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CLINICAL RESEARCH

Progressive IgA Nephropathy Is Associated With Low Circulating MBL-Associated Serine Protease-3 (MASP-3) and Increased Glomerular Factor H–Related Protein-5 (FHR5) Deposition

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Introduction: IgA nephropathy (IgAN) is characterized by glomerular deposition of galactose-deficient IgA1 and complement proteins and leads to renal impairment. Complement deposition through the alternative and lectin activation pathways is associated with renal injury.

Methods: To elucidate the contribution of the lectin pathway to IgAN, we measured the 11 plasma lectin pathway components in a well-characterized cohort of patients with IgAN.

Results: M-ficolin, L-ficolin, mannan-binding lectin (MBL)-associated serine protease (MASP)-1 and MBL-associated protein (MAp) 19 were increased, whereas plasma MASP-3 levels were decreased in patients with IgAN compared with healthy controls. Progressive disease was associated with low plasma MASP-3 levels and increased glomerular staining for C3b/iC3b/C3c, C3d, C4d, C5b-9, and factor H–related protein 5 (FHR5). Glomerular FHR5 deposition positively correlated with glomerular C3b/iC3b/C3c, C3d, and C5b-9 deposition, but not with glomerular C4d. These observations, together with the finding that glomerular factor H (fH) deposition was reduced in progressive disease, are consistent with a role for fH deregulation by FHR5 in renal injury in IgAN.

Conclusion: Our data indicate that circulating MASP-3 levels could be used as a biomarker of disease severity in IgAN and that glomerular staining for FHR5 could both indicate alternative complement pathway activation and be a tissue marker of disease severity.

Kidney Int Rep (2018) ■, ■–■; https://doi.org/10.1016/j.ekir.2017.11.015
KEYWORDS: complement; IgA nephropathy; lectin; MBL

IgA nephropathy (IgAN) is a common glomerular pathology that frequently causes renal failure, especially in young people.[1,2] IgAN is characterized by glomerular deposits of galactose-deficient IgA1.[3,4] Although a 4-hit theory is proposed for mesangial IgA deposition,[5] the mechanisms leading to glomerular injury remain poorly understood. The clinical course of IgAN is heterogeneous: after 20 years of follow-up from renal biopsy, up to 40% of patients will reach end-stage renal disease, but 20% of patients will have preserved renal function.[6] Our incomplete understanding of IgAN pathogenesis limits the development of biomarkers allowing the identification of patients who may benefit from immunosuppression and disease-specific therapies.[2,7]

The complement system is an important inflammation-generating arm of the immune system. Complement activation occurs in IgAN.[8] Colocalization of glomerular complement C3c with IgA is present in 90% of cases.[3] Serum levels of activated C3[9] and mesangial C3 deposition[10] correlate with loss of renal function. The degree of complement regulation is also important. Imbalances in plasma factor H (fH), an essential negative regulator of C3 activation, and factor H–related (FHR) proteins 1 and 5, that deregulate fH,

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Received 26 October 2017; revised 16 November 2017; accepted 21 November 2017; published online 29 November 2017

associate with IgAN.[11,12] Complement activation leads to the accumulation of C3 proteolytic fragments, such as C3dg, within glomeruli.[8]

The lectin pathway pattern-recognition molecules include MBL (mannan-binding lectin), L-ficolin (also called ficolin-2), M-ficolin (ficolin-1), H-ficolin (ficolin-3), collectin liver 1 (CL-L1, also called CL-10), and collectin kidney 1 (CL-K1 or CL-11). Following interaction with ligands that include pathogen and/or damage-associated molecular patterns, the pattern-recognition molecules trigger complement activation through complexed serine proteases: MBL-associated serine protease (MASP)-1, MASP-2, and MASP-3. Pattern-recognition molecules also can bind nonenzymatic subunits: MBL-associated protein (MAp) 19 and MAp44. The pathway generates a C3-convertase, termed C4bC2b.[13] The C4b is further processed to C4d. The finding of glomerular C4d in the absence of C1q, the activator of the classic pathway of complement, in IgAN is consistent with lectin pathway activation.[14]

IgAN is characterized by disease flares following respiratory or gastrointestinal tract inflammation[15]; both IgA and the lectin complement pathway are important mediators of innate immunity at these sites. IgAN is associated with higher levels of IgA1 with exposed N-acetyl-galactosamine.[5,16] N-acetyl-galactosamine is a structure that may trigger lectin pathway activation due to interaction of ficolins with patterns of acetyl-groups.[17] Furthermore, MBL binds polymeric IgA and triggers complement activation *in vitro*.[18] Both high and very low MBL levels were associated with poor renal outcomes in a Chinese IgAN population.[19] Roos *et al.*[20] demonstrated glomerular MBL, L-ficolin, MASP1/3, and C4d deposition in 25% of patients with IgAN, which associated with disease severity. This finding is supported by the association of glomerular C4d deposition with poor prognosis in IgAN.[12,21]

We hypothesized that the lectin pathway contributes to glomerular inflammation and disease severity in IgAN. We examined (i) levels of circulating lectin pathway components; (ii) glomerular complement deposition; and (iii) glomerular fH, FHR1, and FHR5 deposition in IgAN. Using a cohort of patients with IgAN stratified into those with either stable or progressive disease, we identified circulating lectin pathway components, glomerular complement protein deposition, and immunohistologic evidence of fH deregulation that correlated with disease severity.

METHODS

Study Cohort and Clinical Measurements

We expanded our previously characterized[11] Causes and Predictors of Outcome in IgA Nephropathy study cohort of patients with biopsy-proven IgAN to 323 patients (Supplementary Figure S4, UK National Research Ethics Service Committee number 14/LO/0155). Progressive disease was defined by at least 1 of the following criteria: (i) end-stage renal disease without histology evidence of a second pathology causing renal impairment; (ii) biopsy evidence of endocapillary hypercellularity, or (iii) cellular and/or fibrocellular crescents; (iv) treatment with immunosuppression for native IgAN; (v) clinical Henoch-Schonlein purpura, unless spontaneous resolution and >20 years of follow-up with "stable" criteria; or (vi) 50% loss of estimated glomerular filtration rate (eGFR) or average annual loss of eGFR of more than 5 ml/min without evidence of a second pathology causing renal impairment. Stable disease was defined as meeting all of the following: (i) urine protein-creatinine ratio less than 100 units or daily proteinuria of less than 1 g/24 hours; (ii) combined Oxford classification[22] MEST (mesangial hypercellularity [M], endocapillary hypercellularity [E], segmental glomerulosclerosis [S], interstitial fibrosis/tubular atrophy [T]) score of less than 3; and (iii) average annual loss of eGFR of less than 3 ml/min per 1.73 m^2. The transplantation cohorts have also been characterized.[11] Control samples were obtained from healthy volunteers. The eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration Creatinine Equation.[23]

Protein Measurements

Levels of MBL,[24] M-ficolin,[25] H-ficolin,[26] CL-L1,[27] CL-K1,[28] MASP-1,[29] MASP-2,[30] MASP-3,[31] MAp19,[32] and MAp44[31] were measured using time-resolved immunofluorometric sandwich-type immunoassays as previously described using "in-house" antibodies. Plasma L-ficolin was measured by enzyme-linked immunosorbent assay (Hycult Biotech, Uden, The Netherlands). Serum IgA and galactose-deficient IgA1 levels were measured by enzyme-linked immunosorbent assay.[33]

Histology

Immunohistochemistry protocols were optimized (Supplementary Figures S5–S7) for formalin-fixed paraffin-embedded renal biopsy tissue with the following antibodies: rabbit polyclonal anti-human C3c (Dako, Glostrup, Denmark), rabbit polyclonal anti-human C4d (DB Biotech, Kosice, Slovakia), mouse monoclonal anti-human factor H (OX-24; Abcam, Cambridge, UK), rabbit polyclonal anti-human C3d (Abcam), mouse monoclonal anti-human C5b9 (Dako), mouse monoclonal anti-human FHR1 (Abnova, Taipei, Taiwan), and rabbit polyclonal anti-human FHR5 (Abnova). The anti-C3c antibody cannot distinguish among C3c, C3b, and iC3b, so we refer to this staining as anti-C3b/iC3b/C3c. We graded antigen-staining

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