MPO-ANCA crescentic glomerulonephritis complicated by membranous nephropathy: MPO demonstrated in epimembranous deposits

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Abstract
An elderly woman presented with haematuria and proteinuria accompanied by elevated serum myeloperoxidase (MPO)-specific anti-neutrophil cytoplasmic antibodies (MPO-ANCA). A renal biopsy revealed mild mesangial proliferation with fibrocellular crescent formation and a membranous glomerular lesion. Immunofluorescence microscopy using FITC-labelled rabbit anti-human MPO antibodies revealed granular MPO deposition along the glomerular capillary walls (GCW) with a staining profile similar to that of glomerular IgG deposition. The one-year follow-up renal biopsy revealed minimal IgG and undetectable MPO deposition. Both MPO and MPO-ANCA might have been responsible for the IgG immune deposits along the GCW in this patient.

Keywords: membranous glomerulopathy; MPO-ANCA; myeloperoxidase

Introduction
Myeloperoxidase (MPO)-specific anti-neutrophil cytoplasmic antibodies (MPO-ANCA)-associated glomerulonephritis (GN) is characterized by immunofluorescence microscopy (IF) or by electron microscopy (EM), as a pauci-immune type of necrotizing and crescentic GN caused by a relative lack of immunoglobulin (Ig) and complement deposition within the kidney [1]. Brons et al. have postulated that immune complexes (ICs) are deposited at a very early phase in the course of ANCA-associated GN and vasculitis and that IC plays a role in initiating the disease process, although immune deposits can be removed from severe inflammatory lesions before they are proven by renal examinations [2]. On the other hand, ICs are found in just over half of renal biopsies with MPO-ANCA-associated GN mostly as segmental or scattered deposition [3]. ICs might potentiate the effect of ANCA in the development of GN and act synergistically with ANCA to produce more severe GN than ANCA-associated GN without IC [3].

Here we described a rare MPO-ANCA-associated GN complicated with membranous glomerulopathy. IF microscopy revealed granular deposition of both IgG and MPO along the GCW. These findings suggest that membranous glomerular lesions can be induced by IC consisting of MPO and MPO-ANCA in MPO-ANCA-associated GN.

Case report
An elderly woman was admitted to our hospital with haematuria and proteinuria and oedema of the lower limbs. She had been diagnosed with hypertension and hyperlipidemia during her early sixties, and treated with a calcium channel blocker and a statin. Urinalysis showed haematuria (sediment, RBC 30–49/high power field) and proteinuria (1.6 g/day). Laboratory tests showed Hb 12.9 g/dL, erythrocyte sedimentation rate 47 mm/h, albumin 3.1 g/dL, creatinine 0.6 mg/dL, BUN 23.7 mg/dL, total-cholesterol 316 mg/dL, triglyceride 181 mg/dL and HDL-cholesterol 52 mg/dL. Levels of IgG, IgA and IgM were 720, 259 and 67 mg/dL, respectively, and those of C3 and C4 were 118.7 mg/dL (normal range, 80–160 mg/dL) and 37.0 mg/dL (normal range, 10–40 mg/dL), respectively. Circulating IC (assessed by C1q binding), cryoglobulin and ANA were negative, whereas rheumatoid factor (60.2 U/mL) and MPO-ANCA (>640 EU) were positive (Figure 1).

A renal biopsy on hospital Day 3 showed mesangial proliferative changes and fibrocellular crescents in 3 of 10 glomeruli (30%) (Figure 2) and light microscopy (LM) revealed concomitant GCW thickening. Routine IF revealed moderate, fine granular IgG and C3 staining along the GCW (Figure 3A) and weak IgM and IgA staining. Glomerular IgG subclass distribution determined by IF as described [4] revealed positive IgG1 and IgG4. Electron-dense...
deposits were located by EM in the subepithelial area of the glomerular basement membrane (GBM) and in the paramesangial area (MN, stage I–II; Figure 3B). Therefore, we diagnosed MPO-ANCA-associated GN complicated with membranous glomerulopathy. We evaluated the association between MPO-ANCA and the membranous glomerular lesion using IF to define the glomerular MPO deposition. Granular MPO staining along the GCW was visualized on glomeruli from the present patient and from others with idiopathic membranous nephropathy and membranous lupus nephritis as controls, using rabbit anti-human MPO antibodies (Calbiochem Corp., La Jolla, CA, USA) labelled with fluorescein isothiocyanate (FITC) and an FITC protein labelling kit (Molecular Probes, Inc., Eugene, OR, USA). The staining profile was similar to that of IgG (Figure 3C). However, MPO deposition was not evident on glomeruli from patients with either idiopathic membranous nephropathy (Figure 4A) or membranous lupus nephritis (Figure 4B) as controls.

Pulse therapy with methylprednisolone (500 mg for 3 days) followed by oral prednisolone (30 mg/day) decreased the proteinuria and levels of serum MPO-ANCA (Figure 1). Although steroid therapy prevented recurrent proteinuria, the MPO-ANCA titre increased again during steroid tapering 1 year later. Increased doses of prednisolone and cyclophosphamide slowly decreased the MPO-ANCA titre and increased the serum creatinine level (Figure 1). A second renal biopsy showed mild mesangial proliferation and fibrous crescents in 20% of glomeruli. Active histological findings were absent compared with the first renal biopsy and IF detected only trace IgG deposition (Figure 3D). Intra-membranous electron dense deposits were evident (MN, stage III) (Figure 3E). Deposition of individual IgG subclasses could not be evaluated because IF staining was very faint. The deposition of MPO along the GCW had almost disappeared (Figure 3F).

**Discussion**

An association between membranous glomerulopathy and ANCA-associated GN is rare. Although several reports have described MPO-ANCA-associated GN complicated with membranous glomerulopathy [5–9], the relationship between these glomerular diseases is largely unknown. As far as we understand, this is the first description of a patient with membranous glomerulopathy complicated by early MPO-ANCA-associated GN in which IF confirmed MPO deposition along the GCW in a similar manner to that of IgG. Consequently, IC comprising MPO and MPO-ANCA

**Fig. 1.** Clinical course.

**Fig. 2.** Fibrocellular crescents in initial biopsy (Periodic acid-Schiff's stain × 80).
MPO-ANCA crescentic glomerulonephritis complicated by membranous nephropathy

Fig. 3. Immunofluorescent and electron microscope findings. First (A–C) renal biopsy and second (D–F) 1 year later. (A) Immunofluorescence microscopy (IF) shows fine granular IgG deposition along glomerular capillary walls (GCW) (× 40). (B) Electron microscopy shows dense deposits in subepithelial space of glomerular basement membrane and paramesangial area (× 7000). (C) Staining with fluorescein isothiocyanate (FITC)-labelled rabbit anti-human myeloperoxidase (MPO) antibodies shows MPO deposition along GCW (× 40). (D) Faint IgG staining along GCW (× 40). (E) Electron dense intra-membranous deposition (× 7000). (F) Undetectable MPO deposition along GCW (× 40).

were probably involved in the pathogenesis of the membranous glomerular lesions.

MPO-ANCA is considered a primary pathogenic factor mainly through augmenting leukocyte-endothelial interactions in necrotizing and crescentic GN, but not in IC-mediated pathogenesis. However, Brower et al. found that severe necrotizing and crescentic GN could be induced by unilateral renal arterial perfusion with a neutrophil extract containing MPO and hydrogen peroxide ($H_2O_2$) in Brown Norway rats in vivo immunized with human MPO [10]. That study suggested that MPO-ANCA can induce IC-mediated GN. Human MPO and rat IgG appeared in the kidney shortly after perfusion, but disappeared when necrotizing glomerular lesions developed. On the other hand, Kobayashi et al. reported that administering rabbit anti-rat MPO antiserum to rats before injection with a nephrotoxic serum induced more severe nephrotoxic serum nephritis (NTN), determined as enhanced polymorphonuclear (PMN) infiltration and fibrin deposition. These findings showed that MPO-ANCA aggravates NTN [11]. In that model, rat MPO was deposited along the GCW only at the early stage (3–15 h after NTN induction) and disappeared at the late stage (14 days). Since MPO is highly cationic, it can bind to anionic surfaces such as GBM or endothelial cells, and
possibly behave as a planted antigen. Although MPO was deposited only at the early phase of experimental GN, it suggests that in some situations such as MPO-ANCA-associated GN, MPO released from PMN could be localized on the GCW, where it could interact with MPO-ANCA. This might explain why membranous glomerular lesions were induced during the course of MPO-ANCA-associated GN in our patient.

Among the glomerular IgG subclasses, only IgG1 and IgG4 were positive. Segelmark et al. found that the serum IgG subclasses of MPO-ANCA consisted mainly of IgG1 and IgG4 [12]. Thus, the distribution of the glomerular IgG subclass in our patient was compatible with a diagnosis of membranous glomerulopathy induced by IgG MPO-ANCA combined with MPO.

Haematuria and proteinuria did not worsen, although the serum MPO-ANCA titre increased once again during steroid tapering. In fact, a second renal biopsy revealed no active histological findings of MPO-ANCA-associated GN. Elevated MPO-ANCA titres usually indicate a clinical flare-up of ANCA-associated diseases. However, this association will mostly likely not occur in about 30% of MPO-ANCA-associated diseases with elevated serum MPO-ANCA titres over 1 or more years of follow-up [13]. Furthermore, positive MPO-ANCA is not always associated with the manifestation of overt diseases associated with MPO-ANCA [14].

One reason why disease progression was suppressed in our patient despite elevated serum MPO-ANCA values could be the effects of the immunosuppressive therapy including prednisolone. Kokolina et al. have reported that the affinity as well as the titre of MPO-ANCA decreases after immunosuppressive therapy in sera from patients with primary vasculitis [15]. Steroid therapy possibly contributes to diminishing the affinity of MPO-ANCA leading to polymorphonuclear leukocyte (PMN) activation, which might be related to MPO release from PMN. Of particular interest is the fact that the glomerular deposition of IgG was minimal in glomeruli when MPO was undetectable, while the stage determined by EM changed from I~II to III. These findings support the notion that the immune deposition in membranous lesions in our patient was formed by IC comprising MPO and MPO-ANCA.

Conflict of interest statement. None declared.

References


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