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Case report

Membranous nephropathy with crescents associated with levamisole-induced MPO-ANCA vasculitis

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ABSTRACT

ANCA-associated vasculitis (AAV) is the most common cause of crescentic rapidly progressive glomerulonephritis (GN). Levamisole used as an adulterant in cocaine is increasingly recognized as a cause of AAV. We report the case of a 50 year old woman with atypical anti-MPO AAV associated with cocaine use and exposure to levamisole. In addition to the clinical and pathologic findings of crescentic GN, the patient also had biopsy evidence of secondary membranous nephropathy (MN). Although AAV and MN have been reported previously in the same patient and both have been induced by drug exposures, this is the first report of MN in a patient with AAV likely induced by levamisole. We suggest that MPO can cause both pauci-immune vasculitis and secondary membranous nephropathy in some cases, as in cases of levamisole-adulterated cocaine use.

1. Introduction

The first description of membranous nephropathy (MN) and concurrent crescentic glomerulonephritis (GN) dates back to 1975. There have been reports of MN with coexistent crescentic GN due to antinuclear antibody (ANA), anti-GBM (glomerular basement membrane) antibodies, and IgA Nephropathy. There have also been many reported cases of MN co-existing with crescentic GN, usually due to ANCA-associated vasculitis (AAV), including granulomatosis with polyangitis (formerly Wegener's), microscopic polyangitis and Eosinophilic Granulomatosis with Polyangitis (Churg-Strauss syndrome).

Recently, levamisole, used as an adulterant in cocaine, has been recognized as an important inducer of anti-MPO vasculitis [1]. Levamisole has been identified as a likely etiologic agent in one patient with anti-MPO vasculitis associated with nephrotic syndrome; however, the kidney was not biopsied in that case [2]. To our knowledge, this report describes the first case of levamisole-induced MPO-ANCA vasculitis with crescentic GN and accompanying MN.

2. Clinical summary

A 50 year old female with past medical history of asthma, hypertension, and rheumatoid arthritis presented for evaluation of rash and renal failure in the setting of cocaine use. A toxicology screen was positive for cocaine and opiates. On physical examination, the patient was afebrile. Skin exam revealed a purpuric and violaceous, non-blanching rash in a retiform pattern with areas of necrosis and infected ulcers located on the helix and earlobes and also on the upper and lower extremities. The patient denied photosensitivity, neurological complaints, symptoms of serositis, oral ulcers, temperature sensitivity, Raynaud's phenomenon, sore throat or dyspnea.

Urine analysis showed dysmorphic red blood cells, and 24 h protein excretion was quantified at 1600 mg. The serum creatinine (Cr) peaked at 194.5 mcmol/L. Both C3 and C4 were low at 0.61 g/L (normal 0.88–2.06 g/L) and 0.09 g/L (normal 0.13–0.75 g/L) respectively. Serologies for Hepatitis B and C were negative. Age-appropriate cancer screening, including colonoscopy, mammogram, pap smear and chest xray were all negative. Patient denied NSAID use. Syphilis was ruled out with a negative rapid plasma reagin. P-ANCA was positive and anti-MPO antibodies were quantified at 134 AU/mL (positive: 26 AU/mL or greater). Further serologic studies were neg-

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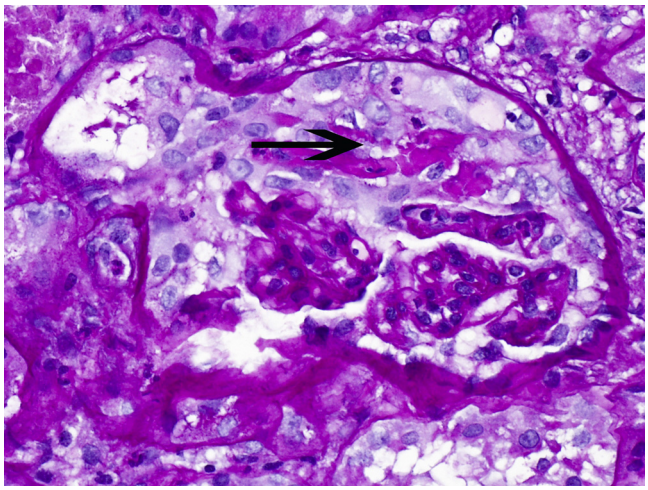

Fig. 1. Glomerulus with fibrin within the urinary space and associated cellular crescent (arrow). PAS 400× magnification.

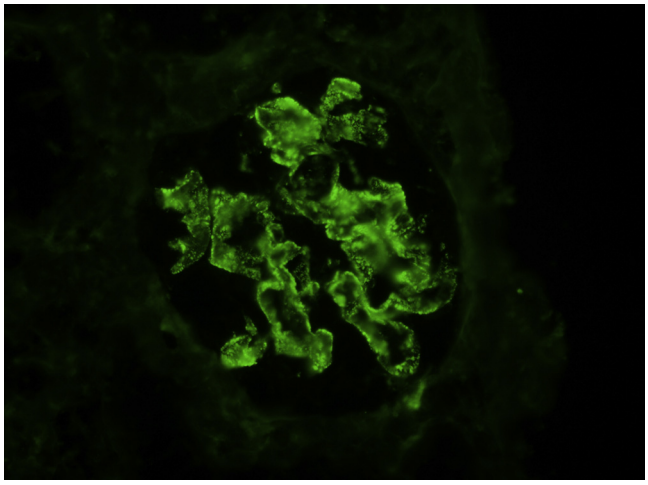

Fig. 2. Immunofluorescence for IgG shows fine granular staining of glomerular capillary loops. IgG 400× magnification.

ative for ANA, dsDNA, HIV, CMV, and EBV. Serology for IgG4-specific and total IgG anti-phospholipase A2 receptor (PLA2R) antibodies was negative. A kidney biopsy was pursued as there was high suspicion of glomerulonephritis.

3. Pathological findings

Kidney biopsy revealed one core of renal cortex with 9–11 glomeruli per level section, of which 0–1 per level section were globally sclerosed. Two glomeruli had segmental sclerosis and one glomeruli contained a small cellular crescent. Biopsy showed features of crescentic vasculitis in addition to membranous nephropathy (Fig. 1). Light microscopy showed mild thickening of basement membranes with spike formation. Capillary loops cut en face showed a moth-eaten appearance by silver stain. Occasional glomeruli also showed segmental endocapillary proliferation. In addition, focal segmental necrotizing lesions with early crescent formation were seen. Immunofluorescence staining revealed a uniform, fine granular capillary loop staining for IgG (2+), IgM (1–2+), C3 (2–3+), C1q (trace),kappa (1+), and lambda (trace to 1+) in a subepithelial pattern characteristic of MN (Fig. 2). Staining for IgA was negative.

Immunofluorescence staining of the renal biopsy for PLA2R was performed (Nephropath laboratory, Little Rock, AK) to help distinguish between primary versus secondary membranous glomerulopathy. Glomerular PLA2R staining was not enhanced in this case. Positive membranous glomerulopathy cases will show a pattern of staining identical to the IgG. The positive control was a confirmed primary membranous case. A negative control consisted of the secondary antibody only.

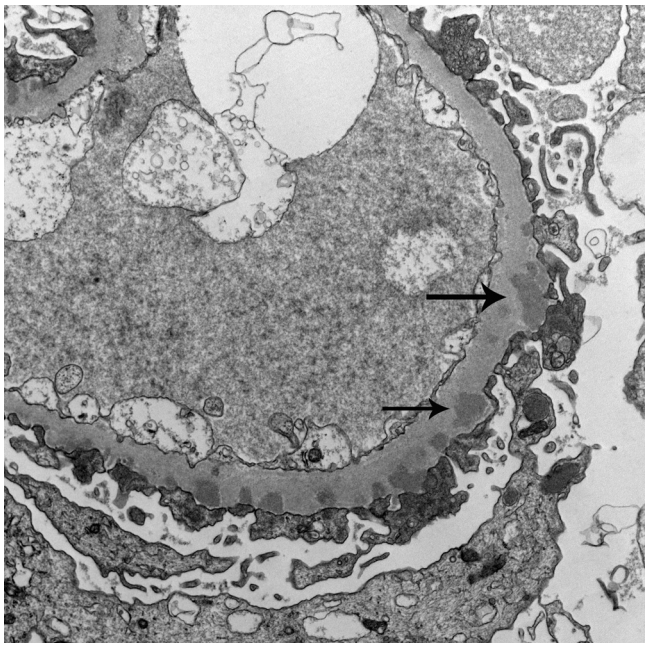

Fig. 3. Numerous small subepithelial immune deposits (arrows) are present along the glomerular basement membrane, with coarsening and effacement of podocyte foot processes. 8200× magnification.

Immunohistochemical staining for MPO and IgG4 was also performed (ARUP laboratory, Salt Lake, UT) and both stains were negative within the glomeruli. Staining for MPO was performed to help determine whether MPO could be detected in the subepithelial immune complexes as a planted antigen. MPO staining uses a rabbit anti-human polyclonal antibody to myeloperoxidase. Spleen tissue constituted positive control. There was no negative control. IgG4 staining was also done by ARUP. A mouse monoclonal antibody against human IgG4 designed to bind to the Fc portion of IgG4 molecules was used for IHC. The positive control was a mixed inflammatory infiltrate with some IgG4 staining cells. There was no negative control.

Anti-PLA2R antibody quantification by ELISA was done by Lawrence H. Beck's laboratory at Boston University School of Medicine. Both IgG4-specific anti-PLA2R as well as total IgG anti-PLA2R were quantified.

Electron microscopy revealed the glomerular capillary loops to be markedly irregular and frequently thickened, due to the presence of numerous subepithelial and intramembranous electron dense immune deposits (Fig. 3). The deposits were associated with remodeling of the GBM, including the formation of epimembranous "spikes" of new basement membrane material between the deposits. Mesangial areas contained increased matrix and cellularity as well as scattered immune deposits (Fig. 4). There was extensive effacement of epithelial cell foot processes with associated microvillous transformation. No significant subendothelial deposits were seen.

4. Discussion

The patient was treated with intravenous methylprednisolone and transitioned to oral prednisone. She was also started on oral

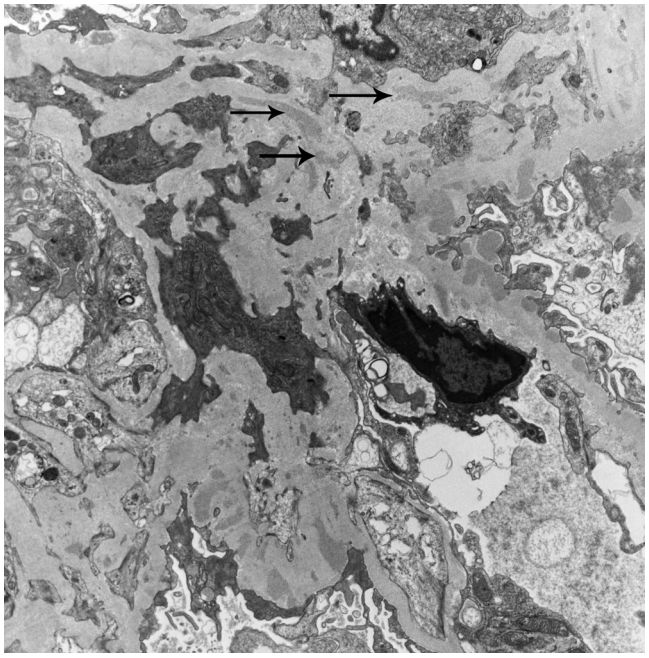

Fig. 4. Scattered electron dense deposits are present within the mesangium (arrows), accompanied by mild increase in mesangial matrix and cell activity. 4200× magnification.

cyclophosphamide, 2 mg/kg/day. She was discharged to a rehabilitation facility 7 days later with a serum creatinine of 159.1 mcmol/L. During her stay at rehab, she developed an abscess of the right foot, and cyclophosphamide was stopped after two weeks of therapy to promote resolution of the abscess. The patient subsequently went into renal remission on monotherapy with oral prednisone, 60 mg daily, for six months. She has now been on maintenance therapy with azathioprine for six months, her current serum creatinine is 70.7 mcmol/L, and spot urinary protein to creatinine ratio is normal (113 mg/g creatinine).

MPO titers also decreased with immunosuppressive treatment. Myeloperoxidase antibody titers were as follows (reference range: 0–19 AU/mL): 134 in January 2014, 17.6 in October 2014, and 17.3 in January 2015.

Elevated MPO antibody titers confirm that ANCA-associated vasculitis (AAV) is the most likely etiology for decreased renal function and the necrotizing and crescentic features of the GN in our patient. In 2008–2009, the drug enforcement agency (DEA) reported that levamisole was found in 69% of cocaine seized by the DEA. The patient's positive cocaine history, purpuric rash with skin necrosis involving the earlobes, anti-MPO ANCA positivity and hypocomplementemia are characteristic features of AAV induced by levamisole.

The mechanisms by which exposure to levamisole leads to AAV have not been defined. Levamisole causes agranulocytosis that may be accompanied by increased release of neutrophil extracellular traps (NETS), composed of a scaffold of chromatin DNA intermingled with histones, MPO, PR3, human neutrophil elastase (HNE) and other components of neutrophil cytoplasmic granules. In addition to directly causing tissue injury, NETs can present constituent molecules like MPO to the immune system and have been implicated in the pathogenesis of MPO-ANCA vasculitis [3].

Alternatively, recent reports have suggested that levamisole may bind as a hapten to self antigens such as MPO causing conformational changes that lead to autoimmunity [4]. Lardinois et al. have recently reported that drugs (including levamisole) that induce ANCA vasculitis form drug-protein adducts after exposure to hypochlorous acid (HOCL). Neutrophil-derived MPO and a halide generate hypochlorous acid which metabolizes these drugs to reactive free radical compounds. These electrophilic compounds may react with MPO to form hapten adducts that can stimulate autoantibody production [5].

In primary MN, an apparent causal etiology is lacking and biopsies reveal a lack of inflammatory changes, basement membrane thickening with spike formation, subepithelial immune complexes, and predominant IgG4 deposition usually accompanied by C3 and C5b-9 [6]. Subepithelial immune deposits result primarily from *in situ* immune complex formation involving podocyte antigens rather than from circulating immune complex trapping [7]. In 70–80% of cases of primary MN, antibodies to PLA2R, which is expressed on the podocyte cell membrane, lead to subepithelial immune deposits when PLA2R is in the proper configuration [8]. Other putative antigens include thrombospondin type-1 domain containing 7A, superoxide dismutase 2, aldose reductase and enolase. Increased staining for PLA2R in glomeruli is also present in patients who have, or had, anti-PLA2R antibody [9].

Secondary MN is characterized by lack of anti-PLA2R antibodies, deposition of IgG1 and IgG3 and usually some electron dense immune deposits in mesangial as well as subepithelial sites [10]. The mechanisms underlying the formation of subepithelial immune deposits in secondary forms of MN are less well understood, but may involve the glomerular deposition of circulating immune complexes (CICs). Low-affinity antigen-antibody interactions may allow immune complexes, initially trapped in a subendothelial position, to dissociate and reform on the abluminal side of the GBM. In secondary MN, circulating, non-renal antigens, primarily cationic proteins, can be "planted" in subepithelial sites leading to *in situ* immune complex formation. Several exogenous and endogenous antigens derived from HBeAg, Hepatitis C, Helicobacter Pylori, tumors and thyroid have been localized in immune deposits in patients with secondary MN [11].

Previously reported cases of MN associated with crescentic GN due to anti-MPO have been claimed to be both primary (although anti-PLA2R antibody has not been documented) and secondary [12]. One must first rule out secondary causes of membranous nephropathy before entertaining primary membranous nephropathy. Serologies for Hepatitis B and C were negative. Age-appropriate cancer screening, including colonoscopy, mammogram, pap smear and chest xray were all negative. Patient denied NSAID use. Syphilis was ruled out with a negative rapid plasma reagin. The absence of staining for IgA, minimal C1q staining, lack of anti-DNA antibodies and clinical picture make lupus nephritis unlikely. The lack of linear glomerular basement membrane (GBM) staining for IgG rules out anti-GBM disease which also causes crescentic GN with increased frequency in primary MN. Both IgG4-specific and total IgG anti-PLA2R antibodies were absent. Staining of the renal biopsy for PLA2R, MPO, and IgG4 were all negative.

Biopsy demonstrated segmental endocapillary proliferation and mesangial immune deposits in addition to the subepithelial immune deposits, spike formation, and fine granular capillary loop immunofluorescence staining. Since this is a mixed lesion with both membranous and ANCA-positive components, the immunopathology is not classic for either. Many studies have reported non-specific immunoglobulin deposition in ANCA-positive vasculitis [13,14]. Mesangial immune complex deposits are frequently seen in secondary MN and, occasionally, in primary MN as well [15]. In glomerulonephritis where there is a substantial increase in glomerular permeability, this can lead to increased trafficking of macromolecules through the mesangium resulting in trapping of immune complexes within the mesangium [16].

The evidence, therefore, strongly suggests that membranous nephropathy, in this case, may be secondary. However, the antigen responsible for the subepithelial immune complex deposits is not apparent. Levamisol, as a hapten, may induce autoimmunity to

self antigens, including proteins present on podocytes. However, MPO could be the putative antigen even though the immunofluorescence staining for MPO is negative. The MPO antigen in the GBM may have been altered or binding sites for the IHC antibody may be masked so that the IHC stain is negative. Alternatively, the MPO antigen may be present in such small quantities that it is below the staining threshold of detection.

Indeed, the coexistence of MN and AAV suggests that both crescentic vasculitis and membranous nephropathy may be an immune reaction to MPO. In AAV, MPO is released from activated neutrophils [17,18]. In normal glomeruli, MPO not only reacts with a halide to produce oxidant injury but has been shown to bind avidly to capillary walls on a charge basis where it could serve as a planted antigen [19]. Hanamura et al. have demonstrated MPO in a subepithelial distribution with membranous nephropathy-like lesions in patients with MPO-ANCA-associated GN [20]. Kawashima et al. have also demonstrated immunopathologic co-localization of MPO, IgG, and C3 in glomeruli in human MPO-ANCA-associated GN [21].

In levamisole-induced AAV, we speculate that the formation of adducts of MPO and levamisole, or one of its metabolites, could not only induce conformational changes that render the native MPO antigenic but also increase its binding to the capillary wall. Anti-MPO antibodies can be reactive with several different epitopes [22]; Levamisole, by forming drug-protein adducts, may induce an antibody reactive only with an MPO variant that is not present in other forms of AAV and has particular affinity for localizing in the subepithelial space. The fact that levamisole-induced AAV has some atypical clinical features, such as the prominence of necrotizing skin lesions, particularly involving the ear, and hypocomplementemia, is consistent with the hypothesis that a different MPO variant might be the target. Both AAV and MN are associated with genetic risk factors that might explain why neither AAV nor MN is present in most patients exposed to levamisole in cocaine [23,24]. Alternatively, the associated MN could involve a different, non-neutrophil derived, antigen or the two glomerular lesions could be independent and not pathogenetically linked.

In summary, the association of exposure to levamisole as an adulterant in cocaine and associated development of a clinically atypical form of anti-MPO vasculitis is increasingly recognized. We report the first case of a coexistant MN in such a patient. We propose that levamisole may relate to the etiology of both diseases by forming MPO-levamisole adducts that not only induce anti-MPO antibody but also serve as planted antigens in the subepithelial space to induce a secondary MN. This case should stimulate careful search for other such patients in which this hypothesis could be further tested.

Support and financial disclosure declaration

None of the authors have any support or financial disclosures to make.

Conflicts of interest

None.

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