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Novel Antigens and Clinical Updates in Membranous Nephropathy

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Abstract

Membranous nephropathy (MN), an autoimmune kidney disease and leading cause of nephrotic syndrome, leads to kidney failure in up to one-third of affected individuals. Most MN cases are due to an autoimmune reaction against the phospholipase A2 receptor (PLA2R) located on kidney podocytes. Serum PLA2R antibody quantification is now part of routine clinical practice because antibody titers correlate with disease activity and treatment response. Recent advances in target antigen detection have led to the discovery of more than 20 other podocyte antigens, yet the clinical impact of additional antigen detection remains unknown and is under active investigation. Here we review recent findings and hypothesize how current research will inform future care of patients with MN.

INTRODUCTION

Membranous nephropathy (MN), an autoimmune kidney disease, is a leading cause of nephrotic syndrome (urinary protein loss >3.5 g/day with edema, hypoalbuminemia, and hyperlipidemia). Disease is diagnosed by histologic evaluation of kidney tissue usually obtained through percutaneous biopsy and, more recently, through noninvasive detection of circulating pathogenic antibodies. Patients who are at moderate to high risk of progressive chronic kidney disease (CKD) or who have life-threatening nephrotic syndrome are treated with immunosuppressive therapy, while others are managed supportively with antiproteinuric medications and lifestyle changes. Diagnosis and management of MN were at a standstill until 2009 when a major antigen involved in disease pathogenesis, the phospholipase A2 receptor (PLA2R), was discovered by Beck et al. (1). Advancements in techniques used for antigen discovery have opened the door for rapid discovery of target antigens, such that now <10% of MN involves an unknown target antigen (2). How antigen discovery will inform patient care is a major developing story. Previous studies on the natural course of MN demonstrated progressive kidney disease and end-stage kidney disease (ESKD) in up to one-third of affected patients, though results from four recent and practice-changing clinical investigations report remission rates as high as 80% (3–6). Here we present an updated review of MN with a focus on the clinical relevance of target antigen discovery.

CLINICAL FEATURES

MN affects people of all races and ethnicities, and the prevalence is estimated at 2–17 cases per million (7–10). Disease morbidity is driven by sequelae of the nephrotic syndrome, and most patients describe an insidious onset of progressive fluid retention in the abdomen, lower extremities, and occasionally around the eyes. Though most patients have preserved kidney function at time of diagnosis, untreated disease can progress to ESKD in up to one-third of patients (7). Several risk models for CKD prediction have been proposed, and the variables most closely associated with risk of kidney failure include nephrotic-range proteinuria (>3.5 g/day), low kidney function at time of diagnosis [estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2], and <50% reduction in nephrotic-range proteinuria after 6 months of conservative/nonimmunosuppressive therapy (11). The 2021 Kidney Disease Improving Global Outcomes (KDIGO) guidelines advise consideration of immunosuppressive treatment in those at risk for kidney failure (11).

DISEASE MANAGEMENT

Supportive treatment for MN is similar to that for most other proteinuric kidney diseases, namely diuretics for fluid retention, RAAS (renin-angiotensin-aldosterone system) blockade for proteinuria reduction, statins in selected cases for cholesterol and proteinuria reduction, blood pressure control, and lifestyle modification (smoking cessation, weight loss, heart-healthy diet). Although SGLT2 (sodium glucose cotransporter 2) inhibitors are associated with proteinuria reduction and eGFR stabilization in the larger category of nondiabetic proteinuric kidney disease, their specific benefit in MN needs further investigation (12).

Management decisions beyond such supportive treatment rely on the suspected etiology responsible for the histologic pattern of MN. Historically, MN was designated "idiopathic" when there was no clinical evidence of another association that might have secondarily led to disease. Secondary associations include systemic autoimmune diseases such as lupus, medications or toxic exposures [e.g., nonsteroidal anti-inflammatory drugs (NSAIDs), mercury salts], solid tumors, and infections such as hepatitis B. In these situations, treatment should be directed at the underlying cause. Idiopathic disease (now better known as primary MN) was presumed to be an autoimmune

response against a target antigen on the podocyte, and therefore definitive treatment has relied on immunosuppression.

Several treatment strategies centered around immunosuppression for treatment of moderate- to high-risk MN have been investigated. None is specific to MN pathogenesis; instead, they dampen the immune response through suppression of T and/or B cells. In recent years, the lead contenders for immunosuppression are (either monotherapy or in combination) glucocorticoids, cyclophosphamide, calcineurin inhibitors, and rituximab. Four major and recent randomized clinical trials (RCTs) have examined these treatments and reported remission rates as high as 80%. In 2017, the GEMRITUX trial (3) compared one cycle of rituximab in combination with non-immunosuppressive antiproteinuric treatment (NIAT) versus NIAT alone in 75 patients with primary MN. There was no difference in the primary outcome of proteinuria reduction at 6 months; however, longer-term follow-up favored rituximab over NIAT with regard to clinical and immunologic disease remission (PLA2R antibody reduction). In 2019, the MENTOR noninferiority RCT (4) evaluated one cycle of rituximab (with additional therapy at 6 months for those in partial remission) versus cyclosporine therapy in 130 patients with MN and nephrotic syndrome. Rituximab was noninferior to cyclosporine for proteinuria reduction at 12 months and superior to cyclosporine for the primary outcome of disease remission at 24 months. In 2021, two RCTs investigated the role of cyclical therapy (cyclophosphamide alternating monthly with corticosteroids for 6 months) versus rituximab. The 2021 the RI-Cyclo pilot RCT (6) investigated one cycle of rituximab versus cyclical therapy in 74 patients with MN and found no difference between the two for the primary outcome of complete remission at 12 months. In contrast, the 2021 STARMEN study (5) compared sequential therapy with tacrolimus followed by one dose of rituximab to cyclical therapy in 86 participants with MN and nephrotic syndrome. Here, cyclical therapy was superior to sequential therapy with tacrolimus and rituximab for the primary outcome of disease remission at 24 months and the secondary endpoint of immunologic remission. Based on these studies, the 2021 KDIGO guidelines (11) recommend either cyclophosphamide-based therapy or rituximab-based therapy for moderate- and high-risk disease, and cyclophosphamide-based therapy for very high-risk disease.

While early treatment and clinical trials relied on a purely clinical outcome (remission of proteinuria, increase in serum albumin, and preservation of eGFR), the identification of target antigens in MN revolutionized the manner in which clinicians and researchers monitor the disease course. The ability to detect and measure specific circulating autoantibodies allows a more rapid assessment of response to treatment, and therefore, quantification of the PLA2R antibody titers has been incorporated as an outcome measure in recent clinical trials and in KDIGO guidelines for clinical care (1, 13).

PLA2R AS THE PROTOTYPICAL TARGET ANTIGEN IN PRIMARY MEMBRANOUS NEPHROPATHY

Discovery

It has become increasingly difficult to remember the days before we had even a single candidate target antigen in what was then known (for this very reason) as idiopathic MN. The decades between the experimental Heymann nephritis rat model of MN, in which the target antigen had been identified as megalin in the 1980s, and the discovery of autoantibodies to PLA2R in 2009 were marked only by the finding that neutral endopeptidase could be a target antigen in rare cases of antenatal maternofetal alloimmune MN (14).

Beck and colleagues used several properties typical of primary MN to identify the first major target antigen (1). Because the IgG subclass that predominated in kidney biopsy was IgG4, they

focused on IgG4 reactivity within MN sera to detect a common band present in protein extracts from normal human glomeruli. The rationale for assuming that the target would be in the normal glomerulus had been established by the paradigm of Heymann nephritis, in which podocyte-expressed megalin was found to lead to in situ formation of immune complexes immediately beneath the podocyte.

Beck et al. (1) then capitalized on the observation that the target antigen (still only characterized as a 180-kDa band, as detected on Western blot) was a glycoprotein and that both the native and the deglycosylated form, which were of distinct molecular sizes, had the same polypeptide backbone that could be queried by mass spectrometry to suggest potential candidates. In addition, the enrichment of the antigen in a microvesicular membrane fraction released from ex vivo sieved human glomeruli allowed substantial enrichment of the protein, which was ultimately found to be the M-type phospholipase A2 receptor (PLA2R) (1, 15). Like megalin in the experimental rat model, PLA2R was found to be expressed by normal human podocytes. In disease, circulating autoantibodies against PLA2R bind to their target on the podocyte, leading to the accumulation of immune complexes containing both the antibody and the antigen.

Pathogenesis

The mechanisms that lead to autoimmunity (loss of tolerance) to PLA2R probably reflect a multi-hit process, much like immunoglobulin A (IgA) nephropathy (16). There is a clear genetic predisposition to the development of primary MN, with a genetic interaction of a locus in or near an enhancer region of the *PLA2R1* gene itself (which may increase expression in certain tissues) and the larger class II major histocompatibility complex locus (17). Variants in the human leukocyte antigen region map to *HLA-DQA1* and *HLA-DRB* loci, creating subtle changes in the peptide binding groove of these antigen presentation molecules that allow presentation of peptides from PLA2R to the immune system. This has recently been confirmed experimentally using PLA2R derived peptides for in vitro binding and stimulation assays (18). The process that leads to presentation of PLA2R by antigen-presenting cells, often in middle age, is not clear, but it may involve tissue inflammation, perhaps originating in the upper airways and lungs.

Autoantibodies (anti-PLA2R) of various specificities and subclasses arise by epitope spreading and maturation of the humoral response (19). With increasing amounts of autoantibody production, the in situ immune complex formation due to presumed shedding of the antigen-antibody complexes from the basal cell surface causes gradual accumulation of the deposits beneath the podocyte (i.e., in a subepithelial position). It is not clear why this occurs in discrete areas, leading to large complexes in some locations but not others. It is important to note that these antigen-containing immune complexes grow over time, enriching the antigens in these deposits (20). Pathologically, these areas serve as factories for the generation of complement activation products, which lead to the insertion of the membrane attack complex C5b-9 into the membrane of the podocytes, as well as the generation of anaphylotoxins C3a and C5a, which appear to activate their cognate membrane receptors of the podocyte (7).

The accumulation of a particular antigen, which seems to be a common process in all subtypes of MN, has been utilized for another purpose—the identification of the enriched target antigen in previously unknown forms of MN. Laser capture microdissection (LCMD) of human glomeruli from tissue sections taken from human MN kidney biopsies allows for the detection by mass spectrometry (MS) of proteins that are present in excess of what is typically found in a normal MN glomerulus. As proof of principle, Sethi et al. showed that PLA2R is specifically enriched by this LCMD-MS technique, but only in cases of PLA2R-associated MN and not in other forms of MN (21). The finding of an unexpectedly enriched protein allows identification of other novel

target antigens, and this technique has been useful for the identification of many of the target antigens discussed in this review.

Clinical Implications

The clinical utility of the identification of PLA2R relates to the newfound ability to detect and measure the titer of anti-PLA2R antibodies in the circulation. Not only is this a specific way to diagnose the presence of PLA2R-associated MN (13), but changes in the titers over time are predictive of the subsequent clinical course (22, 23). Decline and disappearance of anti-PLA2R are a necessary finding before there is significant clinical remission. In a similar fashion, increasing anti-PLA2R predicts a worsened or prolonged clinical course of MN. Monitoring of antibody titers (looking for decline and disappearance) is useful to suggest a spontaneous remission or effective immunosuppression. As an example, anti-PLA2R levels that decrease at 3–6 months after treatment with rituximab and continue to disappear suggest successful treatment (24). A level that stalls at only a 50–60% drop suggests the need for further treatment with the same agent, or a switch to another type of immunosuppression.

There is much clinical experience with the PLA2R and anti-PLA2R system in the diagnosis and monitoring of this major form of MN. Indirect immunofluorescence and enzyme-linked immunosorbent assays are commercially available for PLA2R antibody detection. Current clinical guidelines incorporate PLA2R antibody titers for MN risk stratification, as do various diagnosis, treatment, and monitoring algorithms (11). The clinical utility of PLA2R antibody testing alone for disease diagnosis is controversial, but it is currently reasonable to forgo kidney biopsy only in PLA2R-positive, nondiabetic patients with normal kidney function, in whom no immunosuppression is planned (11, 13).

The hope is that the more recently described antigen-antibody systems (see below) will have the same impact for individuals afflicted with these rarer forms of MN. Several factors have limited early translation, including the only-recent finding of these systems, the rarity of the subtypes (which may limit commercial development of a multitude of serologic assays), and the limited demonstration—often due to very small cohorts of patients—that the newer autoantibodies can have the same prognostic impact on clinical management as do anti-PLA2R antibodies.

PATHOLOGY

From a pathologic perspective and prior to identification of target antigens, kidney biopsies from patients with primary MN were characterized by IgG4 (co)dominant, diffuse and global subepithelial immune deposits without deposits in other locations, a logical morphologic correlate to an antigen-antibody reaction involving a podocyte antigen. As described above, in 2009, these were identified as closely associated with the PLA2R antigen in most cases. In contrast, secondary MN associated with an underlying condition often displayed segmentally (rather than globally) distributed immune deposits, or deposits in other locations such as mesangial, subendothelial, or extraglomerular immune locations. Differences in immunoglobulin (i.e., IgG1 rather than IgG4 dominant) or complement composition by immunofluorescence were also observed.

Growth in the field of non-PLA2R antigens rapidly accelerated around 2018 with the use of LCMD-MS of glomeruli from formalin-fixed paraffin-embedded kidney biopsies, in addition to multipronged serologic techniques for identifying circulating autoantibodies, which together allowed identification of the abundant proteins and antigens in various types of primary and secondary MNs. Utilizing immunohistochemistry and larger cohorts, this antigen-based system has complemented knowledge of morphologic features of primary and secondary MNs and enabled observations linking certain MN antigens, specific underlying etiologies, and clinical

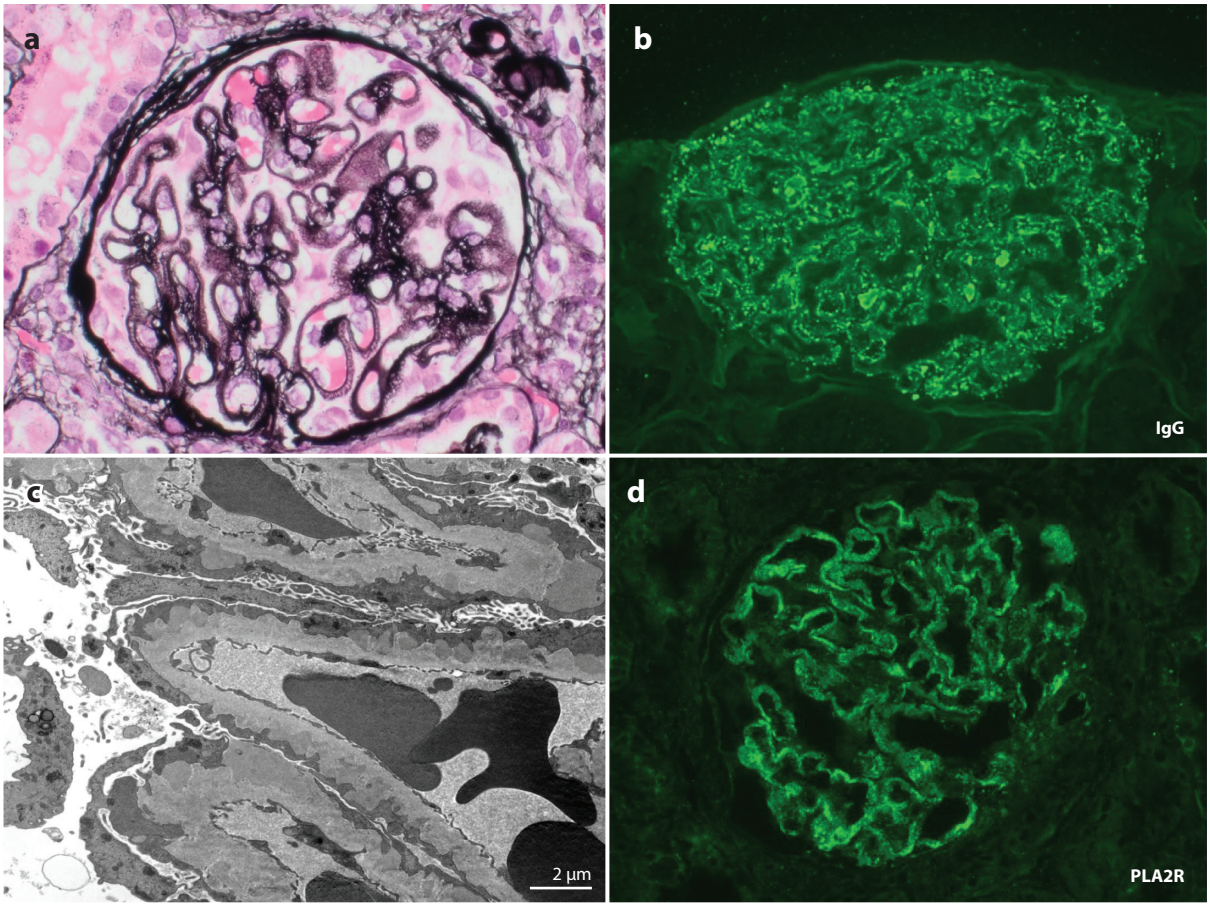

Figure 1

Phospholipase A2 receptor (PLA2R)-associated membranous nephropathy is characterized by (*a*) global "stiff" appearing capillary loops, lucencies in the glomerular basement membrane, and subepithelial "spikes" (Jones silver stain, 400×); (*b*) diffuse granular capillary wall staining for immunoglobulin G (IgG) by immunofluorescence; and (*c*) widespread subepithelial immune deposits with associated diffuse podocyte foot process effacement (transmission electron microscopy, 1900×). No extraglomerular, subendothelial, or mesangial deposits are present. (*d*) Glomerular deposits express the PLA2R antigen.

outcome. It is noteworthy that many of the recently described antigens in MN tend to be proteins highly expressed in the nervous system (25–28), though the mechanisms of autoantibody development remain unknown. Descriptions of the clinicopathologic features of PLA2R (**Figure 1**) and non-PLA2R MN antigens are summarized below and in **Table 1**.

ADDITIONAL ANTIGENS IN MEMBRANOUS NEPHROPATHY

THSD7A

The second MN antigen, identified in 2014, was thrombospondin type-1 domain containing 7A (THSD7A). This form of MN represents 2–5% of MN cases and 8–14% of PLA2R-negative MN cases (29, 30). As with PLA2R, circulating anti-THSD7A antibodies are identifiable in affected patients, and animal models have confirmed that THSD7A autoantibodies are pathogenic and

Table 1 Summary of recently described membranous nephropathy antigens

Antigen	Clinical association	Incidence	Pathology features	Circulating autoantibody detected?
PLA2R	"Primary" with infrequent underlying conditions	70–80% of presumed "primary" MN	Diffuse subepithelial immune deposits, IgG4 (co)dominant	Yes, and commercially available
THSD7A	Malignancy in ~11–20%; "primary"	2–5% of MN; 8–14% of PLA2R-negative MN	Subepithelial immune deposits	Yes
NELL1	Malignancy in 0–33%; lipoic acid use; traditional indigenous medicines including with high mercury content; GVHD; sarcoid	4–6% of MN; 6–21% of PLA2R-negative MN	Segmental subepithelial immune deposits, IgG1 dominant	Yes
Protocadherin 7	Not identified; autoimmune serologies or disease in 4 of 10 reported patients	5.7% of PLA2R-negative MN	Subepithelial deposits, occasional TRIs	Yes
HTRA1	None identified	3.3% of PLA2R-negative MN	Global subepithelial, IgG4 dominant	Yes
Exostosin 1/2 (EXT1/2)	SLE and underlying autoimmunity; possibly better outcome than EXT1/2-negative lupus MN	17–33% of lupus membranous nephritis	Concurrent class 3/4 lupus nephritis in 25%	Not yet reported
NCAM1	Mainly SLE, possible enrichment for neuropsychiatric symptoms	7% of lupus membranous nephritis	Concurrent proliferative lupus nephritis in 25%	Yes
TGFBR3	Mainly SLE	6% of lupus membranous nephritis	Concurrent proliferative lupus nephritis in 30%	Not yet reported
Semaphorin 3B	Children, particularly ≤2 years old	Up to 1% of MN and 5–10% of childhood MN	Global subepithelial, TBM deposits, IgG1 dominant, occasional TRIs	Yes
Netrin G1	None identified	0.2% of MN	Subepithelial immune deposits, IgG4 (co)dominant	Yes
FAT1	HSCT, GVHD	Uniquely in HSCT setting	Subepithelial and TBM immune deposits, IgG4 (co)dominant	Yes
Contactin 1	Chronic inflammatory demyelinating polyneuropathy	Uniquely in inflammatory neuropathies	Subepithelial immune deposits, IgG4 dominant	Yes, can also bind myelinated neural tissue
NDNF	Syphilis	6 of 8 reported patients had syphilis	Stage I subepithelial immune deposits, IgG1 dominant	Yes

Abbreviations: GVHD, graft-versus-host disease; HSCT, hematopoietic stem cell transplant; HTRA1, high-temperature requirement A serine peptidase; NCAM1, neural cell adhesion molecule 1; NDNF, neuron-derived neurotrophic factor; NELL1, neural epidermal growth factor-like 1; PLA2R, phospholipase A2 receptor; SLE, systemic lupus erythematosus; TBM, tubular basement membrane; TGFBR3, transforming growth factor beta receptor 3; THSD7A, thrombospondin type-1 domain containing 7A; TRIs, tubuloreticular inclusions.

induce disease upon binding to the antigen on the podocyte (30–32). Although initially reported as a "primary" MN (29) with coexpression of IgG4, the incidence of neoplasms that are recent or identified soon after biopsy appears higher in THSD7A MN, ranging from 11% (33) to 20% (34) in subsequent cohorts, a rate substantially higher than that seen in PLA2R MN (approximately 4%) (7, 13, 33). Case reports in patients with THSD7A MN have revealed overexpression of the protein by a concurrent malignant (35, 36) or benign (37) tumor. In one of these, overexpression of THSD7A mRNA was also identified in antigen-presenting follicular dendritic cells in a lymph node involved by carcinoma (36), representing a potential mechanism for cancer-associated MN that could be applicable to other MN antigens.

NELL1

Neural epidermal growth factor-like 1 (NELL1) MN (**Figure 2**) is seen in 4–6% (33, 38) of MN cases, or 6–21% (27, 33) of PLA2R-negative cases, and has been associated with a variety of

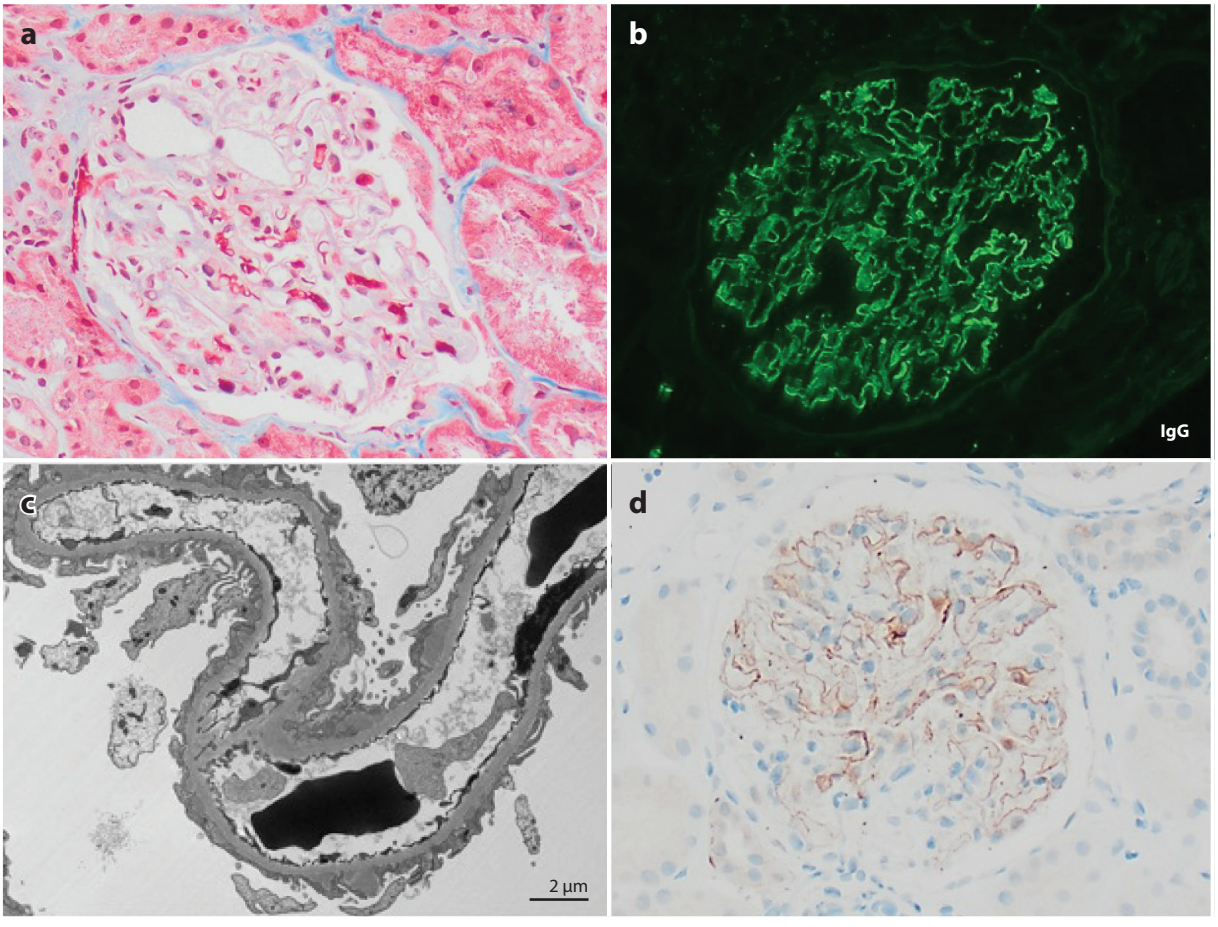

Figure 2

Neural epidermal growth factor-like 1 (NELL1) membranous nephropathy with (*a*) faint subepithelial fuschinophilic immune deposits (trichrome stain, 400×) and (*b*) granular capillary wall staining for IgG by immunofluorescence. (*c*) Electron microscopy shows segmentally distributed subepithelial immune deposits (1900×). (*d*) Glomerular deposits show corresponding capillary loop staining for NELL1 by immunohistochemistry.

underlying conditions. Depending on the cohort, 0% (39) to 33% (33) of patients with NELL1 MN have an underlying malignancy. We and others have identified an association of NELL1 MN with lipoic acid use (40, 41), with resolution of proteinuria in patients who discontinue lipoic acid (40). Other over-the-counter medications and traditional indigenous medicines including those with a high mercury content have been associated with NELL1 MN (42). The NELL1 antigen has been identified in up to 22% of patients with graft-versus-host disease (GVHD)–associated MN (43) as well as sarcoid-associated MN (44). Pathologically, NELL1 MN often has segmentally distributed subepithelial immune deposits, which are IgG1 dominant; focal extraglomerular deposits are seen in a minority of cases. Circulating anti-NELL1 antibodies are present in serum (27, 33), and these patients have a higher rate of clinical remission (nearly 70%) than patients with other forms of MN.

Protocadherin 7

Protocadherin 7 (PCDH7) appears to represent 1.6–2% of MN cases, or 5.7% of PLA2R-negative cases, and is more common in males (25). Positive autoimmune serologies and/or autoimmune disease—including Sjögren's syndrome and sarcoidosis—were identified in 4 of 14 reported patients, and neoplasms in 3 of 14 patients, but clinical associations are otherwise unknown.

HTRA1

Serine protease high-temperature requirement A (HTRA1) was identified as an apparently primary MN from a multi-institutional cohort and accounts for 3.3% of PLA2R-negative MN cases (45). Grouping HTRA1 with the historic categorization of a primary MN, patients in the initial cohort generally lacked underlying malignancy or infection, and immune deposits were often global, subepithelial, and IgG4 dominant (45).

Membranous Lupus Nephritis–Associated Antigens

Membranous lupus nephritis may exist with or without proliferative or sclerosing features of lupus nephritis. Given the recent identification and lack of clinical use of these markers on large cohorts, neither differences in clinical behavior nor the specificity of one of these MN antigens to serve as a harbinger for subsequent development of systemic lupus erythematosus has been established.

EXT1/2

Exostosin 1 and exostosin 2 (EXT1/2)–associated MN (**Figure 3**) is seen in cases of class V (membranous) lupus nephritis and in patients with underlying autoimmunity (21). Among patients with known lupus and membranous lupus nephritis, EXT1/2 is positive in glomerular deposits in approximately 17% (38) to 33% (46) of cases. These patients are generally younger, have lower serum creatinine, are more likely to have proteinuria $\geq$3.5 g, and demonstrate less chronic injury on biopsy than EXT1/2-negative patients. EXT1/2-positive membranous lupus nephritis patients may have a better clinical outcome than EXT1/2-negative patients, although on multivariable analyses in one study, the predictors of ESKD were male sex and global glomerulosclerosis $\geq$25% (46).

NCAM1

Like EXT1/2, neural cell adhesion molecule 1 (NCAM1) is seen particularly in the setting of lupus nephritis with a possible enrichment for patients with neuropsychiatric symptoms (47). NCAM1 may also be seen in 2% of apparently primary MN.

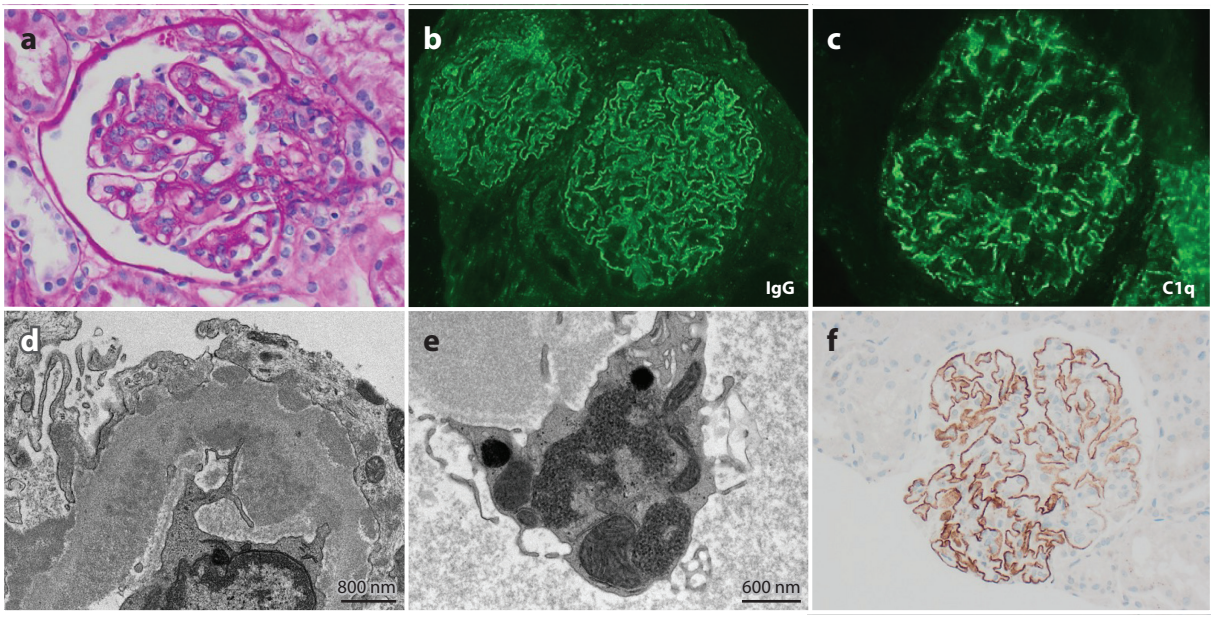

Figure 3

Exostosin 1 and exostosin 2 (EXT1/2)–associated membranous nephropathy in a patient with focally proliferative and membranous lupus nephritis (class IIIA + V), with (*a*) "stiff" appearing capillary loops with focal endocapillary hypercellularity by light microscopy (periodic acid Schiff stain, 200×). By immunofluorescence, there is "full house" granular capillary wall and mesangial staining including (*b*) IgG [also with a speckled "tissue ANA" (antinuclear antibody) staining pattern] and (*c*) C1q. By electron microscopy, (*d*) diffuse subepithelial and occasional mesangial deposits are apparent (6800×), as are (*e*) endothelial tubuloreticular inclusions (9300×). (*f*) Glomerular deposits are positive for EXT1/2 by immunohistochemistry.

TGFBR3

Transforming growth factor beta receptor 3 (TGFBR3)–associated MN is seen in a similar proportion of membranous lupus nephritis patients (6–7%) to NCAM1. The percentage of TGFBR3–associated cases showing concurrent proliferative features (25–29%) is similar to that of both EXT1/2- and NCAM1-positive membranous lupus nephritis (38).

Semaphorin 3B

Semaphorin 3B was described as a target antigen in a multi-institutional MN cohort. It appears to be more common in children, including those ≤2 years of age (26), representing up to 1% of MN cases and 10% of childhood MN cases (38). In another series of childhood MN, PLA2R and EXT1/2 were the most common antigens identified, with semaphorin 3B representing 5% of childhood MN cases (48). Circulating anti–semaphorin 3B antibodies are identified in affected patients, and the disease can recur early posttransplant (49).

Netrin G1

Anti-netrin G1 antibodies and corresponding netrin G1–positive glomerular subepithelial immune deposits have been identified in rare cases of MN (3 cases within international cohorts, approximately 0.2% of MN cases and 0.4% of PLA2R- and THSD7A-negative cases), without known clinical associations to date (50).

FAT1

The protocadherin FAT1 antigen appears uniquely associated with MN that develops in patients with prior hematopoietic stem cell transplant and GVHD (51).

Contactin-1

Contactin-1 represents a potential target antigen in MN associated with inflammatory neuropathies and has specifically been identified in patients with chronic inflammatory demyelinating polyneuropathy (CIDP) (52, 53). Although representing a small subset of MN cases, contactin-1 is particularly intriguing as it identifies a probable shared antigenic target between nerves and podocytes in patients with CIDP. Specifically, anti-contactin-1 antibodies are identified in the sera of affected patients, the contactin-1 antigen is seen in glomerular subepithelial immune deposits (generally IgG4 dominant), and eluted IgG from the kidney binds paranodal (myelinated) neural tissue and colocalizes with contactin-1 (52).

Ongoing Antigen Discovery in Membranous Nephropathy

Application of antigen testing to MN with known underlying conditions is underway with the identification of neuron-derived neurotrophic factor in syphilis-associated MN (54) and of PCSK6 (proprotein convertase subtilisin/kexin type 6) in NSAID-associated MN (55). Other recently discovered minor antigens with no known clinical associations to date include FCN3, CD206, EEA1, SEZ6L2, NPR3, MST1, VASN, CRIM1, EFEMP2, FLRT3, FRAS1, IDE, NLGN3, PGLYRP1, RECK, and SULF1 (2, 56). As detailed above, secondary etiologies do not necessarily align with one antigen, and heterogeneity of MN antigens has also been described in patients with sarcoidosis-associated MN (including PLA2R in 47%, NELL1 in 22%, and others) (44).

HOW NOVEL ANTIGEN DISCOVERY WILL CHANGE CLINICAL PARADIGMS

Incorporating the rewards of progress can benefit from a healthy dose of skepticism, and it should be noted that the clinicopathologic information for most of the non-PLA2R antigens currently comes from relatively few patients. Better data are available for the more common antigens—THSD7A and NELL1—but even among these, the percentage of MNs that are secondary and the strength of associations to specific underlying conditions vary by cohort. Although a few antigens appear uniquely associated with a specific condition, others are associated with multiple underlying etiologies. Similarly, one disease may be associated with a variety of MN antigens. Thus, from a practical standpoint, a diagnosis of a PLA2R-negative MN may prompt a somewhat more precise but overall relatively similar initial clinical workup to exclude secondary etiologies—malignancy, autoimmune disease, infection, drug exposure—as it did prior to identification of many non-PLA2R antigens. Conversely, in the setting of a known secondary etiology, the MN antigen may be of questionable additional clinical value, at least in the absence of established differences in prognosis, response to therapy, or available serologic monitoring. Although circulating autoantibodies to various non-PLA2R antigens have been identified in research laboratories, many must still undergo larger-scale validation required to demonstrate the sensitivity and specificity required for regular clinical use. Thus, while the clinical incorporation of non-PLA2R MN antigen testing may follow the general course of PLA2R, their trajectories will likely differ related to lower overall prevalence of disease in the population and higher rate of confounding variables.

Despite these current limitations, we anticipate that discovery of putative antigens will have some impact on patient care. Several of the newly discovered podocyte antigens are associated

with detectable circulating antibodies, similar to that seen in PLA2R-associated MN. Efforts to correlate circulating antibody titers with disease activity, remission status, and disease recurrence are underway. If successful, then MN may increasingly be detected and monitored through blood tests. Such noninvasive serum antibody testing may become part of a broader diagnostic panel for nephrotic syndrome and potentially reduce the need for diagnostic kidney biopsy in seropositive nephrotic patients. Furthermore, because immunologic remission generally precedes clinical remission, following circulating antibody titers may negate the need to reflexively immunosuppress patients with persistent proteinuria after disappearance of circulating antibodies.

Further, we project that clinical outcomes of MN will improve as our understanding of antigens and associated underlying conditions deepens. For example, supplements, including lipoic acid and mercury-containing compounds, are associated with NELL1 MN. In most cases, discontinuation of these offending agents leads to disease remission, generally without the need for immunosuppressive therapy. Further investigating the strength of reported clinical associations and the mechanisms of disease pathogenesis will help inform whether treating the underlying systemic condition or eliminating the drug/toxin exposure alone will lead to MN remission. Additionally, knowing which MN antigen types are most associated with disease recurrence will impact length of treatment and frequency of medical follow-up. Along these lines, recurrence of MN after kidney transplantation may also differ by MN antigen, and we anticipate that understanding these clinical correlations may influence posttransplant care. Finally, any novel antigen-specific therapies (e.g., induction of tolerance or targeting the pathogenic B cell or plasma cell population) that is successfully developed for the more common PLA2R MN could be adapted for treatment of the less common forms of MN.

In summary, novel antigen discovery in MN has revolutionized our understanding of the disease and redefined its categorization. As with PLA2R-associated MN, we project that several of the newly discovered target antigens will have a significant beneficial impact on patient care through the incorporation of noninvasive diagnosis and targeted evaluation of associated disease.

DISCLOSURE STATEMENT

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