Published in final edited form as:

Semin Nephrol. 2013 November ; 33(6): 531–542. doi:10.1016/j.semnephrol.2013.08.004.

The role of complement in membranous nephropathy

Hong Ma, PhD [Research Assistant Professor], Dana G. Sandor, MD [Post-Doctoral Fellow in Nephrology], and Laurence H. Beck Jr., MD, PhD* [Assistant Professor of Medicine]

Department of Medicine, Renal Section, Boston University School of Medicine

Abstract

Membranous nephropathy (MN) describes a histopathological pattern of injury marked by glomerular subepithelial immune deposits and collectively represents one of the most common causes of adult nephrotic syndrome. Studies in Heymann nephritis, an experimental model of MN, have established a paradigm in which these deposits locally activate complement to cause podocyte injury, culminating in cytoskeletal reorganization, loss of slit diaphragms, and proteinuria. There is much circumstantial evidence for a prominent role of complement in human MN, as C3 and C5b-9 are consistently found within immune deposits. Secondary MN often exhibits the additional presence of C1q, implicating the classical pathway of complement activation. Primary MN, however, is IgG4-predominant and IgG4 is considered incapable of binding C1q and activating the complement pathway. Recent studies have identified the M-type phospholipase A₂ receptor (PLA₂R) as the major target antigen in primary MN. Early evidence hints that IgG4 anti-PLA₂R autoantibodies can bind mannan-binding lectin and activate the lectin complement pathway. The identification of anti-PLA₂R antibodies as likely participants in the pathogenesis of disease will allow focused investigation into the role of complement in MN. Definitive therapy for MN is immunosuppression, although future therapeutic agents that specifically target complement activation may represent an effective temporizing measure to forestall further glomerular injury.

Keywords

membranous nephropathy; phospholipase A₂ receptor; PLA₂R; complement; IgG4

Introduction

Membranous nephropathy (MN) is an immune-mediated glomerular disease and one of the most common causes of the nephrotic syndrome in adults. The term membranous

© 2013 Elsevier Inc. All rights reserved.

*Corresponding Author: Renal Section, 5th floor, Room 504, Evans Biomedical Research Center, 650 Albany Street, Boston, MA 02118, (617) 638-7330, lhbeckjr@bu.edu.

Financial disclosure and conflict of interest statements:

None

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

nephropathy does not refer to a single disease, but rather to a histological pattern shared by a number of separate etiologies. Biopsy features characteristic of MN include the triad of capillary wall thickening visualized by light microscopy (LM), often with spikes and pits revealed by the Jones silver stain ¹, electron-dense subepithelial immune deposits by electron microscopy (EM) ², and a fine granular, peripheral capillary loop staining for IgG by immunofluorescence (IF) ³. Of particular relevance to this article, the IgG is nearly always accompanied by the complement component C3 in the same fine granular pattern by IF.

Membranous nephropathy is a rare disease; its incidence in the Western world is estimated at 1.2 / 100,000 persons/year ⁴. It commonly presents as the nephrotic syndrome, often with a more insidious onset than minimal change disease or primary focal and segmental glomerulosclerosis, which tend to have a more explosive onset. Approximately 20% of the MN cases present with non-nephrotic proteinuria. MN also exists in pediatric populations, but it is a much less frequent cause of the nephrotic syndrome ⁵. Initial manifestations of the disease are related to the nephrotic syndrome: proteinuria, hypoalbuminemia, hyperlipidemia, and edema. MN is more likely than other causes of the nephrotic syndrome to lead to venous thromboembolic events such as deep vein or renal vein thrombosis, and pulmonary embolism ⁶. Although MN may spontaneously remit without treatment, as many as one third of patients have progressive loss of kidney function and may progress to end-stage renal disease (ESRD) at a median of 5 years after diagnosis. In patients who have been transplanted after having reached ESRD due to MN in their native kidneys, the disease can recur, either in a subclinical or overt form, in up to 40% of patients ⁷.

Primary and Secondary MN

Approximately 25% of MN cases, collectively referred to as secondary MN, are associated with a variety of systemic diseases, infections, medications, or exposures (Table 1). The most common secondary causes in the U.S. include systemic lupus erythematosus (SLE), hepatitis B infection, NSAIDs, and malignancy. The remaining 75% of MN cases have traditionally been designated as primary or “idiopathic” MN. Recent investigations from our laboratory have demonstrated that greater than 70% of primary MN cases are associated with circulating autoantibodies directed against the M-type phospholipase A2 receptor (PLA₂R1), a 180-kDa transmembrane glycoprotein expressed on glomerular podocytes (see below) ⁸. Anti-PLA₂R antibodies as well as the presence of the PLA₂R antigen within subepithelial deposits on biopsy are quickly becoming established biomarkers of primary disease. There remains a significant minority of cases of apparent primary MN in which the target antigen remains unidentified.

Distinguishing primary from secondary MN is not always straightforward. The finding of histopathologic features typical of MN with a well-established secondary cause such as lupus or hepatitis B infection is often enough to label that particular case secondary MN. When MN is found in association with other, less common diseases, it is not unusual for the association to be reported in the medical literature, which has led to long tables of secondary associations found in many nephrology textbooks and reviews (see for example the recent KDIGO Clinical Practice Guideline on Glomerulonephritis ⁹). However, a true causal

relationship is not often established and the possibility of two separate but coincidental diseases cannot be easily ruled out.

Despite similar clinical features, the distinction between primary and secondary MN can sometimes be suggested by histopathology, with atypical lesions more frequent in secondary forms. The immune deposits in primary MN tend to be subepithelial and paramesangial exclusively, whereas additional subendothelial and mesangial deposits are more likely to be found in secondary MN. Lupus- and HIV-associated MN may additionally be associated with tubuloreticular inclusions in endothelial cells, a histologic signature of the interferon response ¹⁰.

Important for the subsequent discussions in this article, there are also differences between primary and secondary MN in terms of the immunoglobulins found within the immune deposits as assayed by IF. IgG is the predominant immunoglobulin found within immune deposits in primary MN. Secondary forms, especially lupus MN, may reveal IgG, IgA, and IgM by IF. IgG subclasses can also help to distinguish primary from secondary MN, although most renal pathology services do not routinely characterize the IgG subclasses. Primary MN is characterized by IgG4-rich deposits, at times including IgG1, especially very early in the disease process ^{11,12}. In contrast, IgG1, IgG2 and IgG3 are the predominant forms in secondary MN deposits ^{13,14}. Consistent with the inability of the IgG4 subclass to bind and activate the classical complement pathway, C1q is usually absent or found at very low levels in primary MN, while it is more typically present in secondary disease. The finding of a “full house” pattern by IF (*i.e.*, the presence of IgG, IgM, IgA, C3, and C1q) is very specific for secondary MN ¹⁵.

Mechanisms of immune deposit formation

There are several potential mechanisms by which subepithelial immune deposits might form in MN. An intrinsic glomerular antigen located on the foot process of the podocyte can serve as the target antigen for circulating antibodies and lead to *in situ* deposition of immune deposits. This appears to be the main mechanism of deposit formation both in primary human MN and the rat experimental model of Heymann nephritis (see below). In secondary MN, the target antigen is typically extrinsic to the glomerulus. Circulating antigens from a variety of sources may accumulate in the subepithelial space as so called “planted” antigens ¹⁶. In an experimental model of MN, rabbits injected with cationized bovine serum albumin (cBSA) subsequently developed subepithelial deposits and proteinuria ^{16,17}. Since cBSA has low immunogenicity in rabbits and stimulates formation of low affinity antibodies, uncomplexed cBSA can traverse the GBM due to electrostatic interactions ^{18,19}. Subepithelial immune deposits form later, due to antibodies binding this exogenous antigen that has been “planted” in the subepithelial space. Observational studies have uncovered a similar form of cBSA-induced MN in infants who are exposed to this modified dietary antigen ²⁰. Similar electrostatic interactions have been hypothesized for the subepithelial deposition of histone-rich nucleosomes in lupus associated MN ²¹.

A third potential mechanism involves deposition of pre-formed circulating immune complexes (CIC) in a subepithelial position. In MN secondary to hepatitis ²², immune

complexes are observed both in the blood and in GBM. There are also CIC in SLE and lupus nephritis; however, it is not clear why the majority of lupus nephritis involves proliferative glomerulonephritis with primarily mesangial and subendothelial immune complex deposition, whereas class V (“membranous”) lupus nephritis is marked by mainly subepithelial deposits. Some have suggested that the physicochemical properties of the CIC and the affinity or avidity of the antibody-antigen interaction would allow initial deposition in a subendothelial position, with rapid dissolution and reformation on the abluminal side of the GBM.

The Heymann nephritis model of MN

In 1959, Heymann introduced an experimental rat model of MN²³, now known as active Heymann nephritis (HN), that remains the cornerstone of all the investigations towards the understanding of this disease. Lewis rats were immunized by the intraperitoneal route using homologous renal homogenate, together with complete Freund’s adjuvant. The resulting nephropathy was virtually identical to human MN both clinically and histopathologically, with the characteristic triad of LM, IF, and EM indicating formation of subepithelial immune complexes. Subsequent work in this model limited the antigenic activity to a tubule-rich fraction called Fx1A, and, even more specifically, to a tubular brush border fraction termed RTE 5. Fx1A formed immune complexes in the blood, initially suggesting that HN was caused by deposition of CIC. However, human-derived Fx1A was also able to trigger HN in susceptible rats, yet the immune deposits that formed in this model contained only the rat antigens, raising the possibility that HN results not from CIC, but rather due to an *in situ*, autoimmune process²⁴.

Heterologous antibodies against rat renal homogenates were also generated in sheep and rabbits. Passive immunization of rats with these heterologous antibodies could still produce HN, thus excluding cellular immunity as a major component of the disease process²⁵. This latter model, known as passive Heymann nephritis (PHN), is able to generate small subepithelial immune deposits within hours, again suggesting the presence of an intrinsic glomerular antigen. Two research groups independently demonstrated, using isolated or perfused rat kidneys *ex vivo*, a “fixed antigen” located on podocytes^{26,27} and supporting an *in situ* and CIC independent mechanism of immune complex formation. Decades later, the antigen was identified as megalin^{28,29} and its chaperone, receptor associated protein (RAP)³⁰. The fact that HN antigens are located on the podocyte membrane further supported the hypothesis that MN is an autoimmune disease that targets the podocyte.

Heymann nephritis and the paradigm of complement-mediated podocyte injury

Decades of work in the PHN model, as well as with cultured rat glomerular epithelial cells (GEC), have led to a paradigm of how subepithelial immune deposits lead to podocyte injury and proteinuria. Complement-mediated cytotoxicity plays a paramount role in this paradigm, especially the effector function of the terminal complement components C5b-9, also known as the membrane attack complex (MAC). C5b-9 is a macromolecular complex whose formation is initiated by the proteolytic cleavage of C5 to C5b, which subsequently

combines with C6 and C7 to form the C5b6,7 complex and then binds C8 and multiple C9 molecules. Once the full C5b-9 complex forms, it inserts into the lipid bilayer of cell membranes, forming a channel or leaky patch. Although this event is lethal to non-nucleated cells such as red blood cells, membrane insertion of C5b-9 in nucleated cells such as podocytes causes sublethal injury.

Early studies in HN showed the presence of C3 and C5b-9 that co-localized with the immune deposits. Podocytes from the urine of passive HN rats are coated with C5b-9 membrane attack complex³¹, paralleling the finding of membrane attack complexes in the urine of MN patients³². Progression of disease was correlated with ongoing urinary excretion of C5b-9, suggesting continued activation of complement at the GBM. In PHN, depletion of serum complement factor C3 by daily cobra venom injection prevented proteinuria, despite the subepithelial deposition of IgG³³. Consistent with this induced C3 deficiency, the terminal complement components C5b-9 were also absent from the capillary wall deposits³⁴. Similarly, immune deposits induced by anti-Fx1A derivatives unable to activate complement, such as Fab' fragments or the gamma2 fraction of sheep anti-Fx1a, did not result in proteinuria. The critical role of the C5b-9 in causing podocyte injury in this model was first established in isolated PHN rat kidneys perfused with human plasma deficient in C8, an essential component of the MAC³⁵. Furthermore, depletion of C6 with anti-C6 antibodies during PHN induction prevented the development of proteinuria³⁶. In aggregate, these studies demonstrated that complement activation and formation of the MAC is a critical step in causing podocyte injury and proteinuria in this model.

In addition to developing antibodies against megalin and RAP, rats actively immunized with Fx1A also developed antibodies against complement inhibitors present in the antigenic fraction, such as complement receptor 1-related protein y (Crry)³⁷. Such antibodies were shown to neutralize the local complement inhibitors and allow amplification of the complement cascade by unregulated activity of the alternative pathway. Chromatographic depletion of Crry from the Fx1A antigenic fraction prevented the formation of anti-Crry, which allowed full expression of complement regulatory activity and forestalled the formation of C3 deposits and proteinuria without affecting the formation of subepithelial immune deposits. When such rats that had been immunized with Crry-depleted Fx1A were either additionally immunized with recombinant Crry or later passively infused with heterologous anti-Crry antibodies, they become proteinuric similar to the original HN model³⁸. These results indicate that disease activity in MN may depend not only on complement activation, but also on the balance of promoters and inhibitors of ongoing complement activity.

It is known that nucleated cells can survive limited complement injury. Typical defense mechanisms against complement-mediated cytotoxicity include endocytosis and membrane shedding of the MAC³⁹. Such limited injury has been experimentally achieved *in vitro* by exposing GEC to complement-fixing anti-Fx1A heterologous antibodies and a source of active (vs. heat-inactivated) complement factors^{40,41}. This *in vitro* model of PHN has been used in numerous studies to delineate signaling pathways and cellular changes that are due to complement-mediated cytotoxicity. The reader is directed to Table 2 and the chapter by

Takano and colleagues in this issue of *Seminars in Nephrology* for a much more detailed analysis of the proposed cellular mechanisms of complement-mediated injury.

Based on these observations in animal models and *in vitro* systems, a paradigm has emerged that is felt to recapitulate the pathogenesis of human disease. Subepithelial immune complex deposition leads to local complement activation and insertion of the MAC into the podocyte membrane. Of note, the anaphylotoxins and chemoattractants C3a and C5a, as they are formed abluminally, do not lead to the recruitment of inflammatory cells. Given this absence of inflammation, the term membranous nephropathy or glomerulopathy is more accurate than the widely used misnomer “membranous glomerulonephritis.” Because the podocyte is able to protect itself from the ongoing complement-mediated injury by shedding of the MAC, it is not lethally injured. However, a number of signaling pathways are activated (Table 2) that lead to a marked change in podocyte phenotype. In essence, the podocyte assumes a more dedifferentiated phenotype in which it cannot maintain its intricate cytoskeletal architecture, leading to foot process simplification, loss of slit diaphragms and cell-matrix adhesions, and consequent proteinuria^{42,43}. In addition, the dedifferentiated podocyte is stimulated to increase secretion of extracellular matrix molecules^{44,45}, leading to expansion of the GBM between and around the immune deposits.

While HN has been tremendously useful in terms of establishing a paradigm for complement-mediated cellular injury in the pathogenesis of MN, it suffers as a definitive model in that megalin is not expressed on human podocytes⁴⁶. In addition, the IgG subclasses that lead to proteinuria in HN are those able to fix complement, while IgG4, the predominant IgG subclass for primary MN in humans, is unable to fix complement, at least by the classical pathway. Susceptibility to HN is confined to specific rat strains and thus precludes the use of the diverse repertoire of genetically-deficient strains of mice that could otherwise be used to dissect the molecular pathways in HN. Murine models of MN do exist, but none has achieved the utility of the HN model.

Evidence for complement activation in human membranous nephropathy

The studies in HN suggested that subepithelial immune complex-induced complement activation plays an important role in the pathogenesis of MN, resulting in massive proteinuria. Evidence for complement activation in human MN comes mainly from clinical observations. In 1984, C3 deposits were first detected in 8/16 patients with primary MN¹¹. Although C3 deposits (predominantly C3c, a short-lived breakdown product of C3 and therefore a marker of ongoing immune deposit formation) were found in only 50% of the cases, patients with glomerular C3 deposits showed more proteinuria than those lacking glomerular C3 deposits, suggesting the association of complement activation with disease severity in human MN. Currently, using more sensitive immunohistological staining, C3c deposition can be detected in almost all cases of MN^{47,48} and increased levels of C3d, a stable breakdown product of C3, are present in about 70% of patients with MN⁴⁹. As previously mentioned, insertion of the C5b-9 MAC in podocytes is a sublethal event, as the complex can be shed from the cell, and cell membrane repair occurs rapidly. C5b-9 inserted into the membrane of podocytes can also be transported intracellularly and extruded into the

urinary space, where it subsequently appears in the urine. C5b-9 is thus a dynamic marker of ongoing immunological injury ⁵⁰.

Another molecule of the complement system which has been more recently related to MN is C4d. C4d is the breakdown product of C4 generated during activation of classical complement or lectin pathways. C4d is highly stable and binds covalently to cell surfaces. The detection of C4d in antibody-mediated rejection by immunostaining has sparked considerable clinical interest recently ⁵¹. The earliest study to suggest a role for C4d in MN demonstrated the presence of C4d and C4b-binding protein (C4bP) in 11 cases (92%) of MN in close association with the glomerular IgG deposits ⁵². Recently, Val-Bernal reported characteristic granular basement membrane deposition of C4d in 100% of primary MN (31 cases) and pure class V membranous lupus nephritis (5 cases), following fixation in formalin, paraffin embedding, and immunoperoxidase-based detection ⁵³. Using the same immunoperoxidase-based method, Espinosa-Hernandez and colleagues observed C4d deposition in the glomerular basement of 100% of MN patients (21 cases), while results were negative in 100% of cases with minimal change disease (19 cases) ⁵⁴. Suzuki and colleagues have noted that any intrinsic staining in normal glomerulus is virtually undetectable by this immunoperoxidase-based method, which suggests that the C4d glomerular deposition detected in the 100% of MN cases is likely not intrinsic but rather indicates pathogenic complement activation ⁵⁵. Other groups, however, used the absence of C4 as an indicator of primary MN ⁵⁶. This discrepancy may be related to the particular antibody and specificity used for the study. Of note, there is a report of membranous (class V) lupus nephritis with C3 and MAC deposits occurring in a patient with hereditary absence of C4 ⁵⁷, perhaps due to activation of the alternative pathway through a mechanism that bypasses C4 ⁵⁸.

Collectively, these observations in MN combined with studies in experimental MN make it clear that the complement system plays a substantial role in the pathogenesis of human MN. A number of studies have tried to address which specific complement pathway is activated in MN or how different complement pathways might cooperate to form MAC and cause proteinuria. Thus far, reports to clarify the precise complement components found within glomerular deposit have not been consistent, possibly due to the different technologies applied or to different mechanisms underlying a common phenotype of MN. Most reports, however, agree that IgG4 is the predominant IgG subclass in primary MN, that C1q is typically absent, and, at least in recent publications, that C4d is also a universal component of the subepithelial deposits ^{53,54}.

The presence of IgG4 and C4 in the majority of cases of primary MN challenges the paradigm established in experimental HN, as IgG4 is an immunoglobulin unable to activate the classical complement pathway. Because of the typical absence of C1q in primary MN and due to the fact that the alternative pathway alone does not generate C4, the mannan-binding lectin (MBL) pathway has recently emerged as a potential explanation to account for the presence of glomerular C4 in primary MN. Although not routinely assayed on kidney biopsies, several lines of evidence show the occasional presence of MBL in glomerular pathology. MBL has been detected in association with IgA in the mesangial area of patients with IgA nephropathy ⁵⁹⁻⁶¹, and there are even reports of its presence within immune

deposits of patients with primary and secondary MN^{47,62}. Recent work presented at the 2nd International Conference on Membranous Nephropathy in Bergamo, Italy provides further evidence for the presence of IgG4 and MBL in primary MN, as well as the general absence of C1q (Table 3).

The mechanism by which the lectin pathway might be activated in MN is more speculative. MBL typically binds carbohydrate chains terminating in mannose, fucose, or N-acetylglucosamine (GlcNAc), commonly expressed by microbes but not by host mammalian tissues. A report from the rheumatoid arthritis literature⁶³ demonstrated that a proportion of IgG1 molecules lacked a terminal galactose residue on a glycan moiety located on the IgG1 heavy chain. Absence of galactose on both arms of the biantennary N-glycan led to exposure of terminal GlcNAc residues, allowing MBL to directly bind IgG1 and thereby activate complement by the lectin pathway. A hypothesis has been proposed that a proportion of IgA molecules in IgA nephropathy and IgG4 (or other) subclasses in MN may be terminal galactose-deficient and thus be able to bind MBL and activate the lectin complement pathway^{59,64}. The proportion of hypogalactosylated IgG molecules that could potentially bind and activate MBL increases with advancing age⁶⁵, which is an attractive hypothesis for why primary MN typically presents in an older demographic group⁶⁴.

Consistent with the inability of IgG4 to activate the classical complement pathway, no demonstrable deposits of C1q were determined by IF in early reports⁶⁶. However, possibly due to improved sensitivity of detection, more recent studies have showed trace C1q deposits in patients with MN^{12,47}. It should be noted that a recent report suggests that IgG4 may not be the predominant IgG subclass in early deposits of MN¹². By comparing Ehrenreich-Churg stage of MN deposits as assessed by EM with the intensity of IF staining for all four IgG subclasses, IgG1 was demonstrated to be the major subclass in early stage I deposits (64% of cases), whereas IgG4 was predominant in later stages. There was also an inverse relationship between the intensity of glomerular capillary IgG4 and C1q staining. It is therefore possible that IgG1 may initially activate the classical pathway in early disease, with subsequent activation of the lectin and/or alternative pathways during the evolution of the disease course^{67,68}. Treatments targeted to the later stages of complement activation may thus be more effective as therapeutic regimens in primary MN than those targeting any individual pathway.

The predominance of IgG4 in primary MN may have implications other than the question of its capacity to activate a particular complement pathway. IgG4 has two characteristics distinct from other IgG subclasses: a prominent ability to undergo Fab arm exchange⁶⁹ and a non-traditional rheumatoid factor-like activity⁷⁰, both of which may serve to limit immune-mediated pathology *in vivo*. Fab arm exchange occurs by the exchange of a heavy chain and its associated light chain (half molecule) with another heavy-light chain pair from another unrelated IgG4 molecule, producing an asymmetric IgG4 molecule with two distinct antigenic specificities. Because of the dynamic nature of the exchange, these hetero-bivalent antibodies are transient, and under most conditions, IgG4 molecules fail to participate in immune complexes and instead behave as monovalent antibodies. In this manner, IgG4 molecules may limit immune complex formation and play an anti-inflammatory role⁶⁹.

Another property of IgG4 mimics IgG rheumatoid factor (RF) activity by interacting with IgG on a solid support. In contrast to prototypical RF, which binds the IgG Fc segment through its Fab antigen binding region and can create large CIC that massively activate the classical complement pathway, IgG4 binds the IgG Fc domain is through its own Fc segment. In this manner, such Fc-Fc binding may actually prevent inflammatory responses by shielding IgG1 or IgG3 molecules from C1q binding. These interactions appear to be facilitated by antigen-binding; *e.g.*, IgG4 was found to bind to antigen-bound IgG4, but not to IgG4 free in solution ⁷¹. In this manner, it is conceivable that the later predominance of IgG4 within the subepithelial immune deposits of primary MN ¹² might actually represent a protective response that limits cross-linking of antigen(s) and down regulates classical complement activation ⁷². Despite these speculations, the precise role of IgG4 in MN remains undefined.

Human disease: Fetomaternal alloimmune MN

The first intrinsic glomerular protein demonstrated to be a target antigen in human MN was a cell surface protease known as neutral endopeptidase (NEP). In a fascinating case report, a mother genetically deficient in NEP gave birth to an infant who had developed the nephrotic syndrome *in utero*; a kidney biopsy after birth revealed MN ⁷³. This mother had been alloimmunized to NEP during a previous pregnancy which miscarried, generating circulating anti-NEP. These alloantibodies crossed the placenta and targeted NEP on the fetal kidney during her subsequent pregnancy, leading to *in situ* immune deposits and antenatal MN. Biopsy of the infant revealed C3 in the same pattern as the immune deposits. As the inciting antibodies were maternal in nature, the disease resolved once these antibodies were cleared. These investigators have reported several other cases of MN in infants born to mothers deficient in NEP ⁷⁴ and confirmed the presence of IgG1, IgG4, and C5b-9 within the deposits. Consistent with the biopsy findings, most mothers had circulating anti-NEP antibodies of both the IgG4 and IgG1 subclass. Of particular interest is the observation that the single mother who lacked IgG1 anti-NEP and had only low titers of IgG4 anti-NEP gave birth to an infant without detectable proteinuria. The same authors have recently reported another case in which the mother had only IgG1, and not IgG4, anti-NEP and gave birth to a severely affected infant (P. Ronco, American Society of Nephrology Kidney Week 2012). These results would suggest that IgG1, not IgG4, is the pathogenic IgG subclass in this form of MN.

Anti-PLA₂R-associated MN

PLA₂R, a transmembrane glycoprotein expressed by glomerular podocytes, has recently emerged as the leading target antigen in adult primary MN, as circulating anti-PLA₂R antibodies were detected in 70% of a cohort of American MN patients, consisting predominantly of Caucasians and African Americans ⁸. Several studies since then have reported the prevalence of anti-PLA₂R seropositivity in other international populations ⁷⁵⁻⁷⁹, summarized in Table 4. Using a Western blotting method with heightened sensitivity, Qin and colleagues detected anti-PLA₂R autoantibody in as many as 98% of patients with primary MN ⁷⁶. As these autoantibodies are associated with clinically active disease, and decline or disappear with spontaneous or treatment-induced remission, the

difference in prevalence rates in part reflects the percentage with active disease (see Table 4). The target antigen PLA₂R can also be found concentrated within the subepithelial deposits (Figure 1), which serves as a good marker of primary disease, even after immunological remission has been achieved with the disappearance of circulating anti-PLA₂R ⁷⁷.

The pathogenicity of anti-PLA₂R has not been definitively proved, due to a lack of appropriate animal models at the current time. However, collective observations from several independent research groups internationally hint at the direct pathogenicity of anti-PLA₂R autoantibodies. First, PLA₂R is consistently and specifically detected within the immune deposits of primary MN ⁸⁰ and anti-PLA₂R antibodies have been eluted from biopsy tissue of patients with MN ⁸, confirming the glomerular co-localization of antigen and specific anti-PLA₂R antibodies. Second, anti-PLA₂R antibody titers are closely associated with and precede clinical disease activity ⁸¹. Because these autoantibodies are very specific for primary as opposed to secondary MN, even in the setting of identical histological findings and degree of glomerular injury, it appears likely that these antibodies are the cause rather than a consequence of podocyte injury and proteinuria ⁸². Third, patients who are anti-PLA₂R seropositive at the time of transplantation are more likely to develop recurrent MN, suggesting that the recurrence is the result of circulating anti-PLA₂R antibodies binding to PLA₂R antigen on donor podocyte ^{83,84}.

The identification of these likely-pathogenic autoantibodies in the majority of primary MN cases now allows the investigation into the contribution of specific anti-PLA₂R IgG subclasses to disease pathogenesis. In keeping with previous findings in primary MN, anti-PLA₂R autoantibodies are largely IgG4, the least abundant IgG subclass in general, but known to be the predominant subclass in the deposits of primary MN ¹¹. The other three subclasses of circulating anti-PLA₂R tend to be present in variable but lesser amounts ^{8,76}. Given the substantial evidence of complement activation in the glomerular immune deposits in MN, our laboratory has asked whether the MBL complement pathway is a potential pathway by which the IgG4 subclass of anti-PLA₂R could lead to podocyte injury and proteinuria. We have observed, for example, that the purified anti-PLA₂R IgG4 autoantibodies from MN patients have a high ratio of GalNAC to Gal in a terminal position, and thereby can bind to C4 through MBL *in vitro* ⁸⁵.

However, an obligate role of IgG4 in the direct pathogenesis of PLA₂R-related MN is questioned by the occasional absence of IgG4 deposition in glomeruli in some patients with apparent primary MN ^{56,86}. In addition, even in cases of clinically-active MN with circulating anti-PLA₂R, the IgG4 subclass cannot be detected in approximately 5% of patients ^{79,87}. Given the previously-mentioned change from IgG1 to IgG4 predominance in early vs. late deposits ¹², there exists a possibility that the humoral response to PLA₂R also matures from being primarily IgG1-to IgG4-predominant.

Another recent report introduces uncertainty as to the relative importance of any particular complement pathway. An exceptional case of PLA₂R-related MN, associated with a monoclonal anti-PLA₂R autoantibody, was described in a patient whose MN recurred 13 days after transplantation ⁸⁸. Unexpectedly, this monoclonal antibody was not IgG4, but

rather IgG3 with a light chain restriction. The graft biopsy specimen showed granular staining for C3, C5b-9, C1q and IgG3 but not MBL, as had been found in the patient's native kidney. This particular case of recurrent MN suggests that circulating monoclonal anti-PLA₂R IgG3 caused MN by activating the classical complement pathway.

Other antigens in human MN

Anti-PLA₂R antibodies can be detected in approximately 70% or more of primary MN cases, but up to 30% of patients with active disease have no detectable anti-PLA₂R in their serum. It seems likely that there are other, as yet unidentified antigens involved in the pathogenesis of primary MN. A number of intracellular antigens have recently been reported as other targets of circulating antibodies in MN, including aldose reductase, superoxide dismutase, and α -enolase^{89–91}. Several of these, such as aldose reductase and superoxide dismutase, are neoantigens, as they are not expressed to any great extent by the normal podocyte, but are upregulated in the setting of disease. They may also be expressed on the surface of the podocyte with injury, leading to the possibility of *in situ* antibody binding and immune complex deposition. They have all been shown to co-localize with C3 and C5b-9 in the immune deposits of MN. It is not clear if these are primary antigens, or - more likely - new antigenic targets arising from upregulation and exposure from cell injury started by another process, such as anti-PLA₂R⁷². The fact that anti-PLA₂R is more prevalent than any of these other autoantibodies suggests that they are secondary and may result from intermolecular epitope spreading during the evolving humoral immune response.

Recurrent MN

The paradigm of HN informs us that the process of immune complex deposition is a gradual one, and this is reflected by the Ehrenreich-Churg staging of human MN, by which small, exclusively subepithelial immune deposits grow in size with duration or severity of disease, eventually being separated and surrounded by matrix material. Because of the insidious clinical onset of disease, it is difficult to capture the early pathological events in human disease. MN that recurs in the kidney allograft after transplantation provides a window through which to look at these early stages of disease. Recurrent disease may occur in up to 42% of allografts⁷; rates are higher in reports that have included protocol biopsies at set time points after transplantation. It appears that subclinical cases of disease that would not have otherwise been detected are picked up by this early screening.

A recent report by Rodriguez and colleagues define “stage 0” MN as subepithelial deposits that are miniscule or absent by EM, but are detected in a fine granular capillary loop pattern by IF⁹². Such deposits may be found as early as 6 days after transplantation, and contain IgG4 and the PLA₂R antigen⁹³. It is highly likely, as in the case of IgG3k anti-PLA₂R disease described above, that antibody circulating at the time of transplantation rapidly targets PLA₂R on the allograft, initiating the process of *in situ* immune complex deposition in the subsequent days. Transplant immunosuppression may alter the course of disease, as in some cases, circulating anti-PLA₂R rapidly declines and disappears. In addition, complement factor C3 is not as prevalent in recurrent MN as it is in native disease⁹²; the reason for this is unclear. As in native disease, C4d is detected in a fine granular pattern in

recurrent MN (Figure 1). When anti-PLA₂R antibodies persist at high levels despite transplant immunosuppression, the glomerular deposits continue to grow, as evidenced by serial biopsies in the same patient ⁹², and it is more likely that the disease will be detected clinically, by nephrotic or subnephrotic levels of proteinuria. The mean clinical time to recurrence is nearly one year, as opposed to the early subclinical deposits that can be detected within one week by protocol biopsy.

Membranous lupus nephritis

Systemic lupus erythematosus is commonly complicated by lupus nephritis, and approximately 20% of these cases have a predominantly proteinuric form known as class V lupus nephritis, or lupus membranous nephritis (LMN). LMN is one of the major etiologies of secondary MN worldwide. The subepithelial immune deposits that form in LMN could result from dissociation of subendothelial immune complexes with transit across GBM to reform in subepithelial location or from deposition of lupus autoantibodies with affinity for a broad range of self-antigens ^{94,95}. Although the immune complex composition differs, similar to patients with primary MN, complement C3 and C4 are commonly detected in the patients with LMN, indicating that activation of complement participates in human LMN, mediating podocyte injury ^{10,56,96,97}. In contrast to the rare appearance of C1q in primary MN, C1q has been regularly detected in glomerular deposits of LMN and has been suggested as diagnostic marker to distinguish LMN from primary MN. The presence of C1q suggests that activation of the classical complement pathway is involved in LMN. Recently, blocking studies in murine model by treating with the inhibitor specific for the alternative pathway suggest that alternative pathway also plays a major role in LMN, at least in experimental MRL/lpr mice ⁹⁴. Additionally, MBL was detected in 94% of patients with LMN ⁶², hinting again at a possible role of the lectin pathway.

Is there a role for complement inhibition in MN?

Given what appears to be a major role for one or more complement pathways in both primary and secondary MN, why has therapy not focused on inhibition of the complement system? There is only one small study of complement inhibition in MN, and it was never published due to its negative results. Details are available in abstract form and from several reviews on the topic ⁹⁸. The agent used in this trial was eculizumab, a monoclonal antibody that binds C5 and prevents its cleavage, thus limiting formation of the terminal complement components and generation of C5b-9, the membrane attack complex. Treatment with eculizumab over a 16 week period did not lead to a statistically significant reduction of proteinuria compared to the control cohort of untreated patients. However, many commentators have noted that the doses of eculizumab used in this study, in comparison with dosages used in contemporary trials in other diseases, were too low and the treatment duration was too limited.

Due to the importance of complement in the pathogenesis of many diseases, more therapeutic agents are being developed and are in pre-clinical trials. Some of these will be broadly active, such as eculizumab, and target general processes such as the cleavage of C5. Others are being developed as chimeric molecules that will be targeted only to areas in

which there is active complement activation⁹⁹. Although the definitive therapy in MN will be to administer immunosuppressive therapy to bring about the disappearance of circulating autoantibodies (or to treat the underlying systemic disease or exposure in secondary forms), there will be a window period in which circulating antibodies, antigen, or both will continue to lead to ongoing formation of immune deposits and sublethal injury of podocytes via complement activation. Use of complement inhibitory therapeutics in this defined period would allow amelioration of ongoing glomerular injury while the immunosuppressive therapies have time to act.

Conclusions

The pathophysiology of membranous nephropathy has been the focus of research for more than 50 years, and much work has implicated activation of the complement pathways in experimental and human disease. We are fortunate to be experiencing an awakened interest in this disease and its underlying pathogenesis due to the identification of biomarkers such as PLA₂R. With these molecular markers, investigators should be able to probe deeper into the pathogenesis of human disease to see how pivotal a role the complement system plays and whether targeted inhibition of these pathways could lead to improved therapies for this and other glomerular diseases.

Acknowledgments

Financial support for this work:

HM, LHB are supported by R01 DK090029; DGS is supported by T32 DK07053-37

This work was supported by research grants DK090029 and DK097053 and institutional training grant DK07053.

References

1. Jones DB. Nephrotic glomerulonephritis. *Am J Pathol.* 1957; 33:313–29. [PubMed: 13402889]
2. Movat HZ, McGreggor DD. The fine structure of the glomerulus in membranous glomerulonephritis (lipoid nephrosis) in adults. *Am J Clin Pathol.* 1959; 32:109–27. [PubMed: 13670133]
3. Mellors RC, Ortega LG. Analytical pathology. III. New observations on the pathogenesis of glomerulonephritis, lipid nephrosis, periarteritis nodosa, and secondary amyloidosis in man. *Am J Pathol.* 1956; 32:455–99. [PubMed: 13313715]
4. McGrogan A, Franssen CF, de Vries CS. The incidence of primary glomerulonephritis worldwide: a systematic review of the literature. *Nephrol Dial Transplant.* 2011; 26:414–30. [PubMed: 21068142]
5. Menon S, Valentini RP. Membranous nephropathy in children: clinical presentation and therapeutic approach. *Pediatr Nephrol.* 2010; 25:1419–28. [PubMed: 19908069]
6. Kerlin BA, Ayoob R, Smoyer WE. Epidemiology and pathophysiology of nephrotic syndrome associated thromboembolic disease. *Clin J Am Soc Nephrol.* 2012; 7:513–20. [PubMed: 22344511]
7. Dabade TS, Grande JP, Norby SM, Fervenza FC, Cosio FG. Recurrent idiopathic membranous nephropathy after kidney transplantation: a surveillance biopsy study. *Am J Transplant.* 2008; 8:1318–22. [PubMed: 18444918]
8. Beck LH Jr, Bonegio RG, Lambeau G, et al. M-type phospholipase A2 receptor as target antigen in idiopathic membranous nephropathy. *N Engl J Med.* 2009; 361:11–21. [PubMed: 19571279]
9. Kidney Disease: Improving Global Outcomes (KDIGO) Glomerulonephritis Work Group. KDIGO Clinical Practice Guideline for Glomerulonephritis. *Kidney Inter, Suppl.* 2012; 2:139–274.

10. Jennette JC, Iskandar SS, Dalldorf FG. Pathologic differentiation between lupus and nonlupus membranous glomerulopathy. *Kidney Int.* 1983; 24:377–85. [PubMed: 6358633]
11. Doi T, Mayumi M, Kanatsu K, Suehiro F, Hamashima Y. Distribution of IgG subclasses in membranous nephropathy. *Clin Exp Immunol.* 1984; 58:57–62. [PubMed: 6383668]
12. Huang CC, Lehman A, Albawardi A, et al. IgG subclass staining in renal biopsies with membranous glomerulonephritis indicates subclass switch during disease progression. *Mod Pathol.* 2013
13. Haas M. IgG subclass deposits in glomeruli of lupus and nonlupus membranous nephropathies. *Am J Kidney Dis.* 1994; 23:358–64. [PubMed: 8128936]
14. Ohtani H, Wakui H, Komatsuda A, et al. Distribution of glomerular IgG subclass deposits in malignancy associated membranous-nephropathy. *Nephrol Dial Transplant.* 2004; 19:574–9. [PubMed: 14767011]
15. Larsen CP, Messias NC, Silva FG, Messias E, Walker PD. Determination of primary versus secondary membranous glomerulopathy utilizing phospholipase A2 receptor staining in renal biopsies. *Mod Pathol.* 2012
16. Batsford S, Oite T, Takamiya H, Vogt A. Anionic binding sites in the glomerular basement membrane: possible role in the pathogenesis of immune complex glomerulonephritis. *Ren Physiol.* 1980; 3:336–40. [PubMed: 7323427]
17. Border WA, Ward HJ, Kamil ES, Cohen AH. Induction of membranous nephropathy in rabbits by administration of an exogenous cationic antigen. *J Clin Invest.* 1982; 69:451–61. [PubMed: 7056856]
18. Adler SG, Wang H, Ward HJ, Cohen AH, Border WA. Electrical charge. Its role in the pathogenesis and prevention of experimental membranous nephropathy in the rabbit. *J Clin Invest.* 1983; 71:487–99. [PubMed: 6826719]
19. Koyama A, Inage H, Kobayashi M, Narita M, Tojo S. Effect of chemical cationization of antigen on glomerular localization of immune complexes in active models of serum sickness nephritis in rabbits. *Immunology.* 1986; 58:529–34. [PubMed: 3733152]
20. Debiec H, Lefeu F, Kemper MJ, et al. Early-childhood membranous nephropathy due to cationic bovine serum albumin. *N Engl J Med.* 2011; 364:2101–10. [PubMed: 21631322]
21. Schmiedeke TM, Stöckl FW, Weber R, Sugisaki Y, Batsford SR, Vogt A. Histones have high affinity for the glomerular basement membrane. Relevance for immune complex formation in lupus nephritis. *J Exp Med.* 1989; 169:1879–94. [PubMed: 2732675]
22. Takekoshi Y, Tanaka M, Miyakawa Y, Yoshizawa H, Takahashi K, Mayumi M. Free “small” and IgG associated “large” hepatitis B e antigen in the serum and glomerular capillary walls of two patients with membranous glomerulonephritis. *N Engl J Med.* 1979; 300:814–9. [PubMed: 370592]
23. Heymann W, Hackel DB, Harwood S, Wilson SG, Hunter JL. Production of nephrotic syndrome in rats by Freund’s adjuvants and rat kidney suspensions. *Proc Soc Exp Biol Med.* 1959; 100:660–4. [PubMed: 13645677]
24. Edgington TS, Glasscock RJ, Dixon FJ. Autologous immune-complex pathogenesis of experimental allergic glomerulonephritis. *Science.* 1967; 155:1432–4. [PubMed: 4163593]
25. Feenstra K, van den Lee R, Greben HA, Arends A, Hoedemaeker PJ. Experimental glomerulonephritis in the rat induced by antibodies directed against tubular antigens. I. The natural history: a histologic and immunohistologic study at the light microscopic and the ultrastructural level. *Lab Invest.* 1975; 32:235–42. [PubMed: 1113514]
26. Couser WG, Steinmuller DR, Stilmant MM, Salant DJ, Lowenstein LM. Experimental glomerulonephritis in the isolated perfused rat kidney. *J Clin Invest.* 1978; 62:1275–87. [PubMed: 372233]
27. VanDamme BJ, Fleuren GJ, Bakker WW, Vernier RL, Hoedemaeker PJ. Experimental glomerulonephritis in the rat induced by antibodies directed against tubular antigens V Fixed glomerular antigens in the pathogenesis of heterologous immune complex glomerulonephritis. *Lab Invest.* 1978; 38:502–10. [PubMed: 147961]

28. Kerjaschki D, Farquhar MG. The pathogenic antigen of Heymann nephritis is a membrane glycoprotein of the renal proximal tubule brush border. *Proc Natl Acad Sci U S A.* 1982; 79:5557–61. [PubMed: 6752952]
29. Makker SP, Singh AK. Characterization of the antigen (gp600) of Heymann nephritis. *Lab Invest.* 1984; 50:287–93. [PubMed: 6199583]
30. Orlando RA, Kerjaschki D, Kurihara H, Biemesderfer D, Farquhar MG. gp330 associates with a 44-kDa protein in the rat kidney to form the Heymann nephritis antigenic complex. *Proc Natl Acad Sci U S A.* 1992; 89:6698–702. [PubMed: 1495959]
31. Petermann AT, Krofft R, Blonski M, et al. Podocytes that detach in experimental membranous nephropathy are viable. *Kidney Int.* 2003; 64:1222–31. [PubMed: 12969140]
32. Schulze M, Donadio JV Jr, Pruchno CJ, et al. Elevated urinary excretion of the C5b-9 complex in membranous nephropathy. *Kidney Int.* 1991; 40:533–8. [PubMed: 1787650]
33. Salant DJ, Belok S, Madaio MP, Couser WG. A new role for complement in experimental membranous nephropathy in rats. *J Clin Invest.* 1980; 66:1339–50. [PubMed: 7440718]
34. Perkinson DT, Baker PJ, Couser WG, Johnson RJ, Adler S. Membrane attack complex deposition in experimental glomerular injury. *Am J Pathol.* 1985; 120:121–8. [PubMed: 3160245]
35. Cybulsky AV, Rennke HG, Feintzeig ID, Salant DJ. Complement-induced glomerular epithelial cell injury. Role of the membrane attack complex in rat membranous nephropathy. *J Clin Invest.* 1986; 77:1096–107. [PubMed: 3514672]
36. Baker PJ, Ochi RF, Schulze M, Johnson RJ, Campbell C, Couser WG. Depletion of C6 prevents development of proteinuria in experimental membranous nephropathy in rats. *Am J Pathol.* 1989; 135:185–94. [PubMed: 2672823]
37. Quigg RJ, Holers VM, Morgan BP, Sneed AE. Crry and CD59 regulate complement in rat glomerular epithelial cells and are inhibited by the nephritogenic antibody of passive Heymann nephritis. *J Immunol.* 1995; 154:3437–43. [PubMed: 7534798]
38. Schiller B, He C, Salant DJ, Lim A, Alexander JJ, Quigg RJ. Inhibition of complement regulation is key to the pathogenesis of active Heymann nephritis. *J Exp Med.* 1998; 188:1353–8. [PubMed: 9763614]
39. Rus HG, Niculescu FI, Shin ML. Role of the C5b-9 complement complex in cell cycle and apoptosis. *Immunol Rev.* 2001; 180:49–55. [PubMed: 11414362]
40. Quigg RJ, Cybulsky AV, Jacobs JB, Salant DJ. anti-Fx1A produces complement-dependent cytotoxicity of glomerular epithelial cells. *Kidney Int.* 1988; 34:43–52. [PubMed: 3172636]
41. Topham PS, Haydar SA, Kuphal R, Lightfoot JD, Salant DJ. Complement-mediated injury reversibly disrupts glomerular epithelial cell actin microfilaments and focal adhesions. *Kidney Int.* 1999; 55:1763–75. [PubMed: 10231439]
42. Yuan H, Takeuchi E, Taylor GA, McLaughlin M, Brown D, Salant DJ. Nephrin dissociates from actin, and its expression is reduced in early experimental membranous nephropathy. *J Am Soc Nephrol.* 2002; 13:946–56. [PubMed: 11912254]
43. Saran AM, Yuan H, Takeuchi E, McLaughlin M, Salant DJ. Complement mediates nephrin redistribution and actin dissociation in experimental membranous nephropathy. *Kidney Int.* 2003; 64:2072–8. [PubMed: 14633129]
44. Minto AW, Kalluri R, Togawa M, Bergijk EC, Killen PD, Salant DJ. Augmented expression of glomerular basement membrane specific type IV collagen isoforms (alpha3-alpha5) in experimental membranous nephropathy. *Proc Assoc Am Physicians.* 1998; 110:207–17. [PubMed: 9625527]
45. Floege J, Johnson RJ, Gordon K, et al. Altered glomerular extracellular matrix synthesis in experimental membranous nephropathy. *Kidney Int.* 1992; 42:573–85. [PubMed: 1383596]
46. Lundgren S, Carling T, Hjälm G, et al. Tissue distribution of human gp330/megalin, a putative Ca(2+)-sensing protein. *J Histochem Cytochem.* 1997; 45:383–92. [PubMed: 9071320]
47. Segawa Y, Hisano S, Matsushita M, et al. IgG subclasses and complement pathway in segmental and global membranous nephropathy. *Pediatr Nephrol.* 2010; 25:1091–9. [PubMed: 20151159]
48. Endo M, Fuke Y, Tamano M, et al. Glomerular deposition and urinary excretion of complement factor H in idiopathic membranous nephropathy. *Nephron Clin Pract.* 2004; 97:c147–53. [PubMed: 15331938]

49. Zhang R, Zheng ZY, Lin JS, Qu LJ, Zheng F. The continual presence of C3d but not IgG glomerular capillary deposition in stage I idiopathic membranous nephropathy in patients receiving corticosteroid treatment. *Diagn Pathol.* 2012; 7:109. [PubMed: 22909298]
50. Nangaku M, Shankland SJ, Couser WG. Cellular response to injury in membranous nephropathy. *J Am Soc Nephrol.* 2005; 16:1195–204. [PubMed: 15800119]
51. Collins AB, Schneeberger EE, Pascual MA, et al. Complement activation in acute humoral renal allograft rejection: diagnostic significance of C4d deposits in peritubular capillaries. *J Am Soc Nephrol.* 1999; 10:2208–14. [PubMed: 10505698]
52. Kusunoki Y, Itami N, Tochimaru H, Takekoshi Y, Nagasawa S, Yoshiki T. Glomerular deposition of C4 cleavage fragment (C4d) and C4 binding protein in idiopathic membranous glomerulonephritis. *Nephron.* 1989; 51:17–9. [PubMed: 2644563]
53. Val-Bernal JF, Garijo MF, Val D, Rodrigo E, Arias M. C4d immunohistochemical staining is a sensitive method to confirm immunoreactant deposition in formalin-fixed paraffin-embedded tissue in membranous glomerulonephritis. *Histol Histopathol.* 2011; 26:1391–7. [PubMed: 21938676]
54. Espinosa-Hernandez M, Ortega-Salas R, Lopez-Andreu M, et al. C4d as a diagnostic tool in membranous nephropathy. *Nefrologia.* 2012; 32:295–9. [PubMed: 22592419]
55. Suzuki T, Horita S, Kadoya K, et al. C4d Immunohistochemistry in glomerulonephritis with different antibodies. *Clin Exp Nephrol.* 2007; 11:287–91. [PubMed: 18085389]
56. Song YS, Min KW, Kim JH, Kim GH, Park MH. Differential diagnosis of lupus and primary membranous nephropathies by IgG subclass analysis. *Clin J Am Soc Nephrol.* 2012; 7:1947–55. [PubMed: 23024158]
57. Lhotta K, Wurzner R, Rumpelt HJ, Eder P, Mayer G. Membranous nephropathy in a patient with hereditary complete complement C4 deficiency. *Nephrol Dial Transplant.* 2004; 19:990–3. [PubMed: 15031363]
58. Iwaki D, Kanno K, Takahashi M, Endo Y, Matsushita M, Fujita T. The role of mannose-binding lectin-associated serine protease-3 in activation of the alternative complement pathway. *J Immunol.* 2011; 187:3751–8. [PubMed: 21865552]
59. Roos A, Bouwman LH, van Gijlswijk-Janssen DJ, Faber-Krol MC, Stahl GL, Daha MR. Human IgA activates the complement system via the mannan-binding lectin pathway. *J Immunol.* 2001; 167:2861–8. [PubMed: 11509633]
60. Roos A, Rastaldi MP, Calvaresi N, et al. Glomerular activation of the lectin pathway of complement in IgA nephropathy is associated with more severe renal disease. *J Am Soc Nephrol.* 2006; 17:1724–34. [PubMed: 16687629]
61. Endo M, Ohi H, Ohsawa I, Fujita T, Matsushita M. Glomerular deposition of mannose-binding lectin (MBL) indicates a novel mechanism of complement activation in IgA nephropathy. *Nephrol Dial Transplant.* 1998; 13:1984–90. [PubMed: 9719152]
62. Lhotta K, Wurzner R, Konig P. Glomerular deposition of mannose-binding lectin in human glomerulonephritis. *Nephrol Dial Transplant.* 1999; 14:881–6. [PubMed: 10328463]
63. Malhotra R, Wormald MR, Rudd PM, Fischer PB, Dwek RA, Sim RB. Glycosylation changes of IgG associated with rheumatoid arthritis can activate complement via the mannose-binding protein. *Nat Med.* 1995; 1:237–43. [PubMed: 7585040]
64. Salant DJ. Genetic variants in membranous nephropathy: perhaps a perfect storm rather than a straightforward conformeropathy? *J Am Soc Nephrol.* 2013; 24:525–8. [PubMed: 23492731]
65. Puci M, Knezevi A, Vidic J, et al. High throughput isolation and glycosylation analysis of IgG variability and heritability of the IgG-glycome in three isolated human populations. *Mol Cell Proteomics.* 2011; 10:M111.010090. [PubMed: 21653738]
66. Jennette JC, Hipp CG. Immunohistopathologic evaluation of C1q in 800 renal biopsy specimens. *Am J Clin Pathol.* 1985; 83:415–20. [PubMed: 3885712]
67. Thurman JM, Holers VM. The central role of the alternative complement pathway in human disease. *J Immunol.* 2006; 176:1305–10. [PubMed: 16424154]
68. Lachmann PJ. The amplification loop of the complement pathways. *Adv Immunol.* 2009; 104:115–49. [PubMed: 20457117]

69. van der Neut Kolfschoten M, Schuurman J, Losen M, et al. anti-inflammatory activity of human IgG4 antibodies by dynamic Fab arm exchange. *Science*. 2007; 317:1554–7. [PubMed: 17872445]
70. Kawa S, Kitahara K, Hamano H, et al. A novel immunoglobulin-immunoglobulin interaction in autoimmunity. *PLoS One*. 2008; 3:e1637. [PubMed: 18297131]
71. Rispens T, Ooievaar-De Heer P, Vermeulen E, Schuurman J, van der Neut Kolfschoten M, Aalberse RC. Human IgG4 binds to IgG4 and conformationally altered IgG1 via Fc-Fc interactions. *J Immunol*. 2009; 182:4275–81. [PubMed: 19299726]
72. Makker SP, Tramontano A. Idiopathic membranous nephropathy: an autoimmune disease. *Semin Nephrol*. 2011; 31:333–40. [PubMed: 21839366]
73. Debiec H, Guignon V, Mougnot B, et al. Antenatal membranous glomerulonephritis due to anti-neutral endopeptidase antibodies. *N Engl J Med*. 2002; 346:2053–60. [PubMed: 12087141]
74. Debiec H, Nauta J, Coulet F, et al. Role of truncating mutations in MME gene in fetomaternal alloimmunisation and antenatal glomerulopathies. *Lancet*. 2004; 364:1252–9. [PubMed: 15464186]
75. Hoxha E, Harendza S, Zahner G, et al. An immunofluorescence test for phospholipase-A2-receptor antibodies and its clinical usefulness in patients with membranous glomerulonephritis. *Nephrol Dial Transplant*. 2011; 26:2526–32. [PubMed: 21633097]
76. Qin W, Beck LH Jr, Zeng C, et al. anti-phospholipase A2 receptor antibody in membranous nephropathy. *J Am Soc Nephrol*. 2011; 22:1137–43. [PubMed: 21566055]
77. Debiec H, Ronco P. PLA2R autoantibodies and PLA2R glomerular deposits in membranous nephropathy. *N Engl J Med*. 2011; 364:689–90. [PubMed: 21323563]
78. Hofstra JM, Wetzels JF. anti-PLA(2)R antibodies in membranous nephropathy: ready for routine clinical practice? *Neth J Med*. 2012; 70:109–13. [PubMed: 22516574]
79. Hofstra JM, Debiec H, Short CD, et al. Antiphospholipase A2 receptor antibody titer and subclass in idiopathic membranous nephropathy. *J Am Soc Nephrol*. 2012; 23:1735–43. [PubMed: 22956816]
80. Hoxha E, Kneißler U, Stege G, et al. Enhanced expression of the M-type phospholipase A2 receptor in glomeruli correlates with serum receptor antibodies in primary membranous nephropathy. *Kidney Int*. 2012; 82:797–804. [PubMed: 22673885]
81. Beck LH Jr, Fervenza FC, Beck DM, et al. Rituximab-induced depletion of anti-PLA2R autoantibodies predicts response in membranous nephropathy. *J Am Soc Nephrol*. 2011; 22:1543–50. [PubMed: 21784898]
82. Beck LH Jr, Salant DJ. Membranous nephropathy: recent travels and new roads ahead. *Kidney Int*. 2010; 77:765–70. [PubMed: 20182413]
83. Debiec H, Martin L, Jouanneau C, et al. Autoantibodies specific for the phospholipase A2 receptor in recurrent and De Novo membranous nephropathy. *Am J Transplant*. 2011; 11:2144–52. [PubMed: 21827616]
84. Ayalon R, Beck LH, Fervenza FC, Cosio FG, Brennan DC, Salant DJ. Do anti-Phospholipase A2 Receptor Antibodies Predict Recurrence of Membranous Nephropathy after Transplantation? *J Am Soc Nephrol*. 2012; 23:64A.
85. Ma H, Beck LH, Salant DJ. Membranous nephropathy-associated anti-phospholipase A2 receptor IgG4 autoantibodies activate the lectin complement pathway [abstract]. *J Am Soc Nephrol*. 2011; 22:62A.
86. Qu Z, Liu G, Li J, et al. Absence of glomerular IgG4 deposition in patients with membranous nephropathy may indicate malignancy. *Nephrol Dial Transplant*. 2012; 27:1931–7. [PubMed: 21976738]
87. Dähnrich C, Komorowski L, Probst C, et al. Development of a standardized ELISA for the determination of autoantibodies against human M-type phospholipase A2 receptor in primary membranous nephropathy. *Clin Chim Acta*. 2013; 421C:213–8. [PubMed: 23541686]
88. Debiec H, Hanoy M, Francois A, et al. Recurrent membranous nephropathy in an allograft caused by IgG3 targeting the PLA2 receptor. *J Am Soc Nephrol*. 2012; 23:1949–54. [PubMed: 23123401]
89. Bruschi M, Carnevali ML, Murtas C, et al. Direct characterization of target podocyte antigens and auto antibodies in human membranous glomerulonephritis: Alfa-enolase and borderline antigens. *J Proteomics*. 2011; 74:2008–17. [PubMed: 21640210]

90. Murtas C, Bruschi M, Candiano G, et al. Coexistence of Different Circulating anti-Podocyte Antibodies in Membranous Nephropathy. *Clin J Am Soc Nephrol*. 2012; 7:1394–400. [PubMed: 22773590]
91. Prunotto M, Carnevali ML, Candiano G, et al. Autoimmunity in Membranous Nephropathy Targets Aldose Reductase and SOD2. *J Am Soc Nephrol*. 2010; 21:507–19. [PubMed: 20150532]
92. Rodriguez EF, Cosio FG, Nasr SH, et al. The pathology and clinical features of early recurrent membranous glomerulonephritis. *Am J Transplant*. 2012; 12:1029–38. [PubMed: 22233329]
93. Blosser CD, Ayalon R, Nair R, Thomas C, Beck LH Jr. Very early recurrence of anti-Phospholipase A2 receptor-positive membranous nephropathy after transplantation. *Am J Transplant*. 2012; 12:1637–42. [PubMed: 22390840]
94. Sekine H, Watanabe H, Gilkeson GS. Enrichment of anti-glomerular antigen antibody-producing cells in the kidneys of MRL/MpJ-Fas(lpr) mice. *J Immunol*. 2004; 172:3913–21. [PubMed: 15004199]
95. Kotzin BL. Systemic lupus erythematosus. *Cell*. 1996; 85:303–6. [PubMed: 8616885]
96. Yamashina M, Takami T, Kanemura T, Orii T, Ojima A. Immunohistochemical demonstration of complement components in formalin fixed and paraffin-embedded renal tissues. *Lab Invest*. 1989; 60:311–6. [PubMed: 2644486]
97. Chowdhury AR, Ehara T, Higuchi M, Hora K, Shigematsu H. Immunohistochemical detection of immunoglobulins and complements in formaldehyde-fixed and paraffin-embedded renal biopsy tissues; an adjunct for diagnosis of glomerulonephritis. *Nephrology (Carlton)*. 2005; 10:298–304. [PubMed: 15958046]
98. Cunningham PN, Quigg RJ. Contrasting roles of complement activation and its regulation in membranous nephropathy. *J Am Soc Nephrol*. 2005; 16:1214–22. [PubMed: 15800113]
99. Holers VM, Rohrer B, Tomlinson S. CR2-mediated targeting of complement inhibitors: bench-to bedside using a novel strategy for site-specific complement modulation. *Adv Exp Med Biol*. 2013; 735:137–54. [PubMed: 23402024]
100. Cybulsky AV, Quigg RJ, Salant DJ. Experimental membranous nephropathy redux. *Am J Physiol Renal Physiol*. 2005; 289:F660–71. [PubMed: 16159900]
101. Hofstra JM, Beck LH Jr, Beck DM, Wetzels JF, Salant DJ. anti-phospholipase A2 receptor antibodies correlate with clinical status in idiopathic membranous nephropathy. *Clin J Am Soc Nephrol*. 2011; 6:1286–91. [PubMed: 21474589]
102. Gunnarsson I, Schlumberger W, Rönnelid J. Antibodies to M-type phospholipase A2 receptor (PLA2R) and membranous lupus nephritis. *Am J Kidney Dis*. 2012; 59:585–6. [PubMed: 22177657]
103. Svobodova B, Honsova E, Ronco P, Tesar V, Debiec H. Kidney biopsy is a sensitive tool for retrospective diagnosis of PLA2R-related membranous nephropathy. *Nephrol Dial Transplant*. 2012
104. Kanigicherla D, Gummadova J, McKenzie EA, et al. anti-PLA2R antibodies measured by ELISA predict long-term outcome in a prevalent population of patients with idiopathic membranous nephropathy. *Kidney Int*. 2013
105. Coenen MJ, Hofstra JM, Debiec H, et al. Phospholipase A2 Receptor (PLA2R1) Sequence Variants in Idiopathic Membranous Nephropathy. *J Am Soc Nephrol*. 2013; 24:677–83. [PubMed: 23431073]
106. Behnert A, Fritzler MJ, Teng B, et al. An anti-phospholipase A2 receptor quantitative immunoassay and epitope analysis in membranous nephropathy reveals different antigenic domains of the receptor. *PLoS One*. 2013; 8:e61669. [PubMed: 23637879]
107. Oh YJ, Yang SH, Kim DK, Kang SW, Kim YS. Autoantibodies against Phospholipase A2 Receptor in Korean Patients with Membranous Nephropathy. *PLoS One*. 2013; 8:e62151. [PubMed: 23637987]

Figure 1.

Immunofluorescence staining in a case of recurrent membranous nephropathy. Staining for IgG (A) and PLA₂R (B) demonstrates the fine granular, capillary loop staining pattern typical for MN. C3 (not shown) and C4d (C) exhibit a more linear, less discrete pattern in this case. Images (400x) courtesy of Dr. Joel Henderson, Department of Pathology and Laboratory Medicine, Boston University School of Medicine.

Table 1

Causes of secondary MN

Autoimmune diseases:
Systemic lupus erythematosus (class V lupus nephritis)
Rheumatoid arthritis
Autoimmune thyroid disease
Sjögren's syndrome
IgG4-related systemic disease
Infectious agents:
Viral: Hepatitis B, HIV
Bacterial: syphilis
Parasitic: schistosomiasis, malaria
Iatrogenic:
Non-steroidal anti-inflammatory agents
anti-rheumatic drugs: D-penicillamine, gold salts
Mercury-containing skin lightening creams
Malignancy:
Solid tumors (colon, stomach, lung, prostate)
Non-Hodgkin lymphoma
Chronic lymphocytic leukemia

Table 2

Signaling pathways activated by sublethal injury in GEC(modified from ¹⁰⁰)

Increased intracellular calcium (influx and release from stores)
Protein kinases: Receptor tyrosine kinases, protein kinase C, ERK, p38
Phospholipases and arachidonic acid signaling: PLC, cytosolic PLA ₂ , Cox 2
Reactive O ₂ species: NADPH oxidase, lipid peroxidation
Cellular stress and protein degradation: ER stress, misfolded protein response, ubiquitination
Transcription factors: NFκB
Growth factors: PDGF-B, HB-EGF, TGF-β
CDK inhibitors: p21, p27
Cytoskeleton: F-actin disassembly, dissociation from nephrin
Extracellular matrix: collagens, heparan sulfate proteoglycans, MMP-9

Immunofluorescence detection of IgG subclasses and complement components in primary and secondary membranous nephropathy

Table 3

	Total IgG	IgG1	IgG2	IgG3	IgG4
Primary MN	2.88	0.84	0.50	1.26	2.4
Secondary MN	3.00	2.50	2.00	1.00	0
	C1q	Factor B	MBL	C3	# tested
Primary MN	0.04	1.75	0.36	1.76	25
Secondary MN	0.83	1.50	0	2.00	3

Preliminary data generated by Ms. Elena Gagliardini, Mario Negri Institute for Pharmacological Research, Bergamo, Italy; presented May 7, 2011 at the 2nd International Conference on Membranous Nephropathy, Bergamo, Italy. Values are fluorescence intensity on a scale of 0–3.

Table 4Prevalence of anti-PLA₂R autoantibodies in patients with primary membranous nephropathy

Author (Year)	Patients (n)	anti-PLA ₂ R (%)	Assay
Beck (2009) ⁸	37	70%	WB
Hofstra (2011) ¹⁰¹	18	78%	WB
Beck (2011) ⁸¹	35	71%	WB
Debiec (2011) ⁷⁷	42	57%	IFA
Hoxha (2011) ⁷⁵			
•Entire cohort	100	52%	IFA
•Active disease	35	66%	
Qin (2011) ⁷⁶			
•Active disease	60	82% *	WB
•Remission	21	19%	
Bruschi (2011) ⁸⁹	24	58%	WB
Gunnarsson (2012) ¹⁰²	3	100%	IFA
Hoxha (2012) ⁸⁰	88	68%	IFA
Hofstra (2012) ⁷⁹	117	74% 72%	IFA ELISA
Murtas (2012) ⁹⁰	111	60%	WB, IFA
Svobodova (2012) ¹⁰³			
•Active disease	31	65%	IFA
•Remission	37	22%	
Kanigicherla (2013) ¹⁰⁴			
•Active disease	40	75%	
•Partial remission	27	37%	ELISA
•Complete remission	23	10%	
Coenen (2013) ¹⁰⁵	82	52%	IFA
Behnert (2013) ¹⁰⁶	160	53%	ALBIA
Oh (2013) ¹⁰⁷			
•At time of biopsy	100	69%	
•Proteinuria > 3.5 g/g	75	80%	WB
•Remission	19	16%	

WB=Western blot; IFA=Indirect immunofluorescence assay; ELISA=Enzyme-linked immunosorbent assay; ALBIA=addressable laser bead immunoassay

* when tested at 1:10, 98% (59/60) samples were positive by WB