Antineutrophil Cytoplasmic Antibody and Multiple Sclerosis

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INTRODUCTION

Antineutrophil cytoplasmic antibody (ANCA)—associated pauci-immune crescentic glomerulonephritis (PICGN) is a well-known entity that occurs in approximately 7 to 10 people per million per year in the United States.[1] Its presentation can be highly variable, ranging from subacute kidney injury to a rapidly progressive glomerulonephritis. With a typical onset during the fifth to seventh decade of life, it can be associated with an 80% 1-year mortality if left untreated, leading to rapid progression and end-stage renal disease in many patients without prompt diagnosis and intervention with appropriate immunosuppression.[1,2] ANCAs are predominantly IgG autoantibodies directed against the primary granules of lysosomes in certain immune cells and are present in approximately 80% of patients with pauci-immune necrotizing vasculitis.[3] They are known to exist in association with inflammatory demyelinating diseases (IDD), such as multiple sclerosis (MS), optic neuritis, and neuromyelitis optica. In the context of IDD, few studies to date have described the clinical importance of ANCA, and associated renal disease has not been previously reported. We present a case of ANCA-associated PICGN occurring in a patient with a history of MS/optic neuritis.

CASE PRESENTATION

Clinical History and Pertinent Laboratory Data

A 55-year-old woman of white ethnicity with a history of MS in her usual state of health developed subacute kidney injury as seen on routine laboratory tests (creatinine 3.0 mg/dl from baseline of 0.6 mg/dl 9 months prior). She had no personal or family history of kidney disease and denied fevers, chills, joint pain, gross hematuria, rash, cough, and hemoptysis. Physical examination at the time of the nephrology clinic evaluation included a blood pressure of 162/98 mm Hg.

Additional pertinent examination findings included +2 pitting edema of the lower extremities bilaterally. Additional laboratory tests showed blood urea nitrogen (BUN) of 26 mg/dl, serum albumin of 3.6 mg/dl, and mild hypokalemia (Table 1). Urinalysis showed microscopic hematuria (50+ red blood cells per high power field) with spot urine protein/creatinine ratio of 3.34 g/g. Serology showed perinuclear ANCA (p-ANCA) positivity with a confirmative anti-myeloperoxidase (MPO) titer of 32.4. Pertinent negative laboratory values included cytoplasmic ANCA (C-ANCA), antinuclear antibody (ANA), hepatitis C antibody, HIV antigen/antibody, serum/urine protein electrophoresis, and normal complement levels. Renal ultrasound showed normal kidney size and echogenicity without calculi or hydronephrosis.

Renal Biopsy Findings

Renal biopsy was performed, and 25 glomeruli were presented for light microscopy. A total of 12 glomeruli showed cellular or fibrocellular crescents with fibrin deposition (Figure 1). Significant interstitial inflammation with eosinophils was noted, as well as mild tubular atrophy/interstitial fibrosis (Figure 2). Immunofluorescence showed strong fibrinogen staining in fibrocellular crescents, trace positivity for IgG and IgM, but was negative for IgA, C3, C1q, kappa (κ) and lambda (λ) (Figure 3). Electron microscopy showed focal epithelial foot process effacement, focal basement membrane thickening, and mesangial sclerosis.

Clinical Course and Follow-up

Treatment with methylprednisolone 1 g i.v. daily for 3 days was completed, followed by oral prednisone 1 mg/kg daily. She was subsequently treated with rituximab 375 mg/m^2 i.v. weekly for 4 weeks. Of note, her MS had been diagnosed 10 years prior after an episode of acute right-sided optic neuritis. She was

Table 1. Laboratory data of case patient

Laboratory value	Day 0	6 mo	Reference range
BUN (mg/dl)	26	27	6-20
Creatinine (mg/dl)	3.0	1.4	0.5-1.0
Sodium (mg/dl)	137	140	135-146
Potassium (mg/dl)	3.2	2.9	3.5-5.1
Chloride (mg/dl)	95	96	98-107
CO_2 (mg/dl)	27	29	22-32
Anti-MPO (units)	32.4	13.6	<20.1
Albumin (g/dl)	3.6	3.8	3.8-5.0
Urine protein/creatinine ratio (g/g)	3.34	1.37	<0.150
Complement C3 (mg/dl)	108	-	90-180
Complement C4 (mg/dl)	22	-	10-40
ANA	<40	-	<40
Hepatitis C antibody	Negative	-	Negative
HIV antigen/antibody	Negative	-	Negative
Serum protein electrophoresis	Negative	-	Negative
C-ANCA	Negative	-	Negative

ANA, antinuclear antibody; BUN, blood urea nitrogen; C-ANCA, cytoplasmic anti-neutrophil cytoplasmic antibodies; MPO, antimyeloperoxidase.

treated with prednisone initially and then interferon-β 1b (IFN-β 1b) therapy for 10 years with good response. She developed disease progression with new white matter lesions on brain magnetic resonance imaging and was transitioned to teriflunomide therapy approximately 1 month before her kidney injury. Approximately 6 months after her initial kidney injury and 3.5 months after completion of weekly rituximab therapy, her renal function and proteinuria showed marked improvement, and repeat MPO testing was normal.

DISCUSSION

ANCAs target specific cytoplasmic proteins of neutrophils and monocytes in mediation of disease. Major antigenic targets include MPO and proteinase-3 (PR3). MPO-ANCAs are typically more frequent in microscopic polyangiitis (MPA), whereas PR3-ANCA are
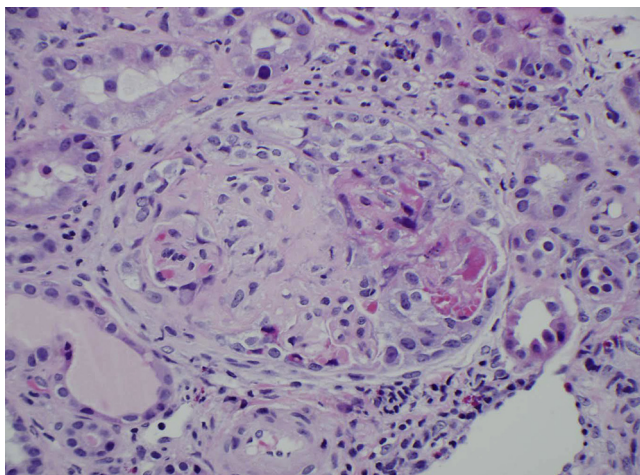

Figure 2. Hematoxylin and eosin stain (original magnification ×400) of renal biopsy sample showing dense interstitial inflammation.

more common in granulomatosis with polyangiitis (GPA).[4] The immune pathogenic effects of ANCA in renal vasculitis have been well demonstrated in animal models.[3] In addition to the kidney, ANCAs can potentially involve the central nervous system (CNS) as part of their systemic pathogenic effects. MS is a well-known and potentially debilitating condition associated with demyelination and chronic inflammation in the CNS white matter. Extensive data from animal models suggest that its pathogenesis is related to an autoimmune process involving T and B lymphocytes and pro-inflammatory cytokines.[5]

ANCA positivity in IDD is known to occur, with a reported incidence of 1% to 7% in retrospective and prospective studies.[6,7] The autoimmunity of ANCA with respect to IDD and their clinical significance have previously been investigated. In a series of 13 patients with clinically definite MS as well as acute transverse myelopathy and severe optic neuropathy,

Figure 1. Hematoxylin and eosin stain (original magnification ×400) of renal biopsy sample showing fibrocellular crescent formation, fibrin deposition, and interstitial inflammation.
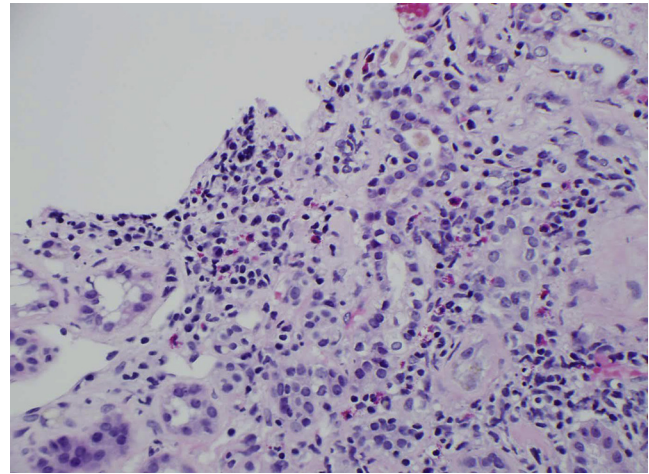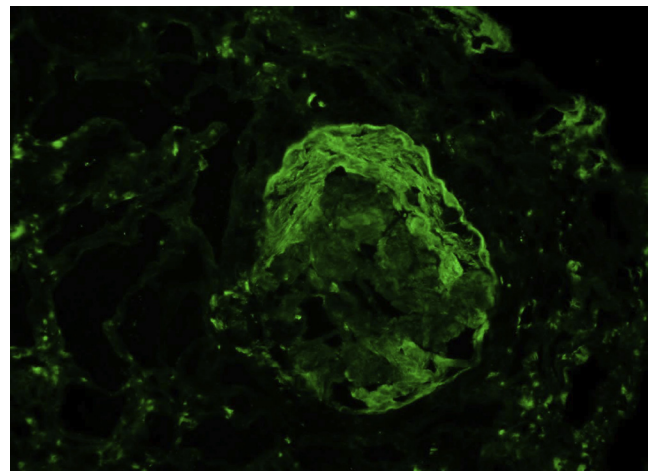

Figure 3. Immunofluorescence showing strong fibrinogen staining in a fibrocellular crescent (original magnification ×400).

Fukazawa *et al.* investigated ANCA serology and found p-ANCA positivity in 46.2% of patients with the optic–spinal form of MS, compared to 0% in those with conventional MS or acute transverse myelopathy ($P = 0.0005$).[8] These data suggest that the presence of ANCA in IDD is not an epiphenomenon but could play a direct role in disease pathogenesis in certain forms of MS. The largest clinical series of ANCA in CNS demyelinating disorders further supported such findings and investigated their clinical significance. Long *et al.* retrospectively investigated 269 patients with IDD who were positive for ANCA and reported 9.5% and 2.3% positivity rates for p-ANCA and C-ANCA, respectively.[6] Patients with spinal cord lesions were more likely to test positive for ANCA. In addition, ANCA-positive patients were older, had longer spinal cord lesions, and had more symptoms of disability at baseline, suggesting an association with worse clinical outcomes in MS patients.[6] Spadaro *et al.* also reported circulating ANCA in 7 of 105 MS patients with various stages of disease.[7] In addition, Nakashima *et al.* examined sera from 98 patients with various CNS disorders, including MS, myelitis, and viral-associated myelopathy, and found p-ANCA in 5 patients, including 2 with MS, and all of whom had subacute myelopathy of varying severity.[9] Overall, these data suggest that ANCAs can not only be present in the context of IDD but can potentially have an association with varying disease severity and clinical outcomes. However, studies investigating the relationship between ANCAs and response to therapy in IDD and risk of ANCA-associated renal disease in such patients are currently lacking.

Investigation of the pathogenic role of ANCAs in various disease processes has become more extensive. The mechanism by which ANCAs exert their effects is not fully understood, but is believed to be related to complex interactions with neutrophils, monocytes, and vascular endothelial cells. Previous studies have suggested that ANCAs indirectly induce a pro-inflammatory state via stimulation of neutrophils to undergo a burst release of reactive oxygen species, subsequently leading to degranulation.[10] To exert their pro-inflammatory effects, ANCAs must recognize and bind their target antigens (i.e,. MPO and PR3). This is accomplished via internalization of the ANCAs by neutrophils or by making the antigens more accessible on the neutrophil surface. When neutrophils are activated by various pro-inflammatory factors, MPO and PR3 translocate from the cytoplasm to the surface of the cell in a process termed priming.[11,12] This neutrophil priming is essential for ANCAs to exert their pathogenic effects, involves various pro-inflammatory mediators (such as tumor necrosis factor [TNF] and interleukin-6), and has been demonstrated *in vitro*.[13] Such molecular events can be triggered by certain infectious stimuli and can precede the clinical manifestations of ANCA-associated disease.[12] Cross-linking of ANCAs with Fc receptors on neutrophils stimulates degranulation and an oxidative burst, which contributes to the development of vasculitis.[11,12] Both ANCA phenotypes (cytoplasmic and myeloperoxidase-specific) can induce cytokine-primed neutrophil activation and the release of primary granule contents in a dose-dependent manner.[10,13,14] TNF-α, a pro-inflammatory cytokine, has also been shown to have a role in the pathogenesis of ANCA-associated disease and can enhance ANCA-induced neutrophil activation.[15] This is thought to be related to increased expression of MPO and PR3 antigens on the surface of unprimed neutrophils after exposure to TNF-α, allowing for increased binding to autoantibodies.[16] Treatment with IFN-ß 1b has been shown to decrease CD3-mediated TNF-α secretion from mononuclear cells.[17] Interestingly, this patient developed ANCA-mediated glomerular injury 1 month after discontinuing IFN-ß 1b therapy, suggesting that unopposed TNF-α secretion may have played a role in the pathogenesis. In addition to PICGN, the renal biopsy sample showed interstitial nephritis, which can occur in the setting of ANCA-associated renal disease, as the immunologic affects are widespread and can involve not only the glomerular microvasculature but all compartments of the kidney.[18] Acute interstitial nephritis due to teriflunomide therapy was also in the differential diagnosis. However, given the positive MPO-ANCAs and crescents seen on the renal biopsy sample, the patient's clinical picture was more consistent with an ANCA-mediated PICGN.

More recent data in animal models suggest that ANCAs play a more direct role and that the presence of anti-MPO alone is enough to induce a PICGN, even in the absence of functional T and B-lymphocytes.[3] *In vivo* evidence to suggest a direct pathogenicity of ANCAs in murine models has been demonstrated. Immunization of MPO knockout mice with mouse-MPO and transfer of anti-MPO splenocytes to wild-type mice and those lacking functional B and T cells elicits a severe, necrotizing glomerulonephritis.[3,19] Despite these data, evidence for direct pathogenicity of ANCAs has been questioned. This is partly due to the fact that healthy individuals can also have natural circulating autoantibodies against MPO and PR3. However, compared with pathogenic ANCAs, studies have shown that these "natural" autoantibodies occur in lower titers, have lower antigen avidity, have less subclass diversity, and are less capable of inducing a neutrophil respiratory burst *in vitro*.[19,20] Earlier data have shown that MPO-ANCAs target not only a single epitope but,

rather, a small number of different epitope regions.[21] A more recent study examining MPO epitope specificity using high-sensitivity epitope excision and mass spectrometry identified 12 specific MPO epitopes that were present exclusively in patients with active disease and 8 that were present in healthy subjects.[19,22] MPO-ANCAs directed against 1 linear epitope were exclusively associated with active disease, and reactivity to this epitope declined with disease remission.[19,22] These findings provide helpful insight into the questions regarding the true pathogenicity of ANCAs. If only a specific subgroup of ANCAs are pathogenic, then the presence of similar natural autoantibodies in healthy individuals does not refute the role of ANCAs as pathogenic.[19]

Based on the paucity of Ig and complement deposition that is classically seen in ANCA-associated glomerulonephritis, these mediators have traditionally not been implicated in the pathogenesis of ANCA-associated vasculitis (AAV). However, emerging data in animal models and clinical observations over the past decade suggest that activation of complement, particularly the alternative complement pathway, plays a crucial role in the development of AAV. The alternative complement pathway, unlike the classical and lectin pathways, is constitutively active and is characterized by spontaneous hydrolysis of complement C3 in the fluid phase rather than on a cell membrane surface. Such a "tick-over" mechanism allows for a low level and steady state of complement activation that does not have any consequences under normal physiologic conditions. Complement C5a, 1 of the end products of the alternative complement pathway, acts as a chemoattractant molecule that can recruit various types of inflammatory cells, such as neutrophils, eosinophils, monocytes, and T-cells.[11] Such a role of complement activation has been implicated in the pathogenesis of AAV. For example, in an experimental murine model of MPO-ANCA, mice deficient in C5 that were pretreated with cobra venom factor to deplete complement failed to develop glomerulonephritis and vasculitis. AAV development in wild-type and C4-deficient mice was comparable, whereas C5-deficient mice were protected from disease.[11,23] In human studies, various complement components have been detected in renal biopsy samples of patients with pauci-immune ANCA-associated glomerulonephritis, including C5b-C9, C3d, and factor B with co-localization in active glomerular lesions.[11,24] Additional studies have shown a positive correlation between deposition of activated factor B (Bb), an essential serine esterase of the alternative complement pathway, and the proportion of cellular crescents, degree of interstitial infiltrate, degree of interstitial

fibrosis/tubular atrophy, and an inverse correlation with the number or normal glomeruli. In addition, urinary levels of Bb, C3a, C5a, and soluble C5b-9 were significantly higher in active disease compared to those in remission.[11,25] Such findings lend evidence to the importance of complement activation in the mediation of ANCA-associated disease. Prior to these observations, the role of complement in the pathogenesis of ANCA vasculitis was not widely recognized, in part due to the lack of complement deposition in the vessel walls in AAV and ANCA-associated PICGN. However, compelling evidence in animal models suggests that the lack of obvious complement and Ig deposition at high levels does not refute its role in the pathogenesis of ANCA-associated disease.[23] The role of complement activation in the pathogenesis of MS has also been suggested by animal models as well as retrospective data in MS patients. For example, in a systematic immunohistochemical analysis of 17 patients with progressive MS and longstanding disease (mean duration 26 $\pm$ 12 years), immune labeling of MS plaques in brain and spinal cord were consistently positive for complement proteins, activators, and regulatory factors (C3, factor B, C1q, C4d, MAC, factor H, C1-inhibitor, clusterin), implicating their role in the pathogenesis of axonal and myelin injury in MS patients.[26] This combination of evidence highlights the importance of complement activation in the pathogenesis of both PICGN and IDD and why the potential for concomitant ANCA-associated renal disease in MS patients should not be overlooked.

The potential similarities in pathogenesis of PICGN and IDD may have their roots in a genetic basis. Several genome-wide association studies have provided helpful insight into the common genetic variants that are shared among several important autoimmune conditions, including type 1 diabetes mellitus (T1D), Graves disease, MS, Crohn disease, and GPA.[27,28] CD226, an important cell membrane—associated protein that is involved in T-cell adhesion and costimulation, belongs to the Ig supergene family of receptors and is expressed on most CD4+, CD8+ and natural killer cells.[27] It is involved in the regulation of T-cell activation and expansion. In autoimmune encephalomyelitis, an animal model of brain inflammation similar to MS, anti-CD226 monoclonal antibody has been shown to delay the onset and reduce the severity of T-cell—mediated autoimmune disease.[29] Furthermore, Gly307Ser, a single nucleotide polymorphism located in the CD226 gene on chromosome 18q22 has shown a significant association with T1D, MS, and rheumatoid arthritis in large panels of populations of white ethnicity.[27] In a separate study evaluating the impact of the CD226 Gly307Ser single-nucleotide polymorphism on the etiology and

Table 2. MS and ANCA: teaching points

- Pauci-immune crescentic glomerulonephritis (PICGN) is a progressive, autoimmune renal disease that is associated with a high mortality rate and that can rapidly progress to end-stage kidney disease without prompt treatment.
- Antineutrophil cytoplasmic antibodies (ANCAs) are predominantly IgG autoantibodies that are commonly seen in patients with PICGN.
- Multiple sclerosis (MS) is a potentially debilitating neurological condition that is associated with demyelinating CNS lesions and chronic inflammation and that can be associated with ANCA in up to 7% of cases.
- In patients with certain forms of MS, ANCAs can potentially contribute to disease pathogenesis and are associated with worse clinical outcomes.
- Certain genetic variants can increase susceptibility to both ANCA-associated vasculitis and central nervous system (CNS) demyelinating disorders
- Future studies are needed to determine whether the presence of ANCA in patients with underlying demyelinating disease can influence susceptibility to clinically significant renal disease.

pathology of GPA, genotyping showed a significant association of the minor allele 307Ser with GPA in a large panel of German patients compared with ethnically matched healthy controls. This was in addition to replicating the association between 307Ser and MS in a similar patient population.[30]

In conclusion, given the known autoimmune and pro-inflammatory mechanisms of these conditions, this case offers some important teaching points (Table 2) and an interesting hypothesis: that the presence of ANCAs in the context of known IDD could potentially influence susceptibility to clinically relevant renal manifestations. However, future studies are needed to substantiate such a potential correlation.

DISCLOSURE

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