The pathogenesis of membranous nephropathy: evolution and revolution

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Purpose of review
The morphological features of membranous nephropathy have been recognized for over five decades, but the pathogenetic mechanisms underlying this lesion in humans have only recently been elucidated. This review analyzes the recent developments in understanding the pathogenesis of the primary and secondary forms of membranous nephropathy.

Recent findings
Seminal studies have identified several autologous antigens that are targets of an autoantibody response in primary membranous nephropathy. The leading candidate autoantigen is M-type phospholipase A2 receptor (PLA2R) protein. Autoantibodies to PLA2R, usually of IgG4 subclass, are found in 70–80% of patients with primary membranous nephropathy, bind to conformational epitopes on PLA2R expressed in the glomerular podocyte, form immune complexes *in situ* and induce proteinuria, mostly likely via local activation of complement. The autoimmune response is governed by genes at the *HLA-DQA1* locus. The level of autoantibody to PLA2R correlates with the severity of the clinical disease and predicts recurrences in renal allografts (at least in some patients). Most forms of secondary membranous nephropathy appear to be due to distinctly different pathogenetic mechanisms.

Summary
The identification of target antigens provides new tools for diagnosis, prognosis and monitoring of therapy in human membranous nephropathy.

Keywords
autoimmunity, membranous nephropathy, phospholipase A2 receptor, recurrent membranous nephropathy

INTRODUCTION

Membranous nephropathy was first delineated as a distinct clinicopathological entity slightly more than 50 years ago [1▪]. For most of these five decades the pathogenesis was uncertain, but aberrant immune processes were strongly suspected to underlie the condition [1▪]. The morphological lesion has two main presentations: first, as a primary disease affecting the glomeruli in the absence of any systemic disease process (such as systemic lupus erythematosus or malignancies) and without known inciting causes (such as drugs or chronic viral infections) [1▪,2]. Primary membranous nephropathy is also referred to as 'idiopathic' membranous nephropathy, although this term has inherent weaknesses when used in a modern framework. The other presentation is as a disease secondary to recognizable systemic disease, inciting factors or cause. Secondary forms of membranous nephropathy are remarkably diverse (see below for a classification system of primary and secondary membranous nephropathy) [2]. There is no *a priori* reason to believe that both primary and secondary membranous nephropathy have similar or even related underlying pathogenetic mechanisms. Experimental work in an animal model of human membranous nephropathy (Heymann nephritis) has clearly shown that the characteristic morphologic feature of membranous nephropathy (subepithelial deposits containing immunoglobulins) is largely evoked by the in-situ formation of immune complexes as circulating antibodies react with

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KEY POINTS

- The pathogenesis of human primary membranous nephropathy has been clarified by recent studies showing the involvement of a podocyte antigen, PLA2R, and an autoantibody response to this autoantigen leading to the in-situ formation of immune complexes, principally (but not exclusively) containing IgG4, in glomeruli.
- Genetic control of the auto-anti-PLA2R is determined by the nature of the conformational epitope on PLA2R, likely controlled by polymorphisms at the *PLA2R* gene locus and by polymorphisms at the *HLA-DQA1* locus.
- Other autoantigens synthesized by the podocyte, arising endogenously from nonglomerular sources or derived exogenously, may also participate in some cases of membranous nephropathy.
- These findings contribute to a rapidly changing and dynamic picture for diagnosis, prognosis and therapeutic monitoring in human membranous nephropathy.

locally synthesized (or artificially planted) antigens [1▪] (see [3▪] for a review). These experimental findings led to the proposition that an autoantibody to a defined native antigen (intrinsic to the glomerular visceral epithelial cell – the podocyte) was the fundamental mechanism involved in the great majority of primary ('idiopathic') membranous nephropathy cases in humans and that primary membranous nephropathy is largely an autoimmune disease. However, until recently the precise nature of the autoantibody and its target antigen was unknown in the vast majority of patients with presumed primary membranous nephropathy. The seminal and breakthrough discovery of M-type phospholipase A2 receptor (PLA2R) protein as the target antigen in human primary ('idiopathic') membranous nephropathy by Beck and coworkers in 2009 paved the way for a revolution in our understanding of this disease [4▪▪,5], and rendered the term 'idiopathic' membranous nephropathy to a domain of lesser relevance, even though the fundamental processes leading to the formation of autoantibody to this target antigen (cause) remain obscure. In addition, it is clear that mechanisms other than binding of anti-PLA2R to its respective target antigen on the podocyte probably explain a minority (perhaps 10–20%) of cases of human membranous nephropathy that are thought to be of a primary nature by conventional morphological and clinical criteria [5–7]. A proposed classification of primary and secondary membranous nephropathy is given below.

(1) Primary membranous nephropathy:
   (a) antiphospholipase A2 receptor (PLA2R) autoantibody positive with IgG4 and PLA2R glomerular deposition (80%+ of cases);
   (b) anti-PLA2R negative with IgG4 glomerular deposition;
   (c) bovine serum albumin–anti-bovine serum albumin 'planted antigen' disease (children only);
   (d) other (antisuperoxide dismutase, aldose reductase, alpha-enolase, megalin) related disease;

(2) Secondary membranous nephropathy
   (a) systemic autoimmune disease (lupus nephritis, mixed connective tissue disease, rheumatoid arthritis);
   (b) chronic infections (hepatitis B virus, hepatitis C virus, syphilis, malaria, leprosy);
   (c) drug-related (NSAID, gold, mercury, penicillamine, probenicid, captopril)
   (d) neoplasia-related (carcinoma, lymphoma, leukemia);
   (e) other (sarcoidosis, sickle cell disease, Kimura's disease, Castleman' disease, chronic thyroiditis, de novo in renal allografts, graft vs. host disease in bone marrow allografts);

(3) Recurrent in renal allograft/isograft:
   (a) some cases may be anti-PLA2R^{+}.

ANTI-PLA2R AUTOANTIBODY-MEDIATED PRIMARY MEMBRANOUS NEPHROPATHY

The nephritogenic antigen (PLA2R1) is synthesized by podocytes and expressed in the membrane surface [4▪▪,8]. PLA2R1 is a 180-KDa polypeptide with a long extracellular domain, consisting of a cysteine-rich head and fibronectin type II-like repeat domains and eight repeated carbohydrate-recognition domains, and short membrane-spanning and intracellular domains [8]. The nephritogenic epitope appears to be located in the methionine-rich terminal extracellular domain and is destroyed by disruption of cysteine–cysteine disulfide bridges [4▪▪]. The conformational nature of the epitope has been confirmed, and its function within the podocyte is uncertain, but studies have raised the possibility that PLA2R may be involved in cellular senescence through the p53 pathway [9]. Antibodies (IgG1 or IgG4 subclass) to this conformational antigen can be detected by Western blot or by other in-vitro assays using recombinant protein in 75–85% of patients with primary ('idiopathic') membranous nephropathy [4▪▪,10]. An immunofluorescent assay may be clinically useful as well

[11]. Only a very small fraction of secondary membranous nephropathy (e.g. hepatitis B viral infection) has detectable anti-PLA2R autoantibodies, except for malignancy-related membranous nephropathy which may have circulating anti-PLA2R autoantibodies in as many as 30% of cases [10]. The factors potentially responsible for 'anti-PLA2R negative' primary membranous nephropathy are numerous and include poor sensitivity of current assays; heterogeneity of the conformational PLA2R epitope among cases of membranous nephropathy; disappearance of the circulating antibody prior to clinical remission (spontaneous or treatment induced); and involvement of other, non-PLA2R1 antigen–antibody systems (e.g. neutral endopeptidase, superoxide dismutase, aldose reductase, alpha-enolase, cationic serum albumin and renal tubular antigens (megalin) [5,6,12▪▪,13–17,18▪▪].

The evidence for pathogenicity of the anti-PLA2R1 autoantibodies in human membranous nephropathy is very strong: Anti-PLA2R autoantibodies can be eluted from diseased kidneys; the anti-PLA2R antibody co-localizes with the PLA2R antigen in glomeruli; recurrence of membranous nephropathy in the transplanted kidney may occur (but not consistently) in the presence of pretransplant anti-PLA2R antibody in the circulation; and anti-PLA2R autoantibody levels correlate with the clinical manifestations of disease and decrease or disappear with remission (spontaneous or treatment-induced) [19▪▪,20▪]. Circulating levels of anti-PLA2R antibody often decline prior to the onset of clinical improvement [20▪]. Discrepancies may exist between the presence of anti-PLA2R autoantibodies in the circulation and the detection of granular deposits of PLA2R antigen in glomeruli (presumably as immune complexes) in membranous nephropathy [21]. Debiec and Ronco [21] studied 42 cases of primary membranous nephropathy: 21 had both anti-PLA2R antibody in circulation and PLA2R antigen in the glomerular deposits; 3 had anti-PLA2R autoantibody in the circulation but no PLA2R detectable in glomerular deposits; and 18 had no circulating anti-PLA2R autoantibody, but 10 of these had PLA2R detectable in the immune deposits. The latter finding could be explained by persistence of immune complexes in subepithelial sites long after the circulating antibody had disappeared.

The control of the nephritogenic immune response in anti-PLA2R antibody positive human membranous nephropathy appears to be under the control of genetic polymorphisms at chromosome 2 (the *PLA2R1* gene locus) and on chromosome 6 (*HLA-DQA1* or other closely contiguous genes at this locus, including *DQA1 05:01*, *DQB1 03:01* or *DQB1 02:01*) [22▪▪]. Presumably these associations depend on binding of immunogenic PLA2R1-derived peptides to HLA-DQA1-expressing antigen-presenting cells leading to activation of T cells and augmentation of autoantibody production. Exactly how single-nucleotide polymorphisms (SNPs) act to define a nephritogenic conformational epitopes on the cysteine-rich domains of PLA2R1 is under intense investigation. Such polymorphisms may explain the inconsistency of recurrence membranous nephropathy in renal transplants even in patients with positive anti-PLA2R autoantibodies [23▪▪,24▪▪]. Theoretically, donor kidneys lacking the relevant *PLA2R1* SNPs might not posses the conformational epitopes and thus be resistant to recurrence. Further research is needed to clarify the role of *PLA2R1* SNPs in recurrent primary membranous nephropathy and *de novo* membranous nephropathy in transplanted kidneys (see below for further discussion).

One of the distinguishing features of membranous nephropathy due to anti-PLA2R autoantibodies is the dominancy of an IgG4 subclass immune response both in circulating antibodies and immunoglobulin deposits in glomeruli, even though autoantibodies of other classes may also appear (usually IgG1) [1▪,25,26▪,27,28,29▪,30]. Similar IgG4 deposits are also seen in recurrences of the primary membranous nephropathy in renal allografts [25]. Recently, in a small cohort ($n = 16$) of apparently primary membranous nephropathy in children, Segawa *et al.* [29▪] found more intense deposition of IgG1, IgG2, IgG4, Factor B and mannose-binding lectin (MBL) in 'global' membranous nephropathy compared to 'segmental' membranous nephropathy. These latter findings have not yet been confirmed in adults with primary membranous nephropathy. Taken together, these observations suggest that primary membranous nephropathy should be regarded as an IgG4-associated disease entity. Th2 cytokine activation appears to promote IgG4 synthesis in primary membranous nephropathy [31]. IgG4 fixes complement poorly via the alternative or classical pathway [32]. It also tends to interfere with the formation of an immune complex lattice when IgG1 antibodies are present [33] and can interfere with complement activation [34]. In this sense, IgG4 might be a 'protective' antibody, whereas other immunoglobulin subclasses might be pathogenic.

In experimental animals, C activation and local formation of the membrane-attack complex (C5b–C9) is a prerequisite for the development of proteinuria in membranous nephropathy, although T-cell activation ($CD8^+$) may also participate in the generation of proteinuria [35,36]. Mannose-binding

lectin (MBL) is commonly co-deposited with IgG in 'global' forms of membranous nephropathy [29▪] and the MBL pathway of complement activation (acting via C4) could theoretically be very important in human membranous nephropathy. In the allogeneic form of infantile membranous nephropathy due to neutral endopeptidase (NEP), alloantibodies of the IgG1 subclass rather than IgG4 subclass alloantibodies may be crucial for development of proteinuria [12▪▪]. Malignancy-related membranous nephropathy [37] is well known to exhibit a deficiency of IgG4 subclass in the immune deposits [26▪,30], yet the clinical and morphological features of membranous nephropathy are identical (or nearly so) to primary membranous nephropathy. The precise role of the subepithelial in-situ deposition of IgG4 subclass anti-PLA2R1 autoantibodies and the generation of abnormal permselectivity of glomeruli in human membranous nephropathy needs much more research.

The recent observation that a large minority (about 30%) of patients with malignancy-related membranous nephropathy have circulating anti-PLA2R1 autoantibodies (by an improved Western blot assay), if confirmed independently, raises important questions regarding the pathogenesis of primary membranous nephropathy [10]. Are the anti-PLA2R antibody-positive cases of malignancy-associated membranous nephropathy also characterized by IgG4 dominant deposits of IgG? This seems unlikely as many studies have shown that malignancy-associated membranous nephropathy is seldom associated with IgG4-dominant IgG deposition [26▪]. Are some malignancies associated with aberrant expression of PLA2R1 nephritogenic epitopes leading to autoimmunity resembling that seen in primary membranous nephropathy? Resolution of the fine structure of the relevant epitopes on PLA2R1 in various disease states will likely resolve this conundrum.

Finally, the stimuli for autoantibody production in PLA2R1-positive human membranous nephropathy remain largely unknown. Is cross-reacting molecular mimicry evoked by exposure to some ubiquitous environmental antigen (e.g. virus) in the presence of a susceptible genetic milieu (e.g. a *PLA2R1* SNP)? If so, the prospect for a preventive strategy for membranous nephropathy looms large on the horizon.

RECURRENCE OF PRIMARY MEMBRANOUS NEPHROPATHY IN RENAL ALLOGRAFTS: IMPLICATIONS FOR PATHOGENESIS

A recurrence of membranous nephropathy in renal allografts performed in patients with membranous nephropathy in their native kidneys has been described for many decades [38]. The frequency of this phenomenon varies between 10 and 40% (depending on the method of ascertainment) and can result in graft loss [23▪▪]. In addition, some patients with diseases other than membranous nephropathy in their native kidneys may develop membranous nephropathy *de novo* posttransplantation [24▪▪], often in association with chronic allograft rejection but not with anti-PLA2R formation [24▪▪]. Patients with anti-PLA2R autoantibodies may be predisposed to such recurrences, but not universally so [23▪▪,24▪▪,39]. A recent seminal study (using protocol or clinically indicated allograft renal biopsies) described the early immunopathologic manifestations of recurrent membranous nephropathy in the renal allograft [23▪▪]. Twenty-one cases of recurrent membranous nephropathy were studied in biopsies taken 0.3–4 months after transplantation. The features of early recurrence of membranous nephropathy were granular glomerular deposition of IgG, kappa and lambda light chains, C4d but usually no C3. Electron-dense subepithelial deposits were observed in 11 of 19 cases studied by electron microscopy. Studies of IgG subclass deposition or MBL were not performed. Control biopsies lacked IgG, C4d, C3 and electron-dense deposits. This very important study raises the issue of a possible role of the MBL pathway of complement activation in the pathogenesis of membranous nephropathy and may encourage the application of complement inhibiting therapy for recurrent disease. It should be noted that the presence of anti-PLA2R autoantibodies prior to transplantation does not always predict recurrence. Whether this is due to the existence of circulating 'nonpathogenetic' anti-PLA2R autoantibodies or to immunogenetic properties of the transplanted kidney cannot be determined at the present time. The absence of C3 deposits in 'early recurrence' of membranous nephropathy is also not easily explained. Recurrences of primary membranous nephropathy are associated with IgG4 deposition, whereas de-novo membranous nephropathy is associated with IgG1 deposition in the glomeruli of allografts [25].

OTHER ANTIGEN–ANTIBODY SYSTEMS IN PRIMARY MEMBRANOUS NEPHROPATHY

Although PLA2R1–anti-PLA2R1 appears to be the major antigen–antibody system operative in primary ('idiopathic') membranous nephropathy, other target antigens and antibodies may also be involved in the pathogenesis of apparently primary membranous nephropathy [5,6,12▪▪,13–17,18▪▪]. In 2002, Debiec *et al.* [12▪▪] first described an alloimmune form of

membranous nephropathy due to antibodies to NEP, mostly in infants and children. Mothers congenitally deficient for NEP who are pregnant with a fetus expressing NEP develop IgG1 and IgG4 anti-NEP alloantibodies, which permeate through the placenta and bind to the native NEP on the fetal podocyte leading to membranous nephropathy, expressed at birth or later. Similar anti-NEP autoantibodies may participate in some cases of membranous nephropathy of adult onset, but this has not been systematically evaluated.

In addition, on rare occasions infants fed cow's milk (containing bovine serum albumin; BSA) may develop membranous nephropathy based on absorption of cationic component of BSA and its subsequent binding to anionic sites in the glomerular capillary wall where it can serve as a 'planted antigen' target for anti-BSA antibodies (to specific epitopes on the BSA molecule) [18▪▪]. This sequence of events has been elegantly described in a small number of infants/children with apparent primary membranous nephropathy, but has not yet been described in adults with apparent primary membranous nephropathy, despite the relative frequency of anti-BSA antibodies in adults.

Additional cases of apparent primary membranous nephropathy involving superoxide dismutase, aldose reductase and alpha-enolase have also been reported, but not independently confirmed [13–16]. Antibodies to alpha-enolase (IgG4 subclass) have been described in membranous nephropathy and eluted from biopsies of patients with membranous nephropathy, but the specificity of this observation has not yet been independently verified [14]. Some of these autoantibodies may represent examples of 'epitope spreading', a common phenomenon in chronic autoimmune diseases. The pathogenetic role of these uncommon autoantibodies in primary membranous nephropathy still remains to be clarified.

ANTIGEN–ANTIBODY SYSTEMS IN SECONDARY MEMBRANOUS NEPRHOPATHY

It has been known for many decades that circulating immune complexes, composed of an exogenous (heterologous) or endogenous (autologous) antigen (not native to the kidney) and its corresponding antibody, can be associated with a lesion of membranous nephropathy in experimental models of disease [1▪,5]. Classically, chronic serum sickness induced by repeated injections of BSA (or bovine gamma globulin) is the model of this disease [40]. Human examples of this model can be cited including cancer-associated membranous nephropathy (neoepitopes of tumor-related antigen); drug-induced membranous nephropathy (mercury or gold); chronic viral diseases (hepatitis B related viral antigen); and thyroid disorders (thyroglobulin) and systemic autoimmune diseases (dsDNA or nucleosomes in lupus erythematosus). The exact mechanisms whereby the circulating immune complexes are concentrated in the subepithelial space of glomeruli are poorly understood. Theoretically, an oligovalent antigen combined with a low-affinity antibody could dissociate and re-associate during migration from the circulation to the subepithelial space and be localized underneath the slit-pore membrane between podocytes. Alternatively, a biophysical attraction (e.g. electrostatic charge interaction) between the antigen and constituents of the basement membrane or podocyte cell membrane might allow certain antigens to 'plant' in subepithelial sites to become targets of circulating antibody (see BSA above). Finally, in rare circumstances megalin (the antigen involved in experimental membranous nephropathy of rats: Heymann nephritis) has been suggested as a target antigen in human disease (such as sickle cell nephropathy, with membranous nephropathy) [17], but megalin is not a target antigen nor do antimegalin antibodies appear in the circulation in primary ('idiopathic') membranous nephropathy [41]. Of note, anti-PLA2R autoantibodies have been observed in sarcoidosis [42] and lupus nephritis [43] as well as in malignancy-related membranous nephropathy [10]. Monoclonal immunoglobulins with unusual charge characteristics may bind to anionic sites in the subepithelial space like cationic BSA [18▪▪,44] and evoke a picture of membranous nephropathy [45].

CLINICAL PERSPECTIVES

The delineation of target antigens (particularly PLA2R) and the immunogenetic foundations of susceptibility will have a transforming effect on diagnosis, prognostic evaluation and therapy of human primary membranous nephropathy, including the possible avoidance of a recurrence of the disease in renal allografts. The in-situ mechanism of formation of subepithelial immunoglobulin containing deposits is well established in membranous nephropathy (particularly in primary membranous nephropathy), but other mechanisms may be operative as well (particularly in secondary membranous nephropathy) (see Fig. 1). Although the shroud enclosing primary membranous nephropathy in mystery has been partially thrown off, much still remains to be discovered. How do the conformational epitopes on native PLA2R become expressed and how do they elicit an autoantibody

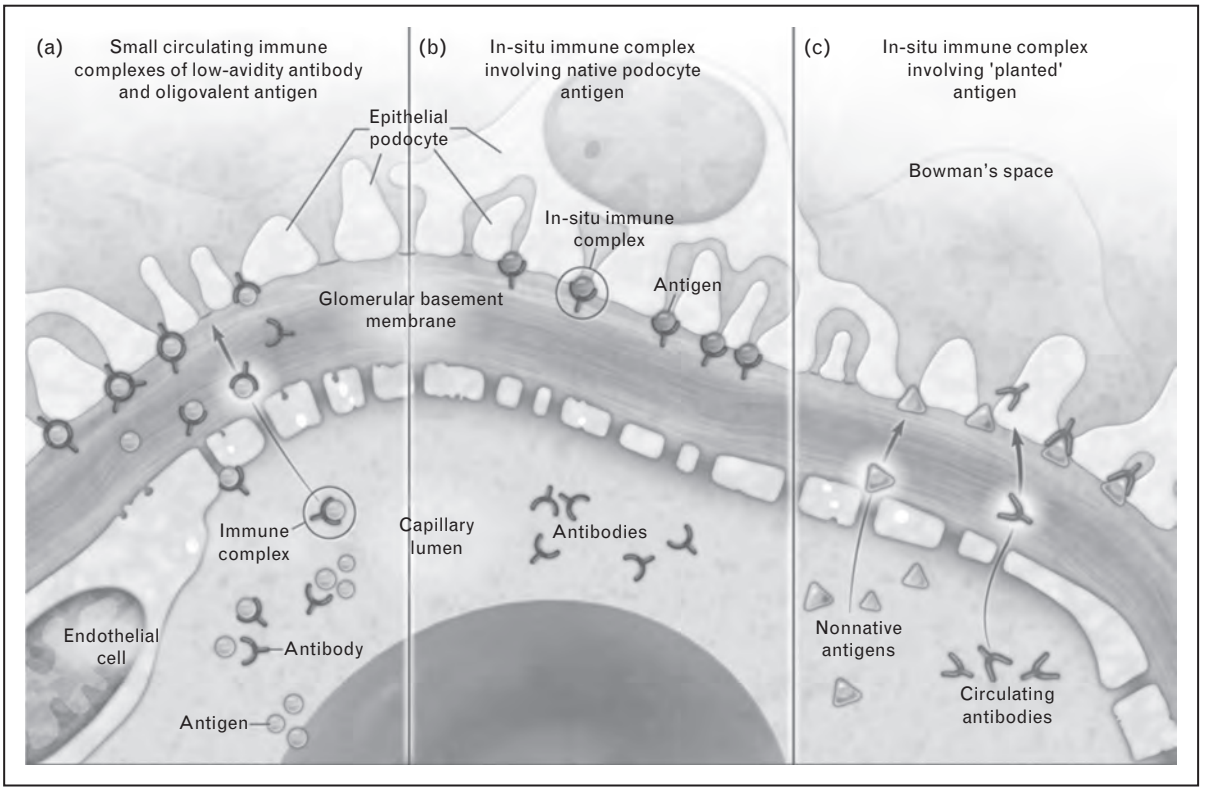

FIGURE 1. (a–c) The immunopathogenetic mechanisms that can be responsible for subepithelial electron-dense deposits containing immunoglobulin (membranous nephropathy). The mechanism shown in (b) [in-situ immune complex involving a native podocyte antigen (PLA2R)] is most common in primary membranous nephropathy. Reproduced with permission [5].

response? What is the molecular nature of the connection between PLA2R1 SNPs and the susceptibility to membranous nephropathy? Why is the autoantibody response so IgG4 subclass dominant in human primary membranous nephropathy? How does the in-situ binding of autoantibody to podocytes lead to a perturbation in glomerular permselectivity (proteinuria)? What are the intermediaries and mediators of podocyte injury in primary membranous nephropathy? What is the spectrum of antigen–antibody systems that operate in primary membranous nephropathy? What pathogenetic mechanisms are responsible for the development of secondary membranous nephropathy?

These and other questions not yet asked will be the focus of future research in membranous nephropathy. The breakthroughs in identifying target antigen in human membranous nephropathy have greatly invigorated the entire research environment of membranous nephropathy, and great advances in our knowledge are expected to occur in the coming decade.

CONCLUSION

Recent advances in our understanding of the pathogenesis of human membranous nephropathy have been both profound and transforming. Clearly, autoantibody formation to PLA2R expressed on the normal glomerular podocyte with in-situ formation of nephritogenic immune complexes is a major factor in primary membranous nephropathy. However, other antigen–antibody systems may also be operative in some cases of primary and many cases of secondary membranous nephropathy, such as malignancy-related membranous nephropathy. A genetically determined predisposition to primary membranous nephropathy disease is now well established. The fine details of autoepitope expression, recognition and autoantibody formation, including the unique predominance of an IgG4 autoantibody response and its genetic control, and the precise mechanisms whereby in-situ formation of immune complexes elicits proteinuria are all under intense investigation. Factors involved in the recurrence of primary membranous nephropathy in renal allografts are increasingly well understood. Much remains to be done, but already new tools for diagnosis, prognosis and therapeutic monitoring of patients with primary membranous nephropathy have been made available. Recurrences of membranous nephropathy in renal allografts may be rendered avoidable. Human membranous nephropathy has evolved from a mysterious disease

of uncertain cause to one with a more defined pathogenetic basis – and the revolution continues.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ■ of special interest
- ■■ of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 341–343).

1. Glassock RJ. The pathogenesis of idiopathic membranous nephropathy: a ■ 50-year odyssey. Am J Kidney Dis 2010; 56:157–167.

An excellent and up-to-date overview of the historical aspect of the pathogenesis of membranous nephropathy.

2. Glassock RJ. Secondary membranous glomerulonephritis. Nephrol Dial Transplant 1992; 7 (Suppl. 1):64–71.
3. Makker SP, Tramontano A. Idiopathic membranous nephropathy: an auto- ■ immune disease. Semin Nephrol 2011; 31:333–340.

An excellent review of the current understanding of the pathogenesis of membranous nephropathy.

4. 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.

A 'classic' paper describing the 'breakthrough' on pathogenesis of human primary membranous nephropathy – a must read.

5. Glassock RJ. Human idiopathic membranous nephropathy – a mystery solved? N Engl J Med 2009; 361:81–83.
6. Ronco P, Debiec H. Antigen identification in membranous nephropathy moves toward targeted monitoring and new therapy. J Am Soc Nephrol 2010; 21:564–569.
7. Ronco P, Debiec H. Membranous glomerulopathy: the evolving story. Curr Opin Nephrol Hypertens 2010; 19:254–259.
8. Lambeau G, Lazdunski M. Receptors for a growing family of secreted phospholipases A2. Trends Pharmacol Sci 1999; 20:162–170.
9. Augert A, Payré C, de Launoit Y, *et al.* The M-type receptor PLA2R regulates senescence through the p53 pathway. EMBO Rep 2009; 10: 271–277.
10. Qin W, Beck LH Jr, Zeng C, *et al.* Antiphospholipase A2 receptor antibody in membranous nephropathy. J Am Soc Nephrol 2011; 22:1137–1143.
11. 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; 26:2526–2532.
12. Debiec H, Guigonis V, Mougenot B, *et al.* Antenatal membranous ■■ glomerulonephritis due to antineutral endopeptidase antibodies. N Engl J Med 2002; 346:2053–2060.

Another 'classic' paper describing alloimmune membranous nephropathy in humans. The first convincing demonstration of an in-situ immune complex pathogenesis of human membranous nephropathy – a must read.

13. Prunotto M, Carnevali ML, Candiano G, *et al.* Autoimmunity in membranous nephropathy targets aldose reductase and SOD2. J Am Soc Nephrol 2010; 21:507–519.
14. 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; 74:2008–2017.
15. Gitlits VM, Toh BH, Sentry JW. Disease association, origin, and clinical relevance of autoantibodies to the glycolytic enzyme enolase. J Invest Med 2001; 49:138–145.
16. Wakui H, Imai H, Komatsuda A, Miura AB. Circulating antibodies against alpha-enolase in patients with primary membranous nephropathy (MN). Clin Exp Immunol 1999; 118:445–450.
17. Strauss J, Pardo V, Koss MN, *et al.* Nephropathy associated with sickle cell anemia: an autologous immune complex nephritis. I. Studies on nature of glomerular-bound antibody and antigen identification in a patient with sickle cell disease and immune deposit glomerulonephritis. Am J Med 1975; 58:382–387.
18. Debiec H, Lefeu F, Kemper MJ, *et al.* Early-childhood membranous nephro- ■■ pathy due to cationic bovine serum albumin. N Engl J Med 2011; 364:2101–2110.

A very important study showing the 'planted antigen' mechanism in rare cases of membranous nephropathy in children.

19. Hofstra JM, Beck LH Jr, Beck DM, *et al.* Antiphospholipase A2 receptor ■■ antibodies correlate with clinical status in idiopathic membranous nephropathy. Clin J Am Soc Nephrol 2011; 6:1286–1291.

A key study showing how anti-PLA2R autoantibody levels relate to clinical features in primary membranous nephropathy.

20. 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; 22:1543–1550.

Another key study showing how one form of treatment (rituximab) results in a lowering of levels of anti-PLA2R autoantibody in membranous nephropathy.

21. Debiec H, Ronco P. PLA2R autoantibodies and PLA2R glomerular deposits in membranous nephropathy. N Engl J Med 2011; 364:689–690.
22. Stanescu HC, Arcos-Burgos M, Medlar A, *et al.* Risk HLA-DQA1 and ■■ PLA(2)R1 alleles in idiopathic membranous nephropathy. N Engl J Med 2011; 364:616–626.

A key study clearly showing an interaction between *PLA2R* polymorphisms and *HLA-DQA1* in determining the predilection for development of primary membranous nephropathy.

23. Rodriguez EF, Cosio FG, Nasr SH, *et al.* The pathology and clinical features of ■■ early recurrent membranous glomerulonephritis. Am J Transplant 2012. doi: 10.1111/j.1600–6143.2011.03903.x. [Epub ahead of print]

A seminal paper on the immunopathological appearance of 'early' recurrent membranous nephropathy in renal allografts.

24. 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; 11:2144–2152.

One of the first detailed studies of anti-PLA2R in recurrent and de-novo membranous nephropathy. Anti-PLA2R autoantibodies do not reliably predict recurrent disease in renal allografts.

25. Kearney N, Podolak J, Matsumura L, *et al.* Patterns of IgG subclass deposits in membranous glomerulonephritis in renal allografts. Transplant Proc 2011; 43:3743–3746.
26. Qu Z, Liu G, Li J,*et al.* Absence of glomerular IgG4 deposition in patients with ■ membranous nephropathy may indicate malignancy. Nephrol Dial Transplant 2011. [Epub ahead of print]

An important study showing that malignancy-associated membranous nephropathy is seldom associated with IgG4 deposition in glomeruli.

27. Omokawa A, Komatsuda A, Nara M,*et al.* Distribution of glomerular IgG subclass deposits in patients with membranous nephropathy and anti-U1 ribonucleoprotein antibody. Nephrol Dial Transplant 2011. [Epub ahead of print]
28. Cravedi P, Abbate M, Gagliardini E, *et al.* Membranous nephropathy associated with IgG4-related disease. Am J Kidney Dis 2011; 58:272–275.
29. Segawa Y, Hisano S, Matsushita M, *et al.* IgG subclasses and complement ■ pathway in segmental and global membranous nephropathy. Pediatr Nephrol 2010; 25:1091–1099.

An important study showing different patterns of deposition of immunoglobulin and complement in 'segmental' compared to 'global' membranous nephropathy in children.

30. Ohtani H, Wakui H, Komatsuda A, *et al.* Distribution of glomerular IgG subclass deposits in malignancy-associated membranous nephropathy. Nephrol Dial Transplant 2004; 19:574–579.
31. Kuroki A, Iyoda M, Shibata T, Sugisaki T. Th2 cytokines increase and stimulate B cells to produce IgG4 in idiopathic membranous nephropathy. Kidney Int 2005; 68:302–310.
32. Aalberse RC, Stapel SO, Schuurman J, Rispens T. Immunoglobulin G4: an odd antibody. Clin Exp Allergy 2009; 39:469–477.
33. Van der Zee JS, van Swieten P, Aalberse RC. Serologic aspects of IgG4 antibodies. II. IgG4 antibodies form small, nonprecipitating immune complexes due to functional monovalency. J Immunol 1986; 137:3566–3571.
34. Van der Zee JS, van Swieten P, Aalberse RC. Inhibition of complement activation by IgG4 antibodies. Clin Exp Immunol 1986; 64:415–422.
35. Cybulsky AV, Quigg RJ, Salant DJ. Experimental membranous nephropathy redux. Am J Physiol Renal Physiol 2005; 289:F660–F671.
36. Penny MJ, Boyd RA, Hall BM. Permanent CD8(+) T cell depletion prevents proteinuria in active Heymann nephritis. J Exp Med 1998; 188:1775–1784.
37. Beck LH Jr. Membranous nephropathy and malignancy. Semin Nephrol 2010; 30:635–644.
38. Ponticelli C, Glassock RJ. Posttransplant recurrence of primary glomerulonephritis. Clin J Am Soc Nephrol 2010; 5:2363–2372.
39. Stahl R, Hoxha E, Fechner K. PLA2R autoantibodies and recurrent membranous nephropathy after transplantation. N Engl J Med 2010; 363:496–498.
40. Dixon FJ, Feldman JD, Vazquez JJ. Experimental glomerulonephritis. The pathogenesis of a laboratory model resembling the spectrum of human glomerulonephritis. J Exp Med 1961; 113:899–920.
41. Whitworth JA, Leibowitz S, Kennedy MC, *et al.* Absence of glomerular renal tubular epithelial antigen in membranous glomerulonephritis. Clin Nephrol 1976; 5:159–162.

42. Knehtl M, Debiec H, Kamgang P, *et al.* A case of phospholipase A2 receptor-positive membranous nephropathy preceding sarcoid-associated granulomatous tubulointerstitial nephritis. Am J Kidney Dis 2011; 57:140–143.

43. Gunnarsson I, Schlumberger W, Rönnelid J. Antibodies to M-type phospholipase A2 receptor (PLA2R) and membranous lupus nephritis. Am J Kidney Dis 2011. [Epub ahead of print]

44. 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; 69:451–461.

45. De Seigneux S, Bindi P, Debiec H, *et al.* Immunoglobulin deposition disease with a membranous pattern and a circulating monoclonal immunoglobulin G with charge-dependent aggregation properties. Am J Kidney Dis 2010; 56:117–121.