Membranous glomerulonephritis: new insights in pathophysiology and therapeutic approach

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ABSTRACT

During the last decade, several major breakthroughs have led to the identification of human podocyte membrane antigens. Experimental involving antipodocyte antibodies in human membranous nephropathy (MN) have opened a new line of thinking about this disease, relating as an autoimmune kidney disease. In this setting, the M-type phospholipase A2 receptor (PLA2R) was identified as the first major antigen target in human primary MN. Studies have demonstrated anti-PLA2R antibodies against PLA2R ranging from 70 to 89% in patients with MN, but not in those with secondary MN. It has been suggested that the serum level of anti-PLA2R could be used for the diagnosis of idiopathic MN and for the monitoring of response to treatment. However, the coexistence of autoantibodies suggests a complex pathogenic pathway that involves different podocyte targets. New experimental models are needed to elucidate the appearance time and the role of each antipodocyte antibody in MN development and progression.

Keywords: autoimmune diseases, glomerulonephritis membranous, receptors, phospholipase A2.

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Glomerulopathies are the third leading cause of chronic kidney disease among those who start dialysis in Brazil.[1] A retrospective epidemiological analysis, involving 9,617 renal biopsies performed in Brazil, revealed membranous glomerulonephritis (MGN) as the second most prevalent primary glomerular lesion reaching 20.7% of all the patients.[2] This glomerulopathy has the histological characteristic of lacking significant hypercellularity, and the electron microscopy shows subepithelial/intramembranous immune deposits (immunoglobulin G and complement), which cause podocyte damage and usually nephrotic syndrome. MGN can take an idiopathic form without any associated disease (70%-80%), or it can be secondary to various clinical conditions, including infections (hepatitis, syphilis), systemic lupus erythematosus, malignancy or it can be drug-induced.[3] Histological features found in electronic analysis and immunofluorescence may be useful in distinguishing idiopathic (primary) from secondary forms of the disease; however, their clinical and laboratory presentations are indistinguishable. The lack of understanding of the mechanisms involved in the pathogenesis of MGN is transmitted to its therapeutic management. To date, nonspecific severity criteria are the hallmarks of adopted treatment approaches.[4]

The understanding of the MGN pathogenic mechanisms is based on the experimental animal studies described by Heymann for over five decades. The Heymann nephritis was produced after injection of kidney extracts in rats. This antigenic preparation caused podocyte glomerular damage and proteinuria similar to human MGN; it also introduced the concept of an autologous autoimmune pathogenic complex associated with the MGN. In the animal model described, podocyte antigenic targets identified as megalin, would be responsible for *in situ* immunocomplexes.[5] However, this antigen is not expressed in human podocytes. From these bases, anti-podocyte antibodies have been extensively investigated. Podocytes

are highly specialized cells and play a crucial role in the glomerular barrier. Changes to its surface molecules may cause an immune response with antibody binding, complement activation and cell damage. Podocyte retraction causes proteinuria, glomerular barrier destruction and starts progression to chronic kidney disease.

Advances in molecular knowledge in the last decade have allowed the identification of podocyte proteins that act as potential antigenic targets for *in situ* formation of immune deposits, explaining the concept of "podocytopathy". In this field of study, human podocyte targets have been identified and held accountable as autoantigens. Two major antigens, both membrane glycoproteins, deserve highlighting: in 2002, Hanna Debiec and Pierre Ronco's group studied a rare form of antenatal MGN and found the neutral endopeptidase (NEP) - metallopeptidase 94 kDa, which is located on the cell surface of podocytes.[6] The disease can be transferred to animals by injecting immunoglobulins extracted from the serum of children (anti-NEP) with this condition, which is an aloimmunization.[7] A second antigen was described in 2009 by Beck *et al.*[8], the M-type A2 phospholipase receptor (PLA2R) - a protein with 185 kDa expressed in human podocytes. The activation of this receptor causes a pathogenic pathway with complement activation and cell damage.

Using immunoenzymatic assays (Western blotting), human glomerular proteins were added to serum samples from patients with idiopathic and secondary MGN, and other glomerulopathies (IgA, diabetic nephropathy). Thus, it was possible to find the specific reactivity against a protein of 185 kDa in 70% of samples from idiopathic MGN. Subsequently, these antigenic targets had their epitopes identified with the same sensitivity to antibodies directed against PLA_2R (anti-PLA_2R), predominantly of subclass 4 (IgG4). Immunohistochemical techniques made it possible to locate the expression of this antigen between the urinary space and the basement membrane prominently in podocytes. After the description of this autoantigen, relevant publications have highlighted the presence of anti-PLA_2R; with specificity ranging from 57% to 89% in patients with idiopathic MGN.[9-12] Anti-PLA_2R positivity was demonstrated (Western blotting) in 81.7% of blood samples from 60 Chinese patients with idiopathic MGN and proteinuria greater than 3.5g/24h.[9]

Deepening the anti-PLA_2R research, Debiec *et al.*[10] studied PLA_2R immune deposits in renal tissue (glomeruli) of 42 patients with MGN without evidence of secondary forms. These patients had blood and tissue samples collected prior to the immunosuppressive therapy. The anti-PLA_2R serum sensitivity and the study of PLA_2R in glomeruli was 57% and 74%, respectively. In 10 anti-PLA_2R serum-negative patients we found PLA_2R glomerular deposits. These observations showed that establishing scenarios: glomerular tissue and serum could stratify different stages of the disease. A quicker serum clearing and its deposition in renal tissue could explain this discrepancy. Thus, we extracted important information that the absence of circulating anti-PLA_2R at the time of biopsy would not rule out the diagnosis of anti-PLA_2R-related MGN. The prospective study carried out in Hamburg by Hoxha *et al.*[11] included 88 patients with histological diagnosis of MGN. In 61 patients (69%), there was a strong positivity for PLA_2R in the glomeruli, with almost identical serum correlation (anti-PLA_2R). Anti-PLA_2R was also correlated with the activity and therapeutic response achieved. It was possible to correlate anti-PLA_2R levels with proteinuria reduction in patients receiving anti-CD20 monoclonal antibody (rituximab), suggesting anti-PLA_2R monitoring as a tool for treatment decision making. High levels of anti-PLA_2R would be associated with disease activity (proteinuria) and increased risk of decline in renal function.[12]

One issue that has also been discussed is the different risks detected in subgroups determined by HLA DQA1, which would expose populations to greater susceptibility to MGN.[13,14] PLA_2R polymorphism can add clarification to this individual MGN susceptibility. The study by Liu *et al.*[15] demonstrated that the rs35771982 SNPs (single nucleotide polymorphisms) would have a more selective expression in Chinese population with MGN. The frequency of the G allele at rs35771982 and the G/G genotype of this SNP are even associated with the low rate of MGN remission. A European study, after isolating the DNA and genotyping 556 patients (French, Dutch and English) with idiopathic MGN, suggested the hypothesis that there would be "high risk" PLA_2R for alloimmunization. These results show a close relationship between idiopathic MGN and HLA-DQA1 and PLA_2R risk alleles.[16]

MGN may recur in up to 42% of renal transplant recipients. The initial symptoms are subtle, but evolve with proteinuria and graft loss.[17] Rituximab is effective in the treatment of post-transplant MGN relapse, including regression of immune deposits.[18] Debiec *et al.*[19] reported a case of post-transplant MGN recurrence, in which the biopsy showed PLA_2R subepithelial glomerular deposits in both the graft and the native kidneys. Treatment with rituximab stabilized proteinuria and serum creatinine levels; in addition, anti-PLA_2R serum levels became undetectable. In this exceptionally early recurrence report, there was IgG3 subclass and also complement activation via MBL (lecithin). The detection of PLA_2R immune deposits in the biopsy samples may be, at present, more sensitive than serological tests to evaluate anti-PLA_2R mediated-MGN.[20]

PLA_2R is naturally expressed on podocyte cell membranes and acts as a phospholipase A2 (PLA_2) receptor. This receptor participates in the regulation of PLA_2R biological responses involving cell proliferation, adhesion, production of lipid mediators, and the release of arachidonic acid. Oncogenetic studies implicate PLA_2R as a multifunctional receptor; changes in the expression of this receptor have a major impact on human cell senescence via generation of reactive oxygen species. The signaling for cell injury could follow the p-53 path, a protein that plays a central role in cellular response, including cell cycle arrest, allowing DNA damage repair or cell death induction.[21]

The harmful steps that follow the link to the target antigen (PLA_2R) would lead to additional expression of a number of secondary cytoplasmic autoantigens (AR-aldose reductase, 2-SOD2 superoxide dismutase and α-enolase) also described in idiopathic MGN without circulating anti-PLA_2R. In 50% of patients with MGN - non-responders or partial responders to therapy, serum anti-AR and anti-SOD2 were found, turning more intriguing the understanding of the MGN autoimmune character. Additional studies will be needed to clarify the stage of appearance and the role of each anti-podocyte antibody on MGN onset and progression.[22]

Given the cumulative evidence, we gathered arguments to elect anti-PLA_2R as a glomerular biomarker. It is measurable, it reports on the state of a pathological process and on the clinical/laboratory response vis-à-vis the drug therapy.[23] However, further prospective studies with different immunoglobulin subclasses are needed in order to elucidate the complement's role, to let us go deeper in the mechanism of this autoimmune disease. It is also necessary to unravel the role of the various antigenic podocyte targets described.[24] Studies with laser microdissection and proteomics will help elucidate the complex intracellular sequence of antigenic stimulation. We may correlate evolutionary MGN stages with the detection of specific antibodies in circulation and proper drug therapy.[25]

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