Myeloperoxidase-Antineutrophil Cytoplasmic Antibody-Associated Glomerulonephritis with Membranous Nephropathy in Remission

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A 47-year-old woman developed pulmonary hemorrhage and an increase in proteinuria during remission of membranous nephropathy. Renal biopsy revealed crescentic glomerulonephritis. She also had a high perinuclear antineutrophil cytoplasmic antibody level, so a diagnosis of myeloperoxidase-antineutrophil cytoplasmic antibody-associated glomerulonephritis was made. After immunosuppressive therapy was started, the pulmonary hemorrhage resolved and her proteinuria decreased. Renal biopsy was repeated after treatment and showed histological improvement. This case suggests that there may be a relationship between membranous nephropathy and myeloperoxidase-antineutrophil cytoplasmic antibody-associated glomerulonephritis. (Internal Medicine 36: 841-846, 1997)

Key words: pulmonary hemorrhage, crescentic glomerulonephritis, nephrotic syndrome

Introduction

In recent years antineutrophil cytoplasmic antibody (ANCA) has been found to be involved in idiopathic crescentic glomerulonephritis and microscopic polyarteritis nodosa (1, 2). Here we report a patient who developed myeloperoxidase-ANCA (MPO-ANCA)-associated glomerulonephritis during remission of membranous nephropathy.

Case Report

A 47-year-old woman presented with hemoptysis on August 10, 1995. The chest X-ray film revealed bilateral infiltration of the middle and lower lung fields (Fig. 1). Anemia and an elevated C-reactive protein level were also detected, so the patient was admitted on August 17, 1995. Proteinuria was first detected in 1990 during a routine physical examination, and was again detected in 1992. The patient was examined at Onomichi General Hospital on May 24, 1992, and was found to have proteinuria (urinary protein: 4.9 g/day) and hypoproteinemia (total protein: 5.9 g/dl; albumin: 2.8 g/dl). A diagnosis of nephrotic syndrome was made and she was admitted on June 20, 1992. Renal biopsy was performed, but there were no

glomeruli in the specimen. Her proteinuria decreased and the levels of total protein, albumin, and total cholesterol became normal, when she was given cocktail therapy (prednisolone 40 mg/day, azathioprine 100 mg/day, dipyridamole 300 mg/day, and dilazep hydrochloride 300 mg/day). Thus she was discharged on October 7, 1992. Prednisolone and azathioprine were tapered, and then all drugs were stopped in March 1993. Because proteinuria (+) still persisted, renal biopsy was repeated on April 5, 1994. There were 13 glomeruli in the biopsy specimen; one was hyalinized and most of the others showed mild mesangial proliferation. There was glomerular basement membrane thickening, and periodic acid methenamine silver staining revealed spikes and vacuolization (Fig. 2). The interstitium showed localized fibrosis and cellular infiltration, and there was associated tubular atrophy. There was also hyaline thickening of the arterioles. Deposition of immunoglobulin (IgG, IgA, and IgM), complement (C3 and C4), and fibrinogen was negative by the fluorescent antibody technique. Electron microscopy revealed low density or granular deposits on the subepithelial aspect of the glomerular basement membrane associated with regeneration of the basement membrane (Fig. 3). A diagnosis of stage III-IV membranous nephropathy was made. The serum protein concentration, total cholesterol level,

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Received for publication January 20, 1997; Accepted for publication July 7, 1997

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Figure 1. The chest X-ray film on August 10, 1995. Bilateral infiltrates are present in the middle and lower lung fields.

and renal function were normal at the time. MPO-ANCA was measured later by enzyme-linked immunosorbent assay (ELISA) of stored serum and was found to be 18 EU (normal: <10 EU).

Findings on admission: Her height was 157.0 cm and her weight was 49.5 kg. The blood pressure was 138/92 mmHg and the pulse rate was 86/min (regular). Weak rales were heard in the bilateral lower lung fields.

Laboratory findings on admission (Table 1): Urinalysis revealed urinary albumin (4+), occult blood (3+), 20–25 red

Figure 2. Light micrograph of renal biopsy tissue obtained on April 5, 1994 (PAM stain, $\times 560$). Spikes and vacuolization of the glomerular basement membrane can be seen.

Figure 3. Electron micrograph of the same tissue ($\times 11,000$). Low density or granular deposits are present on the subepithelial aspect of the glomerular basement membrane associated with regeneration.

Table 1. Laboratory Data on Admission

Urinalysis		Total cholesterol		165 mg/dl
pH	6.0	Fasting plasma glucose		89 mg/dl
Albumin	(4+)	Blood urea nitrogen		18.4 mg/dl
Sugar	(-)	Creatinine		1.1 mg/dl
Occult	(3+)	Uric acid		4.3 mg/dl
Sediment		Na		142 mEq/l
Red blood cell	20-25/HPF	K		3.9 mEq/l
White blood cell	2-3/HPF	Cl		107 mEq/l
Cast	(+)	Ca		4.5 mEq/l
Total daily urinary protein	5.76 g/day	P		3.7 mg/dl
Peripheral blood exam.		Serum exam.		
White blood cell	4,500/ μ l	C reactive protein		2.45 mg/dl
Red blood cell	253 $\times 10^4$ / μ l	Rheumatoid factor		<20.5 IU/ml
Hemoglobin	7.4 g/dl	Wassermann test		(-)
Hematocrit	22.1%	TPHA		(-)
Platelet	50.2 $\times 10^4$ / μ l	HBs antigen		(-)
Erythrocyte sedimentation rate	134 mm/h	HCV antibody		(-)
Coagulation exam.		Immunological study		
Prothrombin activity	100%	Immunoglobulin G		1,980 mg/dl
aPTT	28.5 sec	Immunoglobulin A		475 mg/dl
Fibrinogen	897 mg/dl	Immunoglobulin M		90 mg/dl
s-FDP	<10 μ g/ml	Complement 3		97.8 mg/dl
u-FDP	0.8 μ g/ml	Complement 4		45.1 mg/dl
Blood chemical exam.		Serum complement		44.0 U/ml
Total protein	6.3 g/dl	Anti DNA antibody		4 U/ml
Albumin	2.9 g/dl (46.1%)	Anti nuclear antibody		< $\times 20$
α_1 -globulin	5.3%	P-ANCA (MPO)		297 EU
α_2 -globulin	13.6%	C-ANCA		<10 EU
β -globulin	11.5%	Anti-GBM antibody		(-)
γ -globulin	23.5%	Renal function		
Total bilirubin	0.50 mg/dl	Creatinine clearance		53.8 ml/min
Aspartate aminotransferase	15 IU/l	Serum- β_2 microglobulin		4.7 μ g/ml
Alanine aminotransferase	7 IU/l	Urinary- β_2 microglobulin		830.3 μ g/h
Lactate dehydrogenase	446 IU/l	NAG		1,160.3 mU/h
Zinc turbidity test	8.7 KU	HLA typing	A24 A31 B52 B51 DR2	

s-FDP: serum-fibrin/fibrinogen degradation products, u-FDP: urinary-fibrin/fibrinogen degradation products, aPTT: activated partial thromboplastin time. TPHA: treponema pallidum hemagglutination test, P-ANCA: perinuclear anti neutrophil cytoplasmic antibody, C-ANCA: cytoplasmic anti neutrophil cytoplasmic antibody, GBM: glomerular basement membrane, NAG: N-acetyl- β -D-glucosaminidase.

blood cells per high power field, and a total urinary protein loss of 5.76 g/day, indicating massive proteinuria. She also had anemia and thrombocytosis, an increased erythrocyte sedimentation rate, and an increased fibrinogen level. The total protein level was 6.3 g/dl and the albumin level was 2.9 g/dl, indicating hypoproteinemia, and there was a slight increase in creatinine. There was slight elevation of C-reactive protein and a high IgA level. Antinuclear antibody, anti-DNA antibody, cytoplasmic ANCA, and anti-glomerular basement membrane antibody were normal, but the MPO-ANCA titer was high (297 EU). The creatinine clearance was 53.8 ml/min, indicating decreased renal function.

Hospital course (Fig. 4): Computed tomography (CT) of the chest (August 22, 1995) showed infiltration of both lungs, principally in the middle and lower lung fields (Fig. 5). Bron-

choscopy was performed on August 23. Although no source of bleeding was detected, the bronchoalveolar lavage fluid was bloody and a diagnosis of alveolar hemorrhage was made. Renal biopsy was performed on September 4. Thirty-nine glomeruli were seen in the biopsy specimen; 10 were completely hyalinized and segmental hyalinization was observed in 15 of the other 29. Adhesion of Bowman's capsule and cellular crescents were frequently noted. Cellular infiltration was observed in the interstitium, and there was tubular atrophy and necrosis (Fig. 6). Immunoglobulin, complement, and fibrinogen were all negative by the fluorescent antibody technique. There were no glomeruli detected in the specimen prepared for electron microscopy. A diagnosis of MPO-ANCA-associated glomerulonephritis and pulmonary-renal syndrome was made. Steroid pulse therapy with methylprednisolone (1,000 mg/day)

Figure 4. Clinical course.

Figure 5. Computed tomography of the chest on August 22, 1995. Infiltrates of both lungs are present.

was given for three days starting on August 31, followed by prednisolone (40 mg/day) and cyclophosphamide (50 mg/day). Her pulmonary hemorrhage had resolved when chest X-ray was repeated on September 7, and a chest CT on September 19

Figure 6. Light micrograph of renal biopsy tissue obtained on September 4, 1995 (PAM stain, ×450). Cellular crescents are present and spikes are also seen.

confirmed this.

However, massive proteinuria persisted, so steroid pulse therapy was performed a total of three times and the prednisolone dose was increased to 60 mg/day. The patient was also treated with dipyridamole (300 mg/day) and warfarin potassium (1 mg/day). Serial MPO-ANCA determinations showed a

value of 101 EU on September 14, which fell to 19 EU on October 19 and to less than 10 EU thereafter. Her proteinuria also gradually diminished and repeat renal biopsy was performed on February 20, 1996. There were 22 glomeruli in the biopsy specimen, 10 of which were hyalinized. The other 12 glomeruli showed mild mesangial proliferation, but no crescent formation. Cellular infiltration was observed in the interstitium as well as tubular atrophy and necrosis, but these changes were milder than in the previous biopsy specimen. Immunoglobulin, complement, and fibrinogen were not detected. Since histological improvement was observed, the patient was discharged on March 11, 1996.

Discussion

ANCA was discovered in the serum of a patient with necrotizing glomerulonephritis by Davies in 1982 (3). It was later identified in patients with idiopathic necrotizing crescentic glomerulonephritis, Wegener's granulomatosis, and microscopic polyarteritis nodosa. ANCA is an IgG autoantibody to antigens in the cytoplasm of neutrophils, and is classified into cytoplasmic ANCA (c-ANCA) and perinuclear ANCA (p-ANCA) based on the immunostaining pattern. The target antigens of ANCA have been identified, and it has been found that most c-ANCA is directed against a protease (proteinase 3) and has a high specificity for Wegener's granulomatosis. In the case of p-ANCA, the antibody is directed against MPO-ANCA in many patients with microscopic polyarteritis nodosa and allergic granulomatous vasculitis (4–8).

Arimura et al reported a dialysis rate of 47% and a mortality rate of 31% for MPO-ANCA-associated glomerulonephritis, indicating that it has a poor prognosis (9). They cited an age over 50 years, renal failure severe enough to require dialysis, and pulmonary hemorrhage as negative factors. While pulmonary hemorrhage occurred in our patient, she was only 47 years old and her renal function was relatively well maintained, resulting in a good response to immunosuppressive therapy.

About 30% of membranous nephropathy is secondary to another disease (10, 11). When membranous nephropathy was diagnosed in our patient on the basis of renal biopsy findings in April 1993, no immunoglobulin deposits were detected by the fluorescent antibody technique and electron microscopy showed low density or granular deposits, so secondary nephropathy was suspected. Nevertheless, subsequent investigations failed to identify any systemic disease. Immunoglobulins and complement may not have been detected because the biopsy was performed after treatment.

The present patient developed MPO-ANCA-associated glomerulonephritis during remission of membranous nephropathy, but there is no known relationship between these diseases. This is the first report of MPO-ANCA-associated glomerulonephritis and membranous nephropathy in the same patient as far as we could determine from a review of the literature. The association of these two diseases might have been a coincidence, but we assumed that they were related for the following reasons. Membranous nephropathy is an immune

complex (IC) type of nephropathy in which deposits accumulate on the subepithelial aspect of the glomerular basement membrane. It has been suggested that circulating ICs from the blood precipitate in the glomerular basement membrane or alternatively that the ICs are formed locally in the membrane (in situ formation) (12–14). In contrast, MPO-ANCA-associated glomerulonephritis is a pauci-immune type of necrotizing crescentic glomerulonephritis, in which ANCA, cytokines, neutrophil hyperactivation, adhesion molecules, and the ANCA target antigen are thought to be involved (1, 2). ANCA is suggested to bind to its target antigen, which is expressed on the surface of glomerular endothelial cells in response to activation by cytokines, as well as to antigen released by neutrophils and bound to endothelial cells. Thus, it could possibly form in situ ICs and cause endothelial cell injury (15). According to this theory, ICs are formed in situ during the initial stage of the disease and are later destroyed by lysosomes released from ANCA-activated neutrophils. In the present patient, MPO-ANCA was increased slightly (18 EU) when membranous nephropathy was diagnosed in 1994, one year after cocktail therapy was discontinued, suggesting the possibility that ANCA participated in IC formation. It is also interesting that the MPO-ANCA level was increased more than one year before MPO-ANCA-associated glomerulonephritis was diagnosed clinically. Accordingly, this case is suggestive with regard to the pathogenesis of MPO-ANCA-associated glomerulonephritis and its relationship with membranous nephropathy.

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