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Research paper

Cross-reactivity of anti-PLA2R1 autoantibodies to rabbit and mouse PLA2R1 antigens and development of two novel ELISAs with different diagnostic performances in idiopathic membranous nephropathy

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ABSTRACT

About 70% of patients with idiopathic membranous nephropathy (iMN) have autoantibodies to the phospholipase A2 receptor PLA2R1. We screened sera from iMN patients for their cross-reactivity to human (h), rabbit (rb) and mouse (m) PLA2R1 by western blot (WB) and antigen-specific ELISAs. All iMN patients recognized hPLA2R1 and rbPLA2R1 by WB, and a rbPLA2R1 ELISA was as sensitive as the standardized hPLA2R1 ELISA to monitor anti-PLA2R1 in patients with active disease or in drug-induced remission. In contrast, only 51% of patients were reactive to mPLA2R1 by WB, and a maximum of 78% were weakly to highly positive in the mPLA2R1 ELISA, suggesting that iMN patients exhibit different subsets of anti-PLA2R1 autoantibodies against epitopes that are shared or not among PLA2R1 orthologs. In a cohort of 41 patients with a mean follow-up of 42 months from anti-PLA2R1 assay, the detection of anti-mPLA2R1 autoantibodies was an independent predictor of clinical outcome in multivariate analysis ($p = 0.009$), and a ROC curve analysis identified a threshold of 605 RU/mL above which 100% of patients (12 patients) had a poor renal outcome ($p < 0.001$). A similar threshold could not be defined in hPLA2R1 and rbPLA2R1 ELISAs. We conclude that rbPLA2R1 is an alternative antigen to hPLA2R1 to measure anti-PLA2R1 in active disease while mPLA2R1 is a unique antigen that can detect a subset of anti-PLA2R1 autoantibodies present at high levels (>605 RU/mL) only in iMN patients at risk of poor prognosis, and is thus useful to predict iMN outcome.

Abbreviations: ELISA, enzyme-linked immunospecific assay; ESKD, end-stage kidney disease; IIFT, indirect immunofluorescence test; iMN, idiopathic MN; MN, membranous nephropathy; PLA2R1, M-type phospholipase A2 receptor 1; hPLA2R1, human PLA2R1; rbPLA2R1, rabbit PLA2R1; mPLA2R1, mouse PLA2R1; UPCR, urinary protein to creatinine ratio; WB, western blot.

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1. Introduction

Idiopathic membranous nephropathy (iMN) is a common cause of nephrotic syndrome in adults [1–5]. Clinically, iMN is associated with high proteinuria, with about 80% of patients loosing more than 3.5 g of protein per day. During follow-up, spontaneous remission occurs in about one third of patients while end-stage kidney disease (ESKD) with persistent proteinuria develops in another third of cases. The treatment of iMN is controversial [3,6]. KDIGO guidelines recommend supportive symptomatic treatment with renin-angiotensin system-blockade and diuretics in all patients with iMN, but immunosuppressive therapy is only recommended in the case of renal function deterioration or a worsening nephrotic syndrome [7]. Therefore, immunosuppressive treatment is often started only after significant and potentially irreversible complications. On the other hand, an unnecessary early start of immunosuppressive therapy can be futile in patients who might develop spontaneous remission. For these reasons, there is a need for clinical and diagnostic parameters at disease onset and during follow-up that would allow the identification of patients at risk of poor clinical outcome and who should benefit from immunosuppressive versus supportive treatment.

In 2009, Beck et al. identified the M-type phospholipase A2 receptor PLA2R1 as the major podocyte antigen involved in adult iMN [8], with about 70% of iMN patients having circulating autoantibodies against PLA2R1. The presence of anti-PLA2R1 autoantibodies has been widely confirmed in subsequent studies in 60–80% of patients with iMN, as well as in a few cases of apparent secondary MN [9–17]. The pathogenic role of anti-PLA2R1 autoantibodies is not yet proven, but antibody titers, especially IgG4 anti-PLA2R1, have been associated with clinically active disease and response to immunosuppressive therapy [10,13,18]. High titers at early presentation of disease also appear to be predictive of poor clinical outcome [16,19]. However, anti-PLA2R1 activity seems to persist in some patients with apparent clinical remission or low proteinuria [11–13,20] or under renin-angiotensin system-blockade [21].

PLA2R1 is a large transmembrane receptor of 180 kDa with an extracellular region comprising 10 distinct globular domains, each harboring 2 to 3 disulfide bridges [22]. Anti-PLA2R1 autoantibodies in iMN patients were initially detected by western blot (WB) using recombinant human PLA2R1 (hPLA2R1) expressed in HEK293 cells [8]. The study also revealed that patients' autoantibodies only recognize hPLA2R1 under non reducing conditions, indicating that the autoantibodies bind exclusively to the folded protein on one or more conformational epitopes [8]. Anti-PLA2R1 autoantibodies were then measured by indirect immunofluorescence test (IIFT) using HEK293 cells overexpressing hPLA2R1 at the cell surface [10,23], a laser bead immunoassay [24] and ELISA using a recombinant soluble form of hPLA2R1 [13,14]. When compared, the different methods of detection were in good accordance, but with different sensitivities, which might be explained by the fact that the methods are either only semi-quantitative, or use preparations of hPLA2R1 antigen with various levels of folded and reactive protein.

Importantly, all the above methods have used hPLA2R1 as antigen. Hence, no study has evaluated the cross-reactivity of anti-PLA2R1 autoantibodies to other mammalian species of PLA2R1. In this study, we compared for the first time the cross-reactivity of anti-PLA2R1 autoantibodies from a cohort of iMN patients to human (h), rabbit (rb) and mouse (m) PLA2R1 orthologs and observed different cross-reactivities, especially for mPLA2R1. We then established species-specific ELISAs using recombinant, purified and folded PLA2R1 antigens and compared their diagnostic and prognostic performances in a cohort of iMN patients with clinical follow-up.

2. Materials and methods

2.1. Patients

Sera of patients with biopsy-proven iMN and secondary MN were collected from five French nephrology centers. iMN was defined by the absence of secondary MN features, such as positivity for anti-nuclear antibodies, history of hepatitis B or C, cancer or other immune pathologies (cryoglobulinaemia, sarcoidosis, graft versus host disease,...). Sera from a total of 153 patients were collected: 130 with iMN and 23 with secondary MN. In the latter group, two had lupus nephritis, one had cryoglobulinaemia, one had HBV infection, one had HCV infection, and one was co-infected with both HBV and HCV. We also collected 67 sera from disease controls: 34 patients without renal involvement including rheumatoid arthritis, psoriatic arthritis, systemic sclerosis, Sjoegren's syndrome and 33 patients with other glomerular diseases such as IgA nephropathy, ANCA-positive systemic vasculitis, lupus nephritis (type V), focal segmental glomerulosclerosis, membranoproliferative glomerulonephritis, minimal change disease and Henoch Schoenlein purpura. Finally, we collected 67 sera from healthy blood donors. Consents were obtained from all patients. Proteinuria levels were classified in three stages: nephrotic range proteinuria when UPCR was >3.5 g/g, residual non nephrotic proteinuria when UPCR was between 0.5 and 3.5 g/g, and low proteinuria when UPCR was <0.5 g/g. Active disease was defined as a UPCR >3.5 g/g or eGFR <60 mL/min/1.73 m^2 and remission was defined as a UPCR <3.5 g/g and eGFR >60 mL/min/1.73 m^2.

2.2. Expression of PLA2R1 orthologs as folded antigens

The cDNAs coding for full-length membrane-bound hPLA2R1, rbPLA2R1 and mPLA2R1 with an HA tag added to the C-terminal end were transiently transfected into HEK293 cells as described previously [25–27]. Three days after transfection, cells were lysed and centrifuged to prepare the cytosolic and microsomal fractions. The microsomal fraction was then solubilized with detergents and centrifuged to obtain detergent-soluble and detergent-insoluble fractions. The expression of PLA2R1 orthologs was validated in the 4 fractions by WB using specific antibodies (Figs. 1 and 2A). See Supplementary information online for more details.

2.3. Western blot analyses

The different forms of recombinant PLA2R1 were analyzed by SDS-PAGE gels under reducing and non reducing conditions and western blotting according to the detailed methods described in the online Supplementary information. The primary antibodies used to identify folded PLA2R1 in the four fractions of PLA2R1-transfected HEK293 cells were a mouse monoclonal anti-HA (Sigma, St. Louis, USA, working dilution 1:5000), a rabbit polyclonal anti-hPLA2R1 (Atlas Antibodies, working dilution 1:1000), a guinea-pig polyclonal anti-rbPLA2R1 (homemade as described [28], working dilution 1:5000) and a rabbit polyclonal anti-mPLA2R1 (homemade as described [27], working dilution 1:2000). HRP-conjugated goat anti-mouse or anti-rabbit IgG (SouthernBiotech, Birmingham, USA) and goat anti-guinea-pig IgG (Cappel, Organon Teknika) were used as secondary antibody at a dilution of 1:5000. For detection of cross-reacting anti-PLA2R1 antibodies in iMN sera, the three folded PLA2R1 orthologs were run on SDS-PAGE gels under non reducing conditions and mini WBs