Localization of Myeloperoxidase (MPO) in the Glomeruli of Patients with Anti-MPO Antibodies-associated Crescentic Glomerulonephritis

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Summary

In order to clarify the pathogenesis of anti-myeloperoxidase antibodies (anti-MPO ab)-associated crescentic glomerulonephritis (CRGN), localization of MPO in renal glomeruli was examined. The three patients with CRGN and circulating anti-MPO ab studied included two with pauci-immune CRGN and one with Goodpasture's syndrome with anti-glomerular basement membrane (GBM) ab as well as anti-MPO ab. Three patients with CRGN without circulating anti-MPO ab were also studied. MPO was detected along the glomerular capillary wall and in the cytoplasm of recruited neutrophils and mononuclear cells in the three patients with anti-MPO ab-associated CRGN. MPO was not detected along the glomerular capillary wall in the three patients with CRGN without anti-MPO ab.

It was suggested that MPO, which is induced by neutrophil activation by anti-MPO ab, might have an important role in the pathogenesis of anti-MPO ab-associated CRGN.

Introduction

Anti-MPO antibodies (ab), one of the anti-neutrophil cytoplasmic autoantibody (ANCA) subsets, have recently been detected in patients with idiopathic crescentic glomerulonephritis (CRGN) and CRGN with systemic vasculitis[1-13].

It is reported that MPO causes glomerular injury by reacting with H2O2, which is one of the reactive oxygen species derived from activated neutrophils[4]. Although we have already reported the presence of serum MPO in patients with anti-MPO ab-associated CRGN, the role of MPO in patients with anti-MPO ab-associated CRGN has not been studied.

The purpose of the present study is to determine the participation of MPO in the glomerular injury by proving the localization of the enzyme in patients with anti-MPO ab-associated CRGN.

Key words: Crescentic glomerulonephritis, Anti-neutrophil cytoplasmic autoantibodies, Anti-myeloperoxidase antibodies, Myeloperoxidase.
CRGN.

Methods

1. Patients

Kidney tissues of 3 cases (cases 1-3) with anti-MPO ab-associated related CRGN and 3 cases (cases 4-6) with anti-MPO ab negative CRGN were stained for MPO.

Case 1: A 74 year-old female with rapidly progressive glomerulonephritic syndrome and dyspnea with hemoptysis after upper respiratory tract infection displayed perinuclear ANCA (P-ANCA) by indirect immunofluorescence assay (IIF), an anti-MPO ab titer of 400 EU/ml, but negative anti-GBM ab and immune complexes. Renal and lung biopsies showed CRGN and massive alveolar hemorrhage, respectively. Both displayed pauci-immune deposits of IgG by immunofluorescence microscopy. After steroid administration, the pulmonary hemorrhage improved remarkably, leaving rapidly progressive failure to end stage kidney supplemented by hemodialysis (HD). In spite of uneventful ambulatory maintenance HD for three years and maintaining her anti-MPO ab within the normal range, she was again admitted because of progressive dyspnea with hemoptysis following upper respiratory tract infection. On the second admission the titer of anti-MPO ab was 300 EU/ml and steroid administration was restated. Although methylprednisolone pulse therapy and plasma exchange were performed, respiratory failure progressed rapidly and she died of sepsis. Postmortem examination showed no evidence of systemic vasculitis. Renal biopsy and lung autopsy specimens were stained for MPO.

Case 2: A 61 year-old male presented with rapidly progressive renal and respiratory failure with pulmonary hemorrhage. He exhibited P-ANCA by IIF, an anti-MPO ab titer of 60 EU/ml, positive anti-GBM ab but no circulating immune complexes. In spite of prednisolone therapy and HD, he died of respiratory failure 3 months after admission. Autopsy revealed CRGN and alveolar hemorrhage. Immunofluorescence studies demonstrated linear deposition of IgG along the glomerular and alveolar capillary walls. Renal and lung autopsy specimens were stained for MPO.

Case 3: A 69 year-old male presented with rapidly progressive renal failure after upper respiratory tract infection. P-ANCA was positive by IIF, the anti-MPO ab titer was 60 EU/ml, but anti-GBM ab and immune complexes were not detected. Renal biopsy showed crescentic glomerulonephritis with pauci-immune deposits of IgG by immunofluorescence microscopy. After methylprednisolone pulse therapy, his renal function improved and low dose prednisolon administration has been continued.

Case 4: A 31 year-old female presented with systemic lupus erythematosus with rapidly progressive renal failure. Although anti-nuclear and anti-DNA ab, as well as a high titer of circulating immune complexes were detected, ANCA, including anti-MPO ab, were not detected by IIF and ELISA. Renal biopsy showed diffuse proliferative lupus nephritis with marked crescent formation around all glomeruli. Immunofluorescence studies showed granular deposition of C3, C4 and IgG along the capillary loops and mesangium. After methylprednisolone pulse therapy, her renal function improved and low dose prednisolon administration has been continued.

Case 5: A 62 year-old female presented with rapidly progressive renal failure after upper respiratory tract infection. ANCA, including anti-MPO and anti-GBM ab as well as immune complexes were not detected. Renal biopsy revealed crescentic glomerulonephritis and no
immune deposits by immunofluorescence. Although methylprednisolone pulse therapy was performed, her renal function deteriorated and maintenance HD has been required.

Case 6: A 23 year-old female presented with systemic lupus erythematosus with rapidly progressive renal failure. Although anti-nuclear and anti-DNA antibodies as well as a high titer of circulating immune complexes were detected, ANCA, including anti-MPO ab, were not detected by IIF and ELISA. Although methylprednisolone pulse therapy was performed, her renal function deteriorated until her death due to massive bilateral pulmonary hemorrhage. Renal autopsy showed diffuse proliferative lupus nephritis with marked cellular crescents of all glomeruli. Immunofluorescence studies showed granular deposition of C3, C4 and IgG along the capillary loops and mesangium.

2. Methods
1) Detection of ANCA by indirect immunofluorescence

The technique described by van der Woude et al\textsuperscript{15} was used. Normal neutrophils from healthy donors were isolated by dextran isopaque gradient, and fixed in 99% ethanol. After 30 min incubation with the patient's serum, the specimens were diluted with PBS at a ratio of 1:20, and the antibodies in neutrophils were examined by fluorescein-isothiocyanate-conjugated rabbit antihuman IgG (Dakopatts Co., Glostrup, Denmark).

2) Detection of anti-MPO ab

Anti-MPO ab were examined by enzyme-linked immunosorbent assay (ELISA) (normal range <10 ELISA unit/ml) using microplates (BioCarb Diagnostics, Lund, Sweden) coated with MPO separated from neutrophils.

3) Staining of MPO in the kidney

Deparaffinized kidney sections were immersed in 5% normal swine serum, and then washed and incubated with rabbit anti-human MPO antiserum (Dakopatts Co., Glostrup, Denmark) for 40 min at 37°C as the first antibody. After incubation with biotinylated swine anti-rabbit immunoglobulin (Dakopatts Co., Glostrup, Denmark) for 30 min at 37°C, sections were covered with alkaline phosphatase conjugated streptavidin for 30 min at 37°C. The sections were visualized in fast red substrate (Nichirei Co., Tokyo) and cross-stained with hematoxylin for light microscopy.

Results

MPO was observed along the glomerular capillary wall and in the cytoplasm of recruited neutrophils and mononuclear cells in the kidney specimens in all 3 patients with anti-MPO ab-associated CRGN (case 1, 2, 3) as shown in fig. 1. In the kidney specimens of the 3 patients with CRGN without anti-MPO ab (cases 4, 5, 6), no MPO was observed along the glomerular capillary wall. MPO was also found along the alveolar capillary wall of 2 patients with anti-MPO ab-associated CRGN with pulmonary hemorrhage (case 1, case 2) as shown in Fig. 2.

Discussion and Conclusion

ANCA were originally described in segmental necrotizing glomerulonephritis and have subsequently been detected in idiopathic CRGN and systemic vasculitis, such as Wegener’s granulomatosis and microscopic polyarteritis, usually associated with pauci-immune CRGN (CRGN with scant immune deposition)\textsuperscript{11-13}. In Japan, ANCA were first described by us\textsuperscript{5} in patients with Wegener’s granulomatosis in 1988.

ANCA are divided into two subsets, perinuclear pattern ANCA (P-ANCA) and diffuse cytoplasmic pattern ANCA (C-ANCA), by
indirect immunofluorescence on alcohol-fixed human neutrophils. It is known that most P-ANCA is antibody to MPO in the neutrophil cytoplasm. Falk and Jennette et al.\textsuperscript{10} reported that ANCA, especially anti-MPO ab, were related to idiopathic CRGN and CRGN associated with systemic vasculitis. We have experienced 25 cases with anti-MPO ab-associated disease, 23 of whom had CRGN, 16 had pauci-immune CRGN and 11 were associated with pulmonary involvement such as pulmonary hemorrhage. Therefore, it is suggested that ANCA, especially anti-MPO ab, may play an important pathogenic role in patients with pauci-immune CRGN and probably in the pulmonary hemorrhage. Although the pathogenic role of anti-MPO ab in CRGN remains unknown, it is reported that anti-MPO ab activate neutrophils and induce oxygen radical release and primary granule degranulation\textsuperscript{10}. It has been reported that MPO, which is the antigen for anti-MPO ab, exists in the primary granule as a neutrophil cationic enzyme which reacts with glomerular polyanion on the glomerular capillary wall. The MPO then causes glomerular injury by reacting with H$_2$O$_2$, which is one of the reactive oxygen species derived from activated neutrophils\textsuperscript{11,12}. We\textsuperscript{11,12} also detected MPO in the serum of patients with anti-MPO ab positive CRGN which was probably released from the neutrophils.

In this study, MPO was detected not only in the cytoplasm of recruited neutrophils and mononuclear cells, but also along the glomerular capillary wall in the kidney specimens of all 3 patients with anti-MPO ab-associated CRGN.

These data suggest that MPO might initiate the basement membrane damage leading to rupture of the GBM via neutrophil activation by anti-MPO ab, as well as pulmonary hemorrhage through a similar mechanism.

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**References**

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