Pathophysiological lessons from rare associations of autoimmune diseases

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Membranous nephropathy (MN) is a non-proliferative glomerular disease characterized by accumulation of immune deposits on the outer aspect of the glomerular basement membrane (GBM) leading to complement activation and proteinuria. Eighty per cent of cases are referred to as ‘idiopathic MN’ (iMN), while ~20% are classified as ‘secondary’ because they occur in patients presenting with associated clinical conditions such as infection (hepatitis B, mostly in children), cancer, systemic lupus erythematosus (SLE) and related systemic autoimmune diseases and drug intoxication.

For a long time, the target antigens have gone unrecognized. However, in the last 10 years, considerable progress has been achieved in the understanding of the pathophysiology of MN, with the identification of several target antigens from the neonatal period to adulthood, which makes it possible to discuss at the molecular level the mechanisms of association with other nephropathies. These advances started in 2002 with the identification by our group of neutral endopeptidase as the responsible enzyme of a stage-like pattern in the majority of patients with early-childhood MN, pointing to immunological heterogeneity of this disease. In MN, target antigens are known, i.e. ANCA-associated vasculitis and anti-GBM disease.

**MN and ANCA-associated necrotizing and crescentic GN**

The association of ANCA-associated necrotizing and crescentic GN (NCGN) and primary MN in the same patient is rare, with only a handful of reports in the literature. In 2009, Nasr et al. \([9]\) reviewed the 14 cases identified from the archives of the Nephropathology Laboratory of Columbia University between January 2000 and February 2008. Cases of SLE were excluded. In 13 patients, MN and NCGN were diagnosed simultaneously at the time of renal biopsy, while in the last patient, biopsy-proven MN preceded the development of NCGN by 7 months. The same chronology was observed in 9 of the 10 patients reported before Nasr’s study. In the patient reported herein, it is likely but not histologically proven that MN preceded NCGN \([8]\). The majority of patients presented with rapidly progressive GN and nephrotic-range proteinuria. All biopsies showed features of MN.

In this issue of the journal, Surindran et al. \([8]\) report the interesting association of PLA2R1-positive MN with anti-neutrophil cytoplasmic antibodies (ANCA)-associated glomerulonephritis (GN). Light microscopy showed cellular crescents with occasional necrosis of the glomerular tuft and mildly thickened GBM with numerous subepithelial electron deposits by electron microscopy. Granular deposits of C3 were seen by immunofluorescence while IgG could not be studied in the absence of glomeruli. Is this association a coincidence or are there common mechanisms or pathways involved? We will discuss this issue in MN associated with two different autoimmune diseases where target antigens are known, i.e. ANCA-associated vasculitis and anti-GBM disease.
positive in three but without anti-dsDNA antibodies. Twenty of 22 patients had normal levels of complement; the 2 patients with hypocomplementaemia had a negative anti-dsDNA antibody and no evidence of SLE, hepatitis, mixed connective tissue disease or polymyositis. No testing was performed for anti-PLA2R1 antibodies because this study was published before the identification of PLA2R1 [3]. Several links may exist between anti-PLA2R1 and anti-MPO antibodies and the related diseases. Firstly, in the patients with preceding MN, one can speculate that, following the initial injury induced at the podocyte surface by anti-PLA2R1 antibodies, complement activation and oxygen derivative production, intracellular proteins and cryptic epitopes might be exposed and induce a second wave of immunization (Ronco, Nat Reviews, in press). This hypothesis is supported by the finding that a proportion of patients with iMN have circulating antibodies to cytoplasmic antigens such as aldose reductase, superoxide dismutase and alpha-enolase, apparently with the corresponding antigen in glomerular immune deposits [5, 6]. These ubiquitous enzymes are not, or only very weakly, expressed on the membrane of normal podocytes but they can be induced in vitro by oxidative stress [5]. We identified some of the antigens involved in metabolic pathways, cytoskeleton architecture and cell stress (Debiec and Ronco, unpublished). Although myeloperoxidase is mostly localized in polymorphonuclear leukocytes, shared epitopes may exist between podocyte peroxidases released by injury and myeloperoxidase. The above pathophysiologic scenario may have occurred in a case reported by Kanahara et al. [10] showing MN in the first biopsy with a low MPO-ANCA titre and a year later, crescentic GN with an elevated ANCA level.

Secondly, myeloperoxidase released from activated neutrophils may be trapped within the GBM [11] and might contribute to the formation of subepithelial immune deposits and local modification of proteins potentially leading to the appearance of neo-antigens [12]. Myeloperoxidase has indeed been detected in the so-called MN-like deposits in patients with ANCA-associated NCGN [13]. In 6 of 17 cases of ANCA-associated GN with granular deposition of IgG along the GBM, double-labeling immunofluorescence showed partial colocalization of MPO and IgG in the GBM and the mesangium, which was not seen in a control. Immunoelectron microscopy revealed that ~50% of the MPO was colocalized with electron-dense deposits. Thyroid peroxidase was also detected in subepithelial immune deposits. Thyroid peroxidase was also detected in subepithelial immune deposits (Debiec and Ronco, unpublished). Although myeloperoxidase is mostly localized in polymorphonuclear leukocytes, shared epitopes may exist between podocyte peroxidases released by injury and myeloperoxidase. The above pathophysiologic scenario may have occurred in a case reported by Kanahara et al. [10] showing MN in the first biopsy with a low MPO-ANCA titre and a year later, crescentic GN with an elevated ANCA level.

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This case and those reported in the literature further illustrate that association of rare autoimmune diseases is not uncommon [19] pointing to genetic predisposition and/or common environmental trigger (Figure 1). In patients with iMN, our GWAS study revealed a highly significant association with HLA-DQA1 alleles, with the risk of developing iMN being multiplied by 80 in individuals homozygous for the most significantly associated SNPs [4]. Genetic factors also purportedly contribute to ANCA-related diseases as evidenced by reports that disease occurs in siblings [20] and within families [21, 22]. Four SNPs in the CD18 gene showed significant associations with MPO-ANCA vasculitis [23], while the DRB1*15 allele was shown to be a risk factor for PR3-ANCA disease in African Americans [24]. The protein DRB1*1501 binds with high affinity to amino acid sequences of sense-PR3, purportedly an antigen epitope, suggesting that this allele contributes to the pathogenesis of PR3-ANCA disease. One can speculate that a rare combination of alleles of the HLA-class II immune response genes involved in the presentation of epitopes to the immune system is responsible for the coexistence of MN with ANCA-related NCGN.

On the other hand, the role of environmental factors should not be neglected because their role is clearly established in ANCA where infections and exposure to silica are well-known triggers [25] and MN where the role of heavy metals has been demonstrated [26] As a matter of fact, genetic and environmental factors may be associated. It is noteworthy that there are three examples of MN-associated with ANCA-related NCGN in patients treated with penicillamine or propylthiouracil [27, 28].

**MN and anti-GBM nephritis**

Although ANCA-related NCGN superimposed on MN is exceptionally rare, the combination of anti-GBM nephritis and MN, albeit also rare, is well recognized, as recently reviewed by Basford et al. [29]. An accurate diagnosis relies on a combination of renal biopsy findings and serological testing. More than 25 cases have been reported (review in [29]). MN can precede (mostly in middle-aged or older patients) or follow (mostly in younger patients) the GBM nephritis or be discovered simultaneously (most patients with varying ages).

Anti-GBM nephritis is caused by antibodies to the non-collagenous domain NC1 of the alpha-3 and alpha-5 chains of Type IV collagen [30]. The antibodies recognize two conformational epitopes, which are normally hidden. Recently, a sulphilimine bond was identified within the hexamer formed by contiguous NC1 domains [31]. When the bond is intact, the epitopes are not accessible. When it is broken, the epitopes undergo a change of conformation, which makes them accessible to pathogenic antibodies. In some patients with MN and the allele HLA-DRB1*1501 which is a
major risk allele for anti-GBM nephritis (review in [32]), immune complex deposition on the outer aspect of the GBM may unmask cryptic epitopes or cause release of damaged GBM antigens into the circulation. Conversely, in those patients with an initial anti-GBM nephritis, subsequent formation of subepithelial immune complexes may result from increased expression, change of conformation or release of PLA2R1 [33] or other podocyte antigens leading to a second wave of immunization or from binding of anti-GBM antibodies to freed GBM antigens within the capillary walls. The latter scenario is supported by observations made by Druet’s group showing that in rats injected with mercury chloride, a linear fluorescence along the GBM precedes the occurrence of granular subepithelial immune deposits [34]. In summary, although rare and even exceptional apart from SLE, the association of MN with autoimmune crescentic GN is most likely not a mere coincidence. A predisposing genetic background, more specifically a more rare combination of immune response gene alleles, may favour the development of an immune response directed against several antigens either simultaneously or subsequently. We think that, thanks to the recent description of target epitopes at the molecular level in several autoimmune glomerulopathies, such rare associations of glomerular disease may contribute to a better understanding of the pathophysiology of antibody-mediated GN.

Conflict of interest statement. None declared.

(See related article by Surindran et al. Coexistence of ANCA-associated glomerulonephritis and anti-phospholipase A2 receptor antibody-positive membranous nephropathy. Clin Kidney J 2012; 5:162–165)

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