Does the presence of ANCA in patients with ulcerative colitis necessarily imply renal involvement?

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Abstract

Background. ANCA are thought to play a pathogenic role in renal vasculitis. ANCA may also be detected in patients with diseases not usually associated with renal pathology, such as ulcerative colitis. Our study was conducted to determine if the presence of ANCA in patients with ulcerative colitis is associated with renal pathology.

Methods. Eight ANCA-positive and five ANCA-negative patients with a histological and endoscopic diagnosis of active ulcerative colitis were investigated. Repeated complete urinalyses and determination of microalbuminuria and creatinine clearance were performed. Serum IgG and IgA ANCA were evaluated in all patients by indirect immunofluorescence and ELISA, and when detected the antibodies were further characterized by alpha granules preparation, myeloperoxidase, lactoferrin, and cathepsin G.

Results. In both ANCA-positive and ANCA-negative patients renal function was normal or near normal and urinalyses (including microalbuminuria) failed to disclose any abnormalities. ANCA exhibited a perinuclear pattern in all ANCA-positive patients. Interestingly, none of the ANCA-positive patients had antibodies to myeloperoxidase or to alpha granules which are usually found in the sera of patients with ANCA-associated vasculitis, and only one had antibodies to lactoferrin. The ANCA specificity remained undetermined in the remaining seven patients. At the end of the 1-year observation period, all ANCA-positive patients remained ANCA-positive without developing symptoms, signs or laboratory abnormalities consistent with renal involvement.

Conclusions. Renal damage was not observed in ANCA-positive patients with ulcerative colitis even after 1 year of follow-up, suggesting that the ANCA found in these patients do not share the antigenic targets with the ANCA commonly found in renal vasculitis. Therefore the potential of ANCA of inducing renal lesions (if any) is dependent on their own antigenic specificity.

Key words: vasculitis; myeloperoxidase; alpha granules; IgG ANCA; IgA ANCA

Introduction

Antinuclear cytoplasmic antibodies (ANCA) are found in a spectrum of diseases ranging from renal-limited processes (i.e. necrotizing glomerulonephritis) to widespread systemic vascular inflammation including polyarteritis nodosa and Wegener's granulomatosis [1-3]. These autoantibodies are thought to play an important role in the pathogenesis of renal vasculitis. This is suggested by recent experimental studies [4-7] and by the clinical evidence that their titre correlates with the disease course [8-9] and rises before clinical relapse [10]. However, in the last few years, ANCA have been detected in diseases not typically associated with renal involvement [4,11-17]. As a consequence of their pathogenic potential, one might expect that the presence of ANCA would result in renal vasculitis even in diseases not generally associated with a renal disorder. We investigated this hypothesis by evaluating renal laboratory parameters in ANCA-positive patients affected by ulcerative colitis.

Subjects and methods

Patients

Seventeen patients, less than 65 years of age, with clinical, endoscopic and histological diagnosis of ulcerative colitis were initially included in the study. All patients were tested for serum ANCA. The clinical history and the laboratory investigations from these patients were carefully reviewed in order to exclude those with conditions (other than vasculitis) known to cause renal insufficiency and/or urinary abnormalities. Four patients had a history consistent with nephrolithiasis (n=2, both ANCA negative) or moderate to severe

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arterial hypertension \( (n = 2, \text{both ANCA negative}) \), and therefore only 13 patients were selected for the study.

**Laboratory investigations**

All patients underwent complete routine laboratory investigations. Renal investigations included repeated analysis of midstream urine and determination of creatinine clearance and microalbuminuria (i.e. albuminuria in the range of 15–150 μg per min). Serum ANCA were evaluated in all patients.

**Analytical techniques**

Urinalyses were performed according to Miller [18]. The patients were prepared by overnight abstention from fluids. Only the samples with a specific gravity greater than 1014 were examined because a negative test on a dilute sample does not exclude renal disease. Glucose, protein, haemoglobin, ketone bodies, bilirubin, nitrates, and pH were evaluated with Multistix (Ames). The urinary sediment was examined after centrifugation of the samples for 5 min at 1500 r.p.m. The entire pellet was placed on a slide under a coverslip. Casts were counted in the entire sample and cells were counted in 10 vertical and 10 horizontal high-power fields \((40 \times)\). The following values were considered as normal: white blood cells: 0–4; red blood cells 0–4; casts: 0–60 [18]. Microalbuminuria was detected by a turbidimetric method (Cobas Miraplan Roche, Italy).

Serum ANCA were determined as follows. Sera of patients and controls were obtained and stored at \(-20 \degree C\) until assay. Control sera were obtained from 20 unaffected blood donors.

The sera were tested for ANCA by enzyme-linked immunosorbent assay (ELISA). Normal neutrophils were isolated from peripheral blood of healthy donors by Ficoll-Hypaque (Pharmacia, Sweden) (specific gravity 1.080) density centrifugation followed by dextran (Pharmacia, Sweden) sedimentation for 70–80 min. The supernatant containing the neutrophils was separated and washed, and the red blood cells were removed by hypotonic lysis. A monolayer of 200 000 neutrophils was air dried, fixed by ethanol, air dried again, and blocked with 0.25% bovine serum albumin in phosphate-buffered saline, pH 7.38. Sera were tested at a 1:100 dilution and bound antibodies were detected with alkaline phosphatase conjugated antihuman IgG (IgG-ANCA) and anti-human IgA (IgA-ANCA) (Sigma, Italy).

The test was considered positive (i.e. the sample contained ANCA-positive patients respectively). The titre of ANCA (expressed as optical density) averaged 0.63 ± 0.28 and 0.042 ± 0.02 in ANCA-positive and ANCA-negative patients respectively. The titre was not correlated with the severity of the intestinal disease (Table 2). The immunofluorescence examination of the sera fully confirmed the ELISA results, showing a perinuclear pattern in all positive cases.

All patients had normal renal function, creatinine clearance averaged 86 ml/min ± 7 and 90 ml/min ± 6 in ANCA-positive and in ANCA-negative patients respectively. The analyses of urine failed to disclose any abnormalities. Specific gravity averaged 1.020 (range 1.016–1.030). In all cases pH was below 7. The urinary erythrocyte counts averaged 1.3 ± 0.3 and 0.8 ± 0.4 per high-power field in ANCA-negative and ANCA-positive patients respectively. The dipstick test was negative in all patients. Microalbuminuria was not detected in any patient.

**Follow up**

All patients were followed up in our clinic for 1 year. They underwent quarterly physical examinations, routine laboratory investigations, and assessment of the activity of ulcerative colitis.

**Statistical analysis**

Unpaired Student's t-test and Fisher's exact test were used as appropriate to compare ANCA-positive and ANCA-negative patients. The data are expressed as means ± SD.

**Results**

**Basal control**

The clinical data of the patients are summarized in Table 1. The intestinal disease was categorized according to the Truelove's criteria [24]. The sera from eight patients were positive for IgG-ANCA. There was no discrepancy between the ELISA and IFI tests. None of them had antibodies to proteinase-3 or myeloperoxidase and only one had antibodies to lactoferrin. The titre of ANCA (expressed as optical density) averaged 0.63 ± 0.28 and 0.042 ± 0.02 in ANCA-negative and ANCA-positive patients respectively. The titre was not correlated with the severity of the intestinal disease (Table 2). The immunofluorescence examination of the sera fully confirmed the ELISA results, showing a perinuclear pattern in all positive cases.

All positive samples on ELISA were examined by indirect immunofluorescence according to the standard procedure delineated at the first ANCA workshop [23].

**Table 1. Clinical and laboratory data of patients**

<table>
<thead>
<tr>
<th>ANCA+</th>
<th>ANCA-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (n)</td>
<td>8</td>
</tr>
<tr>
<td>Mean age (range)</td>
<td>36.8 (16–64)</td>
</tr>
<tr>
<td>Male/female (n)</td>
<td>6/2</td>
</tr>
<tr>
<td>RCI disease activity (n)</td>
<td></td>
</tr>
<tr>
<td>Remission</td>
<td>3</td>
</tr>
<tr>
<td>Mild</td>
<td>4</td>
</tr>
<tr>
<td>Moderate</td>
<td>1</td>
</tr>
<tr>
<td>Severe</td>
<td>0</td>
</tr>
<tr>
<td>Unable to evaluate</td>
<td>0</td>
</tr>
</tbody>
</table>

*Laboratory data: No patients had Cl Cr < 70 ml/min, microhaematuria, proteinuria, cylindruria, or microalbuminuria.*
the ANCA detected in patients with ulcerative colitis or can be attributed to structural differences between biopsy). Therefore our observations do not support a role for ANCA in the pathogenesis of renal damage. In order to test this possibility, extrarenal diseases [11-17], no study has hitherto been able to detect late onset of renal vasculitis, since both clinical and laboratory signs of this disease can be very mild or absent in the prodromal phase. Although ANCA remained persistently positive during the follow-up, none of the ANCA-positive patients developed renal insufficiency or even minor urinary abnormalities (the absence of laboratory evidence of renal disease excluded any ethical indication for renal biopsy). Therefore our observations do not support a role for ANCA in the pathogenesis of renal damage or can be attributed to structural differences between the ANCA detected in patients with ulcerative colitis and those detected in patients with renal vasculitis [29-32]. The first possibility is quite unlikely. There is a growing body of evidence supporting the pathophysiological potential of ANCA in renal vasculitis. A recent study demonstrated that the lysosomal membrane glycosylation h-lamp-2 in neutrophil granulocytes and a 130 kDa membrane protein on the cell surface of renal microvascular endothelial cells are autoantigenic targets for ANCA in patients with necrotizing and crescentic glomerulonephritis [33]. Thus it is probable that the autoantibodies found in patients with renal vasculitis do not share this reactivity. Indeed, antibodies directed against myeloperoxidase and proteinase-3, usually detected in patients with renal vasculitis [34], but absent in our ANCA-positive patients—as well as in other series of patients with ulcerative colitis [31]—seem to be crucial to promote the ANCA-dependent cellular damage. Myeloperoxidase and proteinase-3, both of which are highly cationic, may bind non-covalently to the surface of endothelial cells [4,26-27]. In this situation they can be recognized and bound by ANCA, thus allowing further binding of ANCA, neutrophils and endothelium [35]. Neutrophil adhesion to ANCA-Fc portions is very important because it could (a) further activate neutrophils and cause direct injury of the endothelium, and (b) enhance complement-dependent cytotoxicity [4,35-38]. Therefore if ANCA have no specificity for myeloperoxidase and proteinase-3, as in our patients with ulcerative colitis, their pathogenic potential could be very low. The nature of the potential targets of ANCA in patients with ulcerative colitis or other extrarenal diseases has to be defined by more elaborate studies with techniques such as immunoblotting or immunoprecipitation.

In conclusion, no renal damage was observed during a 1-year observation period in ANCA-positive patients with ulcerative colitis, thus excluding a nephrotic role for the ANCA in this disease. This observation, however, does not conflict with other studies suggesting a role for ANCA in the pathogenesis of renal vasculitis, because the different antigenic specificity between ANCA found in renal vasculitis and those found in ulcerative colitis could fully account for their different nephrotic potential.

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