Frontiers in Nephrology

Membranous Nephropathy: A Long Road but Well Traveled

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J Am Soc Nephrol 16: 1184–1187, 2005. doi: 10.1681/ASN.2005010087

My job as Guest Editor of this symposium is to put the four separate articles that it contains into context and tie them together as one cohesive feature. To do that, I am going to describe (admittedly from a very personal perspective, because I have walked this road!) the highlights of our journey with this disease over the past half century or so.

The evolution of our understanding of idiopathic membranous nephropathy (MN) is, at least to me, one of the most intriguing stories in the area of glomerular disease. It has paralleled advances in renal science over the past five decades beginning with the immunofluorescence and electron microscopic analysis of renal biopsy tissue that began in the 1950s and first opened the field of immunopathology that allowed exploration of the immune mechanisms that cause these diseases. It has progressed through the cell culture era of the 1980s and into the current era of molecular biology and genetics with a focus on podocyte biology. Each of these periods has opened our eyes to yet another new and often unexpected aspect of this common and fascinating disease. Our thinking about the pathogenesis of MN has evolved from an initial view of the disease as the classic human equivalent of chronic serum sickness to our current belief that MN, like most immune glomerular diseases, is instead a manifestation of an immune response to self antigens, in this case ones expressed on the podocyte cell membrane.

My own journey with MN began in the early 1970s. As a new and untested young physician-scientist just starting an independent laboratory in a sea of renal physiologists, I was challenged by the late Norman Levinsky to produce a proteinuric rat with normal renal function suitable for micropuncture studies of the mechanisms of sodium retention in nephrotic syndrome (1). The model of MN described by Walter Heymann (see below) seemed to fit that description (2). As we studied these animals with Heymann nephritis more closely (3), however, several aspects of the glomerular lesion seemed to me inexplicable by the paradigms of the times (4). If the glomerular immune deposits represented circulating immune complex trapping, then why were they so exclusively subepithelial and not in the mesangial and subendothelial sites, where circulating complexes more typically localize? And why do these deposits cause such massive proteinuria in the absence of any histologic evidence of tissue injury or inflammatory cells? Pursuing the answers to these questions has occupied me, a large number of fellows and technicians, and many other laboratories for more than three decades in a quest that still goes on. The disease and the models that reproduce it have launched the careers of a number of renal scientists, including some of the other participants in this Frontiers in Nephrology, who continue to tread a path that yields new and unexpected revelations at each turn. I have organized this Frontiers in Nephrology on MN to update what I believe are the most salient of these observations and the ones that are most active and interesting at the present time.

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The hallmark of MN is the presence of multiple, finely granular, electron-dense deposits exclusively along the subepithelial surface of the glomerular capillary wall between podocyte foot processes (3). This pattern of immune deposition was first described in the human disease in 1956 by Mellors and Ortega (5), who studied patients with idiopathic nephrotic syndrome using immunofluorescence and electron microscopy. The first experimental reproduction of this immunopathologic pattern was reported by Germuth *et al.* (6) in their studies of chronic serum sickness in rabbits induced by immunization with foreign proteins such as BSA. The mechanisms that lead to formation of subepithelial immune deposits were defined further in the 1960s by Dixon *et al.*, who observed that formation of deposits that contain exogenous antigens at a subepithelial site occurred predominately when there was antigen excess and the presence of relatively small, soluble immune complexes in the circulation (7). However, unlike human MN, immune deposits in the serum sickness models were always present in mesangial and subendothelial locations as well. On the basis of these studies, it was long believed that the subepithelial immune

Published online ahead of print. Publication date available at www.jasn.org

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ISSN: 1046-6673/1605-1184

deposits in MN represented the passive trapping of preformed immune complexes from the circulation, with the glomerulus playing no active role in the process except as a filter (4).

In 1959, Heymann *et al.* (2) in Cleveland first described another model of MN in rats induced by immunization with a tissue antigen fraction derived from proximal tubular brush borders (Fx1A). In this model, subsequently referred to as Heymann nephritis, the morphology more closely resembled human MN because the deposits were exclusively subepithelial in location. Later studies of Heymann nephritis identified small amounts of tubular antigen in immune complex form in the circulation and in the glomerular deposits themselves, reinforcing the prevailing thought that the deposits resulted from passive trapping of circulating complexes (8).

The so-called "active" Heymann nephritis model took several weeks to develop after immunization, rendering it almost impossible to use for studies of the mediation of immune injury in an era before the development of knockout and transgenic animals. Thus, efforts were made in several laboratories to induce the model more rapidly by passively transferring heterologous antibodies to the brush border (anti-Fx1A) into normal animals. Success in reproducing the subepithelial deposits passively was first reported by Feenstra *et al.* (9). We described the first passive Heymann nephritis (PHN) model in which antibody deposition induced heavy proteinuria within only 3 to 4 d, thus finally enabling studies to be conducted of the mediation of glomerular injury in MN (10). However, in the 1970s, all of us still believed that we were inducing the passive trapping of small, soluble immune complexes formed in the circulation despite that the massive antibody excess that we created by administering large amounts of antibody was unlikely to produce such complexes.

The first insight into how the deposits in MN actually did develop followed studies to answer the question of whether glomeruli that were altered to produce massive proteinuria still trapped immune complexes. We found that when glomeruli were made permeable to protein by previous administration of aminonucleoside of puromycin, administration of anti-Fx1A antibody no longer induced any subepithelial deposits, demonstrating that some intrinsic property of the glomerulus itself was essential for deposit formation to occur (11). Within months, Van Damme *et al.* (12), in Hoedemaeker's laboratory, using an *ex vivo* perfusion system, and our group, using a physiologically intact isolated perfused kidney system (13), reported that perfusion of anti-Fx1A antibody IgG into bloodless glomeruli produced clear subepithelial immune deposits identical to those that followed intravascular antibody administration to the intact animal. These studies thus established that the deposits that are so characteristic of MN result not from circulating immune complex trapping but from direct, or *in situ*, binding of IgG antibody to native glomerular antigens, presumably expressed on the membrane of podocyte foot processes, where the deposits first form (14). Later, Adler *et al.* (15), working in Border's laboratory, showed that in the BSA serum sickness models, it was the electrical charge on the antigen rather than antigen:antibody ratios or complex size that seemed to be the most important determinant of where deposits formed. Subepithelial deposits formed only when antigens were small and cationic, allowing localization, or "planting," in the subepithelial space, where deposits then presumably formed *in situ*.

The nature of the glomerular antigen that is involved in formation of subepithelial deposits in Heymann nephritis was systematically tracked down by Kerjaschki and Farquhar in the late 1970s and 1980s and shown to be a combination of megalin and a receptor-associated protein (RAP) in the podocyte membrane (and tubular brush border), now referred to as the Heymann nephritis antigenic complex. This story was well reviewed recently (16). In this Frontiers in Nephrology, I have chosen not to review this topic again but rather to jump directly to where the story is today. The article by Ronco *et al.* that follows summarizes the fascinating recent observations of his group documenting that mothers who lack a podocyte membrane protein, neutral endopeptidase, develop antibodies to this protein when pregnant with a normal fetus and that these antibodies cross the placenta and induce typical MN in the fetus (17). This finding represents the first convincing evidence that at least some cases of human MN involve mechanisms that are identical to those defined in Heymann nephritis. This work is an elegant example of how the trained scientist with a prepared mind can capitalize on a seemingly irrelevant event, the birth of a nephrotic infant, to make observations that clarify the pathogenesis of a major adult disease. My expectation, now that the link between Heymann nephritis and MN has finally been established after 20 years of searching for it, is that there now will be a rapid expansion of knowledge in this area with discovery of more such patients and more podocyte antigens that participate in this disease. This should lead soon to assays of pathogenic antibodies and much better approaches to treatment and follow-up than we currently have.

Clarification of what the immune deposits in MN represented, however, did not clarify why patients who developed them became so severely nephrotic, particularly because the histopathology of MN was free of inflammation and looked more like minimal-change nephrotic syndrome (with which it was often confused before routine immunofluorescence and electron microscopy were performed) than it did like anti–glomerular basement membrane (GBM) disease, the prototypical antiglomerular antibody disease. Why did these two diseases, with seemingly similar mechanisms underlying them, look so dramatically different both clinically and morphologically? The development of the passive model of Heymann nephritis with heavy proteinuria enabled studies to be done to answer this question. At first glance, the Heymann models looked very similar to models of anti-GBM nephritis in guinea pigs described in the 1970s, models in which glomerular antibody deposition also resulted in heavy proteinuria despite the absence of any significant changes in the glomerulus by light microscopy (a scenario that we recognize today probably represented a predominance of antibodies to the podocyte rather than to GBM) (18). In the guinea pig models, complement activation and deposition did not occur, and proteinuria was completely complement independent. Convinced that the Heymann models were probably similar, we were stunned to find

that complement-depleting PHN rats did not just reduce proteinuria but also totally abolished it, despite subepithelial deposition of large quantities of antibody (19). Because this was true despite the total absence of neutrophils or other signs of inflammation, it seemed probable that glomerular injury involved some other function of the complement system than the only one known to cause tissue injury at that time—the generation of chemotactic factors that attract neutrophils (20). Subsequent studies that selectively depleted components of the distal complement cascade, leading to membrane attack complex formation, or that used genetically complement-deficient serum and animals, showed that it was not generation of soluble chemotactic factors but formation of the C5b-9 complex that inserted into the lipid bilayer of podocyte cell membranes as deposits were formed in the isolated kidney (21) and later in the intact animal (22) that led to the loss of the glomerular barrier to protein filtration.

These findings in experimental MN have generated many subsequent studies to define the consequences of C5b-9 insertion into podocytes that account for the proteinuria and glomerular sclerosis that develop in MN. Sublytic C5b-9 leads to cell activation and to an array of cellular changes in the podocyte that seem to explain many of the classic features of MN—the proteinuria, lack of inflammation, lack of cell proliferation, loss of podocytes, thickening of GBM, and spike formation. The article in this Frontiers in Nephrology by Nangaku, Shankland, and myself reviews and updates what we have learned to date about why sublytic C5b-9 attack on podocytes leads to proteinuria, nephrotic syndrome, and all of the clinical and morphologic findings that characterize MN in humans.

Pathogenic C5b-9 attack on podocytes or other nucleated cells requires that a more recently appreciated defense system against complement attack, at both the circulatory and the cellular levels, fail. Complement regulatory proteins have moved center stage in the past decade as players in a number of complement-mediated glomerular diseases, including membranoproliferative glomerulonephritis, hemolytic-uremic syndrome (note the outstanding review of this by Noris and Remuzzi in the April 2005 issue of *JASN*), and, of course, MN.

Quigg and colleagues (23) were the first to connect complement regulatory proteins to MN when they demonstrated that the antibody used to induce proteinuria in the PHN model contained antibodies to Crry, a cell-bound complement regulatory protein expressed on rat podocytes, and when this antibody was removed, the anti-Fx1A no longer induced proteinuria. So it is not just antibody deposition, complement activation, and C5b-9 that are required to induce experimental MN; it also is necessary to disable the regulatory proteins that defend cells such as podocytes from these mediators (24). Can we treat MN by upregulating podocyte expression of these proteins or even administering soluble or cell-bound versions of them? This, of course, is exactly where the therapeutic "frontier" is in MN in 2005, with a number of laboratories and biotechnology companies actively pursuing this question for application to a number of diseases in which complement activation plays a role (25). Quigg and Cunningham provide an excellent overview of this very promising area of research in their article in this Frontiers in Nephrology.

From the end, we return to the beginning of the Frontiers in Nephrology on MN. In general, *JASN* tries to introduce each Frontiers in Nephrology with a Disease of the Month-type paper to update the clinical reader and provide a stimulus to learn more about research that is ongoing to better understand and treat the human disease. For this Frontiers in Nephrology, Dr. Dan Cattran, who continues to be the most active clinical investigator studying treatment of this disease, has provided a clear and current overview of approaches to therapy of MN. Considerable success has been achieved in this area with conventional steroid and cytotoxic drug therapy, and newer agents offer promise (26). Those who study therapy of MN are faced with two major problems that compromise this effort and can be overcome only by continued progress in understanding the mechanisms of the disease discussed in the three other articles. One problem is that, unlike diseases such as lupus nephritis and anti-GBM disease, we still have not identified the pathogenic antibody in MN. There is therefore no way that therapeutic success can be assessed and patients be rationally selected for immunosuppressive treatment or followed up. The complement studies have offered some surrogates for assessing immune disease activity (glomerular C3 deposition, urinary C5b-9, urinary podocytes, etc.) that are discussed in the article by Nangaku *et al*., but these are indirect, unvalidated in humans, and not readily available. What we need is the antibody.

That leaves only clinical parameters to follow, another problem because of the unique pathobiology of MN. If kidneys with fully developed MN are transplanted into normal hosts, thus mimicking the ideal therapy by totally eliminating the immune response, then the subepithelial immune deposits resolve but the proteinuria does not—at least not for many weeks or months (27,28). This phenomenon, coupled with the very indolent course of the disease and that a majority of patients with MN do not progress, means that we likely treat many patients who do not have active disease at all despite their nephrotic syndromes. Cattran offers a very well-validated way of calculating the risk for progression and an algorithm for approaching the therapy of patients with MN that clinicians should note and use to minimize overtreatment with toxic drugs.

Of course, we need to do better. My hope is that the series of articles included in this Frontiers in Nephrology on MN will help readers to better understand this unique disease and think about it from a more pathophysiologic perspective, even perhaps to persuade some that its remaining mysteries, both clinical and scientific, could be as interesting to explore for them as they have been for those of us who have already traveled this road and are writing about these experiences here.

References

1. Bernard DB, Alexander EA, Couser WG, Levinsky NG: Renal sodium retention during volume expansion in experimental nephrotic syndrome. *Kidney Int* 14: 478–485, 1978
2. 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* 100: 660–664, 1959
3. Couser WG, Stilmant MM, Darby C: Autologous immune complex nephropathy. I. Sequential study of immune complex deposition, ultrastructural changes, proteinuria, and alterations in glomerular sialoprotein. *Lab Invest* 34: 23–30, 1974
4. Wilson CB, Dixon FJ: Immunopathology and glomerulonephritis. *Annu Rev Med* 25: 83–98, 1974
5. Mellors RC, Ortega LG: Analytical pathology. III. New observations on the pathogenesis of glomerulonephritis, lipid nephrosis, periarteritis nodosa, and secondary amyloidosis in man. *Am J Pathol* 32: 455–499, 1956
6. Germuth FG, Pace MG, Tippett JC: Comparative histologic and immunologic studies in rabbits of induced hypersensitivity of the serum sickness type. II. The effect of sensitization to homologous and cross-reactive antigens on the rate of antigen elimination and the development of allergic lesions. *J Exp Med* 101: 135–150, 1955
7. Dixon FJ, Feldman JD, Vazquez JJ: Experimental glomerulonephritis. The pathogenesis of a laboratory model resembling the spectrum of human glomerulonephritis. *J Exp Med* 113: 899–920, 1961
8. Glassock RJ, Edgington TS, Watson JI, Dixon FJ: Autologous immune complex nephritis induced with renal tubular antigen. II. The pathogenetic mechanism. *J Exp Med* 127: 573–588, 1968
9. Feenstra K, van den Lee R, Greben HA, Arends A, Hoedemaeker PJ: Experimental glomerulonephritis in the rat induced by antibodies directed against tubular antigens. I. The natural history: A histologic and immunohistologic study at the light microscopic and the ultrastructural level. *Lab Invest* 32: 235–242, 1975
10. Salant DJ, Darby C, Couser WG: Experimental membranous glomerulonephritis in rats. Quantitative studies of glomerular immune deposit formation in isolated glomeruli and whole animals. *J Clin Invest* 66: 71–81, 1980
11. Couser WG, Jermanovich NB, Belok S, Stilmant MM, Hoyer JR: Effect of aminonucleoside nephrosis on immune complex localization in autologous immune complex nephropathy in rats. *J Clin Invest* 61: 561–572, 1978
12. 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* 38: 502–510, 1978
13. Couser WG, Steinmuller DR, Stilmant MM, Salant DJ, Lowenstein LM: Experimental glomerulonephritis in the isolated perfused rat kidney. *J Clin Invest* 62: 1275–1287, 1978
14. Couser WG, Salant DJ: *In situ* immune complex formation and glomerular injury. *Kidney Int* 17: 1–13, 1980
15. 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* 71: 487–499, 1983
16. Kerjaschki D: Pathomechanisms and molecular basis of membranous glomerulopathy. *Lancet* 364: 1194–1196, 2004
17. Debiec H, Guigonis V, Mougenot B, Decobert F, Haymann JP, Bensman A, Deschenes G, Ronco PM: Antenatal membranous glomerulonephritis due to anti-neutral endopeptidase antibodies. *N Engl J Med* 346: 2053–2060, 2002
18. Couser WG, Stilmant MM, Jermanovich NB: Complement-independent nephrotoxic nephritis in the guinea pig. *Kidney Int* 11: 170–180, 1977
19. Salant DJ, Belok S, Madaio MP, Couser WG: A new role for complement in experimental membranous nephropathy in rats. *J Clin Invest* 66: 1339–1350, 1980
20. Couser WG, Baker PJ, Adler S: Complement and the direct mediation of immune glomerular injury: A new perspective. *Kidney Int* 28: 879–890, 1985
21. Cybulsky AV, Quigg RJ, Salant DJ: The membrane attack complex in complement-mediated glomerular epithelial cell injury: Formation and stability of C5b-9 and C5b-7 in rat membranous nephropathy. *J Immunol* 137: 1511–1516, 1986
22. Baker PJ, Ochi RF, Schulze M, Johnson RJ, Campbell C, Couser WG Depletion of C6 prevents development of proteinuria in experimental membranous nephropathy in rats. *Am J Pathol* 135: 185–194, 1989
23. Schiller B, He C, Salant DJ, Lim A, Alexander JJ, Quigg RJ: Inhibition of complement regulation is key to the pathogenesis of active Heymann nephritis. *J Exp Med* 188: 1353–1358, 1998
24. Quigg RJ: Complement and the kidney. *J Immunol* 171: 3319–3324, 2003
25. Hsu SI, Couser WG: Chronic progression of tubulointerstitial damage in proteinuric renal disease is mediated by complement activation: A therapeutic role for complement inhibitors? *J Am Soc Nephrol* 7[Suppl 2]: S186–S191, 2003
26. Cattran DC: Mycophenolate mofetil and cyclosporine therapy in membranous nephropathy. *Semin Nephrol* 23: 272–277, 2003
27. Makker SP, Kanalas JJ: Course of transplanted Heymann nephritis kidney in normal host. Implications for mechanism of proteinuria in membranous glomerulonephropathy. *J Immunol* 142: 3406–3410, 1989
28. Pruchno CJ, Burns MM, Schulze M, Johnson RJ, Baker PJ, Alpers CE, Couser WG: Urinary excretion of the C5b-9 membrane attack complex of complement is a marker of immune disease activity in autologous immune complex nephritis. *Am J Pathol* 138: 203–211, 1991