Pathogenic significance of ANCA in a patient with crescentic glomerulonephritis, bone marrow granulomata, and linear staining pattern along the glomerular basement membrane with ANCA by indirect immunofluorescence

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Introduction

Necrotizing vasculitis of the kidney usually presents as crescentic (rapidly progressive) glomerulonephritis. Three patterns of vascular injury are described: (1) immune-complex deposition exemplified by systemic lupus erythematosus, (2) antibody binding directly to the glomerular capillary wall as observed in Goodpasture’s syndrome, and (3) ‘pauci-immune disease’ typically noted in antineutrophil cytoplasmic antibody (ANCA) associated vasculitis [1].

Two subtypes of ANCA are known, based on indirect immunofluorescence staining pattern. C-ANCA refers to granular staining of the cytoplasm of neutrophils and monocytes. The main target antigen of this antibody is a 29-kDa serine protease known as proteinase 3 or PR3, and it is located in the primary granules of neutrophils. C-ANCA has 90% specificity for Wegener’s granulomatosis. P-ANCA refers to a perinuclear staining pattern, an artifact of the staining technique. The target antigen of p-ANCA, usually myeloperoxidase (MPO), also a constituent of primary granules, migrates towards the nucleus after the granule membranes are made permeable with ethanol fixation. The disease associations with p-ANCA are less specific; high titres of this antibody are found in microscopic polyarteritis, Churg–Strauss syndrome, and idiopathic crescentic glomerulonephritis. P-ANCA occurs in considerably lower frequency in Wegener’s granulomatosis [2–4].

The pathogenic role of ANCA in necrotizing vasculitis has been a matter of controversy. We present a case where a direct role is suggested for ANCA in injury to vascular endothelium, similar to that observed in Goodpasture’s syndrome.

Case report

A previously well 70-year-old female presented with a 6-week history of weight loss, arthralgias, headaches, night sweats, anorexia, and weakness. She had a 1-week history of sacral oedema; and had developed a cough, productive of a small amount of green sputum, 2 days prior to admission. She denied any fever or chills, skin rash, shortness of breath, wheeze, haemoptysis, sinus trouble, chest pain of either visceral or pleuritic nature, or gross haematuria.

The physical examination revealed a thin woman in no distress. The temperature was 36.7 °C, blood pressure 110/80 mmHg, and respiratory rate 18 per minute. Head and neck exam revealed only mild conjunctival pallor. Her chest was clear and cardiac exam was normal aside from minimal sacral oedema. The rest of her physical examination was unremarkable.

Laboratory evaluation revealed: Hgb 118 g/l, WBC 9.4 × 10^9/l, platelets 410 × 10^9/l, ESR 101 mm/h; sodium 134 mmol/l, potassium 6.2 mmol/l, chloride 107 mmol/l, bicarbonate 19 mmol/l, urea 19.3 mmol/l, creatinine 635 μmol/l, calcium 1.96 mmol/l, phosphorus 3.11 mmol/l, albumin 22 g/l; ALT, AST, and alkaline phosphatase were normal; 24-h urinary protein excretion was 980 mg; serum IgG was 21.50 g/l, IgA 1.80 g/l, IgM 1.20 g/l; immuno-electrophoresis showed a polyclonal IgG-kappa spike in serum; complement levels (C3, C4) were normal; Rheumatoid factor, antinuclear antibody, anti-DNA antibody, and anti-GBM antibody (by both immunofluorescence and radioimmunoassay) were negative; p-ANCA was positive at 1:160 titre, c-ANCA was negative.

Bone marrow and kidney biopsies were performed. The patient was treated initially by pulse solumedrol 1 g daily for 3 days followed by prednisone at 1 mg/kg. Upon availability of biopsy results, oral cyclophosphamide was added at 2 mg/kg to the corticosteroid regimen. Her systemic symptoms resolved, but renal function and histology did not respond to this
ANCA-positive, anti-GBM negative necrotizing vasculitis

treatment and she developed end-stage renal disease, requiring initiation of haemodialysis. She is alive and continues to receive dialysis to the present date.

Methods

Histological study of the bone marrow aspirate and biopsy were performed. The kidney biopsy specimens were studied by light- and electron-microscopy and indirect immunofluorescence, using routine techniques. Both p- and c-ANCA titres were determined by standard techniques utilizing indirect immunofluorescence on ethanol fixed human leukocyte preparations. ELISA anti-MPO and anti-PR3 antibody assays were not available at the time of this study. Standard indirect immunofluorescence and radioimmunoassay techniques were used for detection of anti-GBM antibody.

Sections of the patient’s kidney biopsy were incubated at room temperature with four sera including her own, and three control sera that were normal (ANCA and anti-GBM negative), p-ANCA positive/anti-GBM negative, and c-ANCA positive/anti-GBM negative. Sections of kidney tissue with normal histology, obtained from other source, were also incubated with these four sera. These eight preparations were studied for the staining patterns on indirect immunofluorescence.

Results

The bone marrow specimen consisted of a single core of tissue measuring 1.0 and 0.5 cm in length respectively. Each section included eight to ten glomeruli of which two were globally sclerosed and the rest all showed cellular crescents with proliferation of epithelial cells mixed with eosinophilic fibrin-like material and PMN’s. Necrosis of capillary loops was present in some areas. There was marked, diffuse mixed interstitial infiltrate composed of plasma cells, lymphocytes, some eosinophils, and few polymorphonuclear cells (Figure 2). Some nodular areas suggesting granulomas were also noted and showed epithelioid histiocytes with central necrosis and polymorphonuclear cells (Figure 3). Many tubules contained red cells and cellular casts. The blood vessels were unremarkable.

Electron-microscopic examination revealed one glomerulus with segmental collapse of a capillary loop, extensive epithelial cell proliferation and red blood cells and fibrin in Bowman’s space. No electron-dense deposits were noted and the basement membrane was unremarkable. Marked mixed inflammatory infiltrate was seen in the interstitium. Routine immunofluorescence revealed a faint, diffuse and global, linear IgG staining pattern along the glomerular capillary wall but not the tubular basement membrane or interstitial vessels. There were numerous inflammatory cells in the interstitium reacting with IgM, IgE, C3, C4, C1q and C5. Granular deposits of C3 were noted in some interstitial vessels and glomerular capillaries.

The additional immunofluorescence studies revealed the following: The preparations consisting of the patient’s kidney biopsy incubated with her serum, as well as those incubated with control p-ANCA and c-ANCA positive sera, showed a diffuse and global linear pattern of staining of ANCA along the glomerular capillary wall (Figure 4). None of the preparations consisting of normal kidney sections demonstrated

Fig. 1. Bone marrow biopsy. Marrow interstitial infiltrate with granuloma formation. (H&E × 160).
staining for ANCA, nor did we observe such pattern when normal control serum was incubated with patient’s kidney biopsy specimen.

**Discussion**

Our patient presented with p-ANCA-positive, anti-GBM negative necrotizing vasculitis, manifesting as crescentic/rapidly progressive glomerulonephritis, with associated granulomatous inflammation of the renal parenchyma and bone marrow. To our knowledge, bone marrow granulomata have not been previously reported in association with ANCA positive disease. In a recent report granuloma formation has been described in a patient with anti-GBM disease [5].

Most commonly cited hypothesis for the role of ANCA in the pathogenesis of vascular injury in necrotizing vasculitis is as follows: an undetermined stimulus, perhaps an infection, induces the expression of ANCA target antigens (PR3 and/or MPO) on the neutrophil cell surface, as mediated by inflammatory cytokines, such as IL-1 and TNF-alpha. ANCA then binds to its antigen, inducing degranulation and respiratory burst of the neutrophil. A transduction pathway mediated by phospholipase C is required for this activation.
Cytokines also induce expression of adhesion molecules that allow for the close association of neutrophils and endothelial cells. Intravascular lysis of the neutrophils results in the release of enzymes and oxygen radicals, causing damage to the endothelium [1,3,6].

An alternative hypothesis is that ANCA may participate more directly in endothelial cell damage. Mayet et al. [7] demonstrated expression of PR3 on human endothelial cells. Additionally, they demonstrated a time-dependent translocation of PR3 from the cytoplasm to the cell membrane after the endothelial cells were treated with cytokines. PR3 would thus be exposed to c-ANCA.

In another study, Savage et al. [8] demonstrated that MPO can bind to human endothelial cells, that it remains antigenic and enzymatically active once bound, and could interact with hydrogen peroxide causing endothelial-cell detachment. Cytotoxicity towards endothelial cells in the presence of complement was enhanced when the endothelial cells were precoated with MPO. They postulated that after activation of neutrophils by either p-ANCA or c-ANCA, release of granular contents including PR3 and MPO would occur, and these cationic species would then bind to the anionic surface of the glomerular endothelial cells because of their size and charge selective properties. Circulating ANCA would then preferentially bind to these glomerular bound antigens, enzymatically induce detachment of these cells from the substratum, and fix complement, furthering the vascular injury. The demonstration of MPO within the glomerular basement membrane in p-ANCA positive crescentic glomerulonephritis is compatible with this hypothesis [9].

Our results tend to support either of these latter two hypotheses. We demonstrated a linear staining pattern along the glomerular capillary wall when patient’s serum, and both anti-GBM negative control p-ANCA and c-ANCA sera were incubated with sections of her kidney. There was no staining of the normal kidney sections. These findings suggest that both p-ANCA and c-ANCA are capable of directly binding to glomerular basement membrane in our patient with ANCA-positive vasculitis, and can potentially lead to renal injury by enzyme-induced endothelial cell detachment or complement fixation as postulated by Savage et al. above.

We suspect that this binding may be at least in part related to the charge structure of the individual’s basement membrane and this may explain why not all patients develop renal disease in the presence of circulating ANCA. This conclusion is supported by the fact that when patient’s serum, or control ANCA positive sera, were incubated with normal kidney tissue binding to the glomerular basement membrane did not occur.

The pattern of staining of our specimens closely resembled that of anti-GBM disease. In a study by Bosch et al. [10] ANCA was detected in 32% of sera from patients with known anti-GBM disease. Although the role of ANCA is unclear under these circumstances, it has been suggested that it may play a contributory role in the pathogenesis of anti-GBM disease. These autoantibodies may both be primarily produced, or alternatively primary tissue injury by ANCA can lead to exposure or release of the Goodpasture antigen and secondary anti-GBM antibody formation [11,12]. In our patient anti-GBM was negative by both immunofluorescence and radioimmunoassay; however, it can be argued that the assays used were not sufficiently sensitive to detect such antibodies. Demonstration of similar linear staining along the capillary basement, when either patient’s serum or control ANCA positive/anti-GBM negative sera were used, would tend to
argue against such assumption. Inability to demonstrate a linear staining pattern with normal control serum would rule out non specific bonding to the glomerular basement membrane.

In conclusion, ANCA binding to the glomerular basement membrane as a potential pathogenic mechanism of renal injury was demonstrated in our case report, however, whether this same mechanism is involved in other patients with ANCA-positive crescentic glomerulonephritis remains to be determined in future studies.

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References


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