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Measurement of antibody to poly (adenosine diphosphate-ribose): its diagnostic value in systemic lupus erythematosus

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SUMMARY

Poly (ADP-ribose) and dsDNA binding activity have been measured in sera from 61 patients with systemic lupus erythematosus (SLE) and 188 control sera from 20 normal individuals, 144 patients with clinically similar diseases and 24 patients with drug-induced anti-nuclear antibodies (ANA). Elevated poly (ADP-ribose) binding was not observed with normal sera. Five of 144 samples from diseases entering the differential diagnosis of SLE gave raised poly (ADP-ribose) binding compared with 12 in the ^{125}I-dsDNA binding. Only two of these false positive samples gave elevated binding in the ^{14}C-dsDNA assay. The apparent high specificity of the poly(ADP-ribose) assay was not observed with samples containing drug-induced ANA where 62% had elevated binding values. The frequency with which the poly(ADP-ribose) assay was positive with SLE sera (sensitivity) was lower than either of the dsDNA assays. This low sensitivity and the high rate of false positives in patients with drug-induced ANA limit the value of the poly(ADP-ribose) assay as a diagnostic test for SLE. However the restriction of poly(ADP-ribose) antibody to SLE and patients with drug-induced ANA together with the known role of poly(ADP-ribose) in DNA excision repair suggest that the antibody may be of fundamental significance.

Keywords poly(ADP-ribose) drug-induced anti-nuclear antibodies dsDNA systemic lupus erythematosus

INTRODUCTION

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease and sera from patients with this disease contain antibodies directed against a range of nuclear antigens (Tan, 1982; Bernstein & Hughes, 1983). Raised levels of antibodies against double stranded deoxyribonucleic acid (dsDNA) are almost exclusively confined to SLE (Hughes, 1975) and this is now one of the American Rheumatism Association's (ARA) classification criteria (Tan *et al.*, 1982). Antibodies to other nuclear antigens including single stranded (ss) DNA commonly occur in conditions that are clinically similar to SLE (Lange, 1978; Tan, 1982), hence the diagnostic value of the dsDNA antibody measurement is critically dependent on the purity of the dsDNA used as antigen and its freedom from single stranded regions (Lewkonia *et al.*, 1977).

Antibody to poly(ADP-ribose) was first reported in SLE sera by Kanai *et al.* (1977) and later studies (Okolie & Shall, 1979; Morrow *et al.*, 1982) suggested that measurement of this antibody

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might provide an alternative diagnostic test for the disease. The test was shown to have a specificity and sensitivity comparable to established dsDNA antibody tests but the variety of non-SLE diseases included in these studies was limited. Poly(ADP-ribose) is a naturally occurring polymer which is made up of repeating ADP-ribose units (Hayaishi & Ueda, 1977; Purnell, Stone & Whish, 1980) and although its precise biological function is still unclear it has been implicated in DNA excision repair (Durkacz *et al.*, 1980). The use of poly (ADP-ribose) as antigen would be of practical value as it can be reproducibly synthesized *in vitro* free of nucleic acid and nuclear proteins (Okolie & Shall 1979).

The major aim of this investigation was to examine the diagnostic value of measuring antibody to poly(ADP-ribose) in SLE, with particular emphasis on the specificity of the test when a wide range of sera from conditions clinically similar to SLE were tested. In addition, the sensitivity of the poly(ADP-ribose) antibody test was compared with two established assays for antibody to dsDNA.

MATERIALS AND METHODS

Serum samples. Serum samples were obtained from patients with the following clinical conditions; primary sicca (21), scleroderma (18), mixed connective tissue disease (eight), myositis (11), rheumatoid arthritis (41), juvenile chronic arthritis (25), chronic active hepatitis (10), primary biliary cirrhosis (10) and SLE (61—all fulfilling the revised ARA criteria). Samples from 24 patients with drug-induced ANA (ANA titre $\geqslant 1/64$ in immunofluorescence tests) were also examined. Eight of these patients had the drug-induced lupus syndrome and 16 were asymptomatic. Serum samples were also obtained from 20 normal healthy volunteers (10 male and 10 female) with an age range of 20–63 years. All samples were stored at $-20°C$ prior to testing.

Poly(ADP-ribose) antibody test. ^{3}H-poly(ADP-ribose) was prepared from ^{3}H-NAD with poly (ADP-ribose) polymerase from pig thymus by the method of Okolie & Shall (1979) and purified to remove nucleic acids and nuclear proteins. Poly(ADP-ribose) binding activity was measured using the Farr technique (Farr, 1958). A hundred and fifty-five microlitres of 0·01 M PBS pH 7·3 was added to 40 μl of serum sample which had been heat-inactivated at 56°C for 30 min. Ten microlitres of ^{3}H-poly(ADP-ribose) (approximately 5,000 ct/min) was then added and the reaction mixture was incubated for 1 h at 37°C followed by 48 h at 4°C. An equal volume (200 μl) of cold saturated ammonium sulphate was then added to precipitate antibody bound poly(ADP-ribose) and samples were left on ice for 30 min. Precipitates were washed twice with 50% saturated ammonium sulphate and resuspended in 450 μl of distilled water. After mixing with 3 ml of Lumagel (Lumac Systems Inc.) radioactivity was measured in an LKB Rack Beta Liquid Scintillation Counter. ^{3}H-poly(ADP-ribose) in the precipitate was expressed as a percentage of the total radioactivity added to each tube. A freeze dried positive reference sample which gave approximately 30% binding was included as a control in each run.

dsDNA antibody test. (I) Farr assay using ^{125}I-dsDNA. Antibodies to dsDNA antibody were measured with the DNA antibody kit obtained from Amersham International, UK using the procedure given by the manufacturer. The DNA binding obtained with test sera was calibrated against the reference sera provided (Holian *et al.*, 1975) and a level of 25 units/ml or more was taken as positive. (II) Farr assay using ^{14}C-dsDNA. The DNA binding activity of the SLE sera and selected non-SLE sera was also measured using a modified Farr assay (Pincus *et al.*, 1969). Fifty microlitres (containing 0·1 μg) of *E. coli* ^{14}C-DNA (Amersham International, UK) was added to 50 μl of a 1 : 10 dilution of serum in a 0·05 M borate buffer pH 8. The reaction mixture was incubated for 1 h at 37°C and then left overnight at 4°C. Antibody bound ^{14}C-DNA was then precipitated with 100 μl of saturated ammonium sulphate. After centrifugation, 100 μl of the supernatant was removed and added to 900 μl of borate buffer. The same volume of buffer was added to dissolve the precipitate. After addition of 10 ml of scintillation fluid the radioactivity of both the precipitate and supernatant was counted in a liquid scintillation counter. The percentage dsDNA binding was calculated as follows:

$$\frac{\text{ct/min precipitate} - \text{ct/min supernatant}}{\text{ct/min precipitate} + \text{ct/min supernatant}} \times 100.$$

A value equal to or more than 30% binding was considered positive.

Detection of soluble poly(ADP-ribose)–anti-poly(ADP-ribose) immune complexes. Selected sera were incubated with snake venom phosphodiesterase (Boehringer) to degrade poly(ADP-ribose) and then assayed to determine whether this had increased the poly(ADP-ribose) binding activity. The method followed was that described by Okolie & Shall (1979).

RESULTS

Validation of the poly(ADP-ribose) antibody test and derivation of the 'cut-off' value

The freeze dried reference serum was tested on nine occasions over a period of 6 weeks. The mean binding was 29·7% with a standard deviation of 2·4% and a co-efficient of variation of 8%.

Six selected SLE sera with a range of ^{14}C-dsDNA binding values and 10 non-SLE sera were tested in a preliminary study to determine the cut-off value and the results obtained are shown in Fig. 1. On the basis of these results samples give a value of equal to or greater than 20% binding were considered positive in the poly (ADP-ribose) antibody test.

Specificity of poly(ADP-ribose) antibody test

Sera from 20 normal blood donors and 144 patients with diseases clinically similar to SLE (total 164) were evaluated in the poly(ADP-ribose) and ^{125}I-dsDNA binding assays to determine the specificity of the former. The results obtained in each test system are shown in Fig. 2. The poly(ADP-ribose) binding assay was found to be more specific than the ^{125}I-dsDNA antibody test. Twelve out of 164 (7·3%) control sera gave elevated dsDNA binding whereas only five of these (3%) were positive in the test for antibody to poly(ADP-ribose). Three of the five samples giving false positives in the poly(ADP-ribose) antibody test were also positive in the ^{125}I-dsDNA antibody test; the remaining two were negative.

All samples giving false positive results (14) were coded and re-examined in the ^{14}C-dsDNA antibody test which is reputed to be a more specific test for SLE. Only two were positive in this assay, and these gave high binding values in all three test systems. The other false positive samples

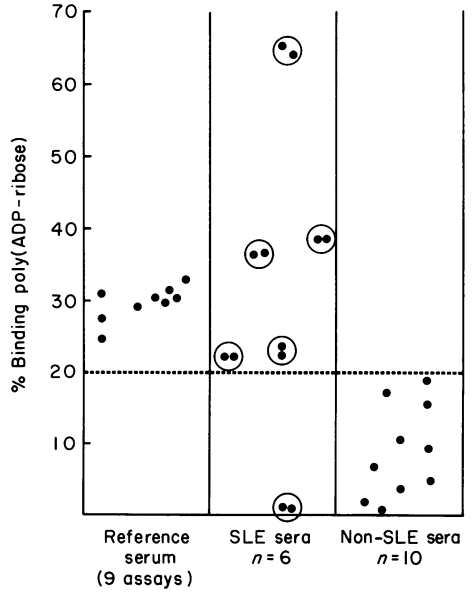

Fig. 1. Validation of poly(ADP-ribose) antibody test. Ringed values were obtained in assays performed on different days.

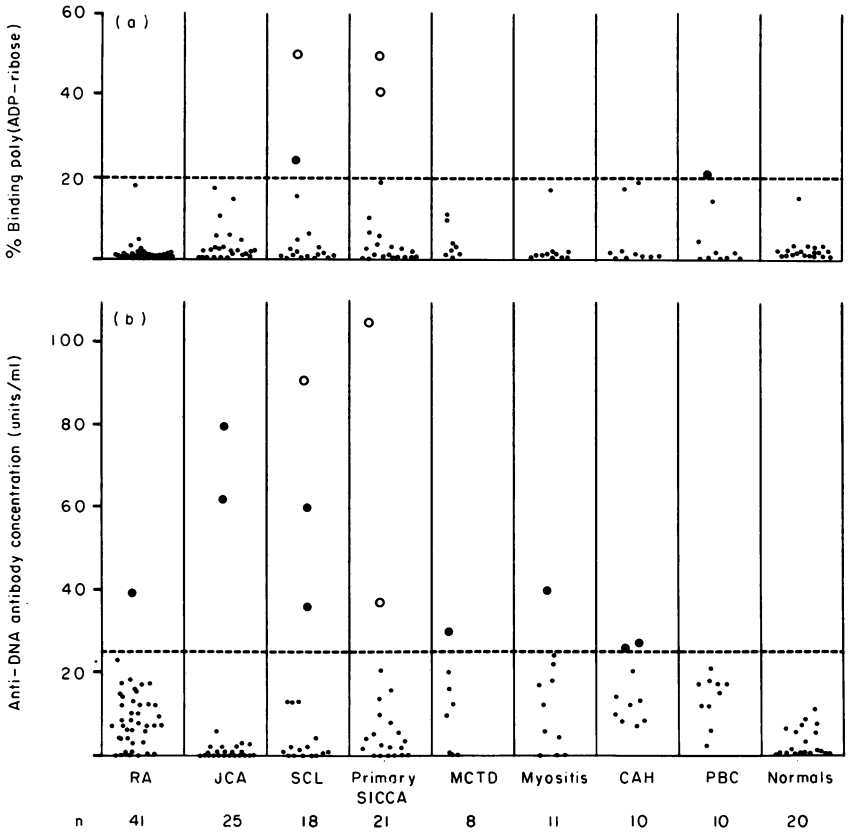

Fig. 2. (a) ^{3}H-poly(ADP-ribose) and (b) ^{125}I-dsDNA binding values in serum samples taken from 20 normal individuals and 144 patients with clinically similar diseases. Open circle = samples showing elevated values in both tests. RA = rheumatoid arthritis; JCA = juvenile chronic arthritis; SCL = scleroderma; MCTD = mixed connective tissue disease; CAH = chronic active hepatitis; PBC = primary biliary cirrhosis.

from patients with juvenile chronic arthritis, myositis, MCTD, primary sicca, scleroderma, chronic active hepatitis, primary biliary cirrhosis and rheumatoid arthritis were all negative.

Sensitivity of the poly(ADP-ribose) antibody test

As the poly(ADP-ribose) antibody test gave few false positive results on a comprehensive range of non-SLE sera and was more specific than the ^{125}I-dsDNA antibody test, we went on to examine its sensitivity as a test for SLE. Serum samples from 61 patients with SLE were tested in the poly(ADP-ribose) binding assay and the two tests for antibody to dsDNA antibody. The results are shown in Fig. 3. The frequency with which the poly(ADP-ribose) antibody test was positive in known SLE sera was less than that of either test for antibody to dsDNA. Forty-six (75%) and 43 (70%) of the sera were positive in the ^{125}I-dsDNA and ^{14}C-dsDNA antibody tests respectively whilst only 33 (54%) were reactive in the poly(ADP-ribose) antibody test.

The agreement between values obtained in the two dsDNA antibody tests was good but five samples gave discordant results. Four samples were positive in the ^{125}I-dsDNA antibody test alone and a further one sample was positive in the ^{14}C-dsDNA test only. The binding values of these samples were in the region of the cut-off levels. Thirty-nine of the SLE sera gave the same result in the ^{14}C-dsDNA and poly(ADP-ribose) antibody tests giving an overall agreement of 63·9% but there were two discordant groups which were positive in one test and negative in the other. Sixteen samples were positive in the ^{14}C-dsDNA antibody test and negative in the poly(ADP-ribose)

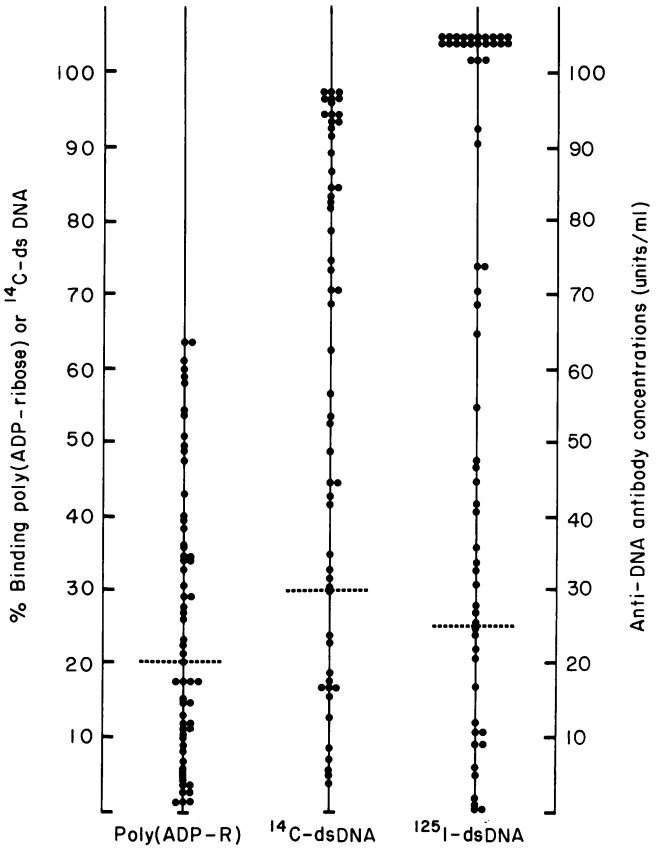

Fig. 3. Comparison of poly(ADP-ribose) and dsDNA binding values in 61 SLE patients.

antibody test. Seven of these gave ^{14}C-dsDNA binding levels in excess of 75% but only four came from patients with clinically active disease. The remaining nine samples gave ^{14}C-dsDNA binding values between 31 and 71%, five of these gave a binding of less than or equal to 45%. Six SLE samples were negative in the ^{14}C-dsDNA binding assay but were positive in the poly(ADP-ribose) binding assay. Only one of these patients had clinically active disease.

Ten of the 16 SLE samples which were negative in the poly(ADP-ribose) assay but gave elevated dsDNA binding, six SLE sera which had elevated values in both assays and 14 non-SLE sera were treated with snake venom phosphodiesterase to degrade antibody complexed poly(ADP-ribose) and then retested for poly(ADP-ribose) antibody. Minimal differences in the binding values of untreated and enzyme treated samples were observed excluding the possibility that immune complex formation was responsible for low poly(ADP-ribose) binding seen with some SLE sera.

Antibodies to poly(ADP-ribose) in patients with drug-induced ANA

Treatment with certain drugs is known to induce an illness with many features of SLE including ANA (Russell, 1981) but elevated dsDNA binding is rarely observed (Mansilla-Tinoco *et al.*, 1982). Sera from 24 patients who had developed ANA during therapy with hydralazine, penicillamine or prizidolol (an anti-hypertensive agent under trial) were examined in the poly(ADP-ribose) and ^{125}I-dsDNA antibody tests (Fig. 4). Eight of the patients had clinical features of the drug-induced lupus syndrome of whom only one (penicillamine-induced) had elevated ^{125}I-dsDNA binding. By contrast seven of the eight were positive in the poly(ADP-ribose) antibody test. None of the samples taken from asymptomatic patients gave elevated ^{125}I-dsDNA binding but eight (50%) were positive

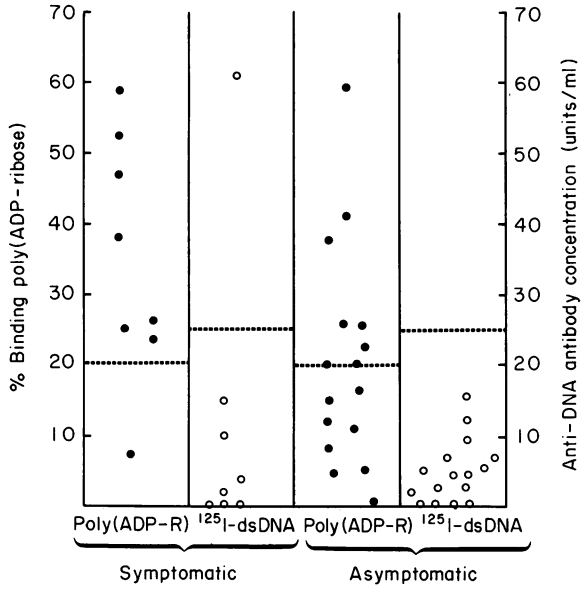

Fig. 4. Occurrence of antibodies to poly(ADP-ribose) and dsDNA in symptomatic and asymptomatic patients with drug-induced ANA.

in the poly(ADP-ribose) antibody test. To determine whether the elevated poly(ADP-ribose) binding was specifically related to administration of the drug, sequential samples from three patients on prizidolol were examined and the results are shown in Fig. 5. Samples taken prior to drug treatment gave low values in both tests. The levels of poly(ADP-ribose) antibody rose in all patients following drug administration, reaching positive levels in two. A decrease in response was seen in a patient in whom treatment was discontinued. A small rise in dsDNA antibody was detected in two of the three patients but levels remained in the negative range.

DISCUSSION

The detection and quantitation of antibodies to dsDNA is of considerable clinical value in the diagnosis and management of SLE (Hughes, 1975, Swaak *et al.*, 1982). The Farr assay using

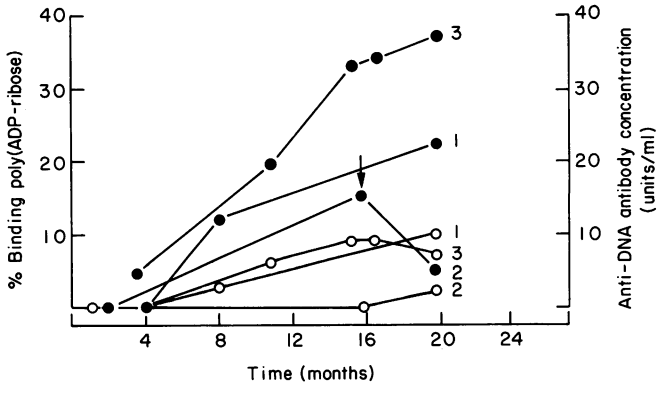

Fig. 5. Development of poly(ADP-ribose) and dsDNA antibody response following drug administration. ↓ Treatment withdrawn: Closed circle = poly (ADP-ribose) antibody; open circle = dsDNA antibody.

iodinated dsDNA is widely used for this measurement but the specificity for SLE is critically dependent upon the purity of the dsDNA preparation employed (Aarden, 1977). Contamination with other nuclear antigens or exposure of regions of ssDNA as a result of radiolysis (Aarden, Lakmaker & Feltkamp, 1976) can give rise to false positive results in conditions which are clinically similar to SLE (Lange, 1978).

Two reports have suggested that the measurement of antibody to poly(ADP-ribose) may provide an alternative discriminatory test for SLE (Okolie & Shall, 1979, Morrow *et al.*, 1982). Poly(ADP-ribose) can be reproducibly prepared and, unlike dsDNA, there is no evidence that new antigenic determinants are introduced through handling or storage. In the current study the specificity of poly(ADP-ribose) binding was examined initially with sera from 20 normal individuals and 144 patients who had diseases that enter the differential diagnosis of SLE. Twelve of these patients (7·3%) were positive in the ^{125}I-dsDNA binding assays compared with only five (3%) in the poly(ADP-ribose) antibody test. Hence the test using poly(ADP-ribose) was reactive with fewer samples taken from conditions clinically similar to SLE than the conventional test using ^{125}I-dsDNA. Three of the false positive results were common to both tests. One patient had primary sicca syndrome; another, who was elderly, had sicca syndrome with Raynaud's phenomenon and a stroke; the third had scleroderma involving the trunk and was unusual in that Raynaud's phenomenon was absent. Subsequent studies showed that antibody to poly(ADP-ribose) was present in a high proportion of sera containing drug-induced ANA suggesting that the poly(ADP-ribose) binding assay may be insufficiently specific in practice as drug-induced autoantibodies are not uncommon.

Although the comparison of the ^{14}C- and ^{125}I-dsDNA antibody tests was limited, the ^{14}C-dsDNA assay was found to be more specific for SLE. The greater specificity may be due to a lower frequency of single stranded regions in the antigen. Exposure of single stranded regions could result from greater radiation damage by ^{125}I gamma emissions than ^{14}C beta emissions (Aarden *et al.*, 1976).

Excluding our results with drug-induced ANA sera, the specificity of the poly(ADP-ribose) antibody test is rather better than that reported by Okolie & Shall (1979). These authors found that three out of 34 (7%) of non-SLE patients gave greater than 20% binding, the same cut-off as we have chosen. Morrow *et al.* (1982) using a lower threshold of 5% found that seven out of 117 (6%) of non-SLE sera gave positive binding values. We have observed a decline in binding after prolonged storage of the antigen (6 months) and this is related to cleavage of the poly(ADP-ribose) polymers into smaller units (unpublished data). The use of stored antigen could explain the low values in non-SLE sera reported by Morrow *et al.* (1982) as well as their compressed range of binding values in SLE.

The poly(ADP-ribose) and dsDNA binding values in our non-SLE patients were often higher than those found in the control group of normal healthy individuals. This binding is probably specific and due to low levels of antibody as, in the sequential study of patients in whom ANA were induced by prizidolol therapy, a rise in ANA titre was paralleled by a rise in both poly(ADP-ribose) and DNA binding.

The sensitivity of the poly(ADP-ribose) test was less than that of either dsDNA test: 54% for the poly(ADP-ribose) antibody test compared with 75% and 70% for the ^{125}I- and ^{14}C-dsDNA antibody tests, respectively. This result with poly(ADP-ribose) is close to the sensitivity of 51% reported by Okolie & Shall (1979) but their conclusion that the test was more sensitive was based on the finding that only 36% of 33 SLE patients were positive in the ^{125}I-dsDNA antibody test. Morrow *et al.* (1982) using a cut-off value of 5% binding found that 73% of SLE patients were positive in the poly(ADP-ribose) antibody test and only 58% of these were positive in the ^{125}I-dsDNA antibody test. In both these reports the frequency of anti-dsDNA antibodies in SLE is lower than in our study and in other published reports (Hughes, 1975; Griffiths *et al.*, 1977). It is conceivable that differences in the ^{125}I-dsDNA antigen used may account for these differing results.

Seven patients with SLE of varying clinical activity had high ^{14}C-dsDNA binding values, in excess of 75%, but poly(ADP-ribose) binding of less than 20%. Conversely elevated poly(ADP-ribose) binding with normal ^{14}C-dsDNA binding was observed in six SLE patients five of whom had clinically inactive disease. Low poly(ADP-ribose) binding in these sera was not due to the presence

of immune complexes. Recent work with murine monoclonal anti-DNA autoantibodies (Schwartz, 1982) has shown that they may react to a varying degree with phospholipids and polynucleotides due to a common phosphodiester linkage and that the range of lupus autoantibodies is more restricted than originally believed. In our study 13 of 61 (21·3%) SLE sera and 14 out of 24 (58·3%) of the sera from patients with drug-induced ANA gave highly discordant results in the two tests. This observation together with the failure of DNA addition to inhibit the measurement of poly(ADP-ribose) antibody (Okolie & Shall, 1979) suggest that the poly(ADP-ribose) and dsDNA tests are measuring antibody directed against different epitopes.

As a test for SLE the measurement of antibodies to poly(ADP-ribose) is more specific and less sensitive than the established dsDNA binding assays if patients with drug-induced ANA are excluded. High levels of poly(ADP-ribose) antibody have been demonstrated in patients with drug-induced ANA for the first time. This finding along with the observed restriction of antibody to SLE patients and those with drug-induced ANA together with the known role of poly(ADP-ribose) in DNA excision repair suggest that further work may establish a fundamental role for this antibody.

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