7-1-2019

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Peroxidasin Is a Novel Target of Autoantibodies in Lupus Nephritis

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Received 20 February 2019; revised 5 April 2019; accepted 8 April 2019; published online 15 April 2019

Kidney Int Rep (2019) **4,** 1004–1006; https://doi.org/10.1016/j.ekir.2019.04.009

Peroxidasin is an animal heme peroxidase with broad tissue distribution, secreted and embedded in basement membranes and implicated in the homeostasis of type IV collagen.[1] Peroxidasin uses bromide ion (Br^-) and hydrogen peroxide (H_2O_2) to generate hypobromous acid (HOBr), which is essential for formation of sulfilimine (S=N) bonds that covalently crosslink type IV collagen molecules at the NC1-NC1 interface.[2] Sulfilimine bonds crosslinking of α345(IV) collagen in the glomerular basement membrane (GBM) confer protection against Goodpasture (GP) disease, an autoimmune kidney disease mediated by autoantibodies binding to sites within the NC1 hexamers of α345(IV) collagen.[3] A recent study reports that approximately 46% of patients with GP disease have autoantibodies to peroxidasin, which inhibit HOBr generation activity.[4] Anti-peroxidasin antibodies, additionally identified in approximately 13% of patients with anti-myeloperoxidase (anti-MPO) ANCA-associated vasculitis (AAV), have been proposed to be a serologic marker of disease in the pulmonary-renal syndrome spectrum.[4] Besides GP disease and AAV, systemic lupus erythematosus (SLE) is another cause of pulmonary-renal syndrome. We tested the hypothesis that anti-peroxidasin antibodies are present in lupus nephritis (LN).

RESULTS

Sera from 32 patients with biopsy-confirmed proliferative or membranous LN (ISN/RPS class III, IV, or V) were analyzed for peroxidasin immunoreactivity by enzyme-linked immunosorbent assay (ELISA) (see Supplementary Material). Anti-peroxidasin IgG antibodies were identified in 2 of 25 (8%) patients with proliferative LN (one each class III and class IV), and also in 3 of 29 (10%) patients with GP disease (Figure 1). The prevalence of anti-peroxidasin antibodies in GP disease in our study is lower than that previously reported (46%),[4] which may be due to demographic differences in the patient population. Alternatively, our immunoassay may be less sensitive owing to technical differences between the assays, in which case the true prevalence of anti-peroxidasin antibodies in LN may be underestimated. Anti-α3NC1 or anti-MPO IgG autoantibodies, which invariably accompanied previously identified anti-peroxidasin antibodies, are sometimes found in patients with LN,[5,6] prompting us to investigate whether these autoantibodies also occurred together with anti-peroxidasin antibodies in LN. However, in our LN cohort, none of the sera contained antibodies binding to purified MPO (Suppolementary Figure S1). Anti-α3NC1 antibodies were also absent in LN, except for q case that did not contain anti-peroxidasin antibodies (Supplementary Figure S2). These findings imply that there are anti-peroxidasin antibodies in a subpopulation of patients with proliferative LN, in the absence of serologic markers of anti-MPO AAV or GP disease.

We further characterized anti-peroxidasin antibodies from LN compared with GP disease. Their ability to bind native peroxidasin was verified by competition ELISA using soluble antigen. Soluble peroxidasin inhibited antibody binding to immobilized peroxidasin in a dose-dependent manner, with similar half-maximal inhibitory concentration for LN and GP sera (Figure 2a). To determine whether the autoantibodies recognize conformational epitopes, we compared

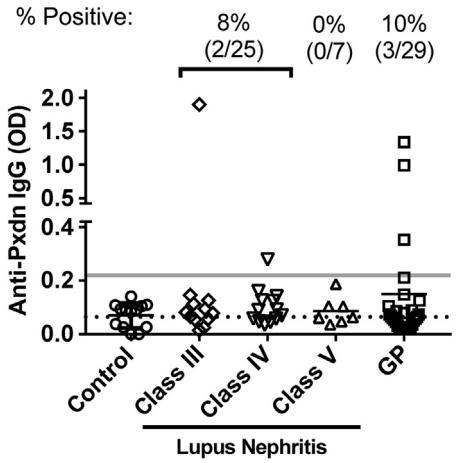

Figure 1. Anti-peroxidasin (anti-Pxdn) IgG autoantibodies are present in sera of lupus nephritis. Immunoreactivity toward peroxidasin was evaluated by enzyme-linked immunosorbent assay in sera from controls ($n = 13$) and patients with proliferative lupus nephritis (class III or class IV), membranous lupus nephritis (class V), and Goodpasture disease (GP). Sera diluted 1:100 were incubated in wells coated with 200 ng purified recombinant peroxidasin, then IgG binding was measured. The positivity threshold (indicated by a gray line) was set at 3 SD above the mean of controls (dotted line). OD, optical density.

IgG binding with native versus misfolded peroxidasin. Decreased binding to misfolded peroxidasin, indicative of conformational epitopes, was observed for anti-peroxidasin IgG from LN but not GP sera (Figure 2b).

DISCUSSION

Our results identify peroxidasin as a novel target of autoantibodies in LN. In contrast to most autoantigens implicated in SLE, peroxidasin is an intrinsic component of basement membranes that provides a target for direct autoantibody binding. Immunofluorescence staining of the renal biopsy from the patient with LN with higher titer of anti-peroxidasin showed strong IgG staining (3+) accompanied by IgA, C1q, C3, kappa, and lambda (2–3+ each) in glomeruli, tubules, interstitium, and vessels, with IgM (2+) additionally found in glomeruli and tubules. Antibodies binding to peroxidasin in the GBM may accelerate or exacerbate glomerular immune complex formation, potentially increasing disease activity or severity. Interestingly, the patient with LN with higher titer of anti-peroxidasin antibodies showed declining renal function (estimated glomerular filtration rate decreased from 96 ml/min per 1.73 m^2 at serum collection to 52 ml/min per 1.73 m^2 at follow-up), but a larger sample size will be required to analyze clinical associations.

Altered crosslinking of type IV collagen has been proposed as a major pathogenic mechanism for anti-peroxidasin autoantibodies from GP sera, based on their ability to inhibit in vitro HOBr generation by

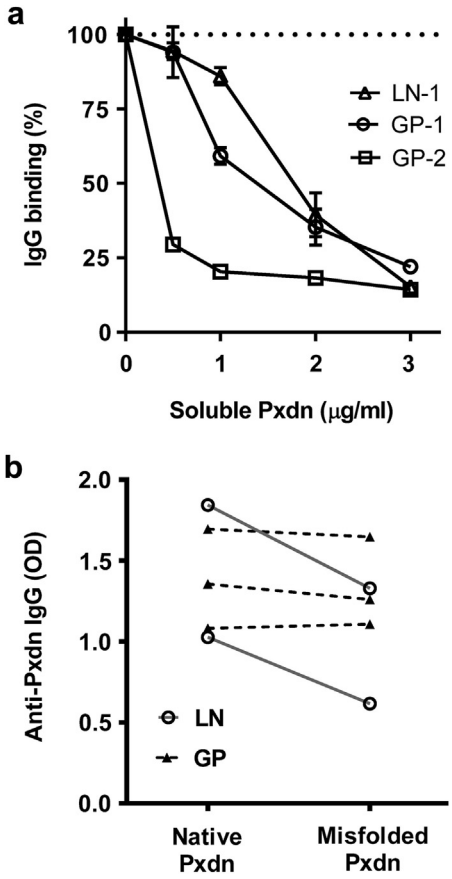

Figure 2. Characterization of anti-peroxidasin (anti-Pxdn) antibodies. (a) The specificity of antibodies for native peroxidasin was verified by competition enzyme-linked immunosorbent assay using soluble antigen. Lupus nephritis (LN) or Goodpasture disease (GP) sera diluted 1:1000 were preincubated for 1 hour at room temperature with the shown concentrations of native peroxidasin (Pxdn), then added to wells coated with 50 ng of recombinant peroxidasin. For each serum, IgG binding was calculated relative to binding in the absence of an inhibitor, which was considered 100% (dotted reference line). Means and SD from 2 independent experiments are shown. (b) Anti-Pxdn antibodies from LN sera recognize conformation-dependent epitopes. A 1:100 dilution of sera containing anti-Pxdn antibodies was applied to wells coated with 200 ng of peroxidasin in phosphate-buffered saline (native Pxdn) or in 3M guanidinium chloride containing 20 mM tris(2-carboxyethyl)phosphine as a reducing agent (misfolded Pxdn). The mean ± SD of the relative decrease in IgG binding to misfolded Pxdn was 34% ± 5% for LN sera compared with 3% ± 5% for GP sera ($P = 0.012$, 2-tailed unpaired t test). OD, optical density.

peroxidasin by 70%.[4] Reduced crosslinking of α345(IV) collagen in the GBM is essential for the pathogenesis of GP disease because: (i) GP autoantibodies bind only to a small subset of α345(IV)NC1 hexamers that are not stabilized by crosslinks,[7] and (ii) sulfilimine bonds lock the epitopes preventing the *in vivo* binding of GP autoantibodies to the GBM.[8] Consistent with a role in promoting GP disease, anti-peroxidasin immunoreactivity has been detected before the onset of clinical disease in approximately half of the prediagnostic cases of GP disease.[4] In analogous manner, continued presence of anti-

peroxidasin antibodies over a period of months (commensurate with the rate of collagen IV turnover) may impair the crosslinking of α345(IV) collagen in LN. An association between LN and GP disease has been reported in 1 retrospective study, in which 9% (14/157) of patients with SLE had detectable levels of anti-GBM (anti-α3NC1) autoantibodies along with antinuclear antibodies, and some of these patients presented with rapid progressive glomerulonephritis or were clinically diagnosed with GP disease.[6] It is plausible that anti-peroxidasin antibodies may be among the factors triggering GP disease in patients with LN, a rare presentation with worse outcomes.

To decrease sulfilimine bond formation, given that already formed bonds are not affected, anti-peroxidasin antibodies must be present when nascent collagen IV molecules assemble within basement membranes. Their impact would be maximal during periods of rapid turnover of basement membranes, such as during fetal development or the formation of feto-maternal interface. Therefore, the potentially harmful role of anti-peroxidasin autoantibodies in lupus pregnancies warrants further investigation. As precedent, autoantibodies to phospholipids or laminin-111 (a component of basement membranes in placenta and developing tissues) may occur in lupus and are associated with adverse pregnancy outcomes, whereas maternal anti-Ro/SS-A and anti-La/SS-B autoantibodies have been implicated in neonatal lupus.[9]

Among limitations, this was an exploratory study in a relatively small number of patients. Our findings should be validated in a larger study, also investigating clinical associations. Due to the small amounts of sera available, we could not isolate sufficient IgG to test whether anti-peroxidasin antibodies inhibit HOBr generation. Definitive proof that anti-peroxidasin antibodies are pathogenic will require future studies in animal models yet to be developed.

DISCLOSURE

All the authors declared no competing interests.

ACKNOWLEDGMENTS

We thank Mary Hoffmann and Diane Salamon for specimen and data procurement. This work was supported by a pilot project (to Dorin-Bogdan Borza) from the Meharry Translational Research Center grant U54 MD0007593 from the National Institute on Minority Heath and Health Disparities. The Kidney Translational Research Center at Washington University School of Medicine was partly supported by the George O'Brien Center for Kidney Disease Research (grant P30 DK079333) and by the Division of Nephrology at Washington University School of Medicine. Additional support was from the Paul Teschan Research Fund grant from Dialysis Clinic Inc, R01 DK-116964 from National Institute of Diabetes and Digestive and Kidney Diseases, and the Burroughs Wellcome Fund Career Award for Medical Scientists (13030995).

SUPPLEMENTARY MATERIAL

Figure S1. Absence of anti-MPO antibodies in sera of patients with LN.

Figure S2. Analysis of anti-α3NC1 antibodies in sera of patients with LN.

Supplementary Methods.

Supplementary material is linked to the online version of the paper at www.kireports.org.

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