Anti-Phospholipase A\textsubscript{2} Receptor Antibodies and the Pathogenesis of Membranous Nephropathy

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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 A\textsubscript{2} receptor (PLA\textsubscript{2}R), 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 PLA\textsubscript{2}R1 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-PLA\textsubscript{2}R 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.

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 nephrilysin) as the target antigen in a rare subset of patients with alloimmune antenatal MN [4, 5]. This finding provided

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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 PLA2RI 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 autoantibodies 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 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.
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 confirmation with the N-terminal cysteine-rich domain pointing outwards from the cell surface, or a bent confirmation 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 ‘conformopathy’. 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 [Ruggenenti 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-
PLA \(_2\) R antibodies based on immunofluorescence of PLA\(_2\)R\(_1\) 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-PLA\(_2\)R for MN is close to 100%. Patients with nephrotic syndrome from other causes or healthy individuals do not have detectable levels of anti-PLA\(_2\)R 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 performed. 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-PLA\(_2\)R 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 PLA\(_2\)R-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 PLA\(_2\)R-related disease, which suggests that the immunologic setting of sarcoidosis and hepatitis B might trigger or enhance immunization against PLA\(_2\)R [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 PLA\(_2\)R antigen in glomerular immune deposits is even more sensitive than that of anti-PLA\(_2\)R 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 PLA\(_2\)R antigen, which suggests that not all antibodies to PLA\(_2\)R are pathogenic [9]. Combined assessment of circulating anti-PLA\(_2\)R antibodies and PLA\(_2\)R 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-PLA\(_2\)R antibodies are correlated with urinary protein excretion and disease activity since they usually disappear during a
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 [Ruggenenti 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, Ruggenenti 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

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References


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