The Dominant Humoral Epitope in Phospholipase A_2 Receptor-1: Presentation Matters When Serving Up a Slice of π

Laurence H. Beck Jr.
Renal Section, Department of Medicine, Boston University Medical Center, Boston, Massachusetts

J Am Soc Nephrol 26: 237–239, 2015.
doi: 10.1681/ASN.2014090877

Phospholipase A_2 receptor-1 (PLA_2R), the major autoimmune target in primary membranous nephropathy,[1] is one of four mammalian members of the mannose receptor (MR) family, which consists of the MR (encoded by the *MRC1* gene), Endo180 (*MRC2*), DEC205 (*LY75*), and PLA_2R itself.[2] An additional member, the FcY receptor, is present in avian species and most closely related to PLA_2R.[3] All are transmembrane glycoproteins with extracellular regions that consist of an N-terminal cysteine-rich (CysR) domain, a fibronectin II-type (FnII) domain, and 8–10 C-type lectin-like domains (CTLDs).

Early studies characterizing the autoantibody–antigen interaction noted that reactivity to PLA_2R was abolished if the Western blot (WB) was performed under reducing conditions,[1] which suggested that disulfide bonds were crucial for stabilizing the conformation of the humoral epitope(s). The fact that other MR family members underwent conformational shifts in response to changes in pH[2,4] also gave credence to a potential conformational epitope in PLA_2R. Finally, the identification of several disease-associated single nucleotide polymorphisms within the *PLA2R1* gene[5–7] raised the possibility that a variant sequence might confer an altered configuration to PLA_2R that might be recognized as foreign by the immune system. However, this speculation has since fallen out of favor, because additional sequence analysis has not identified any nonsynonymous single nucleotide polymorphisms unique to PLA_2R-associated membranous nephropathy.[8]

In this issue of *JASN*, two groups hone in on the location of the major humoral epitope in PLA_2R. Kao *et al.*[9] elegantly show that the epitope is within the three most N-terminal domains of the protein and shares the reduction-sensitive properties initially noted for the entire molecule. Fresquet *et al.*[10] go one step farther to localize the epitope to within the CysR domain. Before these reports, there was limited evidence about the position or number of relevant epitopes within PLA_2R. It had been shown that preincubation of PLA_2R with human autoantibodies did not interfere with binding of a well used commercial antibody generated against an epitope in the CTLD2–3 region.[11] Behnert *et al.*[12] identified several linear peptides within PLA_2R that were reactive with anti-PLA_2R–positive sera in an SPOT assay; several of these peptides were also able to partially inhibit autoantibody binding to the nondenatured molecule.

The core consensus domain of CysR is the ricin B domain, which was first identified as a repeated sugar-binding region in the B chain of the plant-derived toxin ricin. This evolutionarily conserved domain is thought to have arisen from gene triplication of an ancient galactoside-binding peptide, resulting in its current three-lobed (β-trefoil) structure.[13] The ricin B domain exists in several contexts in mammalian proteins—most notably the N-terminal CysR domains of the MR family members and the C-terminal ricin B domains of the numerous polypeptide N-acetylgalactosaminyltransferases present in the Golgi. Both groups exhibit six conserved cysteine residues as opposed to the four residues in ricin B. The crystal structure of the MR CysR reveals three disulfide bonds, two of which are involved in internally stabilizing the individual lobes and the third links the final lobe to the extended structure of the molecule.[14]

Because of differences in nomenclature between the two works[9,10] and for the purpose of describing the various PLA_2R constructs used in these articles, I will abbreviate the CysR (or Ricin B) domain as R, the FnII domain as F, and the CTLD repeats as $C_{x–y}$. The full extracellular domain would, therefore, be designated as $RFC_{1–8}$.

Kao *et al.*[9] have masterfully engineered a full panel of PLA_2R constructs and show that anti-PLA_2R autoantibodies seem to exclusively recognize a reduction-sensitive epitope localized to the three most N-terminal domains, RFC_1. Kao *et al.*[9] could not further delimit the epitope-containing region because of the instability of the individual domains. However, deleting the first CTLD from the otherwise intact extracellular domain (*i.e.*, $RFC_{2–8}$) was sufficient to abolish all autoantibody binding by WB, suggesting an important role for this domain in stabilizing the structure of the humoral epitope. Of interest, introduction of the M292V or H300D risk alleles into CTLD1 did not, within the limits of the assay, affect binding. By artificially introducing proteolytic cleavage sites between the domains of RFC_1, Kao *et al.*[9] could selectively remove domains on either end (CysR or CTLD1); however, the smaller pieces did not retain antigenicity by WB. This line of experimentation also provided

Published online ahead of print. Publication date available at www.jasn.org.

Correspondence: Dr. Laurence H. Beck Jr., Renal Section, Department of Medicine, Boston University Medical Center, Evans Biomedical Research Center, X-504, 650 Albany Street, Boston, MA 02118. Email: laurence.beck@bmc.org

evidence that FnII and CTLD1 might be linked by an interdomain disulfide bridge. Kao *et al.*[9] conclude that the RFC_1 structure is the smallest portion of PLA_2R that allows accurate presentation of the epitope by WB because of an ill-defined interdependency of the three domains. This result was strengthened by the fact that soluble RFC_1 could block autoantibody binding to the full extracellular domain.

The article by Fresquet *et al.*[10] reveals that there are limitations to a comprehensive characterization of antibody–antigen interactions imposed by the typical WB protocol. Before SDS-PAGE, proteins are denatured with heat and detergents, disrupting tertiary structure. This is in contrast to other assay systems, such as ELISA, that allow detection of antigen in a more physiologic native state. Just as the routine performance of WB under reducing conditions may have delayed identification of PLA_2R as the major antigen in membranous nephropathy, the reliance on WB alone to detect discrete epitopes in PLA_2R may have resulted in an incomplete story.

Fresquet *et al.*[10] use a variety of experimental systems to assess autoantibody binding to PLA_2R and ultimately, define the major humoral epitope within the CysR domain proper. Like Kao *et al.*,[9] they were unable to find reactivity by WB in small N-terminal constructs; in their hands, $RFC_{1–3}$ was the smallest construct identified under denaturing WB conditions, because reactivity was lost with $RFC_{1–2}$. However, when these same constructs were assayed under the nondenaturing conditions of a slot blot, anti-PLA_2R autoantibodies detected $RFC_{1–2}$ reactivity and more importantly, the isolated CysR domain. The larger constructs $RFC_{1–3}$ and $RFC_{1–8}$ were able to maintain the structure of the epitope, even in the face of denaturation or reduction, hinting at interdomain interactions within the context of the larger molecule. Experiments using surface plasmon resonance (SPR) reveal remarkably consistent binding affinities of purified anti-PLA_2R to CysR and the larger constructs, strongly suggesting that, for the majority of sera tested by SPR, the autoantibody binds primarily to CysR and not to other locations within the extracellular domain.

In a parallel set of experiments, Fresquet *et al.*[10] perform mass spectrometric analysis of the smallest common proteolytic fragments of PLA_2R reactive with autoantibodies by WB. Fresquet *et al.*[10] find that only two of the identified peptides, both from a similar region of CysR, can significantly inhibit anti-PLA_2R binding to $RFC_{1–3}$. When they add the intervening sequence of PLA_2R to synthesize a 31-residue stretch roughly corresponding to the first lobe within the β-trefoil structure of CysR, Fresquet *et al.*[10] discover that they have recreated the dominant epitope of PLA_2R. It is remarkable that not only can this 31-mer inhibit anti-PLA_2R binding to $RFC_{1–3}$ by 85% but that it also exhibits a binding affinity identical to that of the larger constructs as determined by SPR and has reduction-sensitive properties because of an internal disulfide bond.

Fresquet *et al.*[10] additionally use two techniques to show that, similar to its MR family counterparts, PLA_2R has pH-dependent changes in configuration, behaving as a more compact structure at acidic pH. Although this change does not affect binding of anti-PLA_2R, it is possible that such conformational change, possibly in conjunction with one or more disease-associated sequence variants, could influence processing of PLA_2R within the acidic endosomes and lysosomes of an antigen-presenting cell. Finally, Fresquet *et al.*[10] use transmission electron microscopy and single-particle averaging to generate low-resolution structural models of PLA_2R. There is evidence that CysR and CTLD3 make contact to form a ring-like structure and that the overall PLA_2R structure resembles the Greek character π.

Both articles provide evidence that, in the majority of cases, the epitopic region in the N-terminal portion of PLA_2R explains all of the reactivity of anti-PLA_2R–positive sera.[9,10] In constructs lacking the three N-terminal domains, Kao *et al.*[9] fail to detect reactivity to the residual domains of the molecule. In a cohort of serum samples from patients with primary membranous nephropathy, reactivity to RFC_1 was almost entirely concordant with reactivity to the whole molecule. Although this analysis was performed by WB with the caveats above, it is reassuring that Fresquet *et al.*[10] provide confirmatory data, with one exception: 10% of the subjects in the cohort described by Fresquet *et al.*[10] do not exhibit inhibition by $RFC_{1–3}$ of anti-PLA_2R reactivity with the full molecule, suggesting that one or more epitopes may exist in the more C-terminal portion of the molecule. The location of these minor epitopes and the percentages of PLA_2R-associated cases associated with antibodies to these minor epitopes will need to be better determined. For now, existing immunoassays containing the entire extracellular domain should be sufficient to detect all anti-PLA_2R antibodies. Neither work shows any strong evidence for epitope spreading (*i.e.*, additional subdominant epitopes that might appear later in the course of disease), although this has not been specifically investigated in either of the articles.[9,10]

The end result of these studies is that there is a dominant humoral epitope in the very N-terminal region of PLA_2R with exposure that is clearly affected by experimental conditions and potentially, physiologic conditions as well. Such results move the field forward at a number of levels. By identifying a discrete region that seems to confer the majority of reactivity with anti-PLA_2R autoantibodies, hypotheses about the initial events in the autoimmune process can better be proposed and studied. Fresquet *et al.*[10] note that the nine-amino acid peptide within their 31-mer that itself is capable of inhibiting 47% of binding shares homology with a bacterial enzyme, thus invoking molecular mimicry as a potential initiator of autoimmunity. Although humoral (B cell) epitopes are not equivalent to T cell epitopes, Fresquet *et al.*[10] propose a hypothetical model of how B cells, initially reactive with a specific bacterial antigen, might subsequently internalize and process a similar-appearing PLA_2R, such that specific peptides are loaded into class II HLA molecules encoded by specific risk alleles (*e.g.*, HLA-DQA1) in membranous nephropathy. In addition, if the identified region turns out to be the primary and dominant humoral epitope responsible for disease, a small molecular inhibitor of antigen binding might

be sufficient to neutralize anti-PLA_2R antibodies and render them unable to bind their target antigen.

Both groups are to be congratulated for their meaningful contributions to our evolving understanding of PLA_2R-associated membranous nephropathy, and we look forward to future studies that build on this exciting research.[9,10]

DISCLOSURES

None.

REFERENCES

1. Beck LH Jr., Bonegio RG, Lambeau G, Beck DM, Powell DW, Cummins TD, Klein JB, Salant DJ: M-type phospholipase A_2 receptor as target antigen in idiopathic membranous nephropathy. *N Engl J Med* 361: 11–21, 2009
2. Llorca O: Extended and bent conformations of the mannose receptor family. *Cell Mol Life Sci* 65: 1302–1310, 2008
3. West AP Jr., Herr AB, Bjorkman PJ: The chicken yolk sac IgY receptor, a functional equivalent of the mammalian MHC-related Fc receptor, is a phospholipase A_2 receptor homolog. *Immunity* 20: 601–610, 2004
4. Boskovic J, Arnold JN, Stilion R, Gordon S, Sim RB, Rivera-Calzada A, Wienke D, Isacke CM, Martinez-Pomares L, Llorca O: Structural model for the mannose receptor family uncovered by electron microscopy of Endo180 and the mannose receptor. *J Biol Chem* 281: 8780–8787, 2006
5. Kim S, Chin HJ, Na KY, Kim S, Oh J, Chung W, Noh JW, Lee YK, Cho JT, Lee EK, Chae DW; Progressive Renal Disease and Medical Informatics and Genomics Research (PREMIER) members: Single nucleotide polymorphisms in the phospholipase A2 receptor gene are associated with genetic susceptibility to idiopathic membranous nephropathy. *Nephron Clin Pract* 117: c253–c258, 2011
6. Liu YH, Chen CH, Chen SY, Lin YJ, Liao WL, Tsai CH, Wan L, Tsai FJ: Association of phospholipase A_2 receptor 1 polymorphisms with idiopathic membranous nephropathy in Chinese patients in Taiwan. *J Biomed Sci* 17: 81, 2010
7. Stanescu HC, Arcos-Burgos M, Medlar A, Bockenhauer D, Kottgen A, Dragomirescu L, Voinescu C, Patel N, Pearce K, Hubank M, Stephens HA, Laundy V, Padmanabhan S, Zawadzka A, Hofstra JM, Coenen MJ, den Heijer M, Kiemeney 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* 364: 616–626, 2011
8. Coenen MJ, Hofstra JM, Debiec H, Stanescu HC, Medlar AJ, Stengel B, Boland-Augé A, Groothuismink JM, Bockenhauer D, Powis SH, Mathieson PW, Brenchley PE, Kleta R, Wetzels JF, Ronco P: Phospholipase A_2 receptor (PLA2R1) sequence variants in idiopathic membranous nephropathy. *J Am Soc Nephrol* 24: 677–683, 2013
9. Kao L, Lam V, Waldman M, Glassock RJ, Zhu Q: Identification of the immunodominant epitope region in phospholipase A2 receptor-mediating autoantibody binding in idiopathic membranous nephropathy. *J Am Soc Nephrol* 26: 291–301, 2015
10. Fresquet M, Jowitt TA, Gummadova J, Collins R, O'Cualain R, McKenzie EA, Lennon R, Brenchley PE: Identification of a major epitope recognized by PLA2R autoantibodies in primary membranous nephropathy. *J Am Soc Nephrol* 26: 302–313, 2015
11. Hoxha E, Kneißler U, Stege G, Zahner G, Thiele I, Panzer U, Harendza S, Helmchen UM, Stahl RA: Enhanced expression of the M-type phospholipase A_2 receptor in glomeruli correlates with serum receptor antibodies in primary membranous nephropathy. *Kidney Int* 82: 797–804, 2012
12. Behnert A, Fritzler MJ, Teng B, Zhang M, Bollig F, Haller H, Skoberne A, Mahler M, Schiffer M: An anti-phospholipase A_2 receptor quantitative immunoassay and epitope analysis in membranous nephropathy reveals different antigenic domains of the receptor. *PLoS ONE* 8: e61669, 2013
13. Rutenber E, Ready M, Robertus JD: Structure and evolution of ricin B chain. *Nature* 326: 624–626, 1987
14. Liu Y, Chirino AJ, Misulovin Z, Leteux C, Feizi T, Nussenzweig MC, Bjorkman PJ: Crystal structure of the cysteine-rich domain of mannose receptor complexed with a sulfated carbohydrate ligand. *J Exp Med* 191: 1105–1116, 2000

See related articles, "Identification of the Immunodominant Epitope Region in Phospholipase A_2 Receptor-Mediating Autoantibody Binding in Idiopathic Membranous Nephropathy," and "Identification of a Major Epitope Recognized by PLA2R Autoantibodies in Primary Membranous Nephropathy," on pages 291–301 and 302–313, respectively.

A Breath of Fresh Air for Diabetic Nephropathy

Volker H. Haase*†

*Division of Nephrology and Hypertension, Departments of Medicine, Cancer Biology, and Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, Tennessee, and †Medicine and Research Services, Department of Veterans Affairs Hospital, Tennessee Valley Healthcare System, Nashville, Tennessee

J Am Soc Nephrol 26: 239–241, 2015.
doi: 10.1681/ASN.2014080754

Diabetic nephropathy (DN) is a leading cause of CKD and a common complication in patients with type 1 or type 2 diabetes mellitus. The disease, as it progresses through defined morphologic and clinical stages, frequently leads to ESRD. Despite certain therapeutic interventions that slow its progression (such as blockade of the renin-angiotensin axis and strict BP, lipid, and glycemic control), the risk of advancing to ESRD remains high. Innovative therapeutic interventions are urgently needed to halt the development and progression of this devastating disease, which is responsible for approximately 40% of all new ESRD cases in the United States.[1]

In this issue of *JASN*, Nordquist and colleagues report that activation of the hypoxia-inducible factor (HIF) pathway with cobalt chloride ($CoCl_2$) protects kidneys from DN in a rat model of type 1 diabetes.[2] The investigators used streptozotocin to induce diabetes mellitus and began to treat animals with $CoCl_2$ at the time of streptozotocin administration for 4 weeks. Their study demonstrates that $CoCl_2$ treatment has strong beneficial effects on a functional and morphologic level. By the end of the study, diabetic rats had normal GFR and showed significant

Published online ahead of print. Publication date available at www.jasn.org.

Correspondence: Dr. Volker H. Haase, Department of Medicine, Division of Nephrology and Hypertension, Vanderbilt University Medical Center, S-3223, MCN, 1161 21st Avenue S., Nashville, TN 37232. Email: volker.haase@vanderbilt.edu