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Membranous nephropathy: A fairy tale for immunopathologists, nephrologists and patients

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Abstract

This article reviews the considerable progress which has been made in the recent years in the understanding of the pathophysiology of membranous nephropathy, a model of organ-specific auto-immune disease. It shows how experimental models developed more than 30 years ago have led to the identification of several human antigens including neutral endopeptidase in the neonate, phospholipase A2 receptor and thrombospondin 1 domain 7A in the adult, and cationic bovine serum albumin in children. Thanks to a successful GWAS performed in European Caucasians, the genetics of the disease begins to be understood. These groundbreaking findings already have a major impact on patients' care owing to the development of reliable ELISA and immunofluorescence test for the detection of PLA2R antibodies and of PLA2R antigen screening in biopsies. This review will tell the story from the careful clinical observation of cases to the most recent therapeutic perspectives which have been made possible by these advances.

Advances in medical science often proceed by steps which are highly interdependent. New, groundbreaking findings with important clinical implications often result from the combination of faithful experimental models and careful clinical observations. This is well illustrated by the story of membranous nephropathy which started more than 50 years ago. It is remarkable that in this disease, the experimental models predicted the pathophysiology of the human glomerulopathy. The stories that we will tell in this article are aimed at young clinical investigators who are sometimes reluctant to embark on research projects. We hope that they will convince them that bedside research performed with intellectual curiosity and a bit of chance can lead to significant progress in clinical medicine.

Presentation of the disease

Although membranous nephropathy (MN) is a rare disease, it is a major cause of nephrotic syndrome in the adult, which accounts for about 20% of cases, being only overpassed among non-diabetic glomerular diseases by focal segmental glomerulosclerosis (FSGS) in some ethnic populations (African and Hispanic Americans). MN annual incidence in the adult is 1 in 100,000, accounting for approximately 10,000 new cases in the EU each year (1). The disease affects patients of all ages and ethnic and racial groups but is more common in men than in women (sex ratio, 2:1) with the peak incidence during the fourth and fifth decades of life (2).

In the earliest stage, the glomeruli appear normal by light microscopy, and diagnosis relies only on immunofluorescence and electron microscopy. The next stage is characterized by homogeneous thickening of the capillary wall in sections stained with hematoxylin and eosin or with periodic acid-Schiff reagent. Early projections of the GBM between deposits may then be detected in a characteristic spike-like configuration by silver methenamine staining (Jones' stain). Later on, deposits are incorporated into the GBM and lucencies may appear as immune deposits are resorbed. Glomerular and tubulointerstitial fibrosis develop as the disease progresses. Deposits are formed of immune complexes and also contain C5b-9, the membrane attack complex of complement, which is the major mediator of proteinuria. However, this common histopathological pattern does not refer to a single disease entity. MN can be "idiopathic" (iMN) or primary without any identified cause (70 to 80% of cases), or secondary to various clinical conditions including infections (for example hepatitis B and

syphilis), autoimmune diseases (systemic lupus erythematosus), malignancy and drug intoxication (3, 4).

For a long time, composition of immune complexes was unknown and the pathomechanism of the disease was poorly understood. The molecular pathomechanisms of human MN with the identification of several antigens are summarized on Figure 1A.

A single case can teach us more than large series of patients: from alloimmune neonatal MN to Heymann nephritis and back

In 2000, the case of a neonate with MN was presented at our monthly clinico-pathological conference with pediatrician nephrologists (5). Because of the early occurrence of the disease during antenatal life, it was tempting to speculate that the mother became immunized during pregnancy and that the nephritogenic antibodies had been transferred to the fetus. We suggested such a scenario because it reminded us of the passive model of experimental MN, called Heymann nephritis, which was the theme of the PhD thesis that of one of us (PR) had defended some 20 years earlier.

Actually, Heymann was a pediatrician from Cleveland (Ohio, USA), who established in 1959 a rat model of MN, which was induced by the immunization of Lewis rats with crude kidney extracts (6). Because the disease was induced by fractions of renal brush-border membrane rather than by glomerular extracts, the deposits were initially believed to result from glomerular trapping of circulating immune complexes composed of brush-border-related antigens and the corresponding antibodies. Subsequently, however, the development of passive Heymann nephritis in rats injected with rabbit anti-rat brush-border antibodies, argued against a role for circulating immune complexes. With the use of ex vivo and isolated perfused kidney systems, Van Damme et al. and Couser et al. (7, 8) demonstrated that anti-brush border antibodies bound to an antigenic target located on podocytes, which indicated that the disease was caused by the in situ formation of immune complexes. The autoantigenic target in the rat disease was identified by Kerjaschki and Farquhar in the early 1980's (9, 10) as the podocyte membrane protein now called megalin.

We knew that megalin could not be responsible for human MN because neither megalin had been detected in subepithelial immune deposits, nor circulating anti-megalin antibodies had been found in patients with MN. The nature of the target antigen in our case was suspected

by indirect immunofluorescence examination of rabbit and rat kidney sections incubated with the mother's or the infant's serum. The same pattern of fluorescence as in human kidneys was observed in the rabbit, whereas in the rat, staining was restricted to the cells of Bowman's capsule and to the brush-border of deep cortical segments of the proximal tubule. Because we had previously observed similar interspecies differences with anti-neutral endopeptidase (NEP) antibodies (11), we suspected that NEP was the culprit. Reactivity with NEP was confirmed by immunoprecipitation of rat brush-border extracts with the mother's serum (5, 12). Colocalization of NEP, IgG and C5-b9 within the human immune deposits and transfer experiments, in which a pregnant rabbit was injected with the IgG fraction from the mother, established that the disease was caused by anti-NEP antibodies (5, 13).

Because the mother did not show any renal manifestation even though she had persistently high titers of anti-NEP antibody, we hypothesized that she might be deficient in NEP. This hypothesis was supported by the lack of reactivity of her granulocyte extracts with a panel of anti-NEP antibodies (5). Four additional families with materno-fetal alloimmune MN were identified. All immunized mothers were NEP deficient as a result of the same truncating mutation in exon 7 of the *NEP* gene (12, 14). In the first family, the mother was a compound heterozygote for this mutation and for another truncating mutation in exon 15. Alloimmunization can be triggered by previous spontaneous miscarriages or by the ongoing pregnancy during which the mother's immune system is first exposed to the NEP of paternal origin on syncytiotrophoblastic cells.

Thus, identification of this original mechanism of renal disease was made possible by our previous work on Heymann nephritis and the in-depth analysis of a clinical case which is the human counterpart to passive Heymann nephritis, which enabled us to bridge the gap between the experimental model and the human disease. Although the neonatal MN appears to be very rare, analysis of its pathogenic mechanisms provided the proof of concept that a podocyte antigen could be responsible for human MN, as is the case for megalin in the rat, and laid the foundation for the identification of the phospholipase A2 receptor (PLA2R) involved in adult forms of iMN.

From alloimmunity to autoimmunity and genetics: a two-way approach

The immunopathological approach

The search for antigens in iMN in the adult was unsuccessful for many years. We failed to identify a relevant antigen because we used differentiated human cultured podocytes as starting antigenic preparation and because our mass spectrometry analyser lacked sensitivity. In 2009 and 2014, using the same approach based on microdissection of human glomeruli, proteomic technology and mass spectrometry, two podocyte proteins were identified. The first major autoantigen is PLA2R (15). Circulating autoantibodies to PLA2R were detected in about 70% of patients with iMN. The second autoantigen is thrombospondin type-1 domain-containing 7A (THSD7A), and circulating autoantibodies against this protein were detected in 5 to 10% of the anti-PLA2R negative patients (16). PLA2R and THSD7A were detected in normal human glomeruli, in podocytes, and both antigens co-localized within subepithelial deposits with IgG4. Furthermore, IgG eluted from biopsy samples reacted with recombinant PLA2R or THSD7A. PLA2R and THSD7A have similar structural and biochemical properties. Autoantibodies to both proteins recognize their target antigens only under non-reducing conditions and are predominantly of the IgG4 subclass. Interestingly, the patients have an autoimmune response against either PLA2R1 or THSD7A, but not both. This suggests that PLA2R- and THSD7A- associated MN are two separate molecular entities and that these antigens are primary targets in this disease. An immunodominant epitope region was recently characterized in the 3 most N-terminal domains of PLA2R by two North-American (17) and British (18) groups.

The genetic approach

At about the time when Beck et al described PLA2R, we were chasing gene variants that could explain predisposition to iMN in white Europeans by using a pangenomic approach. We were the co-founders of a European MN consortium that collected patients with iMN in the UK, the Netherlands and France. We performed a genome-wide association study (GWAS) based on comparative hybridization of DNA from patients with iMN and from

ethnically matched controls with chips featuring >300,000 SNPs. SNPs, which stand for single nucleotide polymorphisms, are single base changes in the DNA sequence that represent the most frequent polymorphism observed in humans. We found highly significant associations of iMN with a series of SNPs in the 6p21 *HLA-DQA1* and 2q24 *PLA2R1* loci (19). The *P* values of the top (most significantly associated) SNPs reached 10⁻¹⁰⁰ for *HLA-DQA1* and 10⁻³⁰ for *PLA2R1* despite the small size of the cohorts. The success of this GWAS was explained by the homogeneous nature of the disease, as compared to many other conditions where histological lesions are diverse such as IgA nephropathy or even not defined such as chronic kidney disease in which thousands of patients are required to get significant gene associations. The ethnical match with the healthy controls is another requirement which should be carefully fulfilled.

Carrying the risk alleles of the two genes had an additive effect. Patients with all four risk alleles had an odds ratio close to 80 compared with individuals who had only the protective alleles. These data which were replicated in various cohorts from Europe (20, 21), and Asia (22-24), confirmed by an unbiased holistic approach that PLA2R, the antigen encoded by *PLA2R1*, was a key player in iMN. They further suggested that rare variants in *PLA2R1* might account for a special conformation of the antigen triggering autoimmunity. However, sequence analysis of *PLA2R1* coding sequence failed to identify rare polymorphisms or mutations specific for PLA2R-associated MN (25). It is remarkable that the most significantly associated SNPs in the GWAS are common alleles in the general population but it is still possible that a rare combination of these alleles may trigger the disease. More studies are needed to explain how alleles in the *PLA2R1* and *HLA-DQA1* loci interact with each other to increase susceptibility to MN.

Food and environmental antigens: not gone, just forgotten

An alternative model to Heymann nephritis first described in the rabbit by Border et al (26, 27) three decades ago and later in dogs, mice and rats (28, 29) was induced by injection of repeated doses of cationized bovine serum albumin (BSA) which serves as a planted antigen. Border's experiments were driven by the hypothesis that antigen charge could be a key factor for the formation of sub-epithelial deposits, given the negative charge of the glomerular capillary wall. Only rabbits immunized with cationic BSA were found to have sub-

epithelial deposits of IgG and C3, whereas those receiving anionic or native (neutral) BSA featured mostly mesangial deposits.

Based on these data, we speculated that in children with increased permeability of the intestinal barrier, cationic BSA (cBSA) could also serve as a target antigen for circulating anti-BSA antibodies after deposition in the glomerular capillary wall. We identified children less than 5 years of age with both high serum titre of anti-BSA antibodies (both IgG1 and IgG4) reacting primarily with one peptide region in BSA but not with the homologous peptide in human serum albumin, and elevated levels of circulating BSA which was shown to be cationic (30). In these patients only, BSA could be detected in the absence of PLA2R in immune deposits. Although the source of cationic BSA remains obscure (milk formula, microbiota, intestinal cells), these data confirm the experimental model. Even if BSA is one of the more common diet antigens due to early exposure to cow and beef products, there are other dietary and environmental antigens that may lead to disease by similar mechanism. Other antigens such as hepatitis B and C viral antigens and enzymes used in replacement therapy may be involved in secondary MN (31-33).

Actually, there is a world-wide increasing prevalence of MN among the biopsied patients which is most likely related to pollution factors (Ronco, unpublished). Both air and water polluting compounds could be involved. Whether these compounds trigger auto-immunity against PLA2R or other antigens and whether these compounds could themselves serve as auto-antigens needs further investigations.

Complement activation in membranous nephropathy: still mysteries to be solved

Although MAC is a major mediator of proteinuria, the predominant T-helper-2 immune response leads to the production of IgG4 which do not activate complement by classical and alternative pathways (34, 35). The classical pathway is most likely not a major player because of very low or undetectable amount of C1q in immune deposits, apart from exceptional cases (36). Mannan-binding lectin (MBL) has been identified in the glomeruli of patients with iMN (37). Preliminary studies indicate that anti-PLA2R IgG4 could bind MBL and activate the lectin pathway (38). Because MBL has been shown to bind N-linked sugars on IgG that lack terminal galactose (39), it was suggested that the anti-PLA2R1 IgG4 antibodies might also lack galactose (38). However, we recently observed cases of iMN occurring in patients with complete MBL functional deficiency which was confirmed by

genetic analysis (Bally and Debiec, unpublished observations), thus pointing to a major role of the alternative pathway as also attested by deposits of factor B (40). Genetic polymorphisms resulting in reduced activity of circulating or podocyte complement regulatory proteins as well as intrinsic or acquired defect of complement receptor-1 (CR1), could contribute as well to pathogenesis of MN and account for its varying severity.

Translational research in membranous nephropathy: fast, efficient and paradigm shifting in patient's care

The development of assays of circulating anti-PLA2R antibodies and their transfer to clinical practice has been amazingly fast, since the first immunofluorescence test was commercially available in 2011 (EUROIMMUN AG, Lübeck, Germany), less than 2 years after the description of anti-PLA2R antibodies. This test uses biochips coated with HEK293 cells transfected or not with the full-length human *PLA2R1*cDNA and incubated with the patients' sera (41, 42). The ELISA based on the extracellular domain of human PLA2R enables a more quantitative and faster determination of anti-PLA2R antibodies and shows a good correlation with the immunofluorescence test (43, 44) although the latter may be more sensitive. Detection of PLA2R antigen in immune deposits in biopsy specimens is also possible with the use of commercial antibody after a retrieval step to unmask PLA2R epitopes (45, 46). These tests have induced a paradigm shift in the diagnosis, prognosis and patient monitoring (47). Studies performed in the last 3 years have shown that anti-PLA2R antibodies are specific and sensitive biomarkers of MN. In a recent meta-analysis including 2212 patients, specificity was 99% (95% CI: 96% to 100%) and sensitivity was 78% (95% CI: 66% to 87%) (48). Anti-PLA2R antibodies are neither found in other nephropathies and auto-immune diseases nor detected in healthy individuals. They may occur with a low prevalence in secondary forms (41, 49, 50) where it may be difficult to exclude a pure coincidence of MN with the associated disorder. Their prevalence seems higher in patients with hepatitis B and active sarcoidosis, which suggests that these two diseases may induce or enhance immune response against PLA2R (46, 51, 52). PLA2R antigen can still be detected in deposits in the absence of circulating antibodies (45), even in archival, paraffin-embedded kidney biopsies. Conversely, in some patients, circulating anti-PLA2R antibodies are not associated with PLA2R deposits, suggesting that these antibodies may not be pathogenic. These results lead to recommend a combined serological (antibody) and biopsy (antigen) analysis in all patients

with MN. Detection of antigen in immune deposits also is an important clue to the diagnosis of primary MN (36) where PLA2R is often associated with predominant or exclusive IgG4 deposits (53). However, presence of PLA2R in the deposits is commonly (46, 50, 51).

Antibody levels also predict outcome since high titers are correlated with a lower risk of spontaneous (43) or immunosuppressant-induced (54, 55) remission and a higher risk of emergence of a nephrotic syndrome in non-nephrotic patients (56) and of renal function deterioration (20, 57). The time interval from the start of immunosuppressive treatment to remission is significantly longer in patients with the highest antibody titers (54).

Last but not least, anti-PLA2R antibodies appear to be sensitive markers of treatment efficacy. Partial or complete depletion of anti-PLA2R antibodies precedes renal remission by several weeks or months (55, 58), while re-emergence or increase of these antibodies precedes a renal relapse (55). The time lag of several months from immunological remission (depletion of antibodies) to renal remission is most likely accounted for by deposit remodeling and restoration of the glomerular capillary wall. Furthermore, antibody titer at the end of immunosuppressive treatment predicts occurrence of relapse (59). Because anti-PLA2R antibodies are much more sensitive markers than proteinuria for monitoring disease activity and outcome, they should play an essential part in therapeutic decisions and in the monitoring of patients. This is all the more important that immunosuppressive therapies may have toxic effects, including the risk of cancer in patients treated with alkylating agents (60). We think that the 2012 KDIGO guidelines (61) should be urgently revisited to include anti-PLA2R in the decision algorithm for patients with iMN. We recommend that antibodies be assessed every two months before starting immunosuppressive therapy to avoid unnecessary treatment in patients entering immunological remission, and every month afterward during the first 6 months of immunosuppression.

Therapeutic perspectives: A look into the future

Currently, only non-specific immunosuppressive treatments are proposed to patients with severe nephrotic syndrome persisting more than 6 months despite anti-proteinuric therapy because of frequent occurrence of spontaneous remission within 6 months to one year after diagnosis. New therapeutic options in patients with MN are presented on Figure 1B. Anti-CD20 antibody (rituximab) is a step in the right direction but still about a third of patients with persistent nephrotic syndrome will not respond (54), calling for more specific epitope-

driven therapy based on specific immunoadsorption. Further knowledge of the molecular structure of the “nephritogenic” epitope will enable to design non-peptide antagonists that will serve as baits for antibody decay. However, efficacy of this therapeutic intervention might be limited by epitope spreading. Because proteinuria is partly caused by complement activation leading to podocyte injury, there is still a place for complement antagonists blocking MAC formation and effects, and for cytoprotective therapy favouring restoration of the glomerular capillary wall. Research in this field must certainly be strengthened.

Conclusion

Three ingredients of translational medicine in the field of immune-mediated glomerular diseases have remained constant over the years: the patients, the need for well-characterized relevant models of their diseases, and the contributions of physician-scientists who have accounted for most of the advances described in this issue. The patients and the need for animal models will remain, but the technology to study them will exponentially advance in the next decade. However, clinical investigators are threatened by overload of clinical work due to heavy financial constraints affecting hospital staffs in the Western world and by shortage of funding although both are not restricted to nephrology. Continued progress in the area of renal immunopathology will require the availability and dedication of investigators who fully understand both the tools of basic science and the clinical and pathologic manifestations of human renal diseases.

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References

1. Maisonneuve P, Agodoa L, Gellert R, et al. Distribution of primary renal diseases leading to end-stage renal failure in the United States, Europe, and Australia/New Zealand: results from an international comparative study. *Am J Kidney Dis* 2000; 35: 157-65.
2. Cattran DC. Idiopathic membranous glomerulonephritis. *Kidney Int* 2001; 59: 1983-94.
3. Glasscock RJ. The pathogenesis of idiopathic membranous nephropathy : a 50-year odyssey. *Am J Kidney Dis* 2010; 56 :157-67.
4. Ponticelli C. Membranous nephropathy. *J Nephrol* 2007; 20 : 268-87.
5. Debiec H, Guigonis V, Mougenot B, et al. Antenatal membranous glomerulonephritis due to anti-neutral endopeptidase antibodies. *N Engl J Med* 2002; 346: 2053-60.
6. Heymann W, Hackel DB, Harwood S, et al. Production of nephrotic syndrome in rats by Freund's adjuvants and rat kidney suspension. *Proc Soc Exp Biol Med* 1959; 100: 660-4.
7. Van Damme BJ, Fleuren GJ, Bakker WW, et al. Experimental glomerulonephritis in the rat induced by antibodies directed against tubular antigens. V. Fixed glomerular antigens in the pathogenesis of heterologous immune complex glomerulonephritis. *Lab Invest* 1978; 38: 502-10.
8. Couser WG, Steinmuller DR, Stilmant MM, et al. Experimental glomerulonephritis in the isolated perfused rat kidney. *J Clin Invest* 1978; 62: 1275-87.
9. Kerjaschki D, Farquhar MG. The pathogenic antigen of Heymann nephritis is a membrane glycoprotein of the renal proximal tubule brush border. *Proc Natl Acad Sci* 1982; 79 : 5557-61.
10. Kerjaschki D, Farquhar MG. Immunocytochemical localization of the Heymann nephritis antigen (gp330) in glomerular epithelial cells of normal Lewis rats. *J Exp Med* 1983; 157: 667-86.
11. Ronco P, Ardaillou N, Verroust P, et al. Pathophysiology of the podocyte: A target and a major player in glomerulonephritis. *Adv Nephrol Necker Hosp* 1994; 23: 91-131.
12. Debiec H, Nauta J, Coulet F, et al. Role of Truncating mutations in MME gene in fetomaternal allo-immunization and neonatal glomerulopathies. *Lancet* 2004; 364: 1252-9.
13. Ronco P, Debiec H. Molecular pathomechanisms of membranous nephropathy: from Heymann nephritis to alloimmunization. *J Am Soc Nephrol* 2005; 16: 1205-1213.
14. Vivarelli M, Emma F, Pellé T, et al. Genetic homogeneity but IgG subclass-dependent clinical variability of alloimmune membranous nephropathy with anti-neutral endopeptidase antibodies. *Kidney Int* 2015; 87:602-9.

15. Beck LH Jr, Bonegio RG, Lambeau G, et al. M-type phospholipase A2 receptor as target antigen in idiopathic MN. *N Engl J Med* 2009; 361: 11-21.
16. Tomas NM, Beck LH Jr, Meyer-Schwesinger C, et al. Thrombospondin type-1 domain-containing 7A in idiopathic membranous nephropathy. *N Engl J Med* 2014; 371:2277-87.
17. Kao L, Lam V, Waldman M, et al. Identification of the Immunodominant Epitope Region in Phospholipase A2 Receptor-Mediating Autoantibody Binding in Idiopathic Membranous Nephropathy. *J Am Soc Nephrol* 2015; 26:291-301.
18. Fresquet M, Jowitt TA, Gummadova J, et al. Identification of a Major Epitope Recognized by PLA2R Autoantibodies in Primary Membranous Nephropathy. *J Am Soc Nephrol* 2015; 26:302-13.
19. Stanescu HC, Arcos-Burgos M, Medlar A, et al. Risk HLA-DQA1 and PLA(2)R1 alleles in idiopathic membranous nephropathy. *N Engl J Med* 2011; 364: 616-26.
20. Kanigicherla D, Gummadova J, McKenzie EA, et al. Anti-PLA2R antibodies measured by ELISA predict long-term outcome in a prevalent population of patients with idiopathic membranous nephropathy. *Kidney Int* 2013; 83: 940-8.
21. Bullich G, Ballarín J, Oliver A, et al. HLA-DQA1 and PLA2R1 Polymorphisms and Risk of Idiopathic Membranous Nephropathy. *Clin J Am Soc Nephrol* 2014; 9:335-43.
22. Lv J, Hou W, Zhou X, et al. Interaction between PLA2R1 and HLA-DQA1 Variants Associates with Anti-PLA2R Antibodies and Membranous Nephropathy. *J Am Soc Nephrol* 2013; 24:1323-9.
23. Liu YH, Chen CH, Chen SY, et al. Association of phospholipase A2 receptor 1 polymorphisms with idiopathic membranous nephropathy in Chinese patients in Taiwan. *J Biomed Sci* 2010; 17: 81-8.
24. Kim S, Chin HJ, Na KY, et al. Single nucleotide polymorphisms in the phospholipase A2 receptor gene are associated with genetic susceptibility to idiopathic membranous nephropathy. *Nephron Clin Pract* 2011; 117: c253-c258.
25. Coenen MJ, Hofstra JM, Debiec H, et al. Phospholipase A2 receptor (PLA2R1) sequence variants in idiopathic membranous nephropathy. *J Am Soc Nephrol* 2013; 24: 677-83.
26. Border WA, Ward HJ, Kamil ES, et al. Induction of membranous nephropathy in rabbits by administration of an exogenous cationic antigen. *J Clin Invest* 1982; 69: 451-61.
27. Adler SG, Wang H, Ward HJ, Cohen AH, Border WA. Electrical charge. Its role in the pathogenesis and prevention of experimental membranous nephropathy in the rabbit. *J Clin Invest* 1983; 71 : 487-99.
28. Wright NG, Mohammed NA, Eckersall PD, et al. Experimental immune complex glomerulonephritis in dogs receiving cationized bovine serum albumin. *Res Vet Sci.* 1985; 38 : 322-8.

29. Kobayashi M, Muro K, Yoh K, et al. Effects of FK506 on experimental membranous glomerulonephritis induced by cationized bovine serum albumin in rats. *Nephrol Dial Transplant*. 1998; 13 : 2501-8.
30. Debiec H, Lefeu F, Kemper MJ, et al. Early childhood membranous nephropathy due to cationic bovine serum albumin. *N Engl J Med* 2011; 364: 2101-10.
31. Hunley TE, Corzo D, Dudek M, Kishnani P, et al. Nephrotic syndrome complicating alpha-glucosidase replacement therapy for Pompe disease. *Pediatrics* 2004; 114:e532-5.
32. Debiec H, Valayannopoulos V, Boyer O, Noël et al. Allo-immune membranous nephropathy and recombinant arylsulfatase replacement therapy: a need for tolerance induction therapy. *J Am Soc Nephrol* 2014; 25:675-80.
33. Bhimma R, Coovadia HM. Hepatitis B virus-associated nephropathy. *Am J Nephrol* 2004; 24:198-211.
34. van der Zee JS, van Swieten P, Aalberse RC. Inhibition of complement activation by IgG4 antibodies. *Clin Exp Immunol* 1986;64: 415-22.
35. Aalberse RC, Schuurman J. IgG4 breaking the rules. *Immunology* 2002;105: 9-19.
36. Debiec H, Hanoy M, Francois A, et al. Recurrent membranous nephropathy in an allograft caused by IgG3k targeting the PLA2 receptor. *J Am Soc Nephrol* 2012;23:1949-54.
37. Lhotta K, Würzner R, König P. Glomerular deposition of mannose-binding lectin in human glomerulonephritis. *Nephrol Dial Transplant* 1999;14: 881-6.
38. Ma H, Sandor DG, Beck LH Jr. The role of complement in membranous nephropathy. *Semin Nephrol* 2013 ;33:531-42.
39. Malhotra R, Wormald MR, Rudd PM, Fischer PB, Dwek RA, Sim RB. Glycosylation changes of IgG associated with rheumatoid arthritis can activate complement via the mannose-binding protein. *Nat Med* 1995; 1:237-43.
40. Segawa Y, Hisano S, Matsushita M, et al. IgG subclasses and complement pathway in segmental and global membranous nephropathy. *Pediatr Nephrol* 2010; 25: 1091-9.
41. Hoxha E, Harendza S, Zahner G, et al. An immunofluorescence test for phospholipase-A₂-receptor antibodies and its clinical usefulness in patients with membranous glomerulonephritis. *Nephrol Dial Transplant* 2011; 26: 2526-32.
42. Debiec H, Ronco P. Nephrotic syndrome: A new specific test for idiopathic membranous nephropathy. *Nat Rev Nephrol* 2011; 7:496-8.
43. Hofstra JM, Debiec H, Short CD, et al. Antiphospholipase A2 receptor antibody titer and subclass in idiopathic membranous nephropathy. *J Am Soc Nephrol* 2012; 23: 1735-43.

44. Dähnrich C, Komorowski L, Probst C, et al. Development of a standardized ELISA for the determination of autoantibodies against human M-type phospholipase A2 receptor in primary membranous nephropathy. *Clin Chim Acta* 2013; 421:213-8.
45. Debiec H, Ronco P. PLA2R autoantibodies and PLA2R glomerular deposits in membranous nephropathy. *N Engl J Med* 2011; 364: 689-90.
46. Svobodova B, Honsova E, Ronco P, et al. Kidney biopsy is a sensitive tool for retrospective diagnosis of PLA2R-related membranous nephropathy. *Nephrol Dial Transplant* 2013; 28:1839-44.
47. Ronco P, Debiec H. Pathophysiological advances in membranous nephropathy: time for a shift in patient's care. *The Lancet*, 2015; 385: 1983-92.
48. Du Y, Li J, He F, et al. The diagnosis accuracy of PLA2R-AB in the diagnosis of idiopathic membranous nephropathy: a meta-analysis. *PLoS One* 2014; 9:e104936.
49. Qin W, Beck LH Jr, Zeng C, et al. Anti-phospholipase A2 receptor antibody in membranous nephropathy. *J Am Soc Nephrol* 2011; 22: 1137-43.
50. Knehtl M, Debiec H, Kamgang P, et al. A case of phospholipase A₂ receptor-positive membranous nephropathy preceding sarcoid-associated granulomatous tubulointerstitial nephritis. *Am J Kidney Dis* 2011; 57: 140-3.
51. Larsen CP, Messias NC, Silva FG, et al. Determination of primary versus secondary membranous glomerulopathy utilizing phospholipase A2 receptor staining in renal biopsies. *Mod Pathol* 2013; 26:709-15.
52. Stehlé T, Audard V, Ronco P, Debiec H. Phospholipase A2 receptor and sarcoidosis-associated membranous nephropathy. *Nephrol Dial Transplant*. 2015;30:1047-50.
53. Ronco P, Debiec H. Pathogenesis of membranous nephropathy: recent advances and future challenges. *Nat Rev Nephrol* 2012; 8:203-13.
54. Hoxha E, Thiele I, Zahner G, et al. Phospholipase A2 receptor autoantibodies and clinical outcome in patients with primary membranous nephropathy. *J Am Soc Nephrol* 2014; 25:1357-66.
55. Ruggenti P, Debiec H, Ruggiero B, et al. Anti-PLA2R antibody titer predicts post-rituximab outcome of membranous nephropathy. *J Am Soc Nephrol* 2015; Mar 24.
56. Hoxha E, Harendza S, Pinnschmidt H, et al. PLA2R antibody levels and clinical outcome in patients with membranous nephropathy and non-nephrotic range proteinuria under treatment with inhibitors of the renin-angiotensin system. *PLoS One* 2014; 9:e110681.
57. Hoxha E, Harendza S, Pinnschmidt H, et al. M-type phospholipase A2 receptor autoantibodies and renal function in patients with primary membranous nephropathy. *Clin J Am Soc Nephrol* 2014; 9:1883-90.
58. Oh YJ, Yang SH, Kim DK, et al. Autoantibodies against phospholipase A2 receptor in Korean patients with membranous nephropathy. *PLoS One* 2013; 8 : e62151.

59. Bech AP, Hofstra JM, Brenchley PE, et al. Association of anti-PLA₂R antibodies with outcomes after immunosuppressive therapy in idiopathic membranous nephropathy. *Clin J Am Soc Nephrol* 2014; 9:1386-92.
60. Van den Brand JA, van Dijk PR, Hofstra JM, et al. Long-term outcomes in idiopathic membranous nephropathy using a restrictive treatment strategy. *J Am Soc Nephrol* 2014; 25:150-8.
61. Kidney Disease: Improving Global Outcomes (KDIGO) Glomerulonephritis Work Group. KDIGO clinical practice guideline for glomerulonephritis. *Kidney Int* 2012; Suppl. 2:186-97.

Figure legend

Figure 1. Mechanisms of immune mediated podocyte injury in MN and pathogenesis based therapeutic approach.

- A) The in situ formation of immune complexes is initiated by binding of circulating antibodies to antigens that are endogenous integral membrane proteins of the podocyte, or to exogenous antigens planted in the glomerular basement membrane. Complement activation and formation of the C5b–9 attack complex, which is triggered by immune complex deposition, have major roles in sublethal podocyte injury and proteinuria. Distinct cytoprotective responses are observed over time in cells undergoing endoplasmic reticulum (ER) stress. Prolonged ER stress beyond threshold results in apoptosis.
- B) Optimum therapeutic intervention requires that four injury mechanisms are simultaneously targeted.

Abbreviations: NEP-neutral endopeptidase, PLA₂R-the phospholipase A₂ receptor, THSD7A-thrombospondin type-1 domain-containing 7A, BSA-bovine serum albumin, ERT-enzymes used in enzyme replacement therapy, HepB-hepatitis B antigen.