ARCHIVES OF HELLENIC MEDICINE: ISSN 11-05-3992

LABORATORY PROCEDURE
ΕΡΓΑΣΤΗΡΙΑΚΗ ΜΕΘΟΔΟΣ

The hard way from bench to bedside
History lessons from the pathogenesis of idiopathic membranous nephropathy (MN)

ARCHIVES OF HELLENIC MEDICINE 2020, 37(Suppl 2):125–128
ΑΡΧΕΙΑ ΕΛΛΗΝΙΚΗΣ ΙΑΤΡΙΚΗΣ 2020, 37(Συμπλ 2):125–128

I. Stefanidis,[1]
E. Nikolaou,[1]
F. Karasavvidou,[2]
T. Eleftheriadis,[1]
A. Diamandopoulos[3]

[1]Department of Nephrology, University Hospital of Larissa, School of Medicine, University of Thessaly, Larissa
[2]Laboratory of Pharmacology, University Hospital of Larissa, School of Medicine, University of Thessaly, Larissa
[3]Medical School, National and Kapodistrian University of Athens, Athens, and Louros Foundation for the History of Medicine, Athens, Greece

Membranous nephropathy (MN) is one of the most common causes of adult nephrotic syndrome. Histopathology involves typical subepithelial immuncomplexes, with an obvious pathogenetic role. Today, the study of pathogenesis, which began in 1959, has proven that MN is an organ-specific autoimmune disease. Our aim was to follow and draw some historical lessons from this 60-year long course of studies on MN. Heymann nephritis (HN; 1959) is the classical animal model, in which the pathogenetic role of immuncomplexes in MN was first established. HN is induced by injection in rats of tubules brush border (BB) antigens (active HN) or the corresponding antibodies (anti-BB; passive HN). In 1978, lesions of HN forming ex vivo after anti-BB injection in an isolated perfused rat kidney model, i.e. in the absence of circulating BB antigens, proved that immune-complex formation occurs in situ. In 1982, megalin was identified as the epithelial auto-antigen in HN. However, as megalin could not be detected in human podocytes, pathogenesis of human MN still remained unresolved. In 2002, neutral endopeptidase was identified as the podocyte antigen in cases of antenatal allo-immune human MN, clearly implicating the pathogenetic role of podocyte membrane proteins and in situ immune-complex formation. In the next years, phospholipase A2-receptor and Thrombospondin type-1 domain containing 7A were identified as organ-specific auto-antigens associated with MN. The maxim "sciencia facit altus" could precisely describe the evolution of 60 years of research on the pathogenesis of MN, which was decisively promoted with the breakthroughs made in the last 20 years. This pattern may change as we reach the exciting new scientific era.

Ο δύσκολος δρόμος από τον πάγκο του εργαστηρίου στην κλίνη του ασθενούς. Ιστορικά μαθήματα από τον παθογενετικό μηχανισμό της ιδιοπαθούς μεμβρανώδους σπειραματονεφρίτιδας

Περίληψη στο τέλος του άρθρου

Key words

Heymann nephritis
History of medical research procedures
Megalin
Membranous nephropathy

1. INTRODUCTION

Membranous Nephropathy (MN) is one of the most common causes of adult nephrotic syndrome.[1] It was first described and defined clinically and histopathologically in 1959.[2] Its histopathology involves typical sub-epithelial immune complex deposits and gradual thickening of the basement membrane. These immune complexes activate C5b-9, the complement membrane attack complex, which is the major mediator of proteinuria.

The majority of cases with MN (about 80%) are idiopathic and 20% are associated with autoimmune disease, infection, malignancy or drugs. The clinical course of idiopathic MN is varying and 30% of cases have a spontaneous remission of nephrotic syndrome, while 30% show a progression towards end-stage renal disease within 5–15 years from diagnosis.[3]

Research on the pathogenesis of MN began from the first clinicopathological description[2] and the development of the experimental animal model of Heymann Nepritis (HN) in 1959.[4] From this beginning, the research –still ongoing– formulated three possible mechanisms for the formation of the sup-epithelial immune complexes (fig. 1).

- Preformed circulating immune complexes are entrapped in the sup-epithelial basement membrane
- Circulating pathogenic antigens are planted in the sup-epithelial space, where they bind with autoantibodies to form immune complexes *in situ*

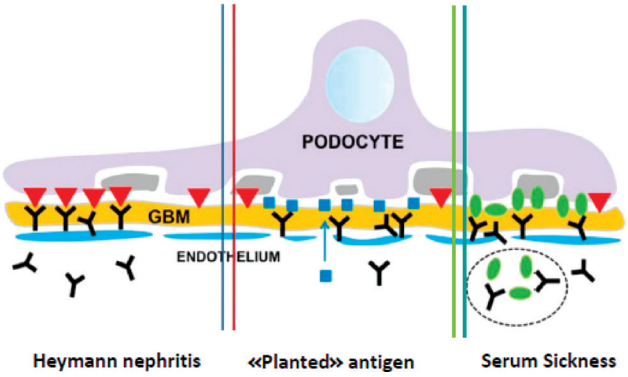

Figure 1. The three possible mechanisms of the sup-epithelial immune complex formation in membranous nephropathy *(see text for details)*.

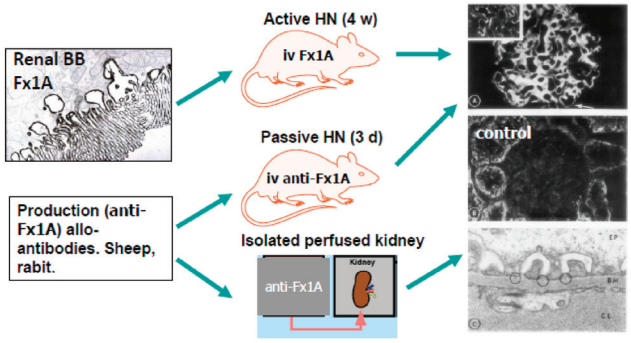

Figure 2. Active Heymann Nephritis (HN), 3–4 weeks after immunisation of Lewis rats by renal brush border (BB) antigen (Fx1A). Passive HN 3 days after injection in Lewis rats of (sheep or rabit) antibodies against rat renal BB antigen (anti-Fx1A). Formation of sup-epithelial immune deposits in an isolated perfused rat kidney after addition in the perfusate of antibodies against rat renal BB (anti-Fx1A).

- Autoantibodies bind to pathogenic antigens inert to the podocyte cell membrane with subsequent in situ sup-epithelial immune complex formation.

Today, research has proven that MN is an organ-specific autoimmune disease.[1] The aim of the present study was to draw some historical lessons from this 60-year long course of studies on MN.

2. HEYMANN NEPHRITIS

Heymann nephritis (HN; 1959) is the classical animal model of MN, in which the pathogenetic role of immune complexes was first established.[4] In the initial experiments by Heymann, a paediatrician from Cleveland (OH, USA), nephritis was induced by immunisation of Lewis rats by whole kidney extracts. From the crude kidney extract only the fractions from the tubules brush border (BB, Fx1A) induced HN (active HN). In addition, passive immunisation with injection of Lewis rats with the corresponding antibodies (anti-BB; anti-Fx1A) induced passive HN. Because HN was not induced by glomerular but rather by tubular BB extracts, the model of active HN indicated that the deposits came from circulating immune complexes. However, the model of passive HN in rats argued against this scenario and supported the possible pathogenetic role of antigens inert to the podocyte's membrane (fig. 1).[5]

This question was answered with certainty only 20 years later (in 1978), when two groups independently showed in an isolated perfused rat kidney model, that HN lesions form *ex vivo* after anti-BB (Anti-Fx1A) injection (fig. 2). The formation of sup-epithelial immune complexes in the absence of circulating BB antigens finally proved that immune-complex formation in HN occurs *in situ*.[6,7] For this reason, research efforts intensified to assess the auto-antigen responsible for HN in rat podocytes.

These efforts finally bore results in 1982. Megalin was identified as the epithelial auto-antigen in HN. These findings, 33 years after Heymann's first description, allowed a significant clarification in the pathogenesis of Heymann nephritis.[8,9] Furthermore, it seemed absolutely justified to hypothesise that human membranous nephropathy would follow a similar pathogenetic pattern with HN, also involving megalin.[9] However, really unexpectedly megalin could not be detected in human podocytes.[1,5]

3. ANTIGENS IN MEMBRANOUS NEPHROPATHY

Megalin is localised in tubular epithelial cells but not in human podocytes.[5] For this reason, the pathogenesis of human MN still remained unclear. For many years, all efforts to assess an auto-antigen in human podocytes with a role in human MN similar to the role of megalin in HN were unsuccessful.[5] This failure was most probably due to the reduced sensitivity of the applied methodology, especially of mass spectrometry, and the use of cultured podocytes as the main source of antigens.

In 2002, neutral endopeptidase was identified as the podocyte antigen in cases of antenatal allo-immune human MN,[10] clearly implicating the pathogenetic role of podocyte membrane proteins and in situ immune-complex formation. This impressive proof that the formation of immune complexes in MN occurs in situ led to the intensification of all research efforts to assess the auto-antigen responsible for MN on human podocytes.

Finally, in 2009 and 2014, two human podocyte proteins were identified as pathogenetic antigens of idiopathic MN. Specifically, phospholipase A2-receptor (PLA2R) and thrombospondin type-1 domain containing 7A (THSD7A) were identified as organ-specific auto-antigens associated with MN.[11,12] A long-standing problem was solved.

The methodological approach was similar to the studies on HN 25 years earlier. It was also based on microdissection of human glomeruli, proteomic technology and mass spectrometry. However, the technological precision of the implemented methods was better in these recent studies and represented a central contributing factor for success. However, the main reason for this success is that non-reducing conditions were applied during the detection procedure. This is based on a simple idea, namely that under non-reducing conditions the disulfide bonds remain intact and protein conformation does not change. Indeed, the antibody reactive epitopes of both antigens involved in idiopathic MN were reduction-sensitive (i.e. conformation-dependent epitopes) and the serum samples from patients with MN did not recognise the antigens under reducing conditions.

4. CONCLUSIONS

Research on the pathogenesis of MN began in 1959 with the description of HN the experimental animal model for MN. Thereafter, the first step forward was to prove the in situ formation of immune complexes in HN. This was achieved by an ex vivo model, applied simultaneously by two scientific groups (1978). The second decisive step was to show that the same principle also applies in human idiopathic MN. Progress came from a rare case of neonatal MN analysed with scrutiny by a specialised group in 2002. The final step was then to identify the specific podocyte auto-antigens in MN, as was already done for HN in 1982. This was achieved thanks to technology and to a very simple idea, namely that the antigen epitopes might be conformation-specific and thus reduction-sensitive (in 2009 and 2014).

The maxim "sciencia facit saltus" could precisely describe the above-described evolution of 60 years of research on the pathogenesis of MN, which was decisively promoted with the breakthroughs made in the last 20 years. This pattern may change, or simply become faster, as we reach the exciting new scientific era.

ΠΕΡΙΛΗΨΗ

Ο δύσκολος δρόμος από τον πάγκο του εργαστηρίου στην κλίνη του ασθενούς. Ιστορικά μαθήματα από τον παθογενετικό μηχανισμό της ιδιοπαθούς μεμβρανώδους σπειραματονεφρίτιδας

Ι. ΣΤΕΦΑΝΙΔΗΣ,[1] Ε. ΝΙΚΟΛΑΟΥ,[1] Φ. ΚΑΡΑΣΑΒΒΙΔΟΥ,[2] Τ. ΕΛΕΥΘΕΡΙΑΔΗΣ,[1] Α. ΔΙΑΜΑΝΤΟΠΟΥΛΟΣ[3]

[1]Νεφρολογικό Τμήμα, Πανεπιστημιακό Νοσοκομείο Λάρισας, Λάρισα, [2]Εργαστήριο Φαρμακολογίας, Πανεπιστημιακό Νοσοκομείο Λάρισας, Λάρισα, [3]Ιατρική Σχολή, Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Αθήνα και Ίδρυμα Λούρου Ιστορίας της Ιατρικής, Αθήνα

Αρχεία Ελληνικής Ιατρικής 2020, 37(Συμπλ 2):125–128

Η μεμβρανώδης νεφροπάθεια (ΜΝ) είναι η πιο συχνή αιτία νεφρωσικού συνδρόμου στους ενήλικες. Η ιστοπαθολογία εμπλέκει τυπικά υποεπιθηλιακά ανοσοσυμπλέγματα, με εμφανή παθογενετικό ρόλο. Σήμερα, η μελέτη της παθογένεσής της, η οποία άρχισε το 1959, έχει αποδείξει ότι η Μεμβρανώδης Νεφροπάθεια αποτελεί μια αυτοάνοση ασθένεια συγκεκριμένων οργάνων. Στόχος μας ήταν να παρακολουθήσουμε και να αντλήσουμε μερικά ιστορικά μαθήματα από την εξηκονταετή πορεία των μελετών πάνω στη Μεμβρανώδη νεφροπάθεια. Η νεφρίτιδα Heymann (HN, 1959) αποτελεί το κλασικό ζωικό μοντέλο στο οποίο καθιερώθηκε για πρώτη φορά ο παθογενετικός ρόλος των ανοσοσυμπλεγμάτων στη ΜΝ. Η νεφρίτιδα Heymann επάγεται με ένεση σε αρουραίους αντιγόνων (ενεργούς HN) ψηκτροειδούς παρυφής (BB) ή των αντίστοιχων αντισωμάτων (αντιγόνα ψηκτροειδούς παρυφής, παθητική νεφρίτιδα Heymann). Το 1978, οι αλλοιώσεις σχηματισμών ex νίνο της νεφρίτιδας Heymann μετά από ένεση αντιγόνων ψηκτροειδούς παρυφής σε ένα απομονωμένο μοντέλο εμποτισμένου νεφρού αρουραίου, δηλ. απουσία κυκλοφορούντων αντιγόνων ψηκτροειδούς παρυφής, απέδειξαν ότι ο σχηματισμός ανοσοσυμπλόκου προκύπτει *in situ*. Το 1982, η μεγαλίνη (megalin) αναγνωρίστηκε ως το επιθηλιακό αυτοαντιγόνο στη νεφρίτιδα Heymann. Ωστόσο, καθώς δεν είναι δυνατή η ανίχνευση της μεγαλίνης στα ανθρώπινα ποδοκύτταρα, η παθογένεση της ΜΝ στον άνθρωπο παρέμενε ανεξήγητη. Το 2002, η ουδέτερη ενδοπεπτιδάση αναγνωρίστηκε ως το αντιγόνο ποδοκυττάρων σε περιπτώσεις προγεννητικής αλλοάνωσης ανθρώπινης ΜΝ, που συνεπάγεται σαφώς τον παθογενετικό ρόλο των πρωτεϊνών

της μεμβράνης ποδοκυττάρων και τον *in situ* σχηματισμό ανοσοσυμπλόκου. Τα επόμενα έτη, ο υποδοχέας Α2 φωσφολιπάσης και η θρομβοσπονδίνη τύπου-1 που περιέχει 7Α ταυτοποιήθηκαν ως αυτοαντιγόνα συγκεκριμένων οργάνων που σχετίζονται με τη ΜΝ. Το αξίωμα *«η επιστήμη κάνει άλματα»* θα μπορούσε να περιγράψει με ακρίβεια την εξέλιξη της εξηκονταετούς έρευνας πάνω στην παθογένεση της ΜΝ, η οποία προχώρησε αποφασιστικά με τις ανακαλύψεις που έγιναν τα τελευταία 20 χρόνια. Αυτό το μοτίβο ενδέχεται να αλλάξει καθώς φτάνουμε στη συναρπαστική νέα επιστημονική εποχή.

Λέξεις ευρετηρίου: Ιστορία της ιατρικής έρευνας, Μεγαλίνη, Μεμβρανώδης νεφροπάθεια, Νεφρίτις Heymann

References

1. RONCO P, DEBIEC H. Membranous nephropathy: A fairy tale for immunopathologists, nephrologists and patients. *Mol Immunol* 2015, 68:57–62
2. JONES DB. Nephrotic glomerulonephritis. *Am J Pathol* 1957, 33:313–329
3. GLASSOCK RJ. Diagnosis and natural course of membranous nephropathy. *Semin Nephrol* 2003, 23:324–332
4. HEYMANN W, HACKEL DB, HARWOOD S, WILSON SG, HUNTER JL. Production of nephrotic syndrome in rats by Freund's adjuvants and rat kidney suspensions. *Proc Soc Exp Biol Med* 1959, 100:660–664
5. GLASSOCK RJ. Human idiopathic membranous nephropathy – a mystery solved? *N Engl J Med* 2009, 361:81–83
6. COUSER WG, STEINMULLER DR, STILMANT MM, SALANT DJ, LOWENSTEIN LM. Experimental glomerulonephritis in the isolated perfused rat kidney. *J Clin Invest* 1978, 62:1275–1287
7. VAN DAMME BJ, FLEUREN GJ, BAKKER WW, VERNIER RL, HOEDEMAEKER PJ. 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, 38:502–510
8. 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 USA* 1982, 79:5557–5561
9. FARQUHAR MG, SAITO A, KERJASCHKI D, ORLANDO RA. The Heymann nephritis antigenic complex: Megalin (gp330) and RAP. *J Am Soc Nephrol* 1995, 6:35–47
10. DEBIEC H, GUIGONIS V, MOUGENOT B, DECOBERT F, HAYMANN JP, BENSMAN A ET AL. Antenatal membranous glomerulonephritis due to anti-neutral endopeptidase antibodies. *N Engl J Med* 2002, 346:2053–2060
11. BECK LH Jr, BONEGIO RGB, LAMBEAU G, BECK DM, POWELL DW, CUMMINS TD ET AL. M-type phospholipase A2 receptor as target antigen in idiopathic membranous nephropathy. *N Engl J Med* 2009, 61:11–21
12. TOMAS NM, BECK LH JRr MEYER-SCHWESINGER C, SEITZ-POLSKI B, MA H, ZAHNER G ET AL. Thrombospondin type-1 domain-containing 7A in idiopathic membranous nephropathy. *N Engl J Med* 2014, 371:2277–2287

Corresponding author:

I. Stefanidis, Department of Nephrology, University Hospital of Larissa, Mezourlo Hill, 411 10 Larissa, Greece
e-mail: stefanid@med.uth.gr