

Projet ANR- 07-Physio -016-01

Acronyme du projet: MN profile

Programme Physio 2007

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Identification of disease-specific antigens and immunodominant peptides in membranous nephropathy: establishment of new prognostic and follow-up biomarkers

CONTEXT and AIM

M-type phospholipase A₂ receptor (PLA_{2R}), a membrane bound podocyte glycoprotein, was recently identified as a major target antigen involved in 70% of adult idiopathic membranous nephropathy (iMN). In addition, the oxidative stress-induced proteins, superoxide dismutase-2 and aldose reductase have been shown to contribute to immune deposits formation in iMN. It seems that multiple antigen-antibody systems could be involved at different time-points and in different categories of patients with so-called "iMN". The identification of all glomerular autoantigens in MN is a crucial step towards understanding the mechanisms involved in the pathogenesis and improving patients' monitoring and care.

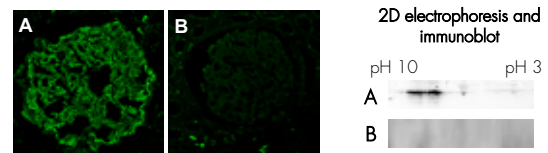
The purpose of our project is to identify pathogenic antigens and immunodominant epitopes involved in the development of membranous nephropathy, and to establish of new prognostic and follow-up biomarkers.

RESULTS

Food antigens in the pathogenesis of children membranous nephropathy

We have identified a new cause of MN in children aged less than 3 years due to immunization against bovine serum albumin (BSA). These patients had both high level anti-BSA antibodies of IgG1 and IgG4 subclasses and circulating cationic BSA. BSA was colocalized with IgG in subepithelial immune deposits. IgG1 and IgG4 eluted from kidney-biopsy specimen had anti-BSA reactivity. These data strongly suggest that in those patients, cationic BSA became planted into the anionic glomerular capillary wall, which led to the subsequent deposition of anti-BSA IgG. These cases appear to be the human counterpart of the experimental models using cationic BSA which led to the concept of antigen planting and subsequent *in situ* formation of immune complexes.

CONCLUSION: These findings thus indicate that the repertoire of antigens involved in MN may include nonglomerular antigens.



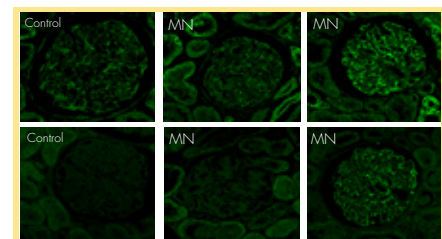
BSA is present in immune deposits of MN patients who had both a circulating cationic BSA and anti-BSA antibodies

Identification of antigens associated with progression of disease in MN

IgG eluted from biopsies of iMN patients but not other renal diseases recognized chaperonin containing α -complex polypeptide (CCT), a stress-inducible protein that acts as molecular chaperon to promote recovery and assist in refolding of proteins denaturated in stress conditions. CCT is weakly expressed in normal glomeruli but its expression was increased on podocytes in renal biopsies (23/35) of iMN patients while being unchanged in other glomerular pathologies. Circulating anti-CCT antibodies were detectable only in the iMN patients. In 20/23 patients with enhanced positive staining for CCT, PLA_{2R} was also detectable in immune deposits, while all patients with weakly expressed CCT had no PLA_{2R} in immune deposits.

CONCLUSION: The immune response against a negative regulator of podocyte injury mediated by initiating events could play an important role in the maintenance and progression of iMN (manuscript in preparation).

CCT
PLA_{2R}



Disease specific peptide epitopes

Conformational epitope on NEP associated with foeto-maternal allo-immunization

Phage display peptide library screened with IgG from mothers with allo-antibodies

1 ESLKEIIVSKLS



2 IPLPPSRPFK



The peptide sequence along with the known 3D structure of NEP allowed to identify conformational epitope formed by non sequential residues on the NEP

They have antigenic and immunogenic properties specific of NEP protein.

These peptides are good candidates for the specific removal of anti-NEP antibodies from sera of NEP deficient pregnant mothers (manuscript in preparation).

Adult form of MN

Initial stage of disease Progressing disease

We have selected several mimotopes for adult patient. Their role in the pathomechanism of MN remains to be established.

Serologic and immunopathologic studies of anti-PLA_{2R} antibodies : relevance for diagnosis and disease monitoring

N	PLA _{2R}	
	Serum reactivity	Biopsy
21	+	+
3	+	-
8	-	-
10	-	+
42	+24 57%	+31 73%

CONCLUSION: Assessment of both circulating PLA_{2R} autoantibody and PLA_{2R} in biopsy may better categorize patients into different groups that have potential prognostic and therapeutic implications

PLA_{2R} in the graft

De novo MN

9/9 tested biopsies
Negative for PLA_{2R}

Recurrent MN

5/10 tested biopsies
Positive for PLA_{2R}

Some patients with PLA_{2R}1-related iMN and anti-PLA_{2R}1 antibodies at the time of transplantation will not develop recurrence

CONCLUSION: PLA_{2R} predictive value should be carefully analyzed in prospective studies

Inserm

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