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CASE REPORT

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Clinicopathological features of antineutrophil cytoplasmic antibodies-associated vasculitis in Japanese patients with IgA nephropathy

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Abstract
Antineutrophil cytoplasmic antibodies (ANCA) have been reported to be associated with systemic vasculitis. However, the roles of ANCA subtypes in patients with IgA nephropathy remain to be fully investigated. We describe three Japanese patients with IgA nephropathy complicated by ANCA-associated vasculitis. Two patients with IgG class ANCA developed rapidly progressive renal failure and demonstrated mesangial proliferation with extensive extracapillary proliferation and segmental glomerular necrosis. One patient with IgM class ANCA showed severe extrarenal symptoms, such as lung fibrosis and neuritis, in addition to glomerular crescent formation. All three patients received immunosuppressive therapies, including corticosteroids and cyclophosphamide. The two patients who received these treatments early showed improvement in urinary protein excretion and renal function, in accordance with a decrease in the serum titer of ANCA. However, one patient in whom serum creatinine was already elevated showed a poor response to the treatment. These results suggest that ANCA subtypes may participate in the pathogenesis of crescent formation in patients with IgA nephropathy, and that early treatment with a combination of methylprednisolone pulse therapy, oral prednisolone, and cyclophosphamide pulse therapy may be beneficial in these patients.

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Introduction

IgA nephropathy is the most common primary glomerulonephritis in the world and usually shows an indolent or slowly progressive course.[1] However, a small number of patients with IgA nephropathy present with a rapidly progressive glomerulonephritis, in which crescent formation is the pathologic hallmark, leading to an unfavorable prognosis.[2–4] The pathogenesis of crescent formation has been mainly thought to be mediated immunologically by immune complexes, including IgA.[3] However, the precise mechanism of crescentic glomerulonephritis in patients with IgA nephropathy has yet to be elucidated.

It has been suggested that crescentic glomerulonephritis may be classified into three subsets: (1) anti-glomerular basement membrane antibody-mediated type (20%); (2) immune complex-mediated type (40%); and (3) pauci-immune type (40%).[5] Recently, antineutrophil cytoplasmic antibodies (ANCA) have been presumed to play an important role in the pathogenesis of crescentic glomerulonephritis in patients with pauci-immune type crescentic glomerulonephritis.[6–9] In addition, ANCA-associated vasculitis may play a crucial role in crescent formation in other immune complex-mediated diseases, such as Castleman's disease, membranous nephropathy, and systemic lupus erythematosus.[10–12] However, the role of ANCA-associated vasculitis in the crescent formation in IgA nephropathy has not been fully elucidated.[13–16]

Here we present reports on three Japanese patients with IgA nephropathy who showed crescentic glomerulonephritis and high ANCA serum titers. Two patients with IgG class of myeloperoxidase (MPO)-ANCA showed mainly renal involvement. However, one patient, with IgM class MPO-ANCA showed severe extrarenal symptoms of systemic vasculitis in addition to crescent formation in glom-

Table 1. Clinicopathological features of our patients

	Patient 1	Patient 2	Patient 3
Clinical features			
Proteinuria (total protein, g/day)	4.3	1.0	ND
Serum Cr (mg/dl)	2.7	0.7	7.5
Extrarenal symptoms	Interstitial pneumonitis	Lung fibrosis, neuritis	None
ANCA specificity (class)	MPO (IgG)	MPO (IgM)	MPO (IgG)
Therapy	PSL, Pulse, CP	PSL, Pulse, CP	PSL, Pulse, HD
Renal outcome (Cr, mg/dl)	1.1	0.8	HD
Pathological features			
Glomerulus			
Crescent	C/FC (72%)	C (13%)	FC/F (20%)
Endocapillary proliferation	+	+	+
Interstitium			
Cell infiltration	Zonal	Patchy	Zonal
Fibrosis	Zonal	Patchy	Zonal
Vasculitis	+	−	+

ANCA, Antineutrophil cytoplasmic antibodies; Cr, creatinine; PSL, prednisolone; pulse, methylprednisolone pulse therapy; CP, cyclophosphamide; HD, hemodialysis; ND, not determined; C, cellular; FC, fibrocellular; F, fibrous; MPO, myeloperoxidase

eruli. All three patients showed decreased disease activity and amelioration of renal dysfunction, concomitantly with a decrease in ANCA titers, through early treatment with immunosuppressive therapies. These results suggest that ANCA-associated vasculitis may be involved in the pathogenesis of crescentic glomerulonephritis in patients with IgA nephropathy.

Case reports

Patient 1

In December 1996, a 67-year-old Japanese woman was admitted because of fever, respiratory symptoms, and renal failure. She had a 3-year history of hypertension, microscopic hematuria, proteinuria, and interstitial pneumonitis. There was no history of purpuric rash, arthralgia, or abdominal pain. On admission, her body temperature was 38.0°C and arterial blood pressure was 142/78 mmHg. There were bilateral Verclo rales and pretibial edema. Chest radiography and computed tomography (CT) scanning showed bilateral interstitial pneumonitis. Urinalysis showed proteinuria (total protein, 1.2 g/day) and microscopic hematuria. Hematological laboratory tests yielded: hematocrit (Ht), 28.7%; red blood cell count (RBC), $3.11 \times 10^6/mm^3$; hemoglobin (Hb), 9.1 g/dl; leucocytosis, 9300 WBC/mm^3, and thrombocytosis $33.4 \times 10^4/mm^3$. Serum creatinine level was 2.1 mg/dl and creatinine clearance (CCr) was 12.5 ml/min. C-reactive protein (CRP) level was 3.8 mg/dl and the erythrocyte sedimentation rate (ESR) was 120 mm/h. Serum total protein was 7.1 g/dl, with an elevated gamma-globulin fraction (22.4%). Hypergamma-globulinemia was found (IgG, 1851 mg/dl; IgA, 492 mg/dl; IgM, 399 mg/dl). Complement components were normal. Anti-glomerular basement membrane (anti-GBM) antibodies were negative. ANCA were detected by enzyme-linked immunosorbent assay (ELISA), using microtiter plates coated with myeloperoxidase extracts or proteinase-3 (BML, Tokyo, Japan). In addition, immunoglobulin isotypes of ANCA were evaluated by indirect fluorescent antibody assay (IFA), by fluorescence microscopy, using ethanol-fixed human neutrophils (Specialty Laboratories, CA, USA). IgG class MPO-ANCA were elevated, at 598 EU. Renal biopsy revealed moderate mesangial proliferative glomerulonephritis associated with cellular, fibrocellular, and fibrous crescents (72% of all glomeruli). Zonal small round-cell infiltration, tubular atrophy, and vasculitis were seen in the interstitium. Immunofluorescence and electron microscopy revealed lumpy deposition of IgA and C3 in mesangial and paramesangial areas, but immune complexes were not detected in the interstitium (Fig. 1 and Table 1). Accordingly, we diagnosed this as ANCA-associated rapidly progressive crescentic glomerulonephritis superimposed on IgA nephropathy. We administered intravenous methylprednisolone pulse therapy (500 mg/day, for 3 days) twice, and oral prednisolone daily (20 mg/day) simultaneously, followed by cyclophosphamide pulse therapy (500 mg/day, for 1 day) twice every 4 weeks. Subsequently, her serum creatinine level improved from 2.7 to 1.1 mg/dl and CCr increased from 12.5 to 48.2 ml/min. In addition, urinary protein excretion decreased, from 4.3 to 1.3 g/day, and hematuria, in terms of RBC numbers in urine, was also alleviated. In addition, her serum titer of MPO-ANCA decreased from 598 to 55 EU concomitantly with the treatment (Fig. 2).

Patient 2

A 69-year-old Japanese man was admitted because of fever, pretibial edema, and weight loss of 3 months, duration, in October 1996. Physical examination revealed a low-grade fever, bilateral Verclo rales, and bilateral hypoesthesia of the lower extremities, manifesting as mononeuritis multiplex. Chest radiography showed severe fibrosis of bilateral lungs. Urinalysis revealed proteinuria 0.7 g total protein day and microscopic hematuria. Hematological results yielded:

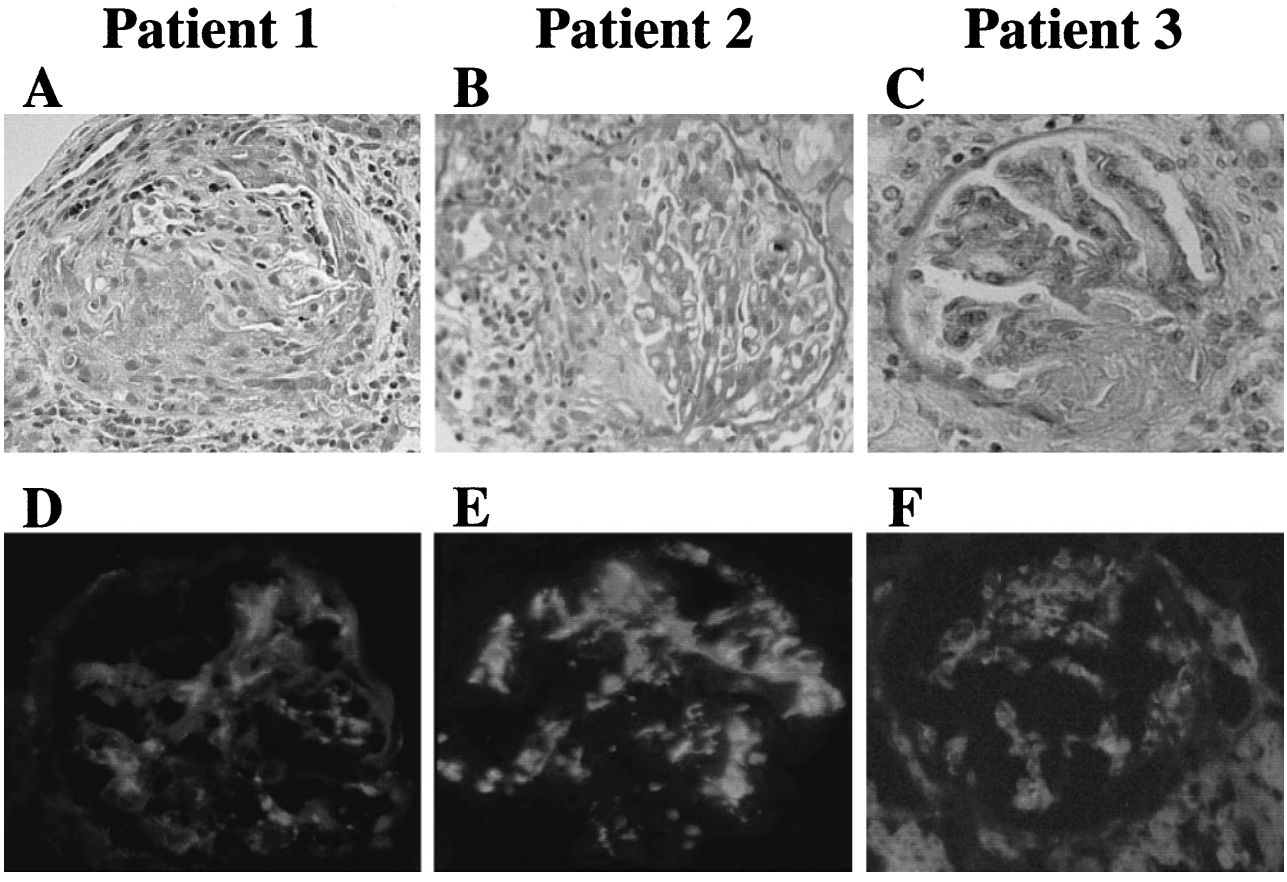

Fig. 1A–F. Light micrographs (**A**, **B**, **C**) and immunofluorescent micrographs of IgA (**D**, **E**, **F**) from patient 1 (**A**, **D**), patient 2 (**B**, **D**), and patient 3 (**C**, **F**). Light microscopic findings showed focal necrotizing glomerulonephritis with small crescents or focal adhesions. **A–C** Periodic acid-Schiff, ×400. **D–F** Immunofluorescence findings revealed predominant mesangial deposits of IgA. ×400

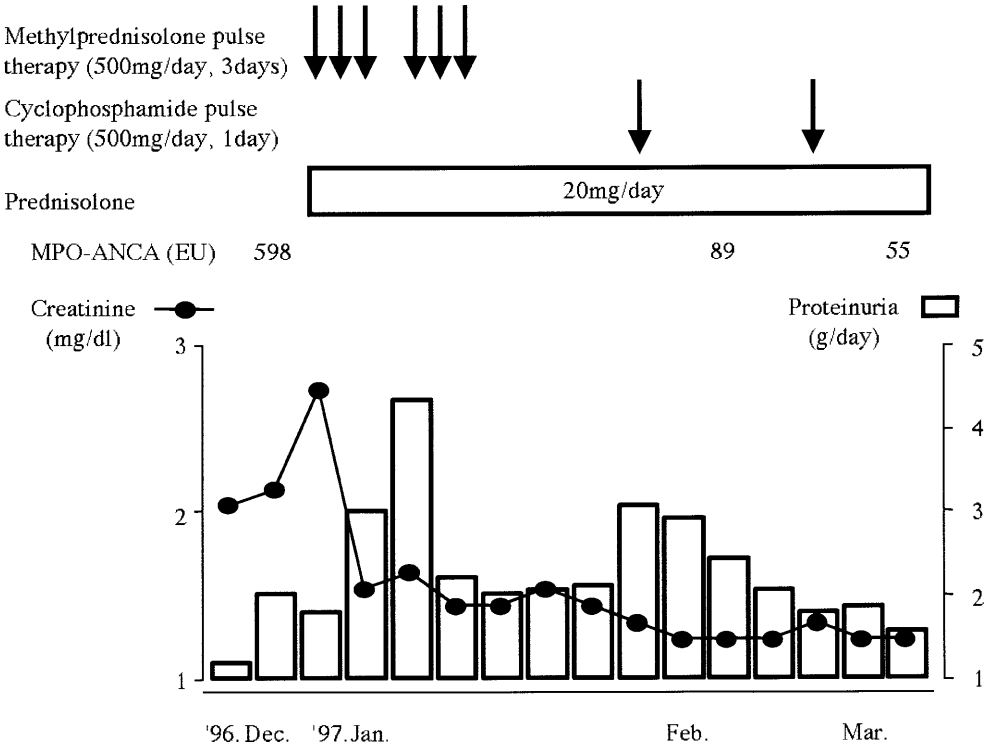

Fig. 2. Clinical course of patient 1. *MPO-ANCA*, Myeloperoxidase-antineutrophil cytoplasmic antibodies

Ht, 29.4%; RBC, $3.14 \times 10^6/mm^3$; Hb, 9.5 g/dl; and thrombocytosis, 38.0×10^4 thrombocytes/mm^3. Serum creatinine level was 0.7 mg/dl and CCr was 63.4 ml/min. CRP was 2.1 mg/dl and ESR was 67/mm. Serum total protein was 6.7 g/dl, with an elevated gamma-globulin fraction (24.8%). Hypergamma-globulinemia was present (IgG, 2344 mg/dl; IgA, 498 mg/dl; IgM, 159 mg/dl). Complement components were normal. Anti-glomerular basement membrane (GBM) antibodies were negative. IgM class MPO-ANCA were elevated, at 93 EU, by ELISA and IFA, as described for patient 1. Renal biopsy revealed moderate mesangial proliferative glomerulonephritis with cellular crescents (13% of all glomeruli), as well as endocapillary proliferation. Patchy cellular infiltration and tubular atrophy were seen in the interstitium, but there was no vasculitis. Immunofluorescence microscopy showed IgA, C3, and IgG deposition within mesangial and paramesangial areas (Fig. 1 and Table 1). We administered methylprednisolone pulse therapy (500 mg/day, for 3 days) twice, followed by oral prednisolone daily (20 mg/day) and cyclophosphamide pulse therapy (500 mg/day, for 1 day). Three months after admission, daily proteinuria had decreased, from 1.0 to 0.1 g, hemoglobin had risen from 9.4 to 12.5 g/dl, and IgG had decreased from 2344 to 1659 mg/dl. The titer of MPO-ANCA had decreased from 93 to 13 EU. In addition, his neurological symptoms gradually resolved. Laboratory results and symptoms have remained stable and his serum titer of ANCA has been under detectable levels for 3 years.

Patient 3

A 83-year-old Japanese woman was admitted because of oliguria in June 1997. She had a 15-year history of hypertension. Her renal function was normal in March 1997 (serum creatinine, 0.8 mg/dl; blood urea nitrogen, 14.3 mg/dl). Physical examination revealed cyanosis and bilateral rales. Chest radiography showed marked cardiomegaly, lung congestion, and pleural effusions. Urinalysis revealed proteinuria and microscopic hematuria. Hematological tests yielded: Ht, 21.3%; RBC, $2.65 \times 10^6/mm^3$; Hb, 7.1 g/dl; and thrombocytosis, 55.2 thrombocytes $\times 10^4/mm^3$. Serum creatinine level was 7.5 mg/dl and blood urea nitrogen was 84.5 mg/dl. CRP was 11.1 mg/dl. Serum total protein was 6.7 g/dl, with an elevated gamma-globulin fraction (31.9%). Complement components were normal, and anti-GBM antibodies and C-ANCA were negative. IgG class MPO-ANCA were elevated, at 77 EU by ELISA and IFA, as described for patient 1. The patient underwent emergency hemodialysis, and methylprednisolone pulse therapy (500 mg/day, for 3 days) was administered twice, followed by oral prednisolone (40 mg/day). Despite the therapy, the patient died of multiple organ failure. Renal histology at autopsy showed focal necrotizing glomerulonephritis with fibrocellular and fibrous crescents (20% of all glomeruli) and interstitial vasculitis. Immunofluorescence microscopy showed glomerular deposition of IgA, C3, and IgM in mesangial and paramesangial areas (Fig. 1 and Table 1).

Discussion

We describe here three Japanese patients with IgA nephropathy complicated by ANCA subtype-associated vasculitis. It is important to recognize that ANCA may be involved in crescent formation in patients with IgA nephropathy. In particular, IgG class ANCA may have played an important role in the pathogenesis of the crescents in our patients. One patient displayed IgM class ANCA, to the best of our knowledge this is the first report of a patient with IgA nephropathy associated with IgM class ANCA. Compared with the patients with IgG class ANCA, the extrarenal symptoms of systemic vasculitis were predominant in this patient. Thus, the immunoglobulin class of ANCA may affect the distribution of systemic symptoms in these patients, as well as the impact of ANCA on crescent formation in IgA nephropathy patients.

ANCA of the IgG isotype, especially P-ANCA directed against MPO, have often been detected and associated with systemic vasculitides, such as microscopic polyangiitis and Wegener's granulomatosis.[17,18] By comparison, IgG class ANCA have rarely been reported in IgA nephropathy (Table 2).[13–16] Taking the findings in our present patients and these reports together, we would emphasize the possibility that patients with IgA nephropathy may also present with MPO-ANCA (especially IgG class)-associated vasculitis when showing rapidly progressive crescentic glomerulonephritis. In the future, it will be necessary to prospectively investigate the involvement of ANCA in the pathogenesis of IgA nephropathy, and their clinical features.

The immunoglobulin isotypes of ANCA have not been extensively reported thus far, and the pathogenetic role of IgM class ANCA therefore remains unclear.[19–21] Although we were unable to detect IgG class ANCA in one of our patients (patient 2), probably because of the early diagnosis and treatment of ANCA-associated vasculitis, our observations suggest that the detection of IgM class ANCA in IgA nephropathy may have a close relationship with the extrarenal symptoms of systemic vasculitis, in addition to the renal involvement. Therefore, it is important for future studies to determine the role of IgM class ANCA in the pathogenesis of crescentic glomerulonephritis in IgA nephropathy, as well as its role in systemic symptoms.

Regarding the precise molecular mechanism of the induction of MPO-ANCA-associated vasculitis, an ANCA-cytokine sequence theory has been advanced, and tumor necrosis factor (TNF)-α, interleukin-8 (IL-8), and intercellular adhesion molecule (ICAM)-1 may play important roles in this sequence.[7–9] We previously reported that TNF-α, IL-8, and ICAM-1 were elevated in patients with acute onset or exacerbation of IgA nephropathy.[22–24] Although we did not evaluate these cytokines in the present patients, it is attractive to speculate that up-regulation of these cytokines and adhesion molecules may induce polyclonal B-cell activation and may amplify ANCA production, which leads to crescent formation with necrotizing vasculitis. The precise molecular mechanism involved in the induction of

Table 2. Reported patients with IgA nephropathy with ANCA-associated vasculitis

Patient no.	Serum Cr (mg/dl)	Extrarenal symptoms	Percentage of crescents	ANCA specificity (class)	Therapy	Renal outcome (Cr, mg/dl)	Reference no.
1	ND	ND	0	MPO (IgG)	ND	ND	13
2	ND	ND	0	MPO (IgG)	ND	ND	13
3	2.3	–	50	MPO (IgG, IgA)	Pulse	HD	14
4	10.8	–	50	MPO (IgG, IgA)	PSL, Pulse, CP, HD	PD	15
5	3	Lung	24	MPO (IgG)	PSL, Pulse, CP, PE, HD	1.2	16
6	6	–	40	MPO (IgG)	PSL, Pulse, CP, HD	2.7	16
7	2.7	–	17	MPO (IgG)	PSL, CP	2.2	16

Cr, Creatinine; PSL, prednisolone; pulse, methylprednisolone pulse therapy; CP, cyclophosphamide; PE, plasma exchange; HD, hemodialysis; PD, peritoneal dialysis; ND, not described

ANCA may be the key to a better understanding of crescent formation in IgA nephropathy.

Immunosuppressive drugs have been reported to have a beneficial effect on the clinical course of crescentic glomerulonephritis, including IgA nephropathy and ANCA-associated vasculitis.[25,26] Allmaras et al.[16] reported that early immunosuppression was effective in patients with IgA nephropathy with IgG class MPO-ANCA and crescentic glomerulonephritis. In fact, two of our patients (patients 1 and 2) who received early treatment with a combination of corticosteroids and cyclophosphamide showed an improvement in renal function in accordance with the decrease in ANCA titers. This may be related to the early phase of the disease, including IgM class ANCA, and active histological changes including cellular crescents. In contrast, renal function failed to improve in one patient (patient 3), who required hemodialysis at initial presentation. These results suggest that early diagnosis and treatment with immunosuppressive agents may be required for a favorable outcome in these patients.

In conclusion, ANCA subtype-associated vasculitis may be involved in the pathogenesis of crescentic glomerulonephritis in patients with IgA nephropathy. Early diagnosis and treatment with immunosuppressive agents may contribute to an improvement in renal function, as well as to improvements in general condition in these patients.

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