Anti-Cardiolipin Antibody and Renal Disease: A Report of Three Cases

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ABSTRACT
Anti-cardiolipin antibodies have been linked to recurrent arterial and venous thrombosis in multiple organs. We present a biopsy-documented report of thrombotic renal disease apparently attributable to circulating anti-cardiolipin antibodies. One patient had primary anti-cardiolipin syndrome, one had mild SLE, and the third had a mild lupus-like syndrome. All three patients had a clinical course dominated by repeated multi-organ system thrombosis. Renal biopsy disclosed thrombosis at the level of the glomerular capillaries, arterioles, and interlobular arteries—similar to that described in other thrombotic microangiopathies. Renal thrombosis was not associated with active endocapillary proliferative lupus nephritis, suggesting a mechanism independent of subendothelial immune deposit injury. Renal presentation was variable, ranging from asymptomatic mild proteinuria to nephrotic-range proteinuria, renal insufficiency, and hypertension.

Key Words: Anti-cardiolipin antibody

In patients with systemic lupus erythematosus (SLE) and the primary anti-cardiolipin syndrome (1–9). While initial identification of anti-cardiolipin antibodies (so-called "lupus anticoagulant") was made in patients with SLE (10, 11), it has become increasingly apparent that a syndrome of recurrent thromboses related to anti-cardiolipin antibodies may also occur in the setting of a lupus-like syndrome which does not fulfill the American Rheumatism Association criteria for SLE (2). Such patients characteristically have a weakly positive ANA and negative titers of anti-DNA antibody. Yet another group of patients with anti-cardiolipin antibody and repeated thromboses lacks serologic and clinical features of SLE altogether; such cases have been termed the primary anti-cardiolipin syndrome or the primary anti-phospholipid syndrome (3).

The presence of anti-cardiolipin antibodies has been linked to recurrent arterial and venous thrombosis in multiple organ systems. Common clinical manifestations include recurrent deep venous thrombosis, placental thrombosis leading to recurrent spontaneous abortions, endocardial disease, livedo reticularis, and a variety of central nervous system complications including migraine, epilepsy, chorea, multi-infarct dementia, and retinal artery occlusions (2, 3, 12–17). Less frequent manifestations include peripheral arterial gangrene, mesenteric artery occlusion, Budd-Chiari syndrome, coronary thromboses, and pulmonary hypertension (2, 18, 19).

To date, the renal thrombotic complications of anti-cardiolipin antibody have not been clearly defined. In part, this is because of the frequent presence of underlying lupus nephritis, in which intravascular coagulation at the level of the glomerular capillaries commonly complicates the more severe, active forms of glomerulonephritis (20–22). Thus, it has been difficult to ascribe such renal lesions directly to the lupus anticoagulant per se, apart from the associated glomerulonephritis. Moreover, no clear association has been established between lupus anticoagulant and other renal thrombotic complications of SLE, including renal vein thrombosis (RVT) (23–27) thrombotic thrombocytopenic purpura (TTP)-like syndrome (28–30), and the non-inflammatory necrotizing "lupus vasculopathy" (31–33). There are no
previous reports of renal disease associated with primary anti-cardiolipin syndrome. We report three biopsy-documented cases of renal disease attributable to anti-cardiolipin antibody, independent of active lupus nephritis. All three cases were characterized by prominent thrombotic lesions in the glomerular capillaries and small arterial circulation of the kidney. One patient had primary anti-cardiolipin syndrome, one had mild SLE, and the third had a mild lupus-like syndrome. In all three patients, the clinical course was dominated by recurrent thrombotic events involving multiple organ systems.

MATERIALS AND METHODS

Anti-cardiolipin antibody was determined by ELISA, (normal IgG, <14 U; normal IgM, <2 U). Lupus anticoagulant was determined by elevated activated partial thromboplastin time. Specimens were then diluted and checked by the tissue thromboplastin inhibitor assay.

Renal biopsies were processed for light microscopy, immunofluorescence microscopy, and electron microscopy by standard techniques. Paraffin sections were stained with hematoxylin-eosin, periodic acid-Schiff, Masson's trichrome, Jones methenamine silver, and the modified Fraser-Lendrum stain for fibrin (34).

CASE REPORTS

Case 1

This 26-year-old white male construction worker was well until age 18 when he developed lower extremity thrombophlebitis, followed by spontaneous axillary vein thrombosis at age 22. In 1986, at age 24, he was first noted to be mildly hypertensive and serum creatinine and urinalysis were reportedly normal. At age 25, he developed recurrent axillary vein thrombosis and fever for which he was hospitalized and begun on anticoagulation. Laboratory work-up disclosed serum creatinine of 1.5 mg/dL; blood urea nitrogen (BUN) of 23 mg/dL; urinalysis, many white blood cells (WBC) and red blood cell (RBC) casts; proteinuria of 4 g/24 h; hematocrit (Hct), 36.3%; WBC, 25,000/mm³. The following tests were negative or normal: ABA, cryoglobulins, hepatitis B surface antigens, and serum complements. A renal biopsy was performed in June 1987, and, because of the question of renal vasculitis, the patient was placed on oral prednisone (60 mg) and Cytoxan (75 mg) daily. In the following months, the patient developed repeated right thigh thrombophlebitis and acute dyspnea and hemoptysis consistent with pulmonary emboli. In September 1987, he was seen in consultation at Columbia Presbyterian Medical Center. Blood pressure was 160/105 mm Hg. Laboratory data included: Hct, 42.3%; WBC, 15,800/mm³; erythrocyte sedimentation rate, 44 mm/h; platelets, 557,000/mm³; BUN, 34 mg/dL; creatinine, 1.5 mg/dL; serum albumin, 3.7 g/dL; serum cholesterol, 293 mg/dL; prothrombin time, 13.3/11.8; and activated partial thromboplastin time, 44.6/29.7. Urinalysis disclosed 4+ proteinuria with many RBC without casts, and 24-h urinary protein was 4.0 g. A chest x-ray disclosed several densities in the left lower lung and vascular thinning, suggestive of pulmonary emboli; this was confirmed by pulmonary angiogram. Coagulation studies performed October 1987 included an antithrombin III level of 104% (normal, 60 to 140), a protein C level of 80% (normal, 60 to 140), and a free protein S level which was decreased (suggesting an acquired deficiency state, probably secondary to nephrotic proteinuria). Venereal Disease Research Laboratories (VDRL) test was positive (1:4), and fluorescent treponemal antibody (FTA) was negative. The patient was found to have anti-cardiolipin antibody (IgG, 50 U), leading to a diagnosis of anti-cardiolipin syndrome.

The renal biopsy contained 15 glomeruli, 3 of which were globally sclerotic. Glomeruli were normal in size. There was mild focal segmental mesangial hypercellularity. In several glomerular capillaries, there was narrow tram tracking of the glomerular capillary wall associated with circumferential mesangial interposition (Figure 1). Mild swelling and proliferation of endocapillary cells was observed segmentally in two glomeruli. A small cellular crescent was noted overlying one of these foci. There was moderate patchy tubular atrophy, interstitial edema, and inflammation. Several terminal branches of the interlobular arteries and arterioles were occluded by intraluminal and intimal Lendrum-positive material consistent with fibrin, associated with swelling or loss of endothelium (Figure 2). By immunofluorescence staining, anti-cardiolipin antibody was found in the glomerular capillaries, mesangial areas, and arterioles, with a pattern consistent with a circulating immune complex. The patient was treated with warfarin sodium and has had no further episodes of thrombophlebitis or pulmonary emboli. He has remained asymptomatic with normal urinary protein for the past 12 months.

Figure 1. Patient in case 1. A representative glomerulus with mild endocapillary proliferation and widespread narrow GBM reduplication. (Jones methenamine silver; magnification, ×400).
cence, there was trace focal staining of glomerular capillary walls and mesangium with antisera to IgG, IgM, C3, C4, and fibrin-related antigen. Four glomeruli studied ultrastructurally disclosed large amounts of electron-lucent, flocculent material widening the subendothelial zones and mesangial matrix (mesangiolysis) (Figure 3). In several capillaries, there was associated peripheral mesangial interposition and mild mesangial hypercellularity. Foot processes were effaced over virtually 100% of the peripheral glomerular capillary surface area. No electron-dense deposits were seen. These biopsy findings were consistent with a thrombotic microangiopathy involving small arteries, arterioles, and glomerular capillaries. There was no evidence of vasculitis or glomerulonephritis of the immune-deposit type.

Case 2

This 38-year-old white female was diagnosed as having thrombophlebitis of the left leg and a pulmonary embolus at the age of 20. Later that year she had a spontaneous abortion, and a false-positive biologic test for syphilis was noted. She then did well until 33 years of age when she developed SLE. This was manifested by grand mal seizures attributed to lupus cerebritis, arthralgias and arthritis, discoid skin lesions, a malar flush, and hypertension. Laboratory evaluation revealed a positive ANA, a low complement level, a plasma creatinine of 1.3 mg/dL, urinalysis with 3+ proteinuria, creatinine clearance of 84 cc/min, 24-h urinary protein between 240 and 1,000 mg, and thrombocytopenia with a platelet count as low as 82,000/mm³. The patient's activated partial thromboplastin time was noted to be 70/35, and a circulating lupus anticoagulant was present. The patient was treated with Plaquinil and increasing doses of corticosteroids with resolution of her signs and symptoms of SLE and return of her activated partial thromboplastin time to normal. The patient was subsequently maintained on low-dose prednisone therapy and did well for over 4 years with <500 mg of proteinuria daily. At the age of 38, she was evaluated for increasing proteinuria of >1 g daily and hypertension. A renal biopsy was performed. Renal function tests revealed a plasma creatinine of 1.1 mg/dL, BUN of 23 mg/dL, urinalysis with 2+ albuminuria, 6–7 WBC/HPF and O RBC/HPF. Activated partial thromboplastin time was 43/30, WBC was 17,100/mm³, Hct was 47%, platelets were 143,000/mm³, serum complement was 237 U (normal, 160 to 210), anti-DNA antibody by ELISA was 722 U/mL (positive, >400 U/mL). Anti-cardiolipin antibody was present (IgM, 6.3 U).

The renal biopsy contained 13 glomeruli. All showed similar changes of mild segmental mesangial hypercellularity (Figure 4). Glomerular basement membranes were mildly thickened with foci of narrow reduplication and rare mesangial interposition. There was focal mild tubular atrophy, interstitial fibrosis, and inflammation and moderate arteriolosclerosis and arteriolosclerosis. By immunofluorescence microscopy, there was trace mesangial and rare segmental glomerular capillary wall staining with antisera to IgM, kappa, lambda, and fibrin-related antigen. The major ultrastructural finding in all six glomeruli studied by electron microscopy was diffuse widening of the lamina rara interna by accumulations of relatively electron-lucent flocculent material (Figure 5). In some areas, there was associated mesangial interposition and glomerular basement mem-

Figure 2. Patient in case 1. Multiple arterioles are occluded by intraluminal fibrin thrombus associated with endothelial swelling and denudation. There is no inflammation of the vessel wall (periodic acid-Schiff; magnification, ×400).

Figure 3. Patient in case 1. Electromicrograph showing marked subendothelial accumulation of electron-lucent flocculent material, without electron-dense deposits. There is marked effacement of foot processes (uranyl acetate, lead citrate; magnification, ×2,000).
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Figure 4. Patient in case 2. Glomeruli display mild mesangial hypercellularity consistent with lupus nephritis class II (hematoxylin and eosin; magnification, ×100).

Figure 5. Patient in case 2. Glomerular capillary walls are thickened by subendothelial electron-lucent material associated with mesangial interposition (uranyl acetate, lead citrate; magnification, ×7,100).

brane thickening and wrinkling. There were rare minute mesangial electron-dense deposits. Two small tubuloreticular inclusions were identified in the glomerular endothelium. There was mild focal foot process fusion affecting approximately 30% of the glomerular capillary surface area. The renal biopsy findings were primarily those of a low-grade coagulopathy affecting glomerular capillaries. The rare minute mesangial deposits were consistent with associated mild, inactive lupus nephritis, World Health Organization (WHO) class II.

Case 3

This 42-year-old white male was noted to have a positive VDRL at age 22, for which he received multiple antibiotic courses. At age 25, he developed claudication and cyanotic toes in his left lower extremity. Angiography disclosed thrombosis of the left popliteal artery, and he underwent a femoral-popliteal bypass. In his late 20s, he was noted to have livedo reticularis, nasal Raynaud’s phenomenon, pulsatile occipital headaches, diplopia, visual blurring, and vertigo. Brain CT scan showed mildly dilated ventricles. At age 41, he had two episodes of loss of consciousness and was admitted to Columbia Presbyterian Medical Center for work-up. There was livedo reticularis of the lower extremities and a prominent apical systolic murmur. The neurologic exam was normal except for impression of rapid recall and associations. Laboratory data revealed the following: WBC, 3,800/mm³; platelets, 103,000/mm³; Hct, 41.8%; creatinine clearance, 95 cc/min; 24-h urinary protein, 1.368 mg; urinalysis, 0 to 1 WBC, no RBC, ANA of 1:40, and anti-DNA antibody by ELISA of 185 U/mL (positive, >400), VDRL was borderline positive, and FTA was negative. The following tests were negative or normal: latex fixation, extractable nuclear antibody, Smith antibodies, lumbar puncture, cryoglobulins, serum protein electrophoresis, protein C and S levels, antithrombin III level, serum complement, human immunodeficiency virus titers. Prot time was 13.8/11.9, and activated partial thromboplastin time was 58.2/29.0. Lupus anticoagulant was positive with anti-cardiolipin IgG of 50 U, IgM of 10 U, and IgA of 7 U. A cerebral angiogram showed irregularities of the right calcarine artery, suggestive of vasculitis. On echocardiogram, there was mild aortic and mitral insufficiency with mild left ventricular hypertrophy. Renal biopsy was performed.

The renal biopsy contained 54 glomeruli, 3 of which were globally sclerotic. There was diffuse mild mesangial hypercellularity. The GBM was mildly thickened with focal small spikes, and GBM vacuolization was noted with the Jones methenamine silver stain. On one edge of the biopsy core, there was a focal scar containing 18 glomeruli, all of which were closely approximated because of marked tubular atrophy (Figure 6). In this area, glomeruli showed ischemic-type retraction of the tuft with GBM thickening and wrinkling and Bowman’s capsular sclerosis. Multiple arterioles and terminal branches of the interlobular arteries in the scarred region were narrowed or occluded by large Lendrum-positive fibrin thrombi in varying stages of organization and recanalization (Figure 7). Fibrin thrombi also oc-
eral capillaries contained electron-lucent flocculent subendothelial material. Visceral cell foot processes were 50% effaced. An interlobular artery was obliterated by an organized thrombus composed of myointimal cells embedded in a granular matrix containing irregularly distributed collagen fibers, without immune deposits. The renal biopsy was felt to be consistent with membranous lupus nephritis, Class V associated with multiple organizing thrombi involving arterioles, arteries, and glomerular capillaries, and secondary renal scarring.

DISCUSSION

The etiology of thrombosis in patients with anti-cardiolipin antibodies has been the object of recent investigations (6, 35–40). Lupus anticoagulants were first recognized in 1952 by Conley and Hartmann (10) by their ability to prolong lipid-dependent coagulation tests, such as the prothrombin time and partial thromboplastin time. While the lupus anticoagulant has an anticoagulant effect in vitro, in vivo it has been found to have procoagulant properties. The responsible agent has been identified as an immunoglobulin, usually of IgG (less commonly, IgM) subclass which binds to negatively charged phospholipids (6). Lupus anticoagulant is believed to be one of many possible antiphospholipid antibodies which are capable of binding cardiolipin and producing a false-positive VDRL. Recent work by Rauch et al. (40) has suggested that these antibodies react specifically with phosphatidyethanolamine in a hexagonal phase and not in a lamellar (bilayer) conformation. This specificity has led to speculation that lupus anticoagulant may form to a neoantigen which results from structural alteration of membrane phospholipids after a yet unrecognized cellular injury. These antibodies presumably prolong the prothrombin time or partial thromboplastin time by interfering with the phospholipid component of the prothrombin activator complex. It has been proposed that their procoagulant effect in vitro may be related to endothelial cell damage by antibody binding to endothelial plasmalemmal phospholipids (6, 39), reduced prostacyclin release by damaged endothelial cells (39), or alterations in platelet function and aggregation properties.

A variety of renal vascular complications has been identified in patients with SLE. These include renal vein thrombosis, vascular immune deposits, noninflammatory lupus vasculopathy, necrotizing vasculitis of the polyarteritis nodosa type, and widespread glomerular and arteriolar thromboses associated with a TTP-like syndrome (20–33). All of these renal vascular lesions, with the exception of vascular immune deposits, have in common the presence of intravascular coagulation with deposition of fibrin at

Figure 6. Patient in case 3. One portion of the renal biopsy showed scarring with tubular atrophy, interstitial fibrosis, and multiple organizing thrombi in interlobular arteries (arrows) (hematoxylin and eosin; magnification, ×100).

Figure 7. Patient in case 3. An interlobular artery contains a recanalized thrombus which resembles the glomeruloid bodies seen in TTP (hematoxylin and eosin; magnification, ×100).

cluded the vascular pole and peripheral capillaries of several glomeruli. By immunofluorescence microscopy, there was 1+ mesangial and focal finely granular glomerular capillary wall staining with antisera to IgG, IgA, C3, kappa, and lambda. Several glomerular capillary and arteriolar lumina stained 1+ for fibrin-related antigen and IgM. Six glomeruli studied ultrastructurally disclosed numerous small mesangial electron-dense deposits. Approximately half of the glomerular capillaries studied also contained small epimembranous and intramembranous electron-dense deposits, some separated by spikes. There were rare endothelial tubuloreticular inclusions. Sev-
different levels of the renal vasculature, giving rise to distinct clinical-pathologic entities with presumably differing pathogeneses, course, and prognosis. In addition, many forms of active necrotizing glomerulonephritis (lupus nephritis, WHO class III or IV) may be complicated by glomerular capillary thrombosis, possibly related to extensive immune deposition and glomerular endothelial injury.

Although renal vascular complications are relatively common in lupus nephritis, and many patients with SLE have a circulating lupus anticoagulant, a clear pathogenetic relationship between lupus anticoagulant and renal thrombotic events has been difficult to establish. While one study reported an association between renal vein thrombosis (RVT) and circulating lupus anticoagulant (23), several other studies have failed to confirm an increased risk of RVT in SLE patients with a circulating lupus anticoagulant (24, 25). More important predisposing factors to RVT in SLE appear to be the nephrotic syndrome and the presence of membranous lupus nephritis (26, 27). Moreover, there has been no definite association between the presence of circulating lupus anticoagulant and the occurrence of noninflammatory lupus vasculopathy, necrotizing vasculitis, or TTP-like syndrome. Doseken et al. (41) described a case of SLE with severe hypertension, rapidly progressive renal failure, and extensive renal arteriolar thrombosis in the setting of only mildly active lupus nephritis and lupus anticoagulant. The same group (42) reported renal thrombosis in 14 of 18 SLE patients with circulating lupus anticoagulant, 5 of whom had only mild mesangial proliferative lupus nephritis. In the study of Kant et al. (20), while glomerular capillary thrombosis was identified in 34 of 105 renal biopsies from patients with SLE, only 7 had an identifiable lupus anticoagulant. Of these 34 SLE patients with glomerular thrombosis, 31 had active proliferative forms of lupus nephritis (class III or IV), in which glomerular fibrin deposition is commonly seen. Thus, while it has been indirectly inferred that SLE patients with circulating lupus anticoagulant may be at increased risk for renal thrombotic events, a definite pathogenetic link has been difficult to establish.

The three cases reported here provide strong evidence that antiphospholipid antibodies may cause renal vascular thrombosis. The first case occurred in a young man with primary anti-cardiolipin syndrome without SLE. Although it could be argued that the renal microthrombosis was secondary to severe hypertension, this is unlikely since the patient’s renal manifestations of severe proteinuria and renal insufficiency preceded the clinical onset of severe hypertension. It is more probable that hypertension was a consequence of renal microthrombosis, with secondary renal ischemia and activation of the renin-angiotensin axis. In the second case, that of a patient with mild lupus nephritis, WHO class IIb, evidence of ongoing low-grade intravascular coagulation was found at the level of the glomerular capillaries, in the morphologic form of widespread accumulation of electron-lucent “Fluffy” material. This material is more electron lucent than are immune deposits and fails to stain with antiserum to Ig and C in immunofluorescence microscopy. It is common in a variety of thrombotic microangiopathies including hemolytic uremic syndrome, TTP, scleroderma, eclampsia, and allograft rejection, where it is believed to represent the products of intravascular coagulation which are subsequently degraded and organized into the glomerular capillary wall (43). Immunopathologic studies have suggested that this material consists, in part, of fibronectin, either produced locally in the glomerulus or deposited from the systemic circulation (44).

In this case, the glomerular subendothelial flocculent deposits could not be attributed to active immune-complex injury to the glomerulus, since, by electron microscopy, there were no peripheral capillary wall immune deposits. In the third case, there was a long-standing history of recurrent deep venous thromboses and central nervous system disease consistent with multiple infarcts. ANA was weakly positive at 1:40, and anti-DNA antibody titers and serum complement levels were normal. Thus, this patient would be best categorized as having a lupus-like syndrome. On renal biopsy, he was found to have a mild membranous lupus nephritis with mesangial and subepithelial electron-dense deposits. In addition, glomerular capillaries, multiple small arteries, and arterioles contained fibrin thrombi in varying stages of organization and recanalization. As in the second case reported here, the thrombosis could not be attributed to a nonspecific immunologic injury since the lupus nephritis was of an inactive membranous type and no vascular immune deposits were identified by electron microscopy or immunofluorescence. Thus, all three cases had morphologic and ultrastructural features that closely resemble those seen in the group of thrombotic microangiopathies exemplified by hemolytic uremic syndrome and TTP (45). These included thrombosis of interlobular arteries, arterioles, and glomerular capillaries, glomerular subendothelial deposits of electron-lucent granular material with focal mesangial interposition, and GBM reduplication, in the absence of subendothelial electron-dense deposits.

In two of our three patients, the clinical renal abnormalities were mild, consisting of asymptomatic subnephrotic proteinuria. Yet, all patients had widespread renal morphologic evidence of ongoing intravascular coagulation. It is possible that a more widespread use of renal biopsy in patients with primary anti-cardiolipin syndrome or mild SLE with lupus...
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