Anti–LAMP-2 Autoantibodies in ANCA-Associated Pauci-Immune Glomerulonephritis

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The association between anti–lysosome-associated membrane protein 2 (LAMP-2) autoantibodies and vasculitis is not new; it dates back to work by Kain et al.1 in 1995 that identified LAMP-2 as a target of antineutrophil cytoplasmic antibodies (ANCA) in 14 of 16 patients with a pauci-immune necrotizing crescentic glomerulonephritis. LAMP-2 makes an intriguing target for ANCA; it is a major constituent of the lysosomal membrane,2 plays a role in autophagy,3 and is involved in HLA class II antigen presentation.4 It is a highly glycosylated protein that is found not only lining neutrophil granules but also on the cell surface, where it is a ligand for E-selectin.5

Taken together, these diverse roles link LAMP-2 to the clearance of intracellular pathogens and the immune response, thereby raising the possibility that anti–LAMP-2 antibodies contribute significantly to the pathogenicity of ANCA. The work by Kain et al.6 addressed this directly in a follow-up 2008 study, showing that the immunization of rats with anti–LAMP-2 IgG was sufficient to precipitate a crescentic glomerulonephritis. They also showed that anti–LAMP-2 IgG activates human neutrophils as efficiently as antiproteinase 3 (anti-PR3) autoantibodies and—unlike anti-PR3 antibodies—anti–LAMP-2 antibodies are sufficient to induce the apoptosis of endothelial cells in vitro.6

Perhaps the most-discussed finding of that study, however, was evidence for molecular mimicry as a potential initiating factor in anti–LAMP-2 autoantibody formation.6 Specifically, an antibody-binding continuous epitope, P41–49 of LAMP-2, was found to share significant homology with the bacterial adhesion protein FimH, expressed on a subset of gram-negative bacteria.6 The fact that the homology should have been with gram-negative bacteria was surprising given the accumulated evidence for Staphylococcus aureus involvement in the pathogenesis of ANCA vasculitis.6 Nonetheless, 9 of 10 rats immunized with recombinant FimH proceeded to develop cross-reactive anti–LAMP-2 antibodies and a pauci-immune glomerulonephritis.6 This finding is important, because although long hypothesized, it represented the first time that molecular mimicry had been directly shown in ANCA; neither of the two other major ANCA classes, anti-PR3 or antimyeloperoxidase, shares homology with known bacterial or viral antigens.

Anti–LAMP-2 autoantibodies have the potential, therefore, to signal a major advance in our understanding of ANCA-associated glomerulonephritis: they are widely prevalent in active disease (14 of 16 patients in ref. 1 and 78 of 84 patients in ref. 6), precipitate pauci-immune glomerulonephritis in a rat model, activate human neutrophils, induce endothelial cell apoptosis in vitro, and bind an epitope on LAMP-2 shared with common gram-negative bacteria.

However, these findings represent the work of one group of investigators and have not been yet been replicated in an independent laboratory; in JASN, the work by Roth et al.8 reports their attempts to replicate these results. In an initial cross-sectional cohort of 103 University of North Carolina patients with ANCA glomerulonephritis, they found an anti–LAMP-2 antibody prevalence of 21.1%,8 substantially lower than the prevalence reported by Kain et al.6 and similar to the prevalence of healthy volunteers and two disease control cohorts, including patients with active urinary tract infection and a variety of non–ANCA–associated glomerulonephritides. In a second cohort of 226 patients from Massachusetts General Hospital with ANCA-associated vasculitis, anti–LAMP-2 antibody prevalence was 21.2%. The work by Roth et al.8 then determines autoantibody reactivity against the LAMP-2 P41–49 epitope possessing FimH homology, again finding a similar prevalence to their control cohort. Finally, they injected WKY rats with antibodies shown to be reactive against LAMP-2 and FimH.8 None of these rats went on to develop clinical or histologic glomerulonephritis.8

In contrast, JASN also contains a report by Kain et al.9 in which their initial findings of high anti–LAMP-2 antibody prevalence in ANCA-associated glomerulonephritis are recapitulated in additional cohorts drawn from three European centers: Vienna, Groningen, and Cambridge. Using three independent assays (ELISA, Western blot, and immunofluorescence), they report an overall frequency of 81% in patients presenting with an ANCA-associated glomerulonephritis, significantly higher than healthy controls and two sets of disease controls, one with a range of non–ANCA–associated renal diseases and another with active systemic lupus erythematosus.9 Together, these findings represent data from an additional 74 patients with untreated, new-onset ANCA-associated disease and 52 patients with treated ANCA-associated disease either during a flare or in remission.9

The discordance between these two studies is concerning, and reconciling them will be critical to determining the role of anti–LAMP-2 antibodies in the pathogenesis and clinical management of ANCA-associated vasculitis.

The two most obvious sources of variation are the patient populations and the assay design, especially the choice of LAMP-2 antigen. A consistent feature of the studies by Kain...
et al.\textsuperscript{6,9} has been that the highest anti-LAMP-2 antibody prevalence is in patients presenting with an untreated, active glomerulonephritis. Moreover, they found that anti-LAMP-2 titers are remarkably sensitive to disease activity and/or immunosuppression to the extent that, in their current report, Kain et al.\textsuperscript{9} find that anti-LAMP-2 antibodies were undetectable in 36 of 37 patients after 1 month of treatment. This finding is relevant, because the cohorts enrolled in the work by Roth et al.\textsuperscript{8} are cross-sectional and representative of a range of disease activities and treatment. Only 15 patients among their University of North Carolina cohort of 103 patients had new-onset disease, of which 7 patients were untreated. Of those 15 patients with new-onset active ANCA glomerulonephritis, 7 patients had detectable anti-LAMP-2 antibodies—two times the anti-LAMP-2 prevalence of the remaining patients with continuing disease. Although this finding fails to achieve statistical significance, it provides a hint that population differences may contribute to the discordant findings. However, even in this subset, the prevalence of anti-LAMP-2 remains substantially lower than the prevalence reported in the work by Kain et al.,\textsuperscript{9} suggesting that additional factors are also involved.

Foremost among these factors is likely to be the different assays used by the two groups. One of the strengths of the work by Kain et al.\textsuperscript{9} is that each sample was tested in triplicate by three independent methods: ELISA, Western blot, and immunofluorescence. These assays were concordant in 71\% of samples tested across all three cohorts.\textsuperscript{9} Their ELISA and Western blot assays used a nonglycosylated form of the LAMP-2 extracellular domain (342 amino acids) expressed in Escherichia coli that was GST-tagged and purified using glutathione sepharose as an antigen substrate; for their immunofluorescence assay, they used a glycosylated form of the full-length LAMP-2 protein expressed in ldlC cells.\textsuperscript{9} It is clear that generation and purification of LAMP-2 was not straightforward: it had to be manufactured in small batches and used promptly to overcome significant issues of degradation and contamination. They also found that commercially available anti-LAMP-2 antibodies showed a lack of specificity for recombinant human LAMP-2, prompting them to source a custom antibody elsewhere. By comparison, for their ELISA, Roth et al.\textsuperscript{8} cloned the extracellular domain of LAMP-2 into human embryonic kidney (HEK) cells, resulting in a soluble, glycosylated form. A second nonglycosylated short form (amino acids 30–127) of LAMP-2 produced using a wheat germ cell-free system was obtained commercially as an ELISA substrate for their Massachusetts General Hospital cohort.\textsuperscript{8} Therefore, the antigen substrates used in the two studies are not identical: to what extent might this difference affect assay performance? Although the HEK-based ELISA used by Roth et al.\textsuperscript{8} and the ELISA used by Kain et al.\textsuperscript{9} agreed perfectly for nine shared samples (four with and five without detectable antibody), a confirmatory Western blot on these same samples using the HEK-derived antigen was negative. Given the question regarding the specificity of commercial anti-LAMP-2 antibodies used as a positive control, it is possible that the Western blot result may reflect differences in the antigen substrate. Anti-LAMP-2 antibodies bind at multiple epitopes; variation in antibody binding at a subset of epitopes, perhaps related to glycosylation status, could also contribute to the apparent lower sensitivity of the HEK-based assay. It is more difficult to draw conclusions regarding the ELISA used in the Massachusetts General Hospital cohort. The reactivity of the shared serum samples using this assay is not reported, and such a comparison would have been useful given that by this assay even positive samples were found to have comparatively low titers.\textsuperscript{6}

More difficult to rationalize are the differences in reactivity against a LAMP-2 P41–49 synthetic peptide and the inability of this peptide to reproduce a clinical phenotype in WKY mice. However, it is notable that the initial report by Kain et al.\textsuperscript{6} used a competition ELISA format, whereas the work by Roth et al.\textsuperscript{8} used a peptide ELISA in which controls as well as patient sera were highly reactive.

In summary, two papers examining the prevalence and significance of anti-LAMP-2 antibodies in ANCA-associated glomerulonephritis with markedly discordant results are published in \textit{JASN}. The report by Kain et al.\textsuperscript{9} adds additional weight and detail to their earlier findings of a greater than 80\% prevalence of anti-LAMP-2 autoantibodies in patients with untreated, active pauci-immune glomerulonephritis.\textsuperscript{1,6} They also replicate their earlier observation that anti-LAMP-2 antibodies fall rapidly with treatment/remission and recur with relapse, a useful property for disease activity monitoring.\textsuperscript{1,6,9} The inclusion of cohorts with non-ANCA-associated renal disease and systemic lupus erythematosus provides additional evidence for the specificity of this autoantibody.\textsuperscript{1,6,9} The report by Roth et al.,\textsuperscript{8} however, tempers these observations with a note of caution. Using similar, but not identical, methodology, they have not been able to replicate those findings.\textsuperscript{8} There are enough differences in the enrolled cohorts and assay design to provide a potential explanation for these results, but a priority in the ongoing evaluation of anti-LAMP-2 antibodies for use as a clinical biomarker needs to be a robust and reproducible assay validated across multiple laboratories. With this assay in place, the role of anti-LAMP-2 antibodies in the pathogenesis and clinical phenotype of ANCA-associated vasculitis also needs additional clarification, particularly given an accumulating body of clinical and genetic evidence suggesting that ANCA-associated vasculitis consists of quite distinct disease entities best defined by their anti-PR3 or antimiylperoxidase ANCA specificity.

\textbf{DISCLOSURES}

None.

\textbf{REFERENCES}


See related articles, “Anti–LAMP-2 Antibodies Are Not Prevalent in Patients With Antineutrophil Cytoplasmic Autoantibody Glomerulonephritis” and “High Prevalence of Autoantibodies to hLAMP-2 in Anti–Neutrophil Cytoplasmic Antibody–Associated Vasculitis,” on pages 545–555 and 556–566, respectively.