Membranous nephropathy and the Henle–Koch postulates

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Antibodies against several podocyte antigens have evolved as markers of diagnosis, disease activity, and prognosis in membranous nephropathy, but their pathogenic role remains debated. Detailed work-up of two cases of primary and secondary membranous nephropathy now supports the concept that thrombospondin type-1 domain-containing 7A autoantibodies are pathogenic.

Reports of two cases of thrombospondin type-1 domain-containing 7A (THSD7A)-associated membranous nephropathy (MN) have shed light on the question of whether THSD7A autoantibodies are merely diagnostic markers of MN or are pathogenic in causing injury to the glomerular filtration barrier. Answering this question is of utmost importance to understand the pathogenesis of a group of diseases for which specific treatments are still lacking.

MN is a frequent cause of nephrotic syndrome in adults. Its diagnosis remains defined by histopathological criteria with uniform thickening of the glomerular capillary caused by sub-epithelial immune complexes. These immune complexes cause podocyte injury characterized by actin-cytoskeleton disorganization and podocyte foot-process effacement, leading to increased permeability of the glomerular filtration barrier and eventually to massive proteinuria and nephrotic syndrome. Secondary MN can develop as a consequence of systemic lupus erythematosus, an infection or a malignancy, suggesting that different autoantigens, microbial antigens or tumour antigens can trigger the disease. Most patients, however, have so-called primary MN in which no underlying condition is identified. Regardless of the underlying aetiology, primary and secondary MN are both driven by an underlying adaptive immune response but the nature of the antigen(s) involved has remained elusive for decades.

Neutral endopeptidase (NEP, also known as nepriysin) was the first podocyte antigen reported to cause prenatal fetomaternal alloimmunization-associated MN, whereas cationic bovine serum albumin can act as an antigen implanted into the glomerular capillary wall in early-childhood MN. In 2009 the phospholipase A2 receptor (PLA2R) was identified as a potential podocyte antigen that drives the majority of cases of adult-onset primary MN. Among cases of MN that are negative for anti-PLA2R IgG, Tomas et al. detected antibodies against another podocyte antigen, THSD7A. PLA2R or THSD7A antibodies are associated with disease activity and prognosis of adult-onset MN but these associations do not necessarily imply that the antibodies are pathogenic. Addressing this question is essential not only to clarify the disease pathogenesis but also in considering therapeutic strategies to target these antibodies. However, proving their pathogenicity is challenging.

Careful clinical observation can provide supportive evidence of causality. For example, the presence and concentration of anti-PLA2R IgG or anti-THSD7A IgG can predict the presence of resolving or persistent MN as well as recurrence of MN after kidney transplantation, in a manner that is conceptually similar to the well-established pathogenic role of antibodies against the Goodpasture antigen NC1 domain of collagen type IV. More evidence has now been provided by Hoxha et al., who followed a patient with anti-THSD7A-related MN with overexpression of TSHD7A protein in a mixed adenoneuroendocrine gall bladder carcinoma. Indeed, TSHD7A was found not only within the tumour and its metastases but also in regional lymph nodes. In the latter, TSHD7A was also found in CD21+ follicular dendritic cells, a cell type responsible for priming autoantigen-specific lymphocytes, a process that ultimately triggers initiation of THSD7A antibody production in paraneoplastic MN. This case not only challenges the idea that anti-THSD7A explicitly identifies primary MN but rather raises the question of whether anti-THSD7A preferably occurs in paraneoplastic MN. The researchers identified malignancies in seven of 25 patients with anti-THSD7A-positive MN but other cohorts have not been examined to confirm this finding. This particular case of paraneoplastic anti-THSD7A-related MN improved upon chemotherapy, which reduced plasma anti-THSD7A antibodies to undetectable levels. Although this finding confirms a strong association between TSHD7A antibodies and MN, it does not prove a pathogenic role. So how does one obtain conclusive proof?

Jacob Henle’s student, Robert Koch, faced the same question when he found acid-fast bacteria in lesions from patients with tuberculosis, which at first represented nothing more than a simple and potentially meaningless association. The subsequently developed (Henle–)Koch postulates define how to test causality for infectious agents.

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causality for infectious agents that are spatially and temporally associated with disease. The same methodological approach can be used to test the pathogenicity of autoantibodies in MN [Fig. 1].

The first postulate requires that the microorganism (or antibody, in the case of MN), is found only in patients with the disease. Indeed, antibodies against NEP, PLA2R, and TSHD7A have exclusively been found in patients with MN and not in healthy individuals, substantiating the first postulate. The second postulate requires that the agent be isolated and purified; indeed, antibodies against NEP, PLA2R, and TSHD7A have been isolated from patient serum and glomerular immune deposits. These antibodies stain podocytes in renal sections or recognize the specific antigen at the molecular level. Although anti-NEP IgG can induce MN in rabbits, anti-PLA2R IgG has not yet been demonstrated to induce MN in animals.

Tomas et al. now provide the first demonstration that purified anti-TSHD7A IgG can induce MN in mice by binding to TSHD7A on podocytes and activating complement. These data fulfill the third Henle–Koch postulate and provide considerably strong evidence that TSHD7A antibodies cause MN. We acknowledge that simply because similar attempts for anti-PLA2R have led to negative results, does not disprove a pathogenic role for anti-PLA2R in MN, as human antibodies can only cause disease in animals with conserved antigen epitopes and identical cell type-specific expression of PLA2R.

Research into human MN has been frustrating for many decades but is now constantly delivering exciting new data that build a pathogenic concept of the various forms of MN. Some conclusions have now become obvious: first, MN is not a single disease but a syndrome that involves different pathogenic pathways in, for example, antenatal MN, early-childhood MN, and various forms of adult MN. Furthermore, secondary MN involves diverse antigens, and antigens such as TSHD7A might be involved in primary as well as secondary MN. Despite these exciting new data on the antigens involved, the mechanisms underlying loss of tolerance and auto-immunization remain elusive and might only be solved by genetic profiling of the patient's immune system.

Robert Koch was a physician–scientist who knew that finding a cure for a disease first requires identifying the underlying pathomechanism and providing functional proof beyond potentially misleading associations. Concepts based on associative data have misled medicine for centuries. Disease concepts of autoimmune disease are notoriously contaminated with associative knowledge, especially data on serum autoantibodies. The (Henle–) Koch postulates serve as a reminder of the levels of evidence that are needed to prove pathogenicity of autoantibodies in MN or in other forms of immune-complex glomerulonephritis. The two reports from this research group are illuminating examples in this context.