Determination of IgA- and IgM-rheumatoid factors in patients with rheumatoid arthritis with and without nephropathy

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Abstract

Objective—To clarify the characteristics and pathogenesis of renal disorders in patients with rheumatoid arthritis (RA).

Methods—In this study, 143 patients with RA were included, from whom 43 with urinary abnormalities were biopsied. Serum rheumatoid factor (RF) concentrations of IgA and IgM isotypes were also measured in these patients by enzyme linked immunosorbent assay.

Results—Light microscopy of renal biopsy specimens showed minor glomerular abnormalities in six patients, mesangial proliferative glomerulonephritis (GN) in 21, membranous nephropathy in seven, renal amyloidosis in seven, and tubulo-interstitial nephritis in two. Twelve patients with mesangial proliferative GN and one with minor glomerular abnormalities were found by immuno-fluorescence microscopy to have abnormalities consistent with IgA GN. Although the concentrations of IgA-RF in patients with IgA GN were slightly raised compared with those with glomerulopathy established by biopsy but not associated with IgA GN, the concentrations of IgA-RF were higher in patients with RA with vasculitis or interstitial pneumonia than those with RA complicated by IgA GN.

Conclusions—Mesangial proliferative GN, including IgA GN, may be a frequent renal lesion in Japanese patients with RA. IgA-RF may play little pathogenetic part in the development of IgA GN in RA.

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Renal amyloidosis1–3 and membranous nephropathy (MN) secondary to treatment with gold or D-penicillamine1–8 have been described as major complications of rheumatoid arthritis (RA). Some renal problems have also been associated with the use of non-steroidal anti-inflammatory drugs (NSAIDs), including interstitial nephritis, papillary necrosis, and nephrotic syndrome.9–13

Although glomerulonephritis (GN) had been thought to be rare in patients with RA,14 mesangial proliferative lesions have been recognised to be the most dominant glomerular abnormality as determined by light, electron, and immunofluorescence microscopy in recent studies.15–17 Accumulated clinical data from several studies have disclosed that the occurrence of isolated mesangio-pat h with RA is not associated with drug treatment.15 18

On the other hand, it has been postulated that rheumatoid factor (RF) may play some pathogenetic part in the development of some types of GN.19–22 More recently, immunological comparison of patients with RA with various forms of nephropathy with those without clinical renal disease showed no difference in RF and immune complex determinations.23

We studied the renal lesions of 43 patients with RA and urinary abnormalities by light, electron, and immunofluorescence microscopy. Concentrations of IgA and IgM isotype RF were also measured in serum samples from these patients to investigate the relation between class specific RFs and renal lesions such as IgA GN.

Patients and methods

Patients

Patients admitted to our clinic from 1979 to 1995 were included in the study. There were 143 patients, 38 men and 105 women, mean age at admission 55.4 (SD 12.6) years (range 20–84). The mean duration of RA before admission was 10.3 (8–7) years (range 6 months–53 years). All patients satisfied the 1987 revised criteria of the American College of Rheumatology for RA.24 Patients with RA who also had systemic lupus erythematosus, mixed connective tissue disease, or progressive systemic sclerosis were excluded from the study.

The radiological progression of RA was determined as advocated by Steinbrocker et al.25 The antirheumatic drugs used before the renal biopsy were surveyed retrospectively from the hospital charts. Intravenous urograms were performed in all biopsied patients and no serious urological abnormalities were detected. Urinary tract infection was ruled out by culture of midstream urine.

RENAL BIOPSY

Renal biopsy was performed because of clinical or laboratory evidence of renal disease, after obtaining each patient’s informed consent. Histological evaluations were performed by light, electron, and immunofluorescence microscopy. Light microscopic evaluation was
done after staining with haematoxylin and eosin, periodic acid Schiff, periodic acid methenamine silver (PAM), PAM Masson, elastica Masson, and Congo red. Immunofluorescence studies were performed on cryostat sections using fluorescein isothiocyanate conjugated antisera to human immunoglobulins (IgG, IgA, and IgM), complement (C3, C4, and C1q), and fibrinogen. In patients with renal amyloidosis, the potassium permanganate reaction was used to distinguish between primary and secondary amyloidosis. The presence of characteristic non-branching fibrils on electron microscopy confirmed the diagnosis of renal amyloidosis.

RENAL FUNCTION AND URINARY EXAMINATION
Renal function at the time of admission was determined from serum creatinine measurements. Loss of renal function was assumed when the serum creatinine exceeded 1-2 mg/dL. Haematuria was defined as >50 red blood cells per high power field on urinalysis. Proteinuria was defined as urinary protein excretion ≥0.3 g/24 h and nephrotic range proteinuria ≥3.5 g/24 h.

IMMUNOLOGICAL DETERMINATIONS
Serum immunoglobulins (IgG, IgA, and IgM) and serum complement factors (C3 and C4) were measured by laser nephelometry. Conventional RF activity was determined by turbidity immunoassay (TIA-RF). TIA-RF activity was defined as abnormal when the serum concentration exceeded 10 IU/ml.

MEASUREMENT OF CLASS SPECIFIC RFs
IgA-RF and IgM-RF were detected by enzyme linked immunosorbent assays (ELISA) as described by Bampton et al. with minor modifications. Briefly, rabbit IgG (R-IgG) was used as antigen and R-IgG (50 µl) at a concentration of 10 µg/ml in 50 mM sodium carbonate buffer pH 9-6 was added to Nunc ELISA microtitre plates. Wells containing only sodium carbonate buffer (50 µl) were prepared as controls. The plates were incubated overnight at 37°C, then washed three times with Tris-HCl buffer (0.15 M Tris, pH 7-6) containing 0-05% Tween 20 and 0-2% gelatin (washing solution).

After IgG coating, bovine serum albumin (20 mg/ml) in washing solution was treated for two hours at room temperature to block non-specific antibody adsorption. After washing three times, serum diluted 1:200 in washing solution was added to each well. The plates were incubated for six hours at 4°C and then washed three times. The peroxidase conjugated second antibody diluted 1:2000 in washing solution was added to each well and the plate incubated at 4°C for two hours. After washing three times with washing solution, 100 µl of substrate solution (o-phenylenediamine) was added. The solution was then incubated for 20 minutes, followed by addition of 100 µl 1 M H2SO4, IgA-RF and IgM-RF were measured in 61 patients and 27 normal controls.

The optical density was measured and the results were compared with the density of normal pooled sera as described by Powell et al. or Gioud-Paquet et al. The results were expressed as:

(mean OD with Ag – mean OD without Ag) test serum/(mean OD with Ag – mean OD without Ag) normal pooled sera

where OD = optical density; Ag = antigen.

Mean (SD) serum IgA-RF and IgM-RF concentrations in 27 normal controls were 1.06 (0.62) and 1.08 (0.51), respectively. The upper limits of the normal range for IgA-RF and IgM-RF were defined by the mean ± 2SD of the normal controls as 2.3 and 2.1 respectively. The same normal pooled sera were used for all assays.

Serum samples were stored at -70°C before use.

STATISTICS
Statistical analysis by Student’s t test and χ2 test was used to assess subgroup differences in age at renal biopsy, duration of RA, stage of RA, immunoglobulins, class specific RFs and TIA-RF, urinalysis findings, and serum creatinine.

Results
Renal biopsy was performed in 43 out of 143 patients. Table 1 shows the biopsy findings. The common nephropathy types included mesangial proliferative glomerulopathy in 21 patients, MN, and secondary amyloidosis in seven. Twelve of 21 patients with mesangial proliferative GN and one with minor glomerular abnormalities were classified as IgA GN, because the main glomerular immunofluorescence finding in the biopsy specimen was diffuse global IgA deposition. There was no significant difference between patients with mesangial proliferative GN and other renal histopathology groups for sex or the use of antirheumatic drugs; non-steroidal anti-inflammatory drugs (NSAIDs) had been used in every patient. Three of seven patients with RA with MN received no antirheumatic drug at any point.

Non-renal extra-articular manifestations, including vasculitis and interstitial pneumonia, were recognised in 23 patients. The histological diagnosis of secondary amyloidosis was made from biopsies of the rectum, upper

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Renal histological classification by light microscopy</th>
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</thead>
<tbody>
<tr>
<td>Classification</td>
<td>Patients (n)</td>
</tr>
<tr>
<td>Minor glomerular abnormality</td>
<td>6</td>
</tr>
<tr>
<td>Mesangial proliferative GN</td>
<td>21</td>
</tr>
<tr>
<td>Amyloidosis</td>
<td>7</td>
</tr>
<tr>
<td>Membranous nephropathy</td>
<td>7</td>
</tr>
<tr>
<td>Tubulointerstitial nephritis</td>
<td>2</td>
</tr>
<tr>
<td>No histological evaluation</td>
<td>100</td>
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α-PC = α-penicillamine.
Values are numbers of patients.
gastrointestinal tract, or abdominal wall fat in 19 other patients who underwent renal histological evaluation. Renal disorders were recognised without histological examination in 12 other patients.

According to clinical and histological findings, the patients were divided into six subgroups: (a) patients with IgA GN (IgA GN; n = 13); (b) patients with secondary amyloidosis (amyloidosis; n = 26); (c) patients with secondary amyloidosis (amyloidosis; n = 26); (d) patients with vasculitis or interstitial pneumonia (vasculitis; n = 23); (e) patients with MN (membranous) (n = 7); (f) patients without clinical renal manifestations (controls; n = 46). Patients with interstitial nephritis (n = 2) and patients with renal disease who underwent no histological evaluation (n = 12) were excluded from this study.

The vasculitis group and amyloidosis group were significantly older than the group with IgA GN but were not significantly different from the controls. The mean duration of RA, however, was significantly longer in the amyloidosis group than in the group with vasculitis, the membranous group, or controls. Further, the mean stage of RA was significantly more advanced in patients with amyloidosis than in any other group (table 2).

Proteinuria was commonly found in patients with amyloidosis (21/26; 81%) and the membranous group (6/7; 86%), but the incidence of proteinuria was relatively low in patients with IgA GN (5/13; 38%) or non-IgA GN (3/14; 21%). Nephrotic syndrome was recognised more often in patients with membranous (4/7; 57%) and amyloidosis (7/26; 27%). Definite haematuria was, however, often seen in patients with IgA GN (13/13; 100%) or non-IgA GN (12/14; 86%), therefore isolated haematuria was prevalent in patients with RA and mesangiotherapy. The incidence of haematuria in patients with amyloidosis was 50% (13/26). Renal dysfunction was often seen (19/26; 73%) in patients with amyloidosis; however, renal dysfunction was infrequent in patients of other groups (table 3).

Table 4 shows serum immunoglobulin concentrations. Mean serum IgG and IgA concentrations were significantly higher in the vasculitis group than in the group with amyloidosis. The concentrations of IgG were also higher in the vasculitis group than in the non-IgA GN group. Mean serum IgA concentrations in patients with IgA GN were comparable with those in other groups.

Table 5 shows the results of comparative studies of class specific and conventional RFs. Mean IgG-RF and IgM-RF concentrations were significantly higher in the vasculitis group than in any other group. The concentrations of TIA-RF were also significantly higher in the vasculitis group than in any other group except for the group with IgA GN. Also, mean IgA-RF concentrations were slightly higher in patients with IgA GN than in the non-IgA GN group.

Discussion
Renal failure has been thought to be one common cause of death in patients with RA, and histologically renal amyloidosis has been perceived as a main cause of renal failure in several studies. Prolonged inflammation accompanied by chronic polyarthritis has been considered to be responsible for the occurrence and progression of secondary amyloidosis in patients with RA. The present study confirmed the concept that patients with longstanding and advanced RA may be prone to secondary amyloidosis, and that secondary amyloidosis may be the major cause of renal failure in these patients, whereas the low IgG concentrations in this group were considered to be connected to the high frequency of profuse proteinuria.

Membranous nephropathy has also been described as a major complication in patients with RA, usually associated with the use of antirheumatic drugs such as gold or D-penicillamine. In our study, antirheumatic drugs were recognised as re-
IgA- and IgM-rheumatoid factors in patients with RA with and without nephropathy

Japanese patients was reported by Hiki et al.55 HLA-DR4 antigen is also thought to be related to the occurrence and progression of RA in several countries.56 57 Therefore, a common pathogenetic basis may exist that explains the concurrence of RA and IgA GN. However, because IgA GN is the most common primary glomerular disease in the Japanese population,55 58 the prevalence of IgA GN in Japanese patients with RA in our study may be related to the high frequency of IgA GN in the Japanese population. This may explain why Korpela et al.59 reported that the frequency of IgA GN in patients with RA was nearly equal to that seen in the general population.

In the present study, renal function was maintained in every patient with mesangio-

pathy except for one patient with IgA GN. Although primary IgA GN was thought to take a benign course, some cases did progress to end stage renal failure after 10 to 20 years.59 From their longitudinal study of patients with RA, Korpela et al.60 also showed that one out of five cases developed renal failure. Therefore, the clinical course of IgA GN in patients with RA may be similar to that of primary IgA GN.

In conclusion, renal histological evaluation showed that mesangial proliferative GN with mesangial IgA deposits was the most common type of nephropathy in Japanese patients with RA. Little correlation was found between mesangial glomerulopathy and the serum concentration of any class of RF. Further study should aim to clarify the role of IgA RF in the development of IgA GN in patients with RA. Although renal dysfunction was infrequent in our patients with RA and mesangial glomerulo-

pathy, careful prospective studies may be necessary for such patients because the natural course and importance of the disease combination are not fully understood.

10 Kleinschmidt D, Landais P, Goldfarb B. Analgesic and non-

11 Sandier D P, Burr F R, Weinberg C R. Nonsteroidal anti-