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Direct characterization of target podocyte antigens and auto-antibodies in human membranous glomerulonephritis: Alfa-enolase and borderline antigens

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ABSTRACT

The identification of glomerular auto-antigens in idiopathic human membranous glomerulonephritis (MGN) is a crucial step towards the definition of the mechanisms of the disease. Recent '*in vivo*' studies demonstrated a heterogeneous composition of glomerular immune-deposits in MGN biopsies only a part of which have been characterized.

We studied with a proteomical approach IgGs eluted from laser capture microdissected glomeruli of 8 MGN patients and showed the existence of other three immune proteins in MGN glomeruli (α-enolase, elongation factor 2 and Glycyl Aminoacyl-tRNA Synthetase). One of these, i.e. α-enolase, fulfilled all criteria for being considered an auto-antigen. Specific IgG_1 and IgG_4 reacting with podocyte α-enolase were, in fact, eluted from microdissected glomeruli and Confocal- and Immuno Electron-Microscopy showed co-localization of α-enolase with IgG_4 and C5b-9 in immune-deposits. Serum levels of anti a-enolase IgG4 were determined in 131 MGN patients and were found elevated in 25% of cases.

Overall, our data demonstrate that glomerular α-enolase is a target antigen of autoimmunity in human MGN. Circulating anti α-enolase auto-antibodies can be detected

Abbreviations: MGN, membranous glomerulonephritis; PLA2r, phospholipase A2 receptor; SOD2, superoxide dismutase 2; AR, aldose reductase; NEP, neutral endopeptidase; HN, passive Heymann nephritis.

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in sera of a significant quota of MGN patients. Like other auto-antigens, α-enolase may be implicated in the pathogenesis of human MGN.

1. Introduction

Primary membranous glomerulonephritis (MGN) is a common cause of nephrotic syndrome in adults. It is the prototype of an autoimmune disease characterized by glomerular sub-epithelial immune-deposits (mainly composed by IgG4 and C5b-9) but its pathogenesis remains unknown. Most of our current knowledge on mechanisms for antibody entrapping are based on experimental models of passive Heymann nephritis (HN) that is induced in susceptible rat strains by injection of heterologous antisera from sheep or rabbit immunized with a crude extract of rat tubular antigen known as Fx1A [1]. Megalin is the podocyte antigen involved in HN; anti-megalin IgG and C5b-9 are major components of immune-deposits [2–6], while the receptor associated protein (RAP), its antibody and clusterin [7–10] are regarded as subordinate players with a somehow unclear role in the pathogenesis of HN. Some of these components have been also detected in human MGN [11,12] but megalin and/or its homologue (LDL-receptor) [13] are not present in human glomeruli suggesting that different podocyte antigens are implicated in human MGN. Although the identification of podocyte antigens is crucial for defining the pathogenesis of human MGN, technology problems mainly concerning dissection and purification of glomeruli from human biopsies have limited the experimental approach for years.

Since 2002, major advances have been achieved based on technology developments. Debiec et al. [14,15] first showed that neutral endopeptidase (NEP) emerges as podocyte antigen in congenital MGN due to maternal NEP deficiency and alloimmunization during pregnancy. This is a rare form of MGN that arises in newborns from a mother carrying a genetic deficiency of NEP: the protein is expressed by the podocytes of the foetus, and NEP deficient mothers, during a previous miscarriage, produced anti-NEP antibodies since their immune system recognized it as a non-self protein.

More recently, two independent groups [16,17] have shown specific IgG_4 against the phospholipase A2 receptor (PLA2R), aldose reductase (AR) and superoxide dismutase 2 (SOD2) in glomerular eluates and in sera of a substantial part of patients with MGN. These Authors could demonstrate glomerular co-localization of IgG_4 with their respective auto-antigens (i.e. PLA2r, AR and SOD2) by double staining and by electron microscopy that suggests an implication of all these antigens in sub-epithelial deposit formation. These are not, however, the unique auto-antigens that induce the autoimmune response. In fact, IgG_4 eluted from glomeruli of MGN patients recognize other proteins that represent good candidates for new auto-antigens (*Ghiggeri, personal observation*). Technology developments based on tissue microdissection and proteomical analysis have revolutionized the strategies for the basic approach to autoimmune glomerular diseases in humans allowing a direct analysis of renal tissue 'in vivo' [18].

In this paper we investigated with an integrated proteomics-pathology approach other proteins recognized by IgG4 in glomeruli. The 'in vivo' model consisting of renal biopsy specimen and serum of a large cohort of patients affected by MGN was utilized in order to gain information directly from human pathology.

2. Materials and methods

2.1. Patients

Overall, 131 MGN patients were included in the study (Table 1) and were utilized for studies on circulating auto-antibodies. Eight patients were also utilized for the proteomics approach to renal biopsies and IMF (Table 2). Criteria for enrollment were: a) a biopsy-based diagnosis of MGN; b) negative tests for serum auto-antibodies (ANA, nDNA, ANCA), for cryoglobulins, and for viral markers (HBsAg, HIV, HCV); c) absence of any previous immunosuppressive treatment with exception of anti-hypertensive drugs. For histological evaluation of kidney disease, Dubosq-Bresil solution-fixed tissues were embedded in paraffin, sectioned and stained with hematoxylin/eosin, Masson's trichrome, silver methenamine and periodic-acid Schiff (PAS). Specimens for classic EM were fixed in Karnovsky

Table 1 – Patient characteristics at the onset of clinical signs of nephrotic syndrome. Data for quantitative variable are given as median and interquartile range due to their non-normal distribution.

Patients	n	Age	Serum creatinine (mg/dl)	Serum cholesterol (mg/dl)	Proteinuria (g/24 h)
Overall	131	61 (48–70)	1 (0.8–1.4)	262 (214–317)	5.8 (3.3–8.8)
Class I	29	59 (42–70)	0.9 (0.7–1)	274 (206–328)	6.1 (2.1–7.3)
Class II	57	64 (50–70)	1.1 (0.8–1.7)	261 (228–310)	6 (4–10)
Class III	28	67 (58–70)	1.2 (0.9–1.4)	228 (174–312)	3.9 (2.6–6.6)
Class IV	17	47 (33–58)	1 (0.8–1.4)	307 (219–328)	6 (4.6–9)