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Analysis of antibodies to RNA in patients with systemic lupus erythematosus and other autoimmune rheumatic diseases

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SUMMARY

The frequency and clinical associations of anti-RNA antibodies measured by ELISA were assessed in 138 patients with systemic lupus erythematosus (SLE). Of the sera from these patients 9·4% had anti-RNA antibodies but no distinguishing features, clinical, serological or immunogenetic, between those with or without these antibodies could be identified. However, investigations of patients with other autoimmune rheumatic diseases did not reveal any anti-RNA positivity, which indicates a marked disease specificity for anti-RNA antibodies in SLE. The initial anti-RNA antibody screen used a soluble yeast extract as test antigen. The positive sera were further tested against a range of RNAs from 10 different types of rat tissue. In essence few differences were observed, suggesting that the anti-RNA response is directed against common, highly conserved epitopes.

Keywords autoantibody anti-RNA antibody SLE

INTRODUCTION

The serum of patients with systemic lupus erythematosus (SLE) is characterized by reactivity to a broad spectrum of nuclear and some cytoplasmic components. Amongst these antibodies those binding to DNA, Sm, RNP, Ro, La, histones and poly(ADP-ribose) have been extensively studied (Morrow & Isenberg, 1987). Some antibodies, notably anti-DNA and anti-Sm, show specificity for SLE (Tan *et al.*, 1982; Isenberg *et al.*, 1987), whilst others are associated with particular disease features. Thus antibodies to Ro are linked to subacute cutaneous lupus (Sontheiner *et al.*, 1982), neonatal lupus (Watson *et al.*, 1984) and 'antinuclear antibody negative lupus' (Maddison, Provost & Reichlin 1982). Anti-La antibodies in lupus patients are usually present in those with coexistent Sjögren's syndrome (SS) (Maddison *et al.*, 1988).

In contrast to these observations the presence of antibodies to RNA in SLE patients has been little studied and, in the published reports, the frequency of anti-RNA antibodies in patients with SLE has ranged from 17% to 80%. The published studies have used different methods and different sources of antigen. Thus the frequency of antibodies against RNA using counter-immunoelectrophoresis was 50% of lupus patients (Alarcon-Segovia *et al.*, 1975), by radioimmunoassay 30·7% (Eilat, Steinberg & Schechter, 1978), by ELISA 50–80% (Gripenber, Pirainen & Linder, 1981; Costa, Pimentel & Monier, 1985) and with the Ouchterlony immunodiffusion method 17·9% (Schur & Monroe, 1969).

We now report an analysis of the frequency and clinical and immunogenetic associations of anti-RNA antibodies in the largest group of SLE patients studied to date. Given a previous report (Alarcon-Segovia *et al.*, 1975) that 100% of scleroderma patients had these antibodies we also report the frequency of anti-RNA antibodies in patients with various autoimmune rheumatic diseases and healthy controls.

The positive sera were identified using yeast RNA and then subsequently tested against other RNAs from various sources to try to identify any differences in RNA epitopes from different tissues of a mammalian species (the rat).

MATERIALS AND METHODS

Serum

Test samples were taken at random from stored sets of serum from every lupus patient ($n = 138$) who had attended the lupus clinic at the Bloomsbury Rheumatology Unit for long-term follow-up. In addition, serial bleeds from several of the patients found to have raised anti-RNA antibodies were tested. Twenty-five patients each with rheumatoid arthritis, SS and scleroderma were used as disease controls. Eighty normal controls (50 female, 30 male) were used to establish a normal range.

Each of the lupus patients met four or more of the American Rheumatism Association's (ARA) revised criteria for the classification of the disease (Tan *et al.*, 1982). The patients with rheumatoid arthritis each met four or more of the revised ARA criteria (Arnett *et al.*, 1988). Those with primary SS were

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diagnosed by the criteria of Isenberg *et al.* (1984a). The scleroderma patients all met the preliminary criteria of the ARA (Masi, Rodnan & Medsger, 1981). Serial samples were selected from five lupus patients to investigate variation in anti-RNA expression, if any, with disease activity.

Amongst the SLE patients, disease activity was graded according to a previously published index (Isenberg *et al.*, 1984b). This has recently been validated with an activity index generated by a more detailed computer-based programme (Symmons *et al.*, 1988). This index was used to designate the patients as inactive (grade 1), mildly (grade 2), moderately (grade 3) or severely active (grade 4). In addition, the particular type of organ or system involved on a cumulative historical basis, namely renal, central nervous system (CNS), joint, skin, pleuropericardial and haematological was noted, having been defined according to criteria published elsewhere (Morrow *et al.*, 1982).

Antigen

The RNA used as screening antigen was a preparation of soluble yeast RNA (Sigma Chemical Co, Poole, UK). Subsequently, the positive sera were tested against mammalian RNA derived from the following rat tissues: brain, epididymis, heart, lactating mammary gland (MG), lung, testis, ovary, liver, spleen and kidney. These were kindly supplied by Dr Alison Moore (Department of Biochemistry and Molecular Biology, UCMSM) and prepared according to the method described by Maniatis (1989).

ELISA

Anti-RNA antibodies were measured by an indirect ELISA method. All diluents and washing solutions were sterilized by autoclaving prior to use. All reagents were added in 100 μl volumes per well. Flat-bottomed, 96-well polystyrene microtitre plates (Nunc Maxisorp plates) were coated with 50 μg/ml poly-L-lysine (Sigma) in phosphate-buffered saline (PBS), pH 7·2, for 1 h at 37°C. Plates were washed three times with PBS and half the plate was coated with 15 μg/ml yeast RNA (Sigma), whilst the other half of the plate was incubated with PBS alone. Where rat RNA was used for coating the plate this was used at a concentration of 7·5 μg/ml. This was done for all plates, which were then left at 37°C for 1 h. They were washed with PBS and blocked with 5% rabbit serum in PBS for 1 h at 37°C. Test and control sera were diluted 1/100 in PBS and added in duplicate to the plates, which were then left overnight at 4°C. These were washed three times with PBS-0·5% Tween (PBS-T) (Sigma) and incubated with a 1/1000 dilution of goat anti-human IgG-alkaline phosphatase (Sigma) for 1 h at 37°C. Unbound conjugate was removed by washing the plates with PBS-T and the substrate, dinitrophenyl phosphate (Sigma) in bicarbonate buffer, pH 9·6, added at 1 mg/ml. The plates were left at room temperature for 20 min before being read using an automated ELISA plate reader (Dynatech MR4000). Optical densities (OD) were measured at 410 nm. Final OD values were determined by subtracting the mean of duplicate readings obtained in the uncoated half of the plate from the corresponding mean values obtained when the plates were coated with RNA, and all the plates were run with the same positive and negative sera control to ensure the results were comparable. Test values were considered positive if they had an OD > mean + 2 s.d. of the mean OD value of 80 normal healthy control values, which were used to establish a normal range for the assay.

RESULTS

The anti-RNA ELISA developed and optimized in our laboratory (data not shown) was used to examine the incidence of anti-RNA antibodies in patients with SLE and other connective tissue diseases. Figure 1 illustrates the distribution of these antibodies in the different disease and control groups that we examined. Of the SLE patients 9·4% were found to be positive for anti-RNA antibodies. In contrast none of the other patients with SS, rheumatoid arthritis or scleroderma had raised levels. Only one normal control was found to exhibit high positive RNA binding.

The clinical manifestations in the population with SLE in general and the cohort with antibodies against RNA can be seen in Fig. 2. We did not find any statistically significant difference in clinical manifestations between the two groups by Chi square analysis. All the patients with anti-RNA were female, without predilection for any ethnic group, their mean age was 41·3 years. We did, however, observe some trends in the anti-RNA antibody-positive group, namely an increased predisposition to renal and cerebral disease but less photosensitivity. In the main these patients tended to have milder disease overall. Thus only one patient was graded 3 and the remainder 1 or 2.

The haematological manifestations in patients with anti-RNA antibodies are the same as in the population without these autoantibodies, as shown in Fig. 3.

The autoantibody, complement and disease activity profile of the anti-RNA-positive patients is shown in Table 1. Ten of

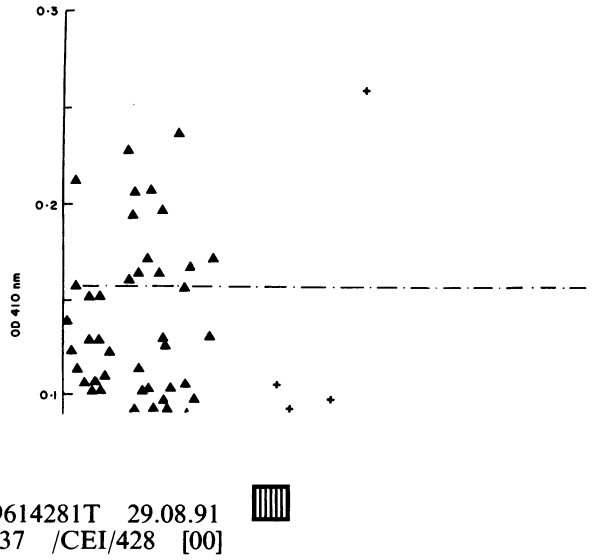

Fig. 1. Anti-RNA antibody level in patient and control sera by ELISA. Sera from systemic lupus erythematosus (▲) ($n = 138$); rheumatoid arthritis (■) ($n = 25$); scleroderma (○) ($n = 25$); Sjögren's syndrome (●) ($n = 25$) and normal healthy controls (+) ($n = 80$) were tested for anti-RNA antibody activity by ELISA. Each point represents a mean of duplicate values. Positive sera were identified as those with optical density (OD) values ≥ the mean OD + 2 s.d. of the normal control population. This is indicated by the horizontal line.

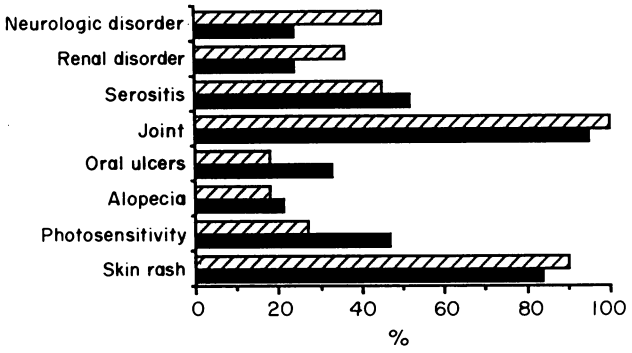

Fig. 2. Frequency of clinical manifestations in systemic lupus erythematosus (SLE) patients with anti-RNA antibody (▨) ($n = 13$) and the other SLE patients without anti-RNA (■) ($n = 125$). Bars represent the percentage of the patients who had these manifestations. We did not find any statistical significance in clinical manifestations between the two groups by Chi square analysis.

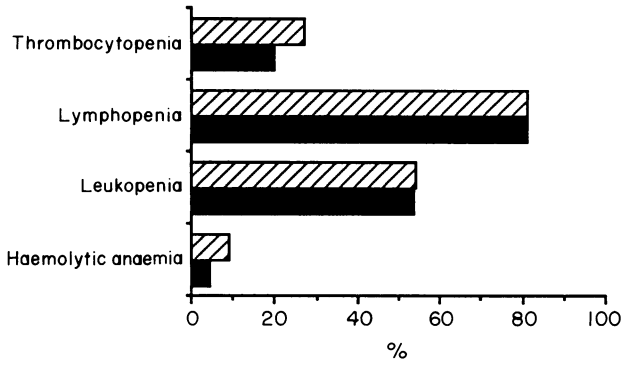

Fig. 3. Frequency of haematological manifestations in systemic lupus erythematosus (SLE) patients with anti-RNA antibody (▨) ($n = 13$) and the other SLE patients without anti-RNA ■ ($n = 125$). Bars represent the percentage of the patients who had these manifestations. We did not find any statistical significance in clinical manifestations between the two groups by Chi square analysis.

the patients with anti-RNA had high titres of anti-nuclear antibodies ($\leq 1:320$). In the anti-RNA-negative population the frequency of anti-RNP antibodies was 15%, anti-Sm 8%, anti-Ro 36%, anti-La 16% and anti-DNA 52% with 32% of patients exhibiting complement deficiency. In the anti-RNA-positive group 15·3% of patients had anti-RNP antibodies, 6% anti-Sm, 23% anti-Ro, 6% anti-La and 46% anti-DNA with 38% who were complement-deficient. The differences are not statistically significant.

Six patients with anti-RNA antibodies did not have a detectable rheumatoid factor (RF) or antibodies to Sm, RNP, Ro, La and DNA, and the complement levels were within the normal range. In contrast six patients had antibodies against DNA plus complement deficiency and only one patient had RF. Three patients had antibodies against Ro, and only one had anti-Sm and anti-RNP antibodies. Another patient had antibodies against RNP, Ro, La and DNA. The distribution of HLA phenotype did not distinguish the RNA antibody-positive and negative populations (data not shown).

Table 1. Serological profile of the anti-RNA-positive patients

Name	ANA	RF	Sm	RNP	Ro	La	DNA	C3	Activity grade
B.C.	1:320	1:160	(−)	(−)	(−)	(−)	(−)	N	2
M.J.	1:80	(−)	(−)	(−)	(−)	(−)	(−)	N	1
S.T.	1:2560	(−)	(−)	(−)	(+)	(−)	(+)	D	2
A.R.	1:40	(−)	(−)	(−)	(−)	(−)	(−)	N	1
F.L.	1:320	(−)	(−)	(−)	(−)	(−)	(+)	D	2
J.S.	1:160	(−)	(−)	(−)	(+)	(−)	(−)	N	1
P.A.	1:2560	(−)	(−)	(−)	(−)	(−)	(+)	D	1
A.O.	1:1280	(−)	(−)	(−)	(−)	(−)	(+)	D	2
J.C.	1:160	(−)	(−)	(−)	(−)	(−)	(−)	N	1
V.S.	1:1280	(−)	(+)	(+)	(−)	(−)	(−)	N	1
L.P.	1:40	(−)	(−)	(+)	(+)	(+)	(+)	D	2
J.A.	1:640	ND	ND	ND	ND	ND	(−)	ND	1
P.S.	1:640	ND	ND	ND	ND	ND	(+)	ND	3

The activity index (see text for details) was used to designate the patients as: 1, inactive; 2, mildly or 3, moderately active. N, normal, D, deficient; ND, not done; ANA, anti-nuclear antibodies; RF, rheumatoid factor.

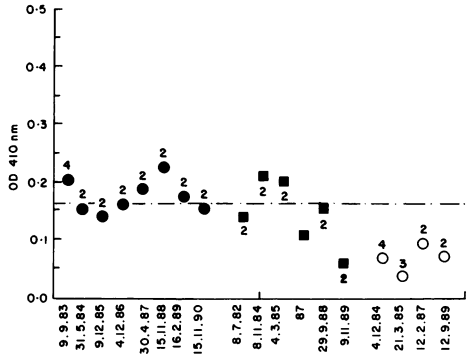

Fig. 4. Anti-RNA antibody level in serial samples of three systemic lupus erythematosus patients. Each point represents a mean of duplicate values on a given day. The activity index (see text for details) was used to designate the patients as 1, inactive, 2, mildly, 3, moderately or 4, severely active. ●, F.L.; ■, S.T.; ○, A.O.

Figure 4 shows the results of the anti-RNA antibody level in three patients with SLE who had raised anti-RNA antibodies in our screening test. We examined serial bleeds in several patients but we did not find any correlation between the grade of activity index and the anti-RNA antibody level. For example, in the first patient (F.L.) the antibody level on 9.9.83, when the patient was severely ill (with active glomerulonephritis and serositis), was lower than that on 15.11.88 when the clinic activity was rated as mild. Similarly in the other two patients shown in Fig. 4 there is no correlation between disease activity and antibody level.

Figure 5 shows the results of the sera tested for binding to RNA from different rat tissues. We observed strong recognition of RNA in one case. This patient demonstrated a very good response against all the types of RNA tested. Two other sera had failed to recognize RNA from testis and liver, one of which also

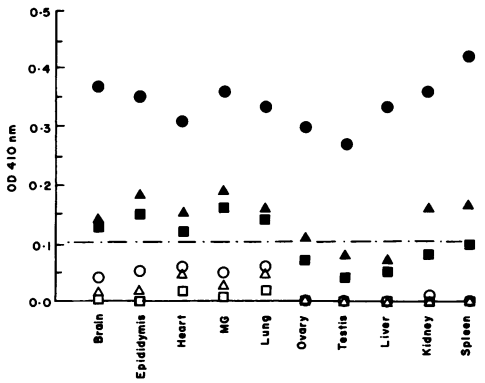

Fig. 5. Anti-RNA antibody binding to rat RNA in three lupus patients with anti-RNA (●, ▲, ■) and three healthy individuals (control) (○, △, □) tested for anti-rat RNA antibody activity by ELISA. Each point represents the mean of duplicate values. MG, Mammary gland.

failed to respond to ovary, kidney and spleen. However, all of the sera tested had a good response against RNA from brain, epididymis, heart, lactating MG and lung.

DISCUSSION

Using an ELISA constructed to remove the effect of non-specific background binding we have determined the frequency of the anti-RNA antibodies in sera from patients with autoimmune rheumatic diseases (SLE, scleroderma, rheumatoid arthritis and SS). Our results indicate that these antibodies are present in 9·4% of SLE but absent in the other diseases. It is possible that our results were low because we used RNA of yeast origin. There is some evidence to suggest that the frequency of antibodies against RNA depends upon the antigen used. Thus using ssRNA from calf liver one group reported that 50% of SLE patients and 100% of patients with scleroderma had anti-RNA antibodies as measured by counter-immunoelectrophoresis (Alarcon-Segovia & Fishbein, 1975). Other authors reported that 22% and 78% of patients with inactive and active SLE, respectively, were positive by ELISA using RNA of viral origin but all scleroderma patients were negative (Eilat *et al.*, 1978). Using yeast RNA Schur & Monroe (1969) did not find any anti-RNA response, but they found that 17·9% of the patients were positive using poly I.C. with the Ouchterlony immunodiffusion method.

There are some reports showing correlation between the occurrence of anti-RNA antibodies and disease activity (Koffler *et al.*, 1971; Eilat *et al.*, 1978; Koffler, Miller & Lahita, 1979; Stetler & Cavallo, 1987). However, in our study there was no correlation between the serological profile and activity (Fig. 4). Moreover our population with anti-RNA positivity had much the same clinical, haematological and immunological features as those SLE patients without these antibodies, though a trend towards increased frequency of the kidney and CNS involvement was observed.

Our results show that sera containing antibodies capable of recognizing RNA from yeast were also able to recognize RNA from different tissues of a mammalian species, the rat. We found a very good response in one patient against RNA from every type of tissue. Another serum showed little response to RNA from ovary, testis, liver, kidney and spleen whilst another did not react with RNA from testis and liver. Nevertheless, these observations imply that the antibodies recognize the same or similar epitopes in the RNA from different sources of considerably different origin.

Do anti-RNA antibodies in patients with SLE recognize a common epitope in different nucleotides or are there in fact several types of antibodies which recognize determinants unique to each nucleotide? Our results favour the first alternative; however, the possibilities are not mutually exclusive.

In conclusion, our findings show that anti-RNA antibodies exhibit a marked disease specificity for SLE. However, they do not offer a means by which different subsets of patients may be defined or the disease activity determined. These antibodies appear to be directed towards highly conserved epitopes on RNA, the exact nature of which remains to be elucidated.

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