Molecular Immunology 68 (2015) 57–62

Contents lists available at ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm

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

Pierre Ronco[a,b,c,*], Hanna Debiec[a,b]

[a] Sorbonne Universités, UPMC Univ Paris 06, Paris F-75005, France
[b] INSERM, UMR_S 1155, Paris F-75020, France
[c] AP-HP, Hôpital Tenon, Department of Nephrology and Dialysis, Paris F-75020, France

ARTICLE INFO

Article history:
Received 5 June 2015
Accepted 2 July 2015

Keywords:
Membranous nephropathy
Podocyte
Antigens
Diagnosis

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.

1. 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 (Maisonneuve et al., 2000). 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 (Cattran, 2001).

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

Abreviations: 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.

* Corresponding author at: Nephrology and Dialysis, Tenon hospital, 4 rue de la Chine, 75020 Paris, France. Fax: +33 1 56 01 69 99.
E-mail address: pierreronco@yahoo.fr (P. Ronco).

http://dx.doi.org/10.1016/j.molimm.2015.07.003
0161-5890/

cause (70–80% 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 (Glassock, 2010; Ponticelli, 2007).

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 Fig. 1A.

1.1. 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 (Debiec et al., 2002). 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 (Heymann et al., 1959). 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. (1978) and Couser et al. (1978) 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 (1982, 1983) in the early 1980's 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 (Ronco et al., 1994), we suspected that NEP was the culprit. Reactivity with NEP was confirmed by immunoprecipitation of rat brush-border extracts with the mother's serum (Debiec et al., 2002; Debiec et al., 2004). 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 (Debiec et al., 2002; Ronco and Debiec, 2005).

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 (Debiec et al., 2002). 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 (Debiec et al., 2004; Vivarelli et al., 2015). 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.

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

2.1. 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 (Beck et al., 2009). Circulating autoantibodies to PLA2R were detected in about 70% of patients with iMN. The second autoantigen is trombospondin type-1 domain-containing 7A (THSD7A), and circulating autoantibodies against this protein were detected in 5–10% of the anti-PLA2R negative patients (Tomas et al., 2014). 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 (Kao et al., 2015) and British (Fresquet et al., 2015) groups.

2.2. 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