Immune Response against Autoantigen PLA2R Is not Gambling: Implications for Pathophysiology, Prognosis, and Therapy

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Membranous nephropathy (MN) is an organ-specific autoimmune disease which targets the kidney glomerulus, resulting in the formation of immune deposits along the glomerular basement membrane, activation of complement, and proteinuria. Although spontaneous remission occurs in up to 40% of patients, 30%–40% of patients will progress to ESRD in 5–15 years, requiring RRT with increased patient comorbidity and substantial economic health care burden. The first major target autoantigen in adult primary MN was identified as the M-type phospholipase A2 receptor (PLA2R), a protein that belongs to the C-type lectin family. PLA2R is a transmembrane glycoprotein composed of a large extracellular portion consisting of an N-cysteine-rich region (CysR), a fibronectin 2 type domain, and a tandem repeat of eight C-type lectin-like domains (CTLDs). The majority of circulating antibodies detected in 75%–80% of patients bind to a conformational, discontinuous epitope stabilized by disulfide bonds. PLA2R discovery was translated very quickly into clinical practice. Simple serologic assays such as the indirect immunofluorescence test and ELISA developed during the past few years provide specific, sensitive, and quantitative measurements of circulating anti-PLA2R antibodies. Anti-PLA2R antibodies are highly specific for MN, not being detected in other nephropathies, autoimmune diseases, or healthy individuals. A number of recent studies further showed that levels of circulating anti-PLA2R antibodies were good prognostic biomarkers and enabled precise monitoring of the response to immunosuppressive treatment. Despite these major advances, there are still many unresolved questions regarding the mechanisms involved in triggering immune response, progression and remission of the disease, and response to therapy. It is still not known why the rate of stable remission does not exceed 70%, irrespective of immunosuppressive treatment. Further molecular insights based on the identification of B and T cell epitopes on PLA2R are required to design more targeted and less toxic therapies and to deliver specific markers of disease initiation and progression.

Recently, a first step in this direction has been taken. In 2015, two independent groups identified an immunodominant epitope region in the PLA2R protein using two different technical approaches. The first epitope region was identified in the three most N-terminal domains of PLA2R by Kao et al. The reduction-sensitive conformational epitope was formed by regions from the CysR and CTLD1 domains brought into contact by the fibronectin 2 type domain. In parallel, Fresquet et al. found that the dominant epitope was exclusively localized to disulfide-bonded peptide within the CysR domain. However, this epitope was maintained only under non-denaturing conditions and the CTLD3 domain was needed for preserving it under denaturing conditions. In both studies, the epitope region in the N-terminal portion of PLA2R explained most of the reactivity of anti-PLA2R antibodies. Surprisingly, at variance with observations made in most autoimmune disorders, epitope spreading was not observed, possibly because studies were performed on a small number of patients or pooled sera.

This discrepancy has been resolved by Seitz-Polski and colleagues in the current issue of the Journal of the American Society of Nephrology. Using nine PLA2R mutants generated by successive deletion of the extracellular domains of the receptor, they confirmed that the CysR region contains the primary dominant epitope, but in addition, they first demonstrated epitope spreading toward the CTLD1 and CTLD7 domains. By using this new approach, they also showed that CysR and CTLD1 are two independent domains recognized by distinct anti-PLA2R autoantibodies.

Seitz-Polski et al. have defined epitope regions of PLA2R autoantigen using recombinant truncated molecules containing approximately 140 amino acid residues. However, such studies do not provide clues on actual contact amino acid residues for antibody paratopes which, in the case of PLA2R, engage conformational structures stabilized by disulfide bonds. Most antibodies produced during immune responses react with conformational epitopes formed from assembled topographic determinants made up of amino acid residues brought into contact on the surface of the molecule during protein folding. Such conformational epitopes can encompass 20–30 amino acids on the surface of the antigenic protein, but...
only a few amino acids within this region can be critical contact residues for antibody binding.\textsuperscript{11,12} Identification of these amino acids critical for binding of autoantibody to an immunodominant nephrilgenic peptide will offer the opportunity for the development of peptide-based immunomodulatory therapies.\textsuperscript{13} An inhibitory effect of orally or nasally administered immunodominant peptides has been reported in several experimental models of autoimmune diseases.\textsuperscript{14,15} Moreover, resolution of the molecular structure of the PLA2R nephrilgenic epitope will help design nonpeptide antagonists that could serve as bait for antibody decay.

How the autoantibody is produced at disease onset remains unclear. PLA2R is weakly expressed in podocytes under normal conditions and is not accessible to the circulating T cells, whose function is to activate the antigen-specific B cells. The molecular analysis presented by Seitz-Polski et al.\textsuperscript{10} should give new impetus to the search for mimicking sequences among antigens of microbial or other environmental origin. Molecular mimicry between these external antigens and PLA2R could lead to production of anti-PLA2R antibodies in patients genetically predisposed to autoimmunity,\textsuperscript{16} and might well account for the increasing incidence of primary MN worldwide. Alternatively, immunization could be due to the unmasking of hidden cryptic epitopes undergoing conformational changes on exposure to toxic environmental factors.

The second important finding of Seitz-Polski et al.\textsuperscript{10} is the correlation of epitope spreading with clinical presentation and outcome as previously observed in many autoimmune diseases and in rat Heymann nephritis, a faithful animal model of human primary MN.\textsuperscript{7,17} They used a soluble form of each domain to develop an epitope-specific ELISA, which allowed characterization of the antibody profile for each patient. They could thus divide their cohort of 69 patients into three epitope-specific subgroups: CysR, CysRCTLD1, and CysRCTLD1CTLD7. The patients with exclusive reactivity with CysR, where the primary dominant epitope is located, had less severe renal disease at the time of diagnosis and a favorable outcome. By contrast, reactivity against CTLD1 and CTLD7 was associated with active disease and was an independent risk factor of poor renal prognosis. Furthermore, follow-up studies showed that epitope spreading was associated with disease worsening, whereas reverse spreading from CysRCTLD1CTLD7 profile back to CysR was associated with a favorable outcome. These observations show that immune response to PLA2R is not gambling, but is an ordered process with prognostic relevance. Epitope spreading also has a therapeutic impact. In patients with the CysRCTLD1CTLD7 profile (as in those with very high titers of anti-PLA2R antibody), it might be unwise to delay immunosuppressive treatment for 6 months, waiting for a problematic spontaneous remission, as recommended by the Kidney Disease Improving Global Outcomes guidelines.\textsuperscript{18} Epitope spreading might be a limitation to decay intervention strategy directed to a single immunodominant epitope because of the possible emergence of additional epitope specificities.

Although Seitz-Polski et al.\textsuperscript{10} provide significant advances in the molecular dissection of PLA2R epitopes, several critical questions still need to be addressed. First, they studied circulating antibodies, but the most relevant antibodies are those which are deposited in the glomerulus. Although the observed clinical correlations suggest that epitope spreading is clinically relevant, these observations should be expanded by analyzing the reactivity of immunoglobulins eluted from a limited number of kidney biopsies from patients with different circulating antibody profiles. Second, it remains to be established whether the intramolecular epitope spreading is relevant to the pathogenesis of MN. Multivalent interactions with several epitopes of PLA2R at the podocyte surface might be required, as observed in Heymann nephritis.\textsuperscript{7,17} The lower level of proteinuria in patients with anti-CysR antibodies only is reminiscent of the coincidence of epitope spreading with onset of proteinuria in rats with Heymann nephritis.\textsuperscript{7,17} However, many antibodies produced during epitope spreading may have no effect, and immunoreactive domains were detected with patients’ sera but not with eluates from kidney. Further studies are needed to examine the pathogenic role of the domain-specific antibodies, including their effect on PLA2R receptor function, such as clearance of proinflammatory soluble PLA2 and activation of signaling pathways leading to podocyte senescence.\textsuperscript{19,20} Third, Seitz-Polski et al.\textsuperscript{10} did not study PLA2R-negative patients. One could speculate that some of these patients could yet have low levels of PLA2R domain-specific antibodies. Fourth, PLA2R is expressed outside the kidney, yet MN is an organ-specific disease. PLA2R is a member of the mannose receptor family and like all members of this family, it might undergo conformational shifts between a more extended conformation and a compact, folded configuration which may be regulated by oligomerization, ligand binding, or pH.\textsuperscript{21} These properties suggest that PLA2R expressed in podocytes could have a unique podocyte-specific conformational structure with the initiating epitope region in the CysR domain being accessible first for antibody binding. Lastly, the possibility of intermolecular spreading (i.e., the production of antibodies to antigens other than PLA2R) should also be investigated, particularly in more severely affected patients.

By identifying a subset of more toxic antibodies, Seitz-Polski et al.\textsuperscript{10} provide both additional clues to the pathogenic effect of PLA2R antibodies and new prognostic biomarkers. Their findings are a step further toward more precision medicine in patients with MN. Further studies are needed to expand their data in prospective cohorts of patients, to further identify B cell and T cell epitopes, and to gain insight into the pathophysiologic consequences of epitope spreading.

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None.

REFERENCES

See related article, “Epitope Spreading of Autoantibody Response to PLA2R Associates with Poor Prognosis in Membranous Nephropathy,” on pages 1517–1533.