Relation of microscopic haematuria in ankylosing spondylitis to circulating IgA containing immune complexes

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SUMMARY Ankylosing spondylitis (AS) is associated with IgA nephropathy. To study the pathogenetic mechanism of this association the presence of haematuria and circulating IgA containing immune complexes (IgA ICs) in 70 patients with AS was determined. In this retrospective study haematuria was present in 15 patients and 25 patients had IgA ICs. Circulating IgA ICs were shown in 9/15 (60%) of the patients with haematuria and in 16/55 (29%) of those without haematuria. These results suggest that IgA ICs in patients with ankylosing spondylitis have a pathogenetic role in causing IgA nephropathy.

Key words: nephropathy, pathogenic role.

Haematuria has been observed in patients with ankylosing spondylitis (AS).^{1,2} Recently several authors reported that IgA nephropathy may be part of the disease spectrum of AS.^{1–8} Primary IgA nephropathy is strongly associated with the presence of circulating IgA containing immune complexes (IgA ICs).⁹ This raises the question of whether nephropathy in AS is also related to the presence of IgA ICs. Conflicting data have been reported on the occurrence of circulating immune complexes in the sera of patients with AS. These differences may be explained by the use of different techniques for the detection of ICs and by differences in disease severity of the patients studied.^{10–17} In this study the presence of circulating ICs was studied with the indirect polymorphonuclear phagocytosis test (IPPT) and the C1q binding assay (C1qBA). The IPPT test allows the detection of different types of IC with one procedure. Previous studies showed a good correlation between the results of the IPPT for IgG and IgA and those of the C1q binding assay and IgA inhibition assay.^{18–20}

We report on the associated presence of haematuria and circulating IgA ICs in a relatively large group of patients with AS.

Patients and methods

Seventy patients satisfying the modified New York criteria for AS²¹ were included in the study. All were patients attending our outpatient clinic over a period of four months, 11 were female. The mean age of the patients was 32 years (range 23–60) and the mean duration of disease after diagnosis was 13 years (range 1–40). The HLA-B27 antigen was present in 65/70 (93%) patients, 54 patients were treated with non-steroidal anti-inflammatory drugs (phenylbutazone in 29 cases, indomethacin in 14, propionic acid derivatives in nine, and tolmetin in two patients). Serum and urine samples were obtained simultaneously. The serum samples used were stored for less than three months and frozen and thawed only once.

Class specific ICs (IgG, IgA, and IgM) were measured by the IPPT^{18–20} and by a modified C1qBA.²² In the IPPT 350 µl of test serum was diluted with 400 µl 0.1 M borate buffered saline, and 6×10^6 polymorphonuclear cells from a single healthy donor were added. The mixture was incubated at 37°C for 90 minutes. The cells were then washed three times and smeared on glass microscope slides, fixed (95% ethanol/5% acetic acid for 15 minutes at –20°C), washed, and stained with fluorescein isothiocyanate (FITC) conjugated goat antihuman IgG (Fc), or FITC conjugated heavy

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chain specific rabbit antibodies against human IgM or human IgA. Slides were then examined by fluorescence microscopy, and the number of cells showing granular fluorescent inclusions was expressed as a percentage after counting 100 cells. The IPPT was defined as abnormal for samples exceeding the mean +2SD of 20 healthy donors. Association between haematuria and circulating IgA ICs was tested by χ^2 test with Yates's correction. Statistical significance regarding erythrocyte sedimentation rate (ESR) was calculated with the Mann-Whitney U test.

Results

Microscopic haematuria (more than five red blood cells per high power field) was found in 15 (21%) of the 70 patients with AS studied. One patient had 2+ proteinuria as measured by stick testing. No other patient had proteinuria, a history of renal disease, or a serum creatinine concentration in excess of 120 $\mu\text{mol/l}$. Two (13%) of these patients were female; all except one male patient were positive for HLA-B27.

The 55 patients without haematuria did not differ from the group with haematuria in age, duration of symptoms, and sex distribution. All had a serum creatinine concentration below 120 $\mu\text{mol/l}$ and none had a significant proteinuria.

The mean ESR in the patient group with haematuria was 25.1 mm/h (range 6–54). This was significantly higher than the 15.3 mm/h (range 1–44) in patients without haematuria ($p=0.02$).

IgA ICs were detected in 25 (36%) of patients with AS, IgG containing ICs in six (9%), and one patient had IgM ICs as measured with the IPPT. The results of the ClqBA were available from 40 patients. The serum of one patient contained ICs as measured by the ClqBA. Circulating IgA ICs were detected in 9/15 (60%) of the patients with haematuria and in only 16/55 (29%) of the patients without haematuria. This difference was statistically significant ($p=0.03$). None of the six patients with circulating IgG ICs as measured by IPPT had IgA ICs and only one of them had haematuria.

Haematuria in the patients with AS studied was not related to current treatment with non-steroidal anti-inflammatory drugs (NSAIDs). The presence of haematuria in patients with AS treated with NSAIDs was also unrelated to any particular kind of drug or the duration of this treatment.

Discussion

These results show that there is an association between microscopic haematuria and circulating IgA ICs in patients with AS. Renal involvement in patients with AS includes amyloidosis²³ and NSAID

related nephropathies such as papillary necrosis and tubulointerstitial nephritis.^{24 25} Older histological studies found glomerulonephritis with renal vasculopathy in AS, and more recent studies report the presence of IgA nephropathy.^{1–8 26}

We were not able to provide histological confirmation of the renal disease in the patients with AS studied. All had normal renal function and kidney biopsies were not performed. These patients, however, had no other diseases known to affect the kidney or evidence of urinary tract disease. The absence of marked proteinuria does not favour the presence of amyloidosis. The absence of a relation between haematuria and NSAID treatment, also found in an earlier study on patients with rheumatoid arthritis,¹ makes NSAID related nephropathies less likely. The high ESR in the patients with AS with haematuria suggests a more active disease than in those without haematuria. More clinical information on disease activity was not available in this retrospective study.

We found haematuria in 15/70 (21%) of patients with AS studied, which agrees with some reports^{2 27 28} but contrasts with others.^{27 28} The presence of IgA ICs in AS has been reported previously,²⁹ but the relation between haematuria and circulating IgA ICs in AS has not been studied before.

IgA nephropathy seems to be part of the disease spectrum of ankylosing spondylitis. In IgA nephropathy clinical and experimental data suggest that circulating IgA ICs lead to mesangial IgA deposits and tissue injury.³⁰ The fact that not all patients with haematuria had IgA ICs may be explained by findings in studies on patients with IgA nephropathy, which showed that these immune complexes can be present intermittently.⁹ Alternatively, the use of more assays available for the detection of IgA ICs may yield a higher percentage of patients with AS and circulating IgA ICs.

The association of IgA ICs and AS, and especially of IgA ICs and AS with haematuria, favours a similar pathogenetic process for the development of IgA nephropathy in a part of the AS population.

Further evidence for this hypothesis could be obtained from a prospective study on haematuria and circulating ICs in patients with AS which should include a full urological investigation, detailed analysis of the IgA containing ICs, and possible renal biopsies.

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