Anti-phospholipase A₂ Receptor Antibody and Immunosuppression in Membranous Nephropathy: More Evidence for Pathogenicity of Anti-phospholipase A₂ Receptor Autoantibodies

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In the 6 years since the discovery of anti-phospholipase A₂ receptor (anti-PLA₂R) antibodies in the majority of patients with primary membranous nephropathy (MN),¹ our understanding of the role of anti-PLA₂R in clinical disease has evolved rapidly. The initial small retrospective clinical studies indicated that anti-PLA₂R levels could be modulated by immunosuppression.¹ ² Levels decreased in advance of levels of proteinuria, with most patients becoming anti-PLA₂R seronegative over 6–9 months followed by remission of proteinuria over 12–24 months. The European MN cohort study showed an association between high levels of anti-PLA₂R and active disease at presentation with less chance of experiencing spontaneous remission.³ The Manchester study reported high anti-PLA₂R levels linked to poor clinical outcome at 5 years.⁴ Using a standard immunosuppression protocol, Bech et al.⁵ have shown that failure to render patients anti-PLA₂R seronegative by immunosuppression therapy is associated with high risk of relapse. A multicenter study from Germany clearly confirms anti-PLA₂R levels as an independent risk factor for not achieving remission of proteinuria.⁶ Importantly, delay in treatment to reduce anti-PLA₂R may risk decline in renal function.⁷ These studies have benefited from ELISA assays⁸–⁹ developed in the last 3 years to provide sensitive, specific, and quantitative assays of anti-PLA₂R, which are more appropriate than Western blotting for routine clinical assay.

The major therapy for MN over the last 25 years has been nonspecific immunosuppression empirically determined using combinations of cyclophosphamide and steroids. Because of a lack of a relevant biomarker of the immunopathology, remission of proteinuria is used as the dominant outcome measure. Although these potent nonspecific drugs may be effective in preventing disease progression in 60%–80% of patients, this comes at the cost of significant morbidity and mortality because of increased risks of infection, malignancy, and cardiovascular events. Most patients experience a remitting/relapsing disease, commonly requiring several courses of immunosuppression, with a subset of patients displaying resistance to immunosuppressive therapy. This experience indicates the need for improved treatment with targeted drugs and better therapy management on the basis of a relevant biomarker. Rituximab, a specific CD20 B cell–depleting mAb, is the first agent representing a new generation of targeted immunosuppressive therapy that has shown promising results in small studies in induction of remission of nephrotic syndrome in two thirds of patients with primary MN. The paper in this issue of JASN by Ruggenenti et al.⁹ is important, because it describes rituximab immunosuppression in a large series of 101 patients with MN treated in a standardized way in a single center in the context of anti-PLA₂R classification and monitoring.

In this study by Ruggenenti et al.,⁹ outcomes of patients with or without detectable anti-PLA₂Rs at baseline were similar. Current evidence suggests that anti-PLA₂R antibodies are of high affinity.¹⁰ Can this property explain the outcome of patients who are seronegative? As anti-PLA₂Rs are secreted and enter the blood circulation, they will bind to target receptors on podocytes and are effectively affinity adsorbed out of the circulation. In patients where the rate of anti-PLA₂R production is low, it is possible that all anti-PLA₂Rs are adsorbed by podocytes in this way to initiate MN pathology and proteinuria, but the patients seem to be seronegative for anti-PLA₂Rs. Only when the rate of kidney sequestration is less than the rate of anti-PLA₂R production will the patient become seropositive. Previous studies have identified that almost 50% of patients with primary MN who are seronegative for anti-PLA₂Rs show PLA₂R antigen in the glomerular basement membrane immune deposits.¹¹ In this paper, Ruggenenti et al.⁹ did not classify kidney biopsy status for PLA₂R, but this explanation may account for some patients who were seronegative and explain the patient who was originally seronegative but became seropositive postrelapse. Recently, another MN antigen, thrombospondin type 1 domain containing 7A insert, has been described that invokes an anti-thrombospondin type 1 domain containing 7A insert antibody that circulates in the blood that can be eluted from kidney biopsies and may
represent an alternative target in 5% of all patients with MN.\textsuperscript{12} Taking into account these possibilities, the similarity in outcome of the patients who were seronegative is not surprising (i.e., they are driven by related anti-podocyte antibody mechanisms).

For patients with anti-\textit{PLA2R}Rs, lower anti-\textit{PLA2R} titer at baseline and full antibody depletion 6 months post-rituximab strongly predicted remission. This key finding places the presence of anti-\textit{PLA2R} antibodies center stage in the pathology of proteinuria. Furthermore, it confirms the study by Bech \textit{et al.}\textsuperscript{5} using a cyclophosphamide protocol that therapy should render the patient anti-\textit{PLA2R} negative to reduce the risk of future relapse. Antibody depletion after treatment implies that, if anti-\textit{PLA2R}Rs are still being produced, they are all deposited in the glomerulus as mentioned previously. Perhaps a nascent podocyte repair mechanism can be induced to encourage remission after the rate of anti-\textit{PLA2R} attack on the podocyte is reduced below a certain threshold? Anti-\textit{PLA2R}Rs modulate podocyte cell biology \textit{in vitro} (P.E.C. Brenchley, unpublished observations), but nothing is known as to how this affects \textit{in vivo}. Interestingly, rituximab may contribute to promoting podocyte repair through not only the immunosuppressive effect by reducing CD20 B cells and anti-\textit{PLA2R} production but also, a direct effect on the podocyte by regenerating normal actin cytoskeleton biology.\textsuperscript{13} The fact that 18 patients in this study achieved persistent antibody depletion and complete remission and never relapsed holds out hope that, with greater knowledge of podocyte repair mechanisms, more patients could be induced to experience complete remission.

Re-emergence of circulating antibodies predicted disease relapse. This finding associating re-emergence of anti-\textit{PLA2R}Rs with disease relapse is as close to proving cause and effect as can be achieved in clinical medicine. The classic experiment where passive transfer of anti-\textit{PLA2R}Rs to a susceptible species to induce proteinuria has not yet been carried out. The reasons for this are several, including low \textit{PLA2R} expression in rat, mouse, and rabbit glomeruli and uncertainty about cross-reactivity of human anti-\textit{PLA2R}Rs on other species \textit{PLA2R}s. So far, attempts to knock in human \textit{PLA2R}s in the mouse and show good podocyte membrane expression have failed, but should this approach be successful, it would be a way of understanding the dynamics, complexity, and chronicity of anti-\textit{PLA2R} immunopathology.

Outcome was independent of \textit{PLA2R}R1 and \textit{HLA DQA1} polymorphisms. The genetic link of \textit{PLA2R} and \textit{HLA DQA1} to primary MN is very strong.\textsuperscript{14} The link is confirmed in Chinese\textsuperscript{15} and American\textsuperscript{16} patients, and both studies show a strong association between high-risk single-nucleotide polymorphisms (SNPs) in both genes and anti-\textit{PLA2R} status. Bech \textit{et al.}\textsuperscript{5} had suggested that these genes might control anti-\textit{PLA2R} production, and indeed, there is accumulating evidence for this. Patients with MN and \textit{HLA} alleles \textit{DQA1}*05:01 and \textit{DQB1}*02:01 are high anti-\textit{PLA2R} producers.\textsuperscript{4} This paper by Ruggenenti \textit{et al.}\textsuperscript{9} confirms the genetic link to anti-\textit{PLA2R} level but not patient outcome, which has been suggested in a Spanish study.\textsuperscript{17} Sequencing of the \textit{PLA2R} gene in patients with MN shows no obvious SNPs that would cause structural amino acid differences in the MN version of \textit{PLA2R} protein.\textsuperscript{18} Recently, the major B cell epitope in \textit{PLA2R} was located in the N-terminal ricin–like domain that is recognized by 90% of anti-\textit{PLA2R} antibodies.\textsuperscript{10} The highest risk SNPs do not link to this epitope (P.E.C. Brenchley, unpublished observation). However, other more likely possibilities are being explored, including a link to the \textit{PLA2R} peptides that are presented to T cells on the high–risk DQ receptor and also, the possibility that patients with MN may express alternatively spliced variants of \textit{PLA2R}Rs.

Measuring circulating anti-\textit{PLA2R}Rs and proteinuria may help in monitoring disease activity and guiding personalized rituximab therapy. The evidence from this study by Ruggenenti \textit{et al.}\textsuperscript{9} supports monitoring of anti-\textit{PLA2R} during immunosuppression. There is a need to move to frequent prospective anti-\textit{PLA2R} monitoring if we are to understand the dynamics of anti-\textit{PLA2R}Rs in response to immunosuppressive treatment. It is already clear that patient anti-\textit{PLA2R} levels respond variably to a standard immunosuppression protocol.\textsuperscript{5} Experience shows that, in some patients, anti-\textit{PLA2R}Rs disappear rapidly over 3 months, but other patients remain anti-\textit{PLA2R} positive for over 1 year. The ability to tailor immunosuppressive treatment dose for an individual rather than rely on a standard regime would be a significant way to reduce the unwanted side effects of immunosuppression. A clinical trial comparing standard therapy with tailored therapy monitored by anti-\textit{PLA2R} measurement could prove such benefit. Because of the significant side effects of existing therapy, a conservative approach has been common practice to avoid the unnecessary treatment of patients who might experience spontaneous remission. Although this delay of 6–12 months is designed to protect against unwanted treatment, evidence now suggests that patients who do not experience spontaneous remission may be exposed to continuously high levels of anti-\textit{PLA2R}Rs and suffer significant decline in renal function.\textsuperscript{7}

It is timely for clinical trial design to incorporate knowledge from anti-\textit{PLA2R} monitoring of patients. Anti-\textit{PLA2R} levels could be used to stratify patients for immediate treatment (high stable levels and unlikely to experience spontaneous remission) versus delayed treatment (low, declining levels and may experience spontaneous remission). After treatment is initiated, the amount and the duration of treatment could be given until the patient is anti-\textit{PLA2R} negative for several months (\textit{i.e.}, titrating the drug dose to achieve anti-\textit{PLA2R} removal). Anti-\textit{PLA2R} monitoring in support of double-blind, randomized clinical trials to compare effectiveness of immunosuppressive agents, such as rituximab, cyclosporin, and cyclophosphamide, should be the next step to improve outcomes in patients with MN.

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REFERENCES


