Anti-Phospholipase A₂ Receptor Antibodies and the Pathogenesis of Membranous Nephropathy

Pierre Ronco^{a–c} Hanna Debiec^{a, b}

^aSorbonne Universités, UPMC Univ Paris 06, UMR-S 1155, ^bINSERM, UMR-S 1155, and ^cAP-HP, Nephrology and Dialysis, Tenon Hospital, Paris, France

Key Words

Heymann nephritis · Membranous nephropathy · Phospholipase A₂ receptor

Abstract

Since the early 2000s, considerable advances have been achieved in the understanding of molecular pathomechanisms of human membranous nephropathy (MN), inspired by studies of Heymann nephritis, a faithful experimental model. These studies led to the identification of neutral endopeptidase, the type-M phospholipase A₂ receptor (PLA₂R), and cationic bovine serum albumin as target antigens of circulating and deposited antibodies in neonatal alloimmune, adult 'idiopathic', and early childhood MN, respectively. A genome-wide association study further showed a highly significant association of the *PLA₂R1* and the *HLA-DQA1* loci with idiopathic MN in patients of white ancestry. The time has come to revisit the spectrum of MN based on the newly identified antigen-antibody systems which should be considered as molecular signatures of the disease, challenging the uniform histological definition. Although some uncertainties remain as to the pathogenic effects of anti-PLA₂R antibodies because of the lack of an appropriate experimental model, the value of these antibodies as biomarkers for diagnosis and disease activity is increasingly being recognized. It

is not exaggerated to state that they have induced a paradigm shift in the monitoring of patients with MN, thus opening a new era of personalized medicine.

© 2014 S. Karger AG, Basel

In the past 10 years, substantial advances have occurred in the understanding of the molecular pathomechanisms of human membranous nephropathy (MN). These advances were inspired by studies of experimental models of MN such as Heymann nephritis [1] and MN induced by cationic bovine serum albumin (BSA) [2]. Studies of Heymann nephritis led to the concept that a podocyte antigen – megalin – could serve as a target of circulating antibodies leading to the in situ formation of immune complexes [3], while cationic BSA-related MN first illustrated the case of 'planted' antigen. In humans, progress started in 2002 with the identification by our group of neutral endopeptidase (also known as neprilysin) as the target antigen in a rare subset of patients with alloimmune antenatal MN [4, 5]. This finding provided

Biologic Treatment in Glomerular Disease
D. Jayne, Cambridge
V. Tesar, Prague

the proof of concept that a podocyte antigen could be involved in human MN, as is the case for megalin in the rat, and laid the foundation for the identification of M-type phospholipase A₂ receptor (PLA₂R), which was the first podocyte autoantigen shown to be associated with idiopathic MN in humans [6]. Genome-wide association studies further showed that single nucleotide polymorphisms in the *PLA₂R1* gene were strongly associated with idiopathic MN [7], which again pointed to the implication of this antigen using an unbiased genetic approach. In addition to podocyte antigens, exogenous antigens such as cationic BSA have also been implicated in some patients with early childhood MN [8]. All of these findings have opened up a new era for the diagnosis and monitoring of MN from early infancy to adulthood.

In this chapter, we will focus on anti-PLA₂R antibodies, discuss their pathogenicity and show how they have induced a paradigm shift in diagnosis, monitoring and even therapy in patients with primary, so-called idiopathic, MN.

First Characterization of Anti-PLA₂R Antibodies in 2009, 50 Years after the Description of Heymann Nephritis

The use of glycoprotein-enriched glomerular extracts from human kidneys as a source of antigens, and of nonreducing conditions for Western blots, enabled the identification of a 185-kDa protein band that was detected in about 70% of patients with idiopathic MN, but not in those with secondary MN and in controls [6]. Reactivity to this protein persisted after N-deglycosylation, but disappeared after reduction of the disulfide bonds. The type-M PLA₂R was identified by mass spectrometry analysis of the reactive band. Furthermore, sera from patients with MN immunoprecipitated PLA₂R from glomerular extracts. Although anti-PLA₂R reactivity was mostly carried by IgG4, other subclasses were also involved, albeit to a lesser extent.

PLA₂R was detected in normal human glomeruli, apparently in podocytes [6]. PLA₂R and IgG4 were found to be colocalized within the subepithelial immune deposits in patients with idiopathic MN. Furthermore, IgG that was eluted from biopsy specimens from these patients reacted with PLA₂R, whereas IgG eluted from biopsy specimens from patients with lupus MN or IgA nephropathy did not. These data suggest that, like Heymann nephritis and alloimmune neonatal MN, autoimmune idiopathic MN involves in situ formation of subepithelial deposits through binding of circulating anti-PLA₂R autoantibod-

Fig. 1. Structural and functional properties of PLA₂R. This receptor is a type I transmembrane glycoprotein composed of a large extracellular portion that consists of an N-terminal cysteine-rich region, a fibronectin-like type II domain (FNII), a tandem repeat of 8 C-type lectin domains (CTLD), a transmembrane domain and a short intracellular C-terminal domain. Known interactions and domains involved are indicated. PLA₂R can function as a positive regulator of PLA₂ by inducing a variety of biological responses, or as a negative regulator through rapid internalization and degradation of PLA₂. The blue loop shows that in the bent configuration, the N terminal domain folds back to interact with C-type lectin-like domains.

ies to podocyte PLA₂R, although this scenario is challenged by the very low level of expression of PLA₂R in normal podocytes [9, 10].

PLA₂R is a type-1 transmembrane receptor for secretory phospholipase A₂ (fig. 1), a member of the mannose receptor family, which also includes the cation-dependent mannose-6-phosphate receptor, the C-type mannose receptor 2, the dendritic cell receptor DEC-205 and the avian yolk sac IgGY receptor FcRY. Several common features are shared by members of this family [11]. First,

all members are recycled between the plasma membrane and the endosomal machinery, leading to internalization of extracellular ligands. Second, they can present with at least two configurations: an extended conformation with the N-terminal cysteine-rich domain pointing outwards from the cell surface, or a bent conformation where the N-terminal domain folds back to interact with C-type lectin-like domains at the middle of the structure, thus affecting ligand binding and oligomerization [12]. This is especially important because the target epitope for circulating antibodies might be accessible in only one of these two configurations. Putative linear epitopes have been identified with the use of a high-throughput capture immunoassay [13]. Further work on identification and modeling of pathogenic PLA₂R epitopes is ongoing in several laboratories.

Predisposing Gene Variants for 'Idiopathic' MN (2011): Unbiased Confirmation of the Role of PLA₂R

The influence of genetic factors is well established both in rats and mice as well as in European Caucasoids who show a strong association of MN with the HLA-B8DR3 haplotype and other HLA class II immune response genes [14]. Genome-wide association studies have described significant associations of the 6p21 *HLA-DQA1* and 2q24 *PLA₂R1* loci with idiopathic MN in patients of white ancestry [7]. Interestingly, carrying the risk alleles of the two genes had an additive effect. Patients with all four risk alleles had an odds ratio of about 80 for the disease compared with individuals who had only the protective alleles. These data were confirmed in ethnically distant populations from Europe and Asia [14]. The finding of common predisposing variants of *PLA₂R1* conserved across these populations and the observation that anti-PLA₂R antibodies were detected in 73% of the patients with the high-risk variants but absent in all carriers of protective genotypes [15] support the role of PLA₂R as a major target autoantigen.

Because of the strong association of *PLA₂R1* single nucleotide polymorphisms with MN in Caucasians, we hypothesized that rare gene variants of the coding sequence could induce an unusual conformation of the antigen/epitopes, which in turn might trigger autoimmunity. By sequencing the exons and contiguous splice sites in 95 Caucasian patients with idiopathic MN, we found only nine rare variants including two new variants in the whole patients' cohort and only four rare variants in a subset of 60 patients with PLA₂R-related MN [16]. These results

exclude a straightforward 'conformeropathy'. Alternative explanations include rare variants in the noncoding regulatory regions which might increase the level of expression of PLA₂R, and/or epigenetic events of infectious or toxic origin which might modify routing and expression level of PLA₂R in podocytes and other cells as well. Autoimmunity most likely results from a combination of those variants and events with predisposing immune response gene variants in the *HLAD* locus encoding specific HLA class II molecules which would present PLA₂R pathogenic epitopes to the host immune system. Idiopathic MN represents a paradigmatic model of autoimmunity where the currently available tools will hopefully enable us to dissect the genetic bases of the triggering events as well as the mechanisms controlling disease outcome.

Are Anti-PLA₂R Antibodies Pathogenic?

Transfer experiments to confirm the pathogenic effects of anti-PLA₂R antibody have been hampered by an apparent lack of expression of PLA₂R in rodent glomeruli. Furthermore, experimental models based on overexpressing PLA₂R in mouse podocytes have thus far failed. However, several lines of evidence suggest that anti-PLA₂R antibodies are pathogenic. The first is the recurrence of MN in kidney transplant recipients with circulating anti-PLA₂R antibodies sometimes only a few days after transplantation [17, 18], although some patients with high titers of anti-PLA₂R antibodies at transplantation will not recur [19]. The recurrence of MN in a kidney transplant recipient caused by a monoclonal IgG3 kappa targeting the PLA₂ receptor provides additional clues to the pathogenic effects of anti-PLA₂R antibodies [20]. Second, there is accumulating evidence of a tight correlation between anti-PLA₂R antibodies and disease activity, remission and relapse, although there are a few outliers that will require further investigation. Even more, the renal outcome of patients treated with rituximab can be independently predicted by the level of residual antibodies after 6 months [Ruggenti et al., unpubl. data].

PLA₂R Antibodies – Less than 4 Years from Description to Clinical Application: A Success of High-Speed Translational Research

Irrespective of the role of PLA₂R in the pathogenesis of MN, anti-PLA₂R antibodies appear to be a very good biomarkers for this disease. Assays of circulating anti-

Fig. 2. Diagnostic tests. Serological assays of circulating anti-PLA₂R antibodies by indirect immunofluorescence and ELISA are now commercially available. Polyclonal and monoclonal anti-PLA₂R antibodies used for the detection of PLA₂R antigen in glomerular deposits in paraffin-embedded biopsy specimens are also commercially available.

PLA₂R antibodies based on immunofluorescence of PLA₂R1 transfected cells and on ELISA are now commercially available (fig. 2). The US Food and Drug Administration recently approved the commercial sale of these assays, and immunofluorescence assays have been available in Europe for more than 3 years. The specificity of anti-PLA₂R for MN is close to 100%. Patients with nephrotic syndrome from other causes or healthy individuals do not have detectable levels of anti-PLA₂R antibodies. The specificity is such that in elderly patients, in those with poor clinical condition or those with life-threatening complications from nephrotic syndrome such as lung emboli, kidney biopsy can be postponed or even not per-

formed. The sensitivity of the test for idiopathic MN has been around 70–80% in all of the studied populations so far. However, a fraction of the antibody-negative patients might have a secondary cause undiagnosed at the time of kidney biopsy.

A low prevalence of anti-PLA₂R antibodies was observed in secondary forms of MN associated with systemic lupus erythematosus, infectious disease, drug intoxication, graft-versus-host disease and malignancy, although in those cases coincidental occurrence of the PLA₂R-related MN and underlying disorder cannot be excluded [14]. There may be exceptions, and indeed patients with MN associated with active sarcoidosis or replicating hepatitis B appear to have a high prevalence of PLA₂R-related disease, which suggests that the immunologic setting of sarcoidosis and hepatitis B might trigger or enhance immunization against PLA₂R [21–23]. Because therapeutic strategies are different for patients with idiopathic and secondary MN, discriminating between these two groups of patients is of utmost clinical importance.

Detection of PLA₂R antigen in glomerular immune deposits is even more sensitive than that of anti-PLA₂R antibodies since this antigen can be detected in antibody-negative patients [9, 23], which could be explained by rapid clearance of circulating antibodies, immunological remission or delayed biopsy after disease onset. This test enables the retrospective diagnosis of MN in archival, paraffin-embedded biopsy specimens, which is crucial for the monitoring of patients who will benefit from a kidney graft. Its positivity also is a strong clue to primary MN [10, 22]. Conversely, circulating antibodies are not always associated with deposits of PLA₂R antigen, which suggests that not all antibodies to PLA₂R are pathogenic [9]. Combined assessment of circulating anti-PLA₂R antibodies and PLA₂R antigen in biopsy specimens might help to better select the patients for appropriate therapy.

Kidney biopsies should also be analyzed for IgG subclass distribution, which varies according to underlying disease. IgG4 is the major deposited subclass in idiopathic MN, where it is associated with variable amounts of IgG1, IgG2 or IgG3. In contrast, in systemic lupus erythematosus and malignancy-related MN, IgG1 and IgG2 are usually the prevailing subclasses, with low or undetectable amounts of IgG4 [14]. The absence of IgG4 in early stages of MN might be a strong predictor of later occurrence of a malignancy [24].

Several studies have indicated that anti-PLA₂R antibodies are correlated with urinary protein excretion and disease activity since they usually disappear during a

Fig. 3. Time-course of anti-PLA₂R antibodies and proteinuria. The figure shows the temporal relationship between proteinuria (clinical disease) and circulating anti-PLA₂R antibodies (immunological disease). Note that in most patients, anti-PLA₂R antibodies precede clinical disease activity and relapse. The time course of antibody titers predicts response to therapy, and consequently the risk of deterioration of renal function.

spontaneous or treatment-induced remission and reappear at relapse [6, 25–27], although there are outliers. Anti-PLA₂R antibodies are also prognostic markers because a high level of these antibodies is associated with a lower chance of spontaneous [26] or immunosuppressive therapy-induced [28] remission, with a lower response rate to rituximab [Ruggenti et al., unpubl. data] and a higher risk of deterioration of renal function [29] (fig. 3).

Quantification of anti-PLA₂R antibodies will most likely become an invaluable tool for the monitoring of disease immunological activity and the titration of immunosuppressive treatments. Antibodies disappear before proteinuria in patients treated with rituximab [25, Ruggenti unpubl. data], which leads one to consider withdrawal of immunosuppressive treatment at the time of immunological remission before renal remission is achieved. The time lag between immunological and renal remission most likely corresponds to the time required for restoration of the glomerular capillary wall. Anti-PLA₂R antibody levels at the end of therapy may also predict the subsequent course. In a small series of 48 patients, 58% of antibody-negative patients were in persistent remission after 5 years compared with none of antibody-positive patients [30]. However, further prospective studies on large cohorts of patients are needed before drawing definitive conclusions. They will also enable the establishment of the meaning and therapeutic implication of the persistence of PLA₂R antigen in immune deposits in repeat biopsies.

Anti-PLA₂R antibodies should be regularly assessed in patients who have received a kidney graft from the time of transplantation. MN is one of the most frequent etiologies of nephrotic syndrome after renal transplantation. Recurrence of MN, which occurs in 10–40% of cases, can result in graft loss. A few reports have described very early recurrence of PLA₂R-related MN, and all such cases

were associated with anti-PLA₂R antibodies [17–20]. Patients with detectable circulating anti-PLA₂R antibodies prior to transplantation might be predisposed to recurrence although not all patients with high titers of antibody will recur [19]. It was recently shown that the titer of IgG4 anti-PLA₂R during follow-up could predict MN recurrence [31]. Because 50–83% of recurrent cases of MN are associated with anti-PLA₂R antibodies, removing these antibodies before transplantation could be a promising therapeutic approach. Search for anti-PLA₂R antibodies in blood and PLA₂R antigen in kidney biopsy specimen was almost always negative in de novo MN, suggesting a different pathophysiological mechanism most likely related to alloimmunization.

Conclusion

The recent breakthroughs in the pathomechanisms of MN have induced a paradigm shift in the diagnosis and monitoring of patients with this disease. Numerous uncertainties still remain as to the genetic and environmental triggering events, the mechanisms of complement activation, and the epitope(s) recognized. However, the spectrum of human MN now needs to be revisited according to the newly identified antigen-antibody systems, which should be considered as molecular signatures of the disease. These signatures, particularly the new tools of PLA₂R serology, offer great promise for a new personalized medical approach which should be taken into account in future clinical practice guidelines.

Disclosure Statement

Roche provided the authors with rituximab for a RCT (Randomized Controlled Trial).

References

- Heymann W, Hackel DB, Harwood S, Wilson SG, Hunter JL: Production of nephrotic syndrome in rats by Freund's adjuvants and rat kidney suspension. *Proc Soc Exp Biol Med* 1959;100:660–664.
- 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.
- 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* 1982;79:5557–5561.
- Debiec H, Guignon V, Mougenot B, Decobert F, Haymann JP, Bensman A, Deschênes G, Ronco PM: Antenatal membranous glomerulonephritis due to anti-neutral endopeptidase antibodies. *N Engl J Med* 2002;346:2053–2060.
- Debiec H, Nauta J, Coulet F, van der Burg M, Guignon V, Schurmans T, de Heer E, Soubrier F, Janssen F, Ronco P: Role of truncating mutations in MME gene in fetomaternal alloimmunization and neonatal glomerulopathies. *Lancet* 2004;364:1252–1259.
- Beck LH Jr, Bonegio RG, Lambeau G, Beck DM, Powell DW, Cummins TD, Klein JB, Salant DJ: M-type phospholipase A₂ receptor as target antigen in idiopathic membranous nephropathy. *N Engl J Med* 2009;361:11–21.
- Stanescu HC, Arcos-Burgos M, Medlar A, Bockenhauer D, Kottgen A, Dragomirescu L, Voinescu C, Patel N, Pearce K, Hubank M, Stephens HA, Laundry V, Padmanabhan S, Zawadzka A, Hofstra JM, Coenen MJ, den Heijer M, Kiemeny LA, Bacq-Daian D, Stengel B, Powis SH, Brenchley P, Feehally J, Rees AJ, Debiec H, Wetzels JF, Ronco P, Mathieson PW, Kleta R: Risk HLA-DQA1 and PLA(2)R1 alleles in idiopathic membranous nephropathy. *N Engl J Med* 2011;364:616–626.
- Debiec H, Lefeu F, Kemper M, Niaudet P, Deschênes G, Remuzzi G, Ulinski T, Ronco P: Early childhood membranous nephropathy due to cationic bovine serum albumin. *N Engl J Med* 2011;364:2101–2110.
- Debiec H, Ronco P: PLA₂R autoantibodies and PLA₂R glomerular deposits in membranous nephropathy. *N Engl J Med* 2011;364:689–690.
- Hoxha E, Kneifler U, Stege G, Zahner G, Thiele I, Panzer U, Harendza S, Helmchen UM, Stahl RA: 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.
- East L, Isacke CM: The mannose receptor family. *Biochim Biophys Acta* 2002;1572:364–386.
- Llorca O: Extended and bent conformations of the mannose receptor family. *Cell Mol Life Sci* 2008;65:1302–1310.
- Behnert A, Fritzler MJ, Teng B, Zhang M, Bollig F, Haller H, Skoberne A, Mahler M, Schiffer M: 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.
- Debiec H, Ronco P: Immunopathogenesis of membranous nephropathy: an update. *Semin Immunopathol* 2014;4:381–397.
- Lv J, Hou W, Zhou X, Liu G, Zhou F, Zhao N, Hou P, Zhao M, Zhang H: Interaction between PLA₂R1 and HLA-DQA1 variants associates with anti-PLA₂R antibodies and membranous nephropathy. *J Am Soc Nephrol* 2013;24:1323–1329.
- Coenen MJ, Hostra JM, Debiec H, Stanescu HC, Medlar A, Stengel B, Groothuisink JM, Bockenhauer D, Powis SH, Mathieson PW, Brenchley PE, Kleta R, Wetzels JF, Ronco P: Phospholipase A₂ receptor (PLA₂R1) sequence variants in idiopathic membranous nephropathy. *J Am Soc Nephrol* 2013;24:677–683.
- Stahl R, Hoxha E, Fechner K: PLA₂R autoantibodies and recurrent membranous nephropathy after transplantation. *N Engl J Med* 2010;363:496–498.
- 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;12:1637–1642.
- Debiec H, Martin L, Jouanneau C, Dautin G, Mesnard L, Rondeau E, Mousson C, Ronco P: Autoantibodies specific for the phospholipase A₂ receptor in recurrent and de novo membranous nephropathy. *Am J Transplant* 2011;11:2144–2152.
- Debiec H, Hanoy M, François A, Guerrot D, Ferlicot S, Johanet C, Aucouturier P, Godin M, Ronco P: Recurrent membranous nephropathy in an allograft caused by IgG3κ targeting the PLA₂ receptor. *J Am Soc Nephrol* 2012;23:1949–1954.
- Knehtl M, Debiec H, Kamgang P, Callard P, Cadranet J, Ronco P, Boffa JJ: A case of phospholipase A₂ receptor-positive membranous nephropathy preceding sarcoid-associated granulomatous tubulointerstitial nephritis. *Am J Kidney Dis* 2011;57:140–143.
- 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* 2013;26:709–715.
- 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* 2013;28:1839–1844.
- Qu Z, Liu G, Li J, Wu LH, Tan Y, Zheng X, Ao J, Zhao MH: Absence of glomerular IgG4 deposition in patients with membranous nephropathy may indicate malignancy. *Nephrol Dial Transplant* 2012;27:1931–1937.
- Beck LH Jr, Fervenza FC, Beck DM, Bonegio RG, Malik FA, Erickson SB, Cosio FG, Catran DC, Salant DJ: Rituximab-induced depletion of anti-PLA₂R autoantibodies predicts response in membranous nephropathy. *J Am Soc Nephrol* 2011;22:1543–1550.
- Hofstra JM, Debiec H, Short CD, Pellé T, Kleta R, Mathieson PW, Ronco P, Brenchley PE, Wetzels JF: Antiphospholipase A₂ receptor antibody titer and subclass in idiopathic membranous nephropathy. *J Am Soc Nephrol* 2012;23:1735–1743.
- 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.
- Hoxha E, Thiele I, Zahner G, Panzer U, Harendza S, Stahl RA: Phospholipase A₂ receptor autoantibodies and clinical outcome in patients with primary membranous nephropathy. *J Am Soc Nephrol* 2014;25:1357–1366.
- Kanigicherla D, Gummadova J, McKenzie EA, Roberts SA, Harris S, Nikam M, Poulton K, McWilliam L, Short CD, Venning M, Brenchley PE: Anti-PLA₂R antibodies measured by ELISA predict long-term outcome in a prevalent population of patients with idiopathic membranous nephropathy. *Kidney Int* 2013;83:940–948.
- Beck AP, Hofstra JM, Brenchley PE, Wetzels JF: Association of anti-PLA₂R antibodies with outcomes after immunosuppressive therapy in idiopathic membranous nephropathy. *Clin J Am Soc Nephrol* 2014;9:1386–1392.
- Seitz-Polski B, Payré C, Ambrosetti D, Albano L, Cassuto-Viguier E, Berguignat M, Jeribi A, Thouret MC, Bernard G, Benzaken S, Lambeau G, Esnault VL: Prediction of membranous nephropathy recurrence after transplantation by monitoring of anti-PLA₂R1 (M-type phospholipase A₂ receptor) autoantibodies: a case series of 15 patients. *Nephrol Dial Transplant* 2014, Epub ahead of print.