and of circulating anti-PLA2R IgG3κ, strongly suggests that this antibody was responsible for the glomerular disease and its recurrence in this patient.

Circulating monoclonal immunoglobulin may recognize self-antigens as shown for IgM anti-IgG in type 2 cryoglobulins, perinuclear ANCA, and anti–glomerular basement membrane antibodies. In this patient, the rate of synthesis of the antibody was probably low because no monoclonal component could be detected in the blood, even by sensitive methods. A similar situation is observed in patients with proliferative GN with monoclonal IgG deposits, which may also recur in the allograft.
however, no target antigen has yet been identified in this entity. It is noteworthy that in the four patients with recurrence, no serum monoclonal component could be detected at the time of first biopsy or at the time of recurrence, although all four patients featured IgG3 deposits (three κ, one λ). IgG3 is the isoform identified in most cases of GN with monoclonal IgG deposits.22,24 This subclass, which accounts for only 2%–8% of serum IgG in normal individuals, is especially prone to self-aggregability via Fc-Fc interactions.25 In addition, compared with other IgG subclasses, it has a slightly higher molecular weight, the greatest complement-fixing ability, and globally more cationic charge, characteristics that may make it intrinsically “nephritogenic.”25,26 Although some

patients with GN with monoclonal IgG deposits have a history of autoimmune disease, none had MN. In our patient, development of MN was probably caused by the anti-PLA2R activity of the IgG3, although deposition of anti-PLA2R antibody might also be enhanced by the physicochemical properties of IgG3.

Our case is unusual among idiopathic MN cases because of signs of activation of the classic complement pathway, such as C1q deposits, associated with C3 and C5b-9 in both native and graft biopsy specimens. There was no evidence of lupus disease. MBL was not found in immune deposits, suggesting that in this case, the MBL pathway was probably not activated, in contrast with recent findings which indicate that anti-PLA2R IgG4 can activate the MBL pathway.27 In addition, MBL was identified in the glomeruli of patients with idiopathic MN.3,28 The high C1q-binding ability of IgG3 probably explains the discrepancy with most common forms of idiopathic MN, in which IgG4 is the prevailing subclass.

Few reports have described very early recurrence of PLA2R-related MN, and all such cases were associated with polyclonal anti-PLA2R antibodies.15–17 Recurrence occurred as early as 6–8 days after transplantation,16,17 suggesting that anti-PLA2R antibodies brought by the recipient’s serum rapidly induced formation of subepithelial deposits in the graft glomeruli, thus reproducing the passive Heymann nephritis model.29,30

Because of the monoclonal nature of the antibody, our observation marks a further step in the demonstration of pathogenicity of anti-PLA2R antibodies.

CONCISE METHODS

Patient

The patient is a 56-year-old man in whom nephrotic syndrome of rapid onset was diagnosed in 1993. Antinuclear antibodies were absent, and serum complement C3 and C4 concentrations were normal. Diagnosis of MN was established on a first kidney biopsy specimen. Light-chain isotype was not determined. At that time, no evidence indicated a secondary cause, although the patient was a heavy smoker (30 pack-years). His medical history included childhood asthma, an acute non-A, non-B, non-C viral hepatitis at age 10 years, and tonsillectomy. Family medical history was unremarkable. Because the serum creatinine level increased to 1.36 mg/dl in July 1994, the patient was treated with steroids and cyclophosphamide according to a revised Ponticelli protocol.31 In May 1995, a second kidney biopsy showed diffuse interstitial fibrosis. Immunosuppressive treatment was stopped. The patient was then lost to follow-up until February 2002, when he was seen with a major episode of volume expansion and dilated cardiomyopathy. Hemodialysis was started. Between 2002 and 2008, no additional immunologic or hematologic disorder was diagnosed. The patient received a kidney transplant in February 2008. Anti-HLA antibodies
Analysis of Renal Biopsy Specimen

The patient’s biopsy specimen was prepared for light, immunofluorescence, and electron microscopy by standard techniques. For detection of IgG subclasses, light-chain isotypes, and complement components, cryosections of the biopsy specimen were incubated with the following antibodies: FITC-conjugated monoclonal antihuman IgG1 (Sigma-Aldrich), IgG2 (Sigma-Aldrich), IgG3 (Sigma-Aldrich), and IgG4 (Sigma-Aldrich) antibodies; rabbit polyclonal antihuman IgG1 (Sigma-Aldrich), IgG2 (Sigma-Aldrich), IgG3 (Sigma-Aldrich), and IgG4 (Sigma-Aldrich) antibodies from mouse were used. For detection of light-chain isotypes, FITC-conjugated rabbit polyclonal antihuman κ or λ light chains were used (Dako).

Acknowledgments

We thank Philippe Fontanges for assistance with the confocal microscope.

The work was supported by grants from Fondation pour la Recherche Médicale (Equipe FRM 2012) and by grant from Agence Nationale pour la Recherche (ANR-07-Physio-016-01).

Disclosures

None.

References


This article contains supplemental material online at http://jasn.asnjournals.org/lookup/suppl/doi:10.1681/ASN.2012060577/-/DCSupplemental.