Myeloperoxidase-antineutrophil cytoplasmic antibody (ANCA)-associated systemic vasculitis developed from ANCA negative renal limited vasculitis

A case report

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Abstract

Rationale: The relationship between antineutrophil cytoplasmic autoantibody (ANCA)-associated vasculitis (AAV) and ANCA-negative vasculitis has not been elucidated.

Patient concerns: A 64-year-old female with edema and proteinuria was admitted. A kidney biopsy indicated focal proliferative nephritis with crescents in 25% of glomeruli. Serum ANCA was negative. Eighteen months later, systemic symptoms emerged and acute kidney injury occurred. Serum ANCA against myeloperoxidase (MPO) turned positive. Repeated kidney biopsy showed more severe lesion than last time. Immunoglobulin (Ig)G was purified from serum obtained before the first kidney biopsy. Weak ANCA which could not be detected in serum was found in IgG.

Diagnoses: MPO-ANCA-associated AAV developed from ANCA-negative renal-limited AAV.

Interventions: The patient was treated with glucocorticoid.

Outcomes: The serum creatinine decreased to 2.17 mg/dL a week later. MPO-ANCA turned negative when re-examined 3 weeks later. No relapse has been observed during follow-up for 6 months.

Lessons: This is the first reported case about the spontaneous transformation from ANCA-negative renal-limited AAV to ANCA-positive systemic vasculitis. There might be a slow process of epitope spreading in the pathogenesis of disease. Physicians should try their best to detect the ANCA in the diagnose and treatment of ANCA-negative AAV.

Abbreviations: AAV = antineutrophil cytoplasmatic antibodies-associated vasculitis, ANA = antinuclear antibody, ANCA = antineutrophil cytoplasmatic antibodies, C3 = complement 3, C4 = complement 4, CRP = C-reactive protein, IF = immunofluorescence, MPO = myeloperoxidase, PBS = phosphate-buffered saline, PR3 = proteinase 3.

Keywords: antineutrophil cytoplasmic antibody negative, case report, epitope spreading, vasculitis

1. Introduction

The relationship between antineutrophil cytoplasmic autoantibody (ANCA)-associated vasculitis (AAV) and ANCA-negative vasculitis has not been elucidated.\textsuperscript{11–41} We reported a 64-year-old woman with proteinuria and normal kidney function. ANCA negative AAV could not be excluded because kidney biopsy indicated focal proliferative nephritis with crescents formation of 25% glomeruli. Treatment with angiotensin receptor blocker alone decreased proteinuria and kept kidney function stable. However, serum myeloperoxidase (MPO)-ANCA turned positive 18 months later. Acute kidney injury also occurred accompanied by extrarenal symptoms. We retrospectively purified immunoglobulin (Ig)G from the serum which was drawn and conserved before the first kidney biopsy and demonstrated the existence of weak ANCA. We believe this is the first reported case about the spontaneous transformation from ANCA-negative renal-limited AAV to ANCA-positive systemic vasculitis. The patient signed an informed consent form at admission to hospitalization. Ethical approval was not necessary because of the routine health care.

2. Case report

A 64-year-old woman arrived at our hospital with a 1-week history of edema. Pertinent physical examination findings were normal except a slight pitting edema of both lower extremities. Chest x-ray and abdominal ultrasonography showed no obvious abnormality. Blood routine and biochemical examinations were
Laboratory test showed serum CRP increased to 5.4 mg/dL. Losartan was stopped and the patient was admitted again. MPO was 64.3 RU/mL (normal range 0.8-20 RU/mL). ANCA was positive. ELISA showed the level of ANCA against myeloperoxidase (MPO) was 2.17 mg/dL (normal range 0.7-1.5 mg/dL). Indirect IF showed perinuclear ANCA was positive.

The patient was discharged with a maintain oral methylprednisolone treatment. MPO-ANCA turned negative when re-examined 6 and 12 months after discharge. ANCA-negative AAV could not be excluded. The patient was treated with intravenous methylprednisolone at a dose of 40 mg/day. The serum creatinine decreased to 2.17 mg/dL a week later. The patient was discharged with a maintain oral methylprednisolone treatment. MPO-ANCA turned negative when re-examined 3 weeks later. No relapse of AAV has been observed during follow-up for 6 months.

To investigate the relationship between twice onsets of the disease, we retrospectively purified IgG with affinity chromatography from the serum which was drawn and conserved before the kidney biopsies. Briefly, the serum was centrifuged for 20 minutes at 10,000 rpm. The supernatant 1 mL was loaded onto a protein-G agarose affinity column (GE Healthcare, USA) with a flow rate 1 mL/min. Bound IgG was eluted with 0.1 mol/L glycine, 0.5 mol/L NaCl (pH 2.7), and dialyzed against 0.01 M phosphate-buffered saline (PBS) (pH 7.4). The purified IgG was then concentrated to a volume of 1 mL with ultrafiltration. Standard IF assays were performed according to the manufacturer’s instructions (Euroimmun, Lubeck, Germany). Serum of the patient with the first and second onset of disease, control serum, purified IgG of the patient with the first and second onset, and purified IgG of control, diluted 1/10 in PBS, were added to slides and incubated for 30 min at room temperature. The slides were washed twice with PBS and fluorescein isothiocyanate-conjugated second antibody was added and incubated for 30 minutes at room temperature. After washing with PBS twice, the slides were sealed with cover slips and observed at an exciting light of 490 nm. The IF test showed the existence of weak ANCA in purified IgG of the patient with the first onset of disease. Furthermore, we found this weak ANCA had a weak ability to bind MPO using ELISA. Briefly, purified human MPO (Merck) was coated to the wells of a polystyrene microtiter plate at 2.0 μg/mL in PBS. Purified IgG at a concentration equivalent to original plasma were diluted at 1:100 with PBS-Tween and incubated at 37°C for 1 hour. Binding was detected with alkaline phosphatase-conjugated goat antihuman IgG (Sigma) at a dilution of 1:1000.
1:20,000. The results were recorded at 405 nm. The results are shown in Figure 2.

3. Discussion

The diagnosis of ANCA-negative AAV is not as easy as ANCA-positive AAV, especially when there is no extrarenal symptoms. Crescent formation without fibrinoid necrosis can also be seen in other glomerulonephritis besides AAV. For this patient, we were not very convinced of the diagnosis of ANCA-negative AAV after the first kidney biopsy since the patient did not have kidney dysfunction, serum ANCA positivity, or extrarenal symptoms. The long-time stability of disease with no need of any immunosuppressive treatment and the negative results of multiple tests of serum ANCA almost made us exclude the diagnosis of AAV. It was the discovery of the weak ANCA in purified IgG that helped us to confirm the diagnosis of ANCA-negative AAV finally. The 2012 revised international Chapel Hill consensus conference nomenclature of vasculitides defined ANCA-negative AAV as a subtype of AAV and emphasized that patients with ANCA-negative AAV might have ANCA that could not be detected with current methods or might have ANCA of as-yet undiscovered specificity.\textsuperscript{6} As far as we know, this might be the first reported case about the spontaneous transformation from ANCA-negative renal-limited AAV to ANCA-positive systemic vasculitis after a long interval.

Some reports indicated that patients with negative ANCA had a greater degree of proteinuria and a higher prevalence of
nephrotic syndrome than patients with positive ANCA. According to previous studies, ANCA-negative AAV has less extrarenal manifestations than ANCA-positive AAV. For the current patient, proteinuria was the sole clinical symptom at first. After the serum MPO-ANCA turned positive, the acute kidney injury occurred and extrarenal symptoms also emerged. This indicated the MPO-ANCA might accelerate the disease. By now, the pathogenesis of AAV has not been elucidated. This is even true for the ANCA-negative AAV. The activation of alternative complement pathway has been considered to be very important in the development of both ANCA-positive and ANCA-negative AAV, but the activation of alternative complement pathway should be secondary to the activation of neutrophils. The neutrophils-activating ability of ANCA has been demonstrated by many in vitro and in vivo experiments. However, the existence of ANCA seems not to be necessary in the pathogenesis of AAV and the initial factor of AAV has not been confirmed yet. Actually, although ANCA can activate neutrophils, it is not the sole reason for the activation of neutrophils in AAV along with the development of examination technique. Although the positivity of ANCA detection in ANCA-negative AAV can be elicited with combined use of different methods, the spontaneous seroconversion of ANCA in ANCA-negative AAV has not been reported by previous studies. For the current patient, since the kidney function was normal and the diagnosis of AAV was not definite, no immunosuppressive treatment was given after the first kidney biopsy. This might be the critical reason for the seropositivity of ANCA after 18 months. To clarify the diagnosis, we purified the IgG from the serum obtained before the first kidney biopsy and found that there was weak ANCA in the IgG which could not be detected in serum. The research of Roth et al. might help to explain this phenomenon. In that study, the researchers found that many patients with ANCA-negative AAV had antibodies recognizing a special linear epitope of MPO (aa 447–459), but the antibodies could only be detected with purified IgG since there were fragments of ceruloplasmin in sera which could interfere the binding between MPO and the antibodies. It is noteworthy that natural anti-MPO antibodies exist in normal people and these natural antibodies only can be detected with purified IgG. So we speculate there should be a slow process of epitope spreading in the pathogenesis of disease for the current patient. Interestingly, although ANCA seemed to be pathogenic for this patient, neither immune complex nor complement deposition was found in 2 kidney biopsies. It should be explained by the fact that the antigen of ANCA exists in neutrophils not in endothelial cells of glomeruli.

In conclusion, this case offers a new clue for the understanding of the relationship between ANCA-negative and ANCA-positive AAV. For some patients, ANCA-negative status might be a transient stage in the development of AAV. Physicians should try their best to detect the ANCA in the diagnoses and treatment of ANCA-negative AAV.

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