Coexistence of Anti-Glomerular Basement Membrane Antibodies and Anti-Neutrophil Cytoplasmic Antibodies in a Child With Human Leukocyte Antigen Susceptibility and Detailed Antibody Description

A Case Report

Li-jun Xie, Zhao Cui, Xiao-yu Jia, Zhi Chen, Xiao-rong Liu, and Ming-hui Zhao

Abstract: Anti-glomerular basement membrane (anti-GBM) disease and anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis both could cause rapidly progressive glomerulonephritis. The coexistence of ANCA and anti-GBM antibodies was known as “double positive,” which was extremely rare in children. We report a pediatric case with coexistence of ANCA and anti-GBM antibodies.

A 6-year-old girl presented with acute renal failure, hematuria, proteinuria, and oliguria. She was double positive of ANCA and specific to myeloperoxidase, and anti-GBM antibodies. Kidney biopsy confirmed linear immunoglobulin (Ig)G deposit along GBM and 100% of crescent formation in glomeruli; among them 83.3% were cellular crescents. Human leukocyte antigen (HLA) gene typing showed DRB1*1501, an allele strongly associated with anti-GBM disease, and DRB1*0405, an independent risk factor for renal failure in patients with ANCA-associated vasculitis. The titer of anti-GBM antibodies was 1:800, and the predominant IgG subclass was IgG1, which was closely related with severe kidney injury and worse outcome. The target antigen of anti-GBM antibodies was restricted on the noncollagen domain 1 of a3 chain of type IV collagen (a3[IV]NC1), with recognitions to both epitopes, E A (a317–31) and E B (a3127–141).

This is the first reported pediatric case with coexistence of ANCA and anti-GBM antibodies, in which the HLA typing and immunologic characters of autoantibodies were identified. The findings on this early-onset patient are meaningful for understanding the mechanisms of both anti-GBM disease and ANCA-associated vasculitis.

INTRODUCTION

Anti-glomerular basement membrane (anti-GBM) disease and anti-neutrophil cytoplasmic antibody (ANCA)-associated systemic vasculitis are each clinically associated with the development of rapidly progressive glomerulonephritis. The concurrence of ANCA and anti-GBM antibodies, known as “double positive,” was seen in 5% to 14% of ANCA-positive patients,1–7 and 21% to 38% of anti-GBM antibodies-positive patients.1–7 These double-positive cases are characteristically older patients and reports in childhood are extremely rare.

Here we report the first pediatric case with coexistence of anti-GBM antibodies and ANCA, in whom the human leukocyte antigen (HLA) gene typing was performed and the immunologic characters of autoantibodies were identified. These findings may provide important information for better understanding of the clinical phenotype and possible mechanism of this rare autoimmune disorder.

CASE REPORT

A 6-year-old Chinese girl was admitted to the hospital with 1 month of edema gradually after catching a cold. She had oliguria with urine volume about 300 mL/day. Urine analysis
showed proteinuria (++++) and microscopic hematuria, without gross hematuria. Urinary protein excretion was 2170 mg/24 h (<150 mg/24h). Serum creatinine (Scr) was 831.7 μmol/L (30~84 μmol/L). Fever, fatigue, emaciation, hemoptysis, or diarrhea was not seen during the course of disease.

Physical examination found pulse 90 beats/min, blood pressure 130/80 mmHg, temperature 36.8°C, and respirations 20 breaths/min. In general, she was weak and anemic. Facial and low extremities edema was remarkable. There was no skin rash, petechia, or cyanosis. Lungs were clear to auscultation.

Laboratory studies showed Scr of 719.0 μmol/L, blood urea nitrogen of 48.0 mmol/L (1.7~7.1 mmol/L), and serum albumin of 30.2 g/L (35~55 g/L). Hemoglobin was 83 g/L (110~160 g/L) and platelet was 381 × 10^9 cells/L (100~300 × 10^9 cells/L). Urine sediment showed red blood cells 136/high-power field. Urinary protein excretion was 1505 mg/24 h. Plasma complements (C)3 was 0.79 g/L (0.85~1.93 g/L) and C4 was 0.244 g/L (0.12~0.26 g/L). Serum immunoglobulin (Ig)G was 7.73 g/L (6.0~13.0 g/L), IgA was 0.947 g/L (1.6~2.2 g/L), and IgM was 2.33 g/L (0.4~1.5 g/L). C-reactive protein was 4.91 mg/L (0.00~8.00 mg/L). Anti-nuclear antibodies were negative. p-ANCA was detected in her serum, with specificity to myeloperoxidase (MPO) and antibody level of 69 RU/mL (<20RU/mL). Anti-GBM antibodies were also positive of 119 RU/mL (<20 RU/mL). Chest computed tomography showed no parenchymal infiltration.

Renal biopsy was performed after admission. Direct immunofluorescence examination showed IgG and C3 linear deposition along GBM. On light microscopy, kidney specimens had 38 glomeruli with 2 sclerotic glomeruli. Glomerular capillary loops of the rest 36 glomeruli were disrupted severely, with 100% of large crescent formation in all glomeruli. Among them, 30 glomeruli had cellular crescents, 6 glomeruli had fibrocellular crescents, and 2 glomeruli had fibrinoid necrosis. Focal lymphocytes and mononuclear cells infiltration was shown in interstitial area with fibrosis. (Figure 1).

She was diagnosed as anti-GBM disease with anti-MPO positivity. Pulse methylprednisolone and plasmapheresis were initiated immediately. She underwent 8 times of plasmapheresis. After 7 times, both serum ANCA and anti-GBM antibodies were undetectable (Figure 2). Simultaneously, she received 3 courses of methylprednisolone pulse therapy followed by full doses of methylprednisolone. Intravenous cyclophosphamide was given twice, 0.12 g and 0.35 g, respectively, but stopped because of severe digestive tract side effect. Unfortunately, her renal function did not recover and she remained hemodialysis-dependent.

The immune characters of circulating autoantibodies in this patient were further investigated. The target antigens, conformational and linear epitopes, antibody titers, and IgG subclasses distribution were examined using enzyme-linked immunosorbent assay (ELISA) as described previously.7,8 The target antigen of anti-GBM antibody was restricted on the noncollagen domain 1 of the α3 chain of type IV collagen (α3IV[NC1]), but negative for α1, α2, α4, or α5(IV)NC1. Her serum antibodies recognized both conformational epitopes E3(IV)[NC1] and E4(IV)[NC1], but not the linear epitopes on α3(IV)NC1. The titer of IgG against α3(IV)NC1 was 1:800, and the predominant subclass of IgG antibodies was IgG1. HLA class II genes were typed by applied biosystems incorporated company (ABI) 3130XL platform using the SeCoreTM Sequencing Kits (Invitrogen, Brown Deer, WI) as described previously.9 The typing of HLA-DRB1, DQA1, DQB1, and DPB1 alleles for this patient revealed DRB1*1501DRB1*0405, DQA1*0102-DQA1*0303, DQB1*0503-DQB1*0501, and DPB1*0201-DPB1*0501.

**DISCUSSION**

Double-positive patients who present with both serum ANCA and anti-GBM antibody are adults, especially older population. In some cases, patients develop vasculitis first and then anti-GBM disease.10 However, the opposite sequence and concurrent cases are also commonly described.11,12 Here we reported a very rare case of pediatric patient with coexistence of MPO-ANCAs and anti-GBM antibodies. The disease course was fulminant without previous history. Renal function lost sharply and 100% of crescent formation at the same age was proven by renal biopsy. All these findings support the speculation that these 2 kinds of autoimmune disorders might arise simultaneously.

The mechanisms behind the development of 2 distinct forms of autoantibodies are unclear. Genetic predisposition is considered as “the first hit” in both ANCA-associated vasculitis and anti-GBM disease. Thus, it was intriguing that our
patient carried HLA DRB1*1501, the prevalence of which in patients with anti-GBM disease was about 3.5 times higher than that in controls. DRB1*1501 encoded β chain of HLA class II molecules, which participated in antigen processing and presenting to CD4+ T cell, resulting in an immune response against GBM. The other HLA DRB1 allele in our patient was DRB1*0405, which has been considered as an independent risk factor for the poor response to treatment and the deterioration of renal function in ANCA-associated vasculitis patients.

The kidney injury and prognosis of double-positive patients are similar to that of patients with antibodies against GBM alone, but much worse than that of patients with ANCA-associated vasculitis. Our patient presented linear IgG deposit on GBM and 100% of crescent formation on the renal biopsy specimens. On diagnosis, the Scr had risen to 831.7 μmol/L and hemodialysis was initiated. Although receiving sufficient plasmapheresis and intensive immunosuppressive agents, she did not recover from acute renal failure. In adults, recovery of renal function in dialysis-dependent patients is exceptionally rare as well as in those with Scr exceeding 600 μmol/L despite undergoing treatment. In children, only 8 cases were reported and the therapy strategy was formulated based on the studies from adults. Our patient showed the same features of kidney injury as the other cases, but her kidney damage was much more severe, which resulted in her end-stage renal disease outcome.

Close relationship between the immune characteristics of autoantibodies and the clinical phenotypes of anti-GBM disease and ANCA-associated vasculitis has been revealed in many studies. Both MPO-ANCAs and anti-GBM antibodies have been proven pathogenic in disease initiation and the characteristics of these antibodies were inferred contributing to the mechanism of kidney injury. Our patient possessed circulating IgG against the target antigen of α3(IV)NC1, with a high titer of 1:800. In adult patients with anti-GBM disease, high titer of anti-GBM antibody is closely associated with more severe kidney injury and worse renal outcome. The titer 1:800 was exactly the mean level of antibody titer in the adult patients who were dialysis-dependent in our previous studies. IgG subclass of serum anti-GBM antibodies was investigated in this case, with restriction to IgG1. IgG1 is the predominant pathogenic antibody in IgG antibodies toward GBM, and is seen in 93.5% of anti-GBM patients who present with renal failure. IgG1 binds C1q efficiently, and therefore activates the classic complement pathway. In addition, the IgG Fc receptors on mononuclear macrophages bind IgG1 strongly. Thus, IgG1 subclass is likely to trigger severe inflammatory damage to the glomeruli.

Several hypotheses have been proposed to explain the coexistence of ANCAcs and anti-GBM antibodies. No cross-reaction has been demonstrated between these 2 autoantibodies at B-cell level. However, the adult double-positive patients have a broader spectrum of anti-GBM antibodies compared with patients with anti-GBM antibodies alone, which might imply that the cross-reactive T-cell responses might initiate the coexistence and develop through epitope-spreading processes.

In the current case, the target antigen of anti-GBM antibody was restricted on α3(IV)NC1 toward both the major epitopes, E2 and E8. The antigen spectrum of antibodies in our patient was not as broad as in the adult counterpart, which suggests a possible different mechanism on the pathogenesis of pediatric patients with double-positive antibodies. Antibody characterization and HLA typing were needed for other pediatric patients with anti-GBM disease, ANCA-associated vasculitis, and their coexistence, which may provide clues on the mechanism and clinical phenotypes of these diseases.

In conclusion, the coexistence of anti-GBM antibodies and ANCAcs is rare in children, but may present a serious kidney injury with poor prognosis. The HLA typing and immunological investigations on these early-onset cases may give lights on the mechanisms of disease pathogenesis, for both anti-GBM disease and ANCA-associated vasculitis.

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