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IgG rheumatoid factors and anti-nuclear antibodies in rheumatoid vasculitis

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SUMMARY

We studied the distribution and characteristics of circulating rheumatoid factors (RF) and anti-nuclear antibodies (ANA) in 30 rheumatoid arthritis (RA) patients who had polyarthritis alone (group I), 28 RA patients with polyarthritis and extra-articular disease (group II), 28 RA patients with systemic vasculitis (group III) and 60 healthy matched controls. IgG RF occurred more frequently and in higher serum titres in group III (100%) than RA patients in group I (40%), or in group II (18%) or in normal controls (5·8%). The serum titre of IgM RF was higher in vasculitis patients than in other RA patients. ANA were found in 74% of all RA patients and although the frequency did not differ in the three patient groups, the serum titre was significantly higher in the vasculitis group. Antibodies to extractable nuclear antigen were found only in group III (18·7%). Antibodies to histones were also more prevalent in group III than in the other RA groups. The serological abnormalities in rheumatoid vasculitis differed quantitatively as well as qualitatively from other RA patients.

INTRODUCTION

Rheumatoid vasculitis is an uncommon but potentially serious extra-articular manifestation of rheumatoid arthritis (RA). In general, the patients have seropositive, nodular and erosive joint disease and often with severe functional class (Mongan *et al.*, 1969; Scott, Bacon & Tribe, 1981). In addition to the elevated serum titre of IgM rheumatoid factors (IgM RF), a number of other serological abnormalities has been associated with rheumatoid vasculitis. As a group they tended to have lower serum complement levels as compared to the general RA patient population (Mongan *et al.*, 1969; Franco & Schur, 1971). They have increased amounts of circulating immune complexes (CIC) measured by serum cryoglobulins and C1q binding assay (Erhardt, Mumford & Maini, 1979; Weisman & Zvaifler, 1975). IgG rheumatoid factors (IgG RF) and 7S IgM have also been found to be more prevalent in patients with rheumatoid vasculitis (Theofilopoulos *et al.*, 1974; Allen *et al.*, 1981; Scott *et al.*, 1981).

The vascular lesion probably results from the deposition of pathogenic antigen–antibody complexes in blood vessels of target organ such as the skin and peripheral nerves (Conn, McDuffie & Dyck, 1972; Rapoport *et al.*, 1980). The composition and nature of the immune complexes are not well understood, however, RF may be important in the formation of the immune complexes. It has not been entirely clear from previous investigations whether RA patients with extra-articular

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manifestations other than necrotizing vasculitis exhibit similar serological abnormalities to some degree. The purpose of our study was to examine the frequency and characteristics of RF and anti-nuclear antibodies (ANA) in patients with rheumatoid vasculitis and compare them to RA patients with articular disease only as well as to RA patients with other types of extra-articular manifestations.

MATERIALS AND METHODS

Patients. We have recruited 86 consecutive patients who fulfilled the American Rheumatism Association criteria for classical or definite RA (Ropes *et al.*, 1958). The patients were selected after a thorough clinical evaluation and review of medical records. The patients were divided into three groups.

Group I. This consisted of 30 RA patients who had clinically active polyarthritis and with no extra-articular disease.

Group II. This was a group of 28 RA patients with active polyarthritis and with evidence of extra-articular disease. The latter was defined as the presence of one or more of the following clinical manifestations: (a) subcutaneous nodules; (b) Sjögren's syndrome; (c) pulmonary lesions such as pulmonary fibrosis, pleural effusion; (d) Felty's syndrome.

Group III. This was a group of 28 patients with RA and systemic vasculitis. The patients had at least one of the following complications from RA: (a) cutaneous infarcts with or without skin ulcerations; (b) acute peripheral neuropathy; (c) peripheral gangrene; (d) scleromalacia perforans.

Sixty age and sex matched healthy human volunteers were also included as controls (group IV).

Twenty-three RA patients in group III received systemic corticosteroids in varying dosages for the vasculitis. Ten patients were on concomitant chrysotherapy. Two patients were treated with a cytotoxic agent for the vasculitis. One patient received d-penicillamine and another patient was given *N*-acetyl-cysteine.

Serological tests

Rheumatoid factors. IgG RF was measured by an indirect immunofluorescent test described in detail by Kallerup, Egeskjold & Grandal (1980). The method utilized rabbit IgG bound to sheep erythrocytes (SRBC) as antigen. Briefly, SRBC were washed with cold saline, fixed in formalin, washed and then stored as a 30% suspension in saline at 4°C. A smear of the SRBC was made on glass slides. A drop of purified rabbit IgG antibody to SRBC was added to sensitize the erythrocytes. The test serum was incubated with the cell smear for 30 min at room temperature. The slide was washed with phosphate-buffered saline 0·01 M pH 7·2 (PBS) and then reacted with fluorescein labelled monospecific rabbit antiserum to human IgG for 30 min. The slides were read with a Zeiss photomicroscope equipped with Ploem incident light illuminator. A positive reaction was indicated by bright green staining of the red cell membrane.

Before testing for IgG RF, each serum specimen was heat inactivated at 56°C for 30 min and absorbed with fresh washed SRBC. The IgM content of the serum was then inactivated by the addition of dithiothreitol (DTT). This procedure eliminated false positive reactions for IgG RF that may have arisen from the binding of non-RF IgG to IgM RF. Briefly, 100 μl of the serum was added to 200 μl of 0·01 M DDT in 0·66 M PBS pH 7·5 and allowed to incubate for 60 min at 37°C. The treated serum was diluted serially with PBS and tested for IgG RF.

Serum antibody to SRBC was prepared by immunization of rabbits with a suspension of SRBC following a schedule described by Garvey, Cremer & Sussdorf (1977). The IgG fraction of the antiserum was isolated by chromatography through DEAE Affi-Gel Blue using a procedure supplied by the manufacturer (BioRad Laboratories, Richmond, California, USA). The purity of the rabbit IgG was confirmed by immunoelectrophoresis against goat anti-rabbit whole serum and polyacrylamide gel electrophoresis. The protein concentration of the IgG anti-SRBC was 10 mg/dl

and was used at an optimal dilution of 1:10 which was determined by chessboard titeration. The $F(ab')_2$ fraction of the IgG antibody was prepared by pepsin digestion (Madsen & Rodkey, 1976).

Fluorescent anti-nuclear antibodies (FANA) were tested by an indirect immunofluorescent test using mouse kidney fixed in acetone as substrate (Quismorio & Friou, 1974). Antibodies to deoxyribonucleoprotein (anti-DNP) were measured by an indirect immunofluorescent spot test (Quismorio & Friou, 1974). The *Crithidia luciliae* immunofluorescent test was used to measure antibodies to double stranded DNA (Beaulieu *et al.*, 1979). Antibodies to ribonucleoprotein, Sm antigen and other extractable nuclear antigens were tested by a haemagglutination test and by Ochterlony test in agarose (Tan & Peebles, 1980). A PBS extract of rabbit thymus acetone powder (Pel-Freeze Biologicals, Rogers, Arkansas, USA) was used as the source of antigen. When a reference serum for anti-RNP antibody (supplied by Centers for Disease Control, Atlanta, Georgia, USA) was tested in our system, the antibody titre obtained was comparable to the known titre of the reference serum. Antibodies to histone were tested by an immunofluorescent test described by Fritzler & Tan (1978). Sera known to contain each of these different types of anti-nuclear antibodies were included in each test run.

Statistical analysis was performed with an Olivetti P652 microcomputer using χ^2 and standard *t*-test.

RESULTS

IgG RFs

The specificity of the immunofluorescent test for IgG RF was established by preliminary experiments. IgG rabbit antibody to SRBC but not normal rabbit IgG gave a positive reaction. When the $F(ab')_2$ fragment of the IgG rabbit to SRBC was used to sensitize the erythrocytes, the fluorescent reaction was abolished. Absorption of positive RA serum with heat aggregated human IgG covalently bound to Sepharose 4B beads inhibited the reaction of IgG RF with the antibody coated SRBC. In every test run, smear of unsensitized SRBC was included as a substrate to eliminate the possibility that the fluorescent reaction was due to the presence of antibody to SRBC in the test serum.

The prevalence of IgG RF in RA patients and healthy controls is shown in Table 1. Forty-four of the 85 RA patients (51·8%) had circulating IgG RF. In contrast, only three of the 52 control subjects were positive (5·77%). The prevalence of IgG RF in the three clinical groups of RA patients was also examined. IgG RF was found to occur more frequently in RA patients with vasculitis (100%) than RA patients in group I (40%, $P < 0.05$) or patients in group II (17·9%, $P < 0.05$).

Table 1. Prevalence of IgG and IgM rheumatoid factors and anti-nuclear antibodies in rheumatoid arthritis and controls

	Rhematoid arthritis				
Test done	Group I	Group II	Group III	Total RA	Normal controls
IgG RF	12/30* (40%)	5/28 (17·8%)	27/27 (100%)	44/85 (51·8%)	3/52 (5·77%)
IgM RF	29/30 (96·7%)	26/28 (92·5%)	27/27 (100%)	82/85 (96·5%)	n.d.†
Fluorescent ANA	20/30 (66·7%)	21/28 (75%)	22/27 (81·5%)	63/85 (74%)	4/60 (6·67%)

* Numerator = number of positive test; denominator = number of subjects tested; percentage in parenthesis.

† Not done.

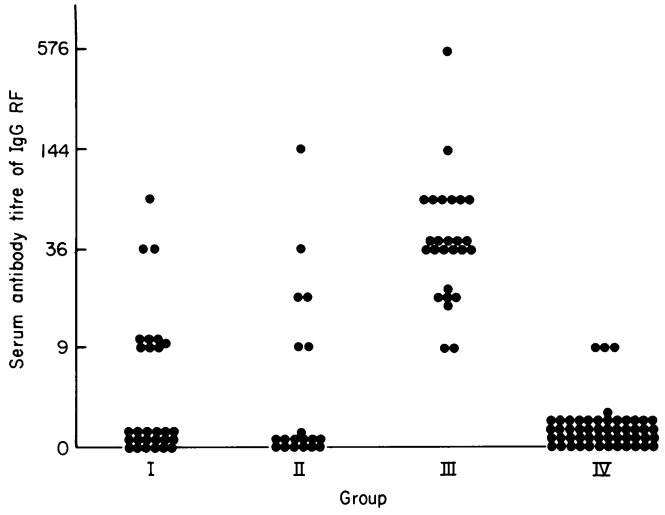

Fig. 1. Serum titre of IgG RF in three groups of RA patients and healthy controls.

The distribution of serum titre of IgG RF in the three groups of RA patients is depicted in Fig. 1. IgG RF titre ranged from 1:9 to 1:576 in the RA patients. In contrast, the three healthy controls who had a positive test had IgG RF titre of 1:9. When the three clinical groups of RA patients were compared, group III patients had significantly higher antibody titre than patients in group I ($P=0{\cdot}001$) as well as patients in group II ($P=0{\cdot}001$).

Latex fixation test for IgM RF

All patients except one in group I and another in group II had positive test for IgM RF as measured by the latex fixation test. The distribution of the serum titres is shown in Fig. 2.

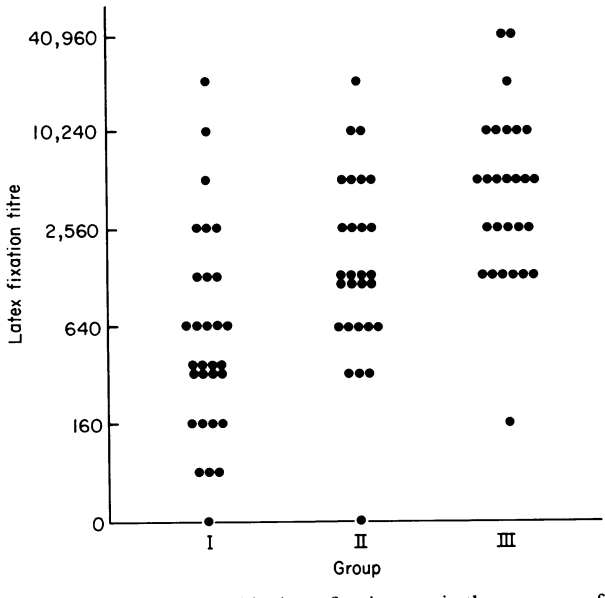

Fig. 2. Serum titre of IgM RF measured by latex fixation test in three groups of RA patients.

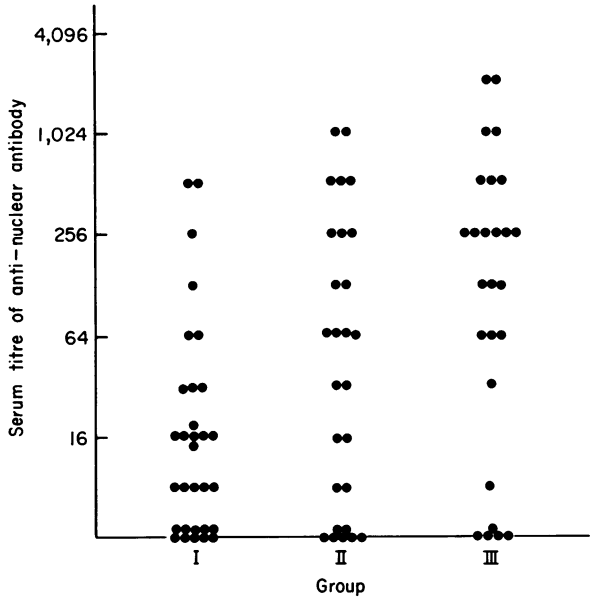

Fig. 3. Serum titre of fluorescent anti-nuclear antibodies in three groups of RA patients.

As a group, RA patients with vasculitis had significantly higher antibody titre (mean titre ± s.e.(mean) = 7,780 ± 743). Group I patients (mean ± s.e.(mean) = 1,800 ± 743) ($P < 0{\cdot}01$) as well as group II patients (mean ± s.e.(mean) = 3,074 ± 822) ($P < 0{\cdot}05$). In contrast, group I patients did not differ from group II patients in the serum titre of IgM RF ($P < 0{\cdot}1$).

Anti-nuclear antibodies

Sixty-three of the 85 RA patients (74%) had positive fluorescent test for ANA. In contrast, only four of 60 healthy subjects tested (6·7%) were positive (Table 1). The prevalence of ANA did not differ in the three groups of RA patients. ANA were found in 66·7% of group I patients, 75% of group II patients and 81·5% of the patients in group III.

The distribution of the serum titre of ANA in the three groups of patients is shown in Fig. 3. The titre ranged from 1:8 to as high as 1:2,048. When the three groups were compared, patients with vasculitis (group III) had significantly higher serum titre of ANA than group I patients ($P < 0{\cdot}01$) but was not different from group II patients ($P > 0{\cdot}1$). There was no difference in serum ANA titre between group III patients and patients in groups I and II combined.

Specificity of anti-nuclear antibodies (Table 2)

Antibodies to ENA were found in 18·5% of RA patients with vasculitis. In contrast, none of the patients in group I or group II were positive. The anti-ENA antibody was found to be reactive with ribonucleoprotein antigen. The haemagglutinating antibody titres ranged from 1:40 to 1:320. The agglutination reaction was abolished completely after ribonuclease digestion of the ENA coated erythrocytes. Moreover, one of five positive sera gave a precipitin reaction on double diffusion against ENA preparation. This showed a line of identity with a reference anti-RNP antiserum. None of the patients had detectable antibodies to double stranded DNA.

Antibodies to DNP were found in equal frequency in the three groups of RA patients. Forty-five per cent of patients in group I and half of the patients in group II as well as group III had antibodies to DNP.

Anti-histone antibodies were found in 10 of 13 patients (77%) with vasculitis. This was more prevalent than in patients in group I (40%, $P < 0{\cdot}05$), in patients in group II (37·5%, $P < 0{\cdot}05$), or both groups without vasculitis combined ($P < 0{\cdot}02$).

Table 2. The specificity of anti-nuclear antibodies in rheumatoid arthritis

ANA	Group I	Group II	Group III
Anti-ENA antibodies	0/11	0/14	5/27 (18·5%)
Anti-DNA antibodies	0/7	0/21	0/27
Anti-DNP antibodies	11/30 (36·7%)	14/28 (50%)	14/27 (50%)
Anti-histone antibodies	4/10 (40%)	3/8 (37·5%)	10/13 (77%)

Numerator = number of positive results.
Denominator = number of patients tested.

DISCUSSION

Theofilopoulos *et al.* (1974) described an association between the presence of IgG RF and the occurrence of vasculitis in RA. IgG RF was present in 67% of their patients with rheumatoid vasculitis and in only 9% of RA patients without vasculitis. Moreover, the serum titre of IgG RF was significantly higher in the former group compared to the latter group of RA patients. Our results using an immunofluorescent test for IgG RF as well as the data of other investigators using sensitive and quantitative radioimmunoassay tests (Carson *et al.*, 1977; Allen *et al.*, 1981) confirmed this association.

A wide variety of laboratory tests has been used to measure IgG RF in serum and joint fluid. Previous workers have found elevated serum level of IgG RF in seropositive RA, seronegative RA as well as in patients with other types of rheumatic disorders such as gout, ankylosing spondylitis (Howell *et al.*, 1972). The apparent ubiquity of IgG RF has raised questions as to its pathogenetic significance. However, it has been pointed out recently that the methods utilized to measure IgG RF in earlier studies lack specificity and sensitivity. To overcome deficiencies of these methods, Hay *et al.* (1975) developed a radioimmunoassay that measured IgG and IgM RFs. Pope & McDuffy (1979) used a modification of this method and found that IgG RF was present in the sera of the majority of patients with seropositive RA. In addition, they also presented data to indicate that the IgG RF values obtained in patients with seronegative inflammatory arthropathies were always low and that these results were due to non-specific adherence of non-RF IgG rather than a true antigen–antibody reaction. This does not imply that IgG RF is specific for seropositive RA because IgG RF has also been found in patients with infectious endocarditis (Carson *et al.*, 1978).

Seventy per cent of RA patients without vasculitis in the present study had positive ANA. The reported prevalence of ANA in RA has been variable in several published series ranging from 10% to 70% (Aitchenson *et al.*, 1979). The variability in prevalence is probably due to multiple factors including patient selection and technical factors such as type of substrate used in the test, characteristics of the fluorescent reagents, etc.

Extra-articular manifestations such as nodules, keratojunctivitis sicca, Felty's syndrome and vascular lesions have been found to be more common among RA patients with positive ANA (Ward *et al.*, 1964). Although the prevalence of ANA in our patients with vasculitis did not differ from RA patients without vasculitis, the antibody titre was significantly higher in the former group compared to the latter. Theofilopoulos *et al.* (1974) reported that while 13% of their patients with vasculitis had positive ANA, only 6% of RA patients without this complication had ANA. In contrast to these results, Mongan *et al.* (1972) found only one of 13 patients with RA vasculitis had positive ANA.

Our study showed that the antigenic specificities of the ANA in patients with vasculitis differed in certain respects from the ANA seen in RA patients without vasculitis. Anti-ENA antibody was detected only in RA patients with vasculitis. The agglutinating antibody titres were significantly lower than those that we have seen in patients with mixed connective tissue disease. Operationally, we called the antibody anti-RNP because of the inhibition of the agglutination reaction by ribonuclease digestion and the presence of precipitating antibody to RNP in one patient. Venables, Erhardt & Maini (1980) found a high prevalence of antibodies to ENA in RA especially in those patients with extra-articular disease. The majority of their patients sera reacted with ENA antigens which resisted ribonuclease digestion but sensitive to trypsin, thus different from what we saw in our patients. In contrast, Aitcheson *et al.* (1980) found anti-RNP and anti-Sm antibodies in 1·7% of their RA patients. It is not clear however, from their report whether patients with rheumatoid vasculitis were included in their study.

The variation in the prevalence of anti-ENA antibodies in different studies of RA patients is probably due to several factors. Antibodies to ENA constitute a very heterogeneous group of antinuclear antibodies. The frequency of each type of anti-ENA antibody in a disease state will depend partly on the sensitivity of the test system used, the source of antigen and the selection of patients. Until highly purified preparations of the different ENA antigens become readily available, many of the questions regarding their frequency as well as their significance will remain unresolved.

Our study shows that the serological abnormalities in patients with rheumatoid vasculitis differ quantitatively as well as qualitatively from RA patients with arthritis only as RA patients with other types of extra-articular manifestations.

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