

## MEASUREMENT OF ANTI-DOUBLE-STRANDED DNA ANTIBODIES IN MAJOR IMMUNOGLOBULIN CLASSES

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A solid-phase radioimmunoassay for quantitating anti-double-stranded deoxyribonucleic acid antibodies (anti-dsDNA) in IgG, IgM and IgA classes has been devised. A distinct feature of the method is an application of polystyrene tubes coated with poly-L-lysine, through which dsDNA could be bound firmly to a solid phase. Studies on patients' sera as well as normal sera revealed that anti-dsDNA was not qualitatively but quantitatively characteristic of systemic lupus erythematosus (SLE) and that IgG anti-dsDNA levels correlated well with the disease activity.

### INTRODUCTION

Antibodies against double-stranded DNA (dsDNA) are thought to be characteristic of systemic lupus erythematosus (SLE) and are generally associated with the disease activity. Measurement of anti-dsDNA gives accurate diagnosis and close monitoring of the course of SLE. In recent years, using radioactively labeled DNA, sensitive techniques such as the ammonium sulfate precipitation method (Wold et al., 1968) and the Millipore filter assay (Ginsberg and Keiser, 1973) have been available for detection of dsDNA binding activity by serum. However, these methods have some disadvantages: they may detect non-specific DNA binding proteins (Aarden et al., 1975) and do not provide any information about immunoglobulin (Ig) classes of the antibodies. There have been accumulated evidences that, as compared with IgM anti-dsDNA, IgG anti-dsDNA is more characteristic of SLE, correlates better with the disease activity (Clough, 1977; Pennebaker et al., 1977) and plays a more important role in the pathogenesis of lupus nephritis (Koffler et al., 1967). Therefore, it is of value to quantitate anti-dsDNA of each Ig class. On the other hand, naturally derived native DNA used in assaying these antibodies are virtually always contaminated with small but immunologically significant amounts of single-stranded DNA (ssDNA) determinant. This may result in frequent occurrence of anti-native DNA antibodies in the diseases other than SLE as well as normals (Hasselbacher and LeRoy, 1974; Rochmis et al., 1974). Purification of native DNA on methylated albumin kieselguhr (MAK) column has been used for improving

the specificity of anti-dsDNA binding assay (Winfield and Davis, 1974; Samaha and Irvin, 1975). Recently, a synthetic polynucleotide, self-complementary alternating copolymer of deoxyadenylate and deoxythymidylate (dA-dT) was proposed to be antigenically equivalent to dsDNA while lacking significant contamination with single-stranded determinant (Steinman et al., 1977). Native DNA treated with endonuclease specific for ssDNA also appears to be ideal dsDNA, although care should be taken to avoid its denaturation during handling and storage.

In the present study, we have devised a solid-phase radioimmunoassay for quantitating anti-dsDNA in the major Ig classes. A distinct feature of this method is an application of polystyrene tubes coated with poly-L-lysine (PLL), through which dsDNA could be bound to a solid phase. After examining technical conditions, sera from patients as well as normals were assayed by this method.

## MATERIALS AND METHODS

### *Patients*

Nineteen patients with SLE, 1 patient with rheumatoid arthritis (RA), 5 patients with progressive systemic sclerosis (PSS), 3 patients with Sjögren's syndrome (SS), 1 patient with mixed connective tissue disease (MCTD) and 1 patient with lupoid hepatitis were subjected to this study. Patients with SLE satisfied the preliminary criteria for the classification of SLE (Cohen et al., 1971), a patient with RA was classical on ARA criteria (Ropes et al., 1958), patients with PSS met Medsger's criteria for PSS (Medsger and Masi, 1971). Sjögren's syndrome was ascertained by a sialography and/or a labial biopsy. MCTD was diagnosed according to Sharp's description (Sharp et al., 1972). Eleven patients with diseases other than SLE were selected on the basis of their having high dsDNA binding activities on the Millipore filter assay.

### *Disease activity score*

At the time sera were drawn, patients with SLE were assigned a disease activity score according to Pennebaker et al. (1977). It represented the sum of the following disease features then present: fever, alopecia, arthritis, serositis, leukopenia, abnormal urinary sediment, erythrocyte sedimentation rate greater than 50 mm/h and CH50 less than 20 units (normal range: 30—45 units). Disease activity score more than one was arbitrarily judged as active.

### *DNA preparations*

[<sup>14</sup>C]DNA was extracted, by the method of Marmur (1961), from nuclei of *E. coli* cultured in the presence of [<sup>14</sup>C]thymine, treated with ssDNA specific endonuclease (nuclease S1, Seikagaku Kogyo Co., Tokyo) and fractionated on MAK column chromatography by the method of Sueoka and

Cheng (1962). Fractions eluted with 0.05 M phosphate buffer, pH 6.7, containing 0.6 M NaCl were used as [ $^{14}\text{C}$ ]dsDNA, whose molecular weight was about  $100 \times 10^4$  as judged on CsCl gradient ultracentrifugation. Its specific activity was 1600 cpm/ $\mu\text{g}$ . [ $^{14}\text{C}$ ]dsDNA did not react with rabbit anti-ssDNA antibodies on the Millipore filter assay.

Calf thymus DNA (CT-DNA) was purchased from Boehringer Mannheim Co., G.F.R. Treatment with nuclease S1 followed by fractionation on MAK column, was carried out to obtain purified CT-dsDNA.

DNA labeled internally with  $^{125}\text{I}$  was obtained from Radiochemical Centre Co., U.K. and treated with nuclease S1 followed by fractionation on MAK.

#### *Millipore filter assay*

DsDNA binding activity by serum was determined with a modification of the Millipore filter method of Ginsberg and Keiser (1973). Five  $\mu\text{l}$  of serum inactivated at  $56^\circ\text{C}$  for 120 min were incubated at  $45^\circ\text{C}$  for 15 min with 195  $\mu\text{l}$  of 0.15 M Tris-HCl buffer, pH 7.6, containing 0.4  $\mu\text{g}$  of [ $^{14}\text{C}$ ]dsDNA. The reaction was stopped by addition of 3 ml of Tris-HCl buffer, and the mixture was passed through a prewetted Millipore filter (HAWP 02500) under gentle suction. After washing two times with Tris-HCl buffer and once with distilled water, the filter was dried, transferred to a glass scintillation vial and measured for radioactivity in a liquid scintillation counter. The result was expressed as per cent [ $^{14}\text{C}$ ]dsDNA retained on the filter.

#### *Immunoglobulins*

IgG was purified from human fraction II of a commercial source (Midorijuji Co., Japan) by DEAE-cellulose chromatography. IgM was prepared from euglobulins of serum from a patient with macroglobulinemia by Sephadex G-200 gel filtration. IgA was prepared from serum of a patient with IgA myeloma by the method of Vaerman et al. (1963). The preparations were tested for purity by Ouchterlony gel precipitation. The concentration of Ig in each preparation was measured by a single radial immunodiffusion method.

#### *Antibodies specific for IgG, IgM and IgA*

Antisera specific for IgG, IgM and IgA (anti-IgG, anti-IgM and anti-IgA, respectively) were purchased from Behringwerke AG, G.F.R. Globulins of antisera, obtained by rivanol fractionation and Sephadex G-25 gel filtration, were applied to Sepharose CL-2B coupled with purified Ig of the corresponding class. Specific antibodies were eluted with 0.5 M glycine-HCl buffer, pH 3.0, immediately mixed with 2.0 M Tris-HCl buffer, pH 8.0 and dialyzed against saline. Purified specific antibodies were then labeled with  $^{125}\text{I}$  by the method of Klinman and Taylor (1969).

#### *Buffers*

0.15 M Tris-HCl buffer, pH 7.6, containing 1% bovine serum albumin (BSA) and 0.1% Triton X-100, was used for incubation in the solid-phase

radioimmunoassay (incubation buffer). 0.15 M Tris-HCl buffer, pH 7.6, containing 0.1% BSA and 0.1% Triton X-100, was used for washing in the solid-phase radioimmunoassay (washing buffer).

*Poly-L-lysine (PLL)*

Poly-L-lysine hydrobromide, type IB, was purchased from Sigma Chemical Co., U.S.A.

*Coating polystyrene tubes with calf thymus dsDNA (dsDNA tubes)*

Polystyrene tubes (11 mm × 80 mm) were purchased from Eiken Kizai Co., Japan. The tubes were filled with 1 ml of 40 µg/ml PLL in Tris-HCl buffer. After 30 min at room temperature, the PLL solution was aspirated and the tubes were washed 3 times with Tris-HCl buffer (PLL tubes). One ml of 10 µg/ml CT-dsDNA in Tris-HCl buffer was next added to the tubes, while to the control tubes was added 1 ml of 10 U/ml heparin in Tris-HCl buffer. After 60 min's rotation at 37°C, the tubes were washed 3 times with Tris-HCl buffer and filled with 2 ml of Tris-HCl buffer containing BSA, i.e., incubation buffer, and rotated at 37°C for 60 min to coat any active sites of the tubes with BSA.

*Radioimmunoassay for anti-dsDNA in major Ig classes (standard assay)*

Two µl of test serum diluted in 1 ml of incubation buffer were added to each of 6 dsDNA-tubes and 6 control tubes. After incubation at 37°C for 120 min, the serum was aspirated and the tubes were washed 3 times with washing buffer. <sup>125</sup>I-labeled anti-Ig (about 10,000 cpm) in 1 ml of incubation buffer was next added so that 2 dsDNA tubes and 2 control tubes received each of specific anti-IgG, anti-IgM and anti-IgA. The tubes were incubated overnight at room temperature and washed 3 times with washing buffer. The radioactivities retained on the tubes were then measured in an auto gamma spectrometer. The results were corrected for non-specific binding of radioactivity by subtracting appropriate control counts and for dilution by multiplying the count by appropriate dilution factor. The amount of anti-dsDNA was expressed as µg/ml, using a conversion factor described in the following standardization of the assay.

*Standardization of the assay*

Purified IgG, IgM and IgA were labeled with <sup>125</sup>I by the method of Klinman and Taylor (1969). After sufficient dialysis, each of them was diluted and specific activity was determined. Three concentrations of each labeled Ig were coupled to polystyrene tubes, 4 of which were for each concentration. The tubes were washed 3 times with washing buffer and treated with incubation buffer to coat any active sites with BSA. To two of the tubes for each concentration was added <sup>125</sup>I-labeled anti-Ig in 1 ml of incubation buffer, while the other tubes (control tubes) were not exposed to <sup>125</sup>I-labeled anti-Ig. After rotating overnight at room temperature, the tubes were

washed 3 times with washing buffer. The amount of Ig coupled to the tubes was determined by radioactive counting of the control tubes. Calculation of specific anti-Ig binding by subtracting radioactivity of the control tubes revealed a conversion factor for each batch of labeled anti-Ig to estimate micrograms of Ig.

## RESULTS

### *PLL concentration used for coating the tubes*

Polystyrene tubes were filled with 1 ml of PLL solution of different concentrations in Tris-HCl buffer. After 30 min at room temperature, the PLL solution was aspirated and the tubes were washed 3 times with Tris-HCl buffer. One ml of [ $^{125}$ I]dsDNA (10  $\mu$ g/ml, 1500 cpm/ $\mu$ g) in Tris-HCl buffer was next added to the tubes. After 60 min rotation at 37°C, the [ $^{125}$ I]-dsDNA solution was aspirated and the tubes were washed 3 times with Tris-HCl buffer. The radioactivities retained on the tubes were then measured in an auto gamma spectrometer. As shown in Fig. 1, more than 5  $\mu$ g/ml of PLL were sufficient for dsDNA coating. For the assay, a concentration of 40  $\mu$ g/ml of PLL was chosen.

### *Antigen concentration used for coating PLL tubes*

One ml of [ $^{125}$ I]dsDNA solution of different concentrations in Tris-HCl buffer was added to PLL tubes. After 60 min rotation at 37°C, the [ $^{125}$ I]-

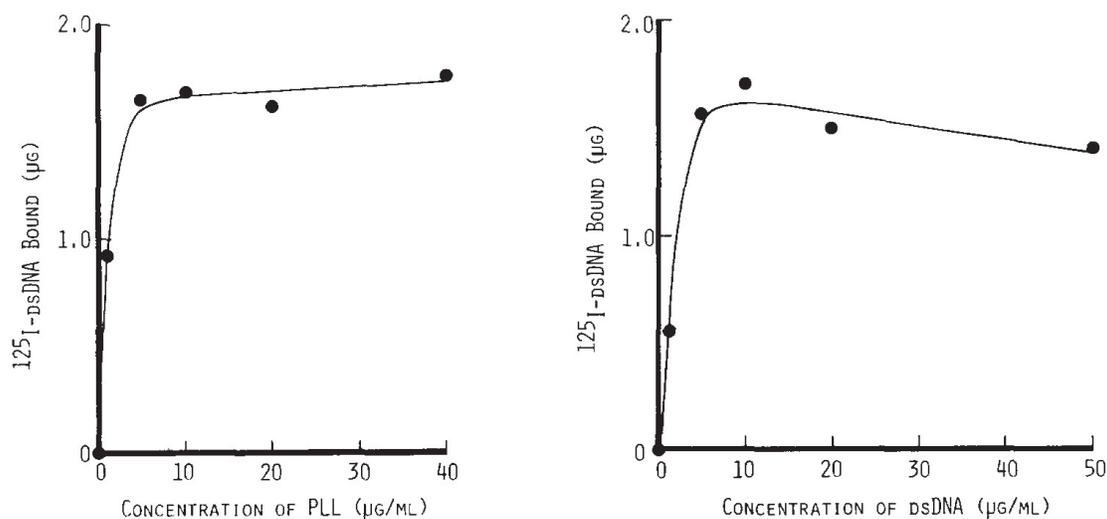


Fig. 1. Concentration of PLL required for coating tubes with [ $^{125}$ I]dsDNA was examined. More than 5  $\mu$ l/ml PLL were sufficient for coating tubes with [ $^{125}$ I]dsDNA. Without PLL [ $^{125}$ I]dsDNA was not bound to the tubes.

Fig. 2. Concentration of [ $^{125}$ I]dsDNA suitable for coating PLL tubes was examined. Efficient coating reached a maximal value at approximately 10  $\mu$ g/ml of [ $^{125}$ I]dsDNA.

dsDNA solution was aspirated and the tubes were washed 3 times with Tris-HCl buffer. The radioactivities retained on the tubes were then measured in an auto gamma spectrometer. As shown in Fig. 2, efficient coating reached a maximal value at approximately 10  $\mu\text{g}/\text{ml}$  of dsDNA.

#### *Stability of dsDNA on a solid phase*

Tubes coated with [ $^{125}\text{I}$ ]dsDNA were rotated with 1 ml of incubation buffer containing different amounts of serum or heparinized plasma at 37°C for 120 min and washed 3 times with washing buffer. The tubes were further treated in the same manner as in the standard assay, except that unlabeled anti-Ig was used and the radioactivities retained on the tubes were measured. As shown in Fig. 3, less than 50  $\mu\text{l}$  of serum eliminated none of the [ $^{125}\text{I}$ ]dsDNA from the tubes during the treatment of the standard assay, while heparinized plasma took off dsDNA from the tubes to a certain degree. This effect was found to be due to heparin by another experiment (data not shown).

#### *Inhibition studies*

Thirty  $\mu\text{l}$  of SLE serum were incubated with 1.5 ml of different concen-

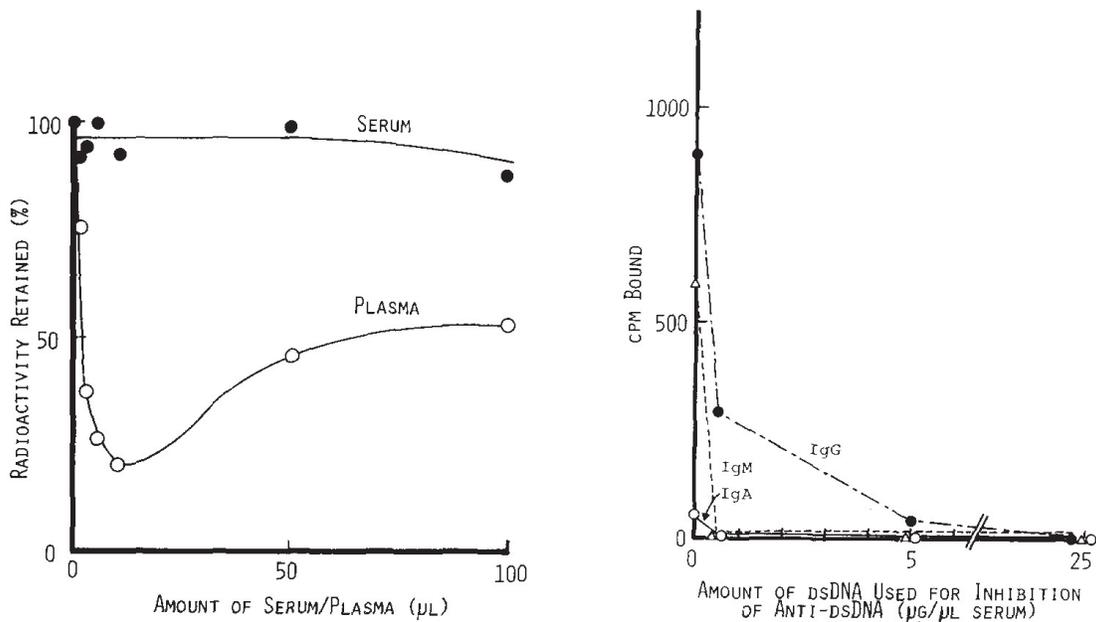


Fig. 3. The stability of dsDNA coupled to a solid phase was examined. Different amounts of serum or heparinized plasma were treated in the same manner as in the standard assay, substituting dsDNA for [ $^{125}\text{I}$ ]dsDNA and [ $^{125}\text{I}$ ]anti-Ig for unlabeled anti-Ig. [ $^{125}\text{I}$ ]dsDNA retained on a solid phase was expressed as per cent radioactivities counted after the treatment.

Fig. 4. Anti-dsDNA levels in the sera absorbed with dsDNA were measured with the solid-phase radioimmunoassay. Anti-dsDNA levels were expressed in cpm.

trations of dsDNA and filtered through a Millipore filter to eliminate dsDNA—anti-dsDNA complexes formed. Aliquots of the filtrates were tested for anti-dsDNA by the standard assay. As shown in Fig. 4, essentially none of anti-dsDNA of the 3 Ig classes was measured in the serum treated with dsDNA of 5  $\mu\text{g}/\mu\text{l}$  serum.

*Kinetics of binding of anti-dsDNA to dsDNA tubes*

Two  $\mu\text{l}$  of SLE serum diluted in 1 ml of incubation buffer were added to dsDNA tubes and were then treated in the same manner as in the standard assay. As shown in Fig. 5, binding of antibodies to the antigen on a solid phase was almost completed within 120 min.

*Kinetics of binding of anti-Ig to anti-dsDNA on dsDNA tubes*

dsDNA tubes, which had been treated with SLE serum, were rotated with  $^{125}\text{I}$ -labeled anti-Ig in 1 ml of incubation buffer at room temperature for 2, 4, 8 and 18 h. The tubes were washed 3 times with washing buffer and the radioactivities were measured. As shown in Fig. 6, a relatively long period was required for sufficient binding of anti-Ig to antibodies on the dsDNA

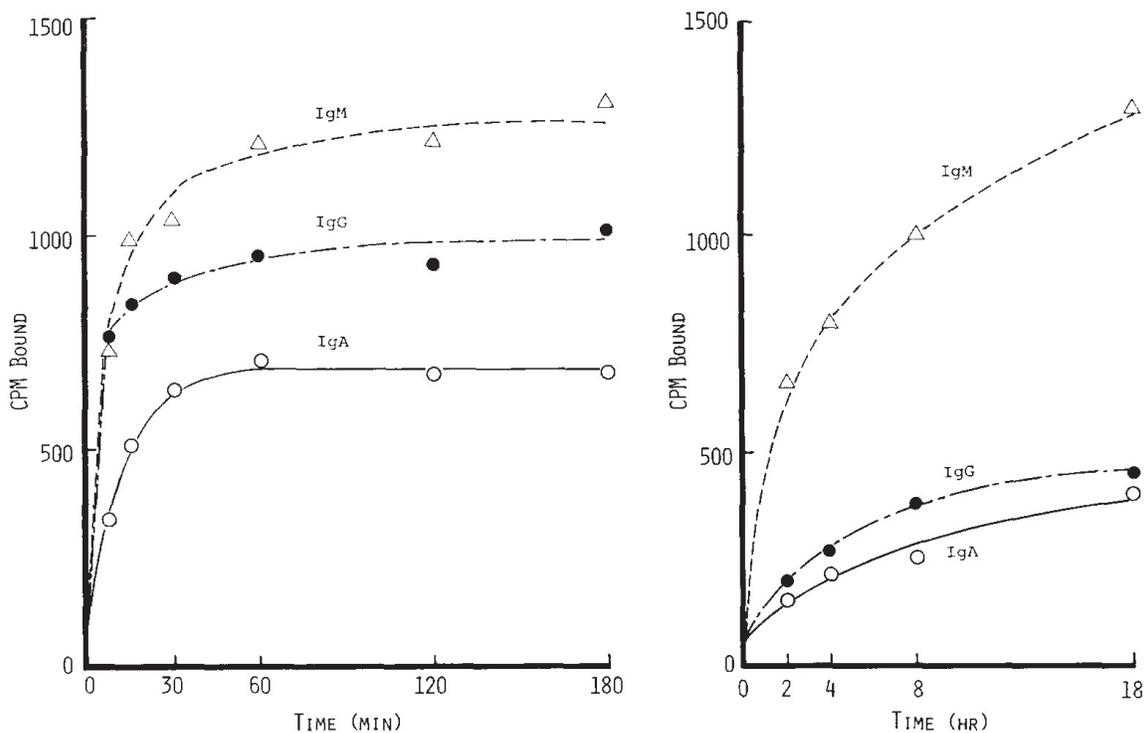


Fig. 5. Kinetics of binding of anti-dsDNA to dsDNA tubes. Binding of anti-dsDNA to dsDNA on a solid phase was almost completed within 120 min.

Fig. 6. Kinetics of binding of anti-Ig to anti-dsDNA on dsDNA tubes. Binding of anti-Ig to anti-dsDNA was not completed in a period examined.

TABLE 1

EFFECT OF RA SERA ON MEASUREMENT OF ANTI-dsDNA LEVELS IN SLE SERUM

	RA sera				nl <sup>a</sup>
	1	2	3	4	
Latex fixation test <sup>b</sup>	40 ×	40 ×	80 ×	320 ×	--
Effect on: IgG anti-dsDNA	0.65 <sup>c</sup>	0.50	0.87	0.93	0.96
IgM anti-dsDNA	0.58	0.30	0.90	0.55	0.89

<sup>a</sup> Normal serum control.<sup>b</sup> Highland's 'RA test' used.<sup>c</sup>  $\frac{\text{anti-dsDNA level in the mixture of SLE and RA sera}}{\text{anti-dsDNA level in SLE serum} + \text{anti-dsDNA level in RA serum}}$ 

tubes. However, an overnight incubation (18 h) was done for practical purposes.

#### *Effect of rheumatoid factor*

Two  $\mu\text{l}$  of SLE serum were mixed with equal amounts of each of 4 RA sera containing rheumatoid factor with various latex fixation titers, and the anti-dsDNA levels in the mixtures were compared with anti-dsDNA levels in SLE serum plus those in RA serum. As shown in Table 1, addition of RA serum decreased both IgG and IgM anti-dsDNA levels.

#### *Studies on patients' sera*

Thirty sera obtained from 19 patients with SLE, 11 sera from 11 patients with diseases other than SLE (others), which had shown significant dsDNA binding activities on the Millipore filter assay, and 8 sera from healthy controls were studied. Twenty-two sera from patients with SLE were obtained during active phase as judged by a disease activity score and 8 sera during inactive phase. The results were shown in Fig. 7 and the statistics in Table 2. Most of the normal sera showed low levels of anti-dsDNA, the majority of which was IgM although IgG and IgA were also detectable. Sera from patients with active SLE contained large amounts of anti-dsDNA distributed among the 3 Ig classes in different proportions, though no significant difference was revealed between IgG and IgM anti-dsDNA levels. Sera from patients with inactive SLE also contained some amounts of anti-dsDNA in the 3 Ig classes. The IgG anti-dsDNA levels were significantly lower than those in active SLE, while IgM anti-dsDNA levels were not significantly different from those in active SLE. Anti-dsDNA levels in sera from diseases other than SLE were elevated predominantly in IgM class, although anti-dsDNA levels in each class were significantly higher than those of normal controls.

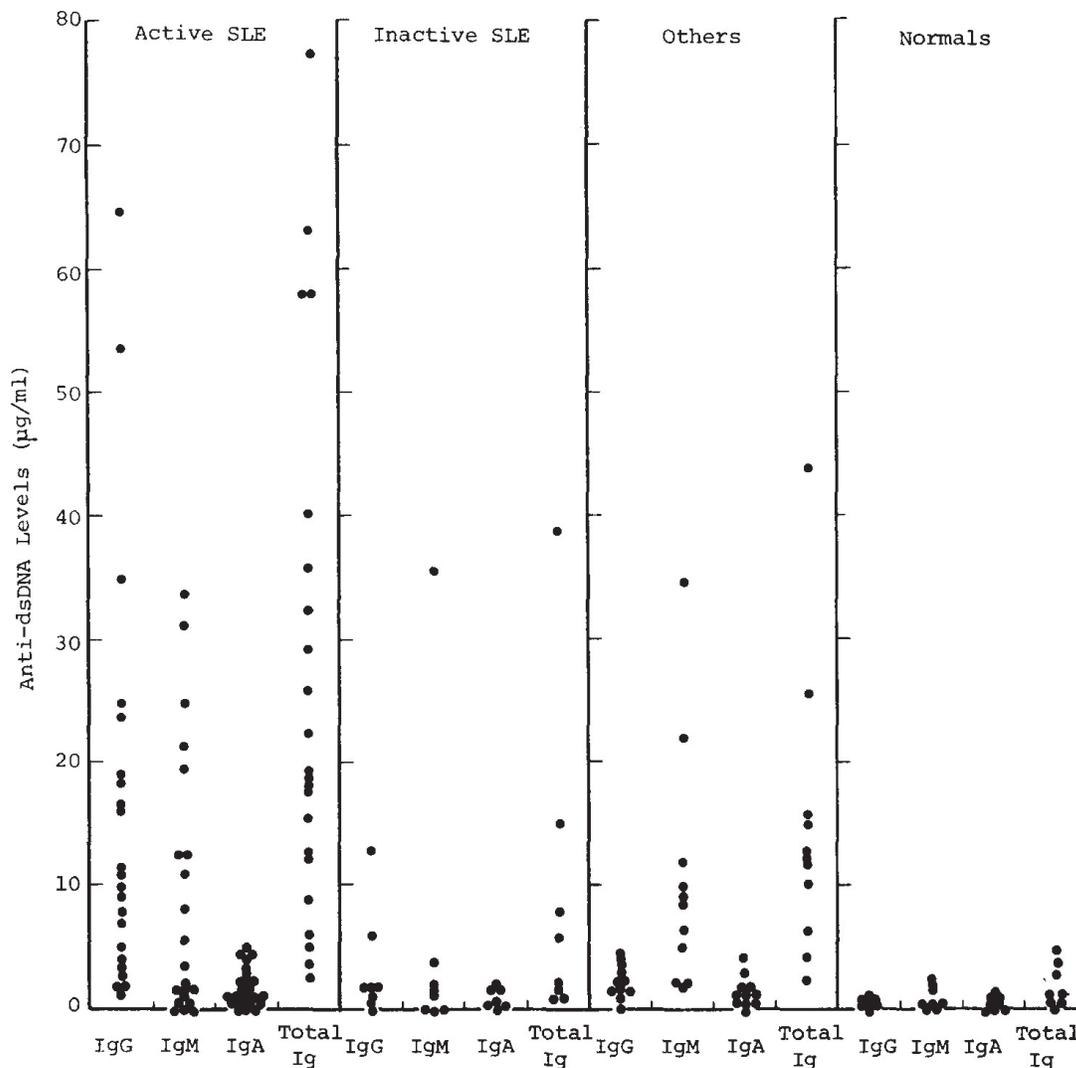


Fig. 7. Results of measurement of anti-dsDNA levels in IgG, IgM and IgA on sera from patients with SLE, those with diseases other than SLE (others), whose dsDNA binding activities were high on the Millipore filter assay, and normal controls. Total anti-dsDNA levels (total Ig), i.e., IgG + IgM + IgA, were also shown.

#### *Comparison with the Millipore filter assay*

Fifty-one sera were examined with both the Millipore filter assay and the method we have developed. The results were shown in Fig. 8. Significant correlations were revealed for IgG and total Ig anti-dsDNA levels, as compared with dsDNA binding activities on the Millipore filter assay. It is noted that 8 sera from patients with high dsDNA binding activities on the Millipore filter assay showed total Ig anti-dsDNA levels below the upper limit of normal, namely,  $4.99 \mu\text{g/ml}$  (the mean plus 2 S.D. for normal sera). On the other hand, none of sera with normal dsDNA binding activities on the Millipore

TABLE 2

## ANTI-dsDNA LEVELS IN PATIENTS WITH SLE, PATIENTS WITH OTHER DISEASES AND NORMALS

Active SLE, n = 22; inactive SLE, n = 8; others, n = 11; normals, n = 8.

		Mean + S.D. <sup>a</sup> ( $\mu\text{g/ml}$ )	Inactive SLE	Others	Normals
IgG	Active SLE	15.8 $\pm$ 16.2	$P < 0.05$ <sup>b</sup>	$P < 0.025$	$P < 0.005$
	Inactive SLE	3.31 $\pm$ 4.02	—	NS <sup>c</sup>	NS
	Others	2.34 $\pm$ 1.15	—	—	$P < 0.05$
	Normals	0.575 $\pm$ 0.277	—	—	—
IgM	Active SLE	8.77 $\pm$ 10.4	NS	NS	$P < 0.05$
	Inactive SLE	5.55 $\pm$ 11.4	—	NS	NS
	Others	10.4 $\pm$ 9.39	—	—	$P < 0.025$
	Normals	0.925 $\pm$ 1.02	—	—	—
IgA	Active SLE	1.91 $\pm$ 1.55	NS	NS	$P < 0.025$
	Inactive SLE	0.750 $\pm$ 0.634	—	NS	NS
	Others	1.47 $\pm$ 1.16	—	—	$P < 0.025$
	Normals	0.425 $\pm$ 0.441	—	—	—
IgG + M + A	Active SLE	26.5 $\pm$ 20.6	$P < 0.05$	NS	$P < 0.005$
	Inactive SLE	9.24 $\pm$ 12.4	—	NS	NS
	Others	14.2 $\pm$ 9.80	—	—	$P < 0.005$
	Normals	1.95 $\pm$ 1.53	—	—	—

<sup>a</sup> S.D. = standard deviation.<sup>b</sup>  $P$  values for significant differences, based on Student  $t$ -test.<sup>c</sup> NS = not significant.

filter assay (less than 10%) showed total Ig anti-dsDNA levels higher than the upper limit of normal.

*Relationship of anti-dsDNA levels to the disease activity of patients with SLE*

Anti-dsDNA levels in each of 3 Ig classes and total Ig were examined for relations to disease activity scores, which patients had at the time sera were drawn (Fig. 9). Statistically significant correlations with disease activity scores were revealed for anti-dsDNA levels in IgG and total Ig.

DISCUSSION

A solid-phase radioimmunoassay or a radioallergosorbent test (RAST) originally described by Wide et al. (1967) provided a highly sensitive method to quantitate antibodies in any of Ig classes, if an antigen is effectively coupled to a solid phase. As to antibodies against DNA, Shimizu et al. (1975) reported the measurement of IgG antibodies to ssDNA by modification of the RAST procedure. Clough (1977) described a radioimmunoassor-

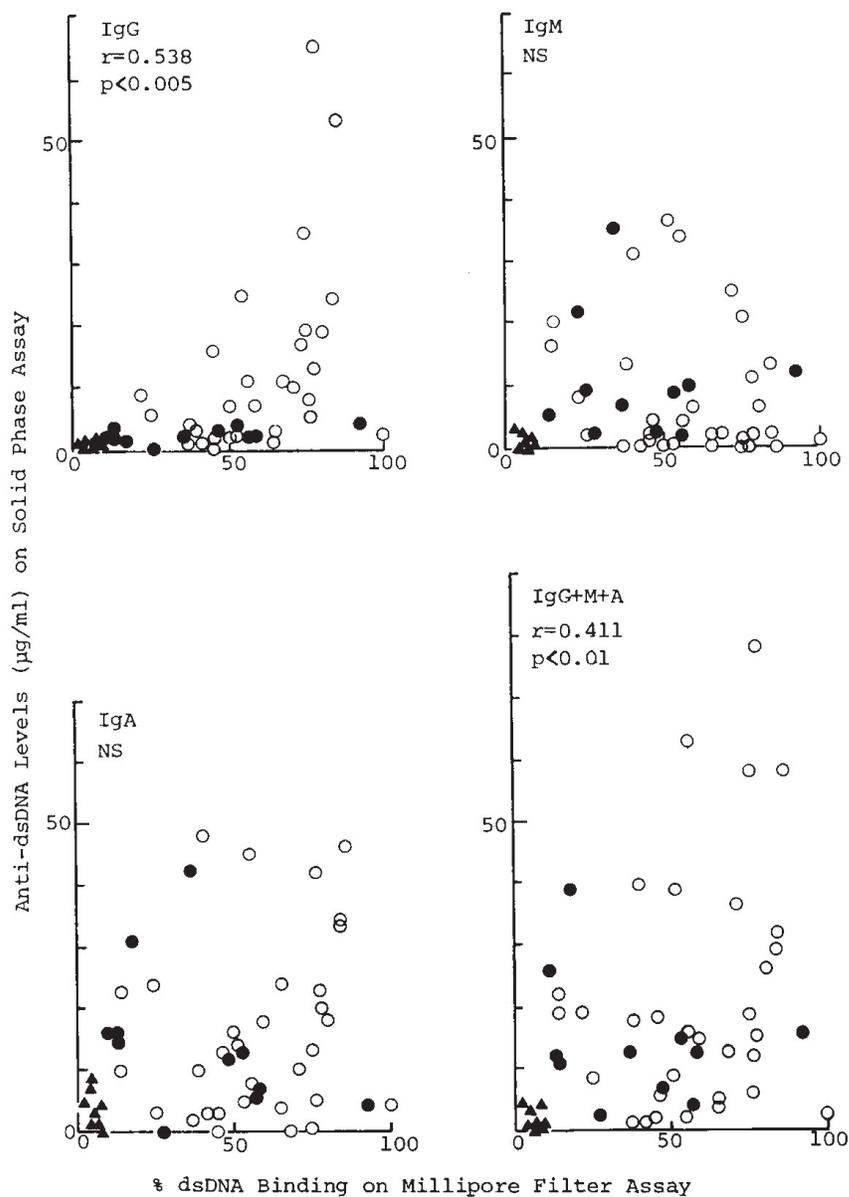


Fig. 8. Anti-dsDNA levels in IgG, IgM and IgA as well as total Ig measured with the solid-phase radioimmunoassay were compared to dsDNA binding activities measured with the Millipore filter assay. ○, SLE; ●, others; ▲, normals. Significant correlations were revealed for IgG and total Ig anti-dsDNA levels as compared to dsDNA binding activities on the same assay.

bent assay for quantitating specific DNA binding Ig in IgG, IgM and IgA class. One of the problems in their techniques is repeated troublesome centrifugations for washing, since antigens are coupled to particles such as microcrystalline cellulose (Shimizu et al., 1975) or formalinized human erythrocytes (Clough, 1977). We tried, therefore, to coat polystyrene tubes with dsDNA, which do not require centrifugations. Although Tan and Epstein (1973)

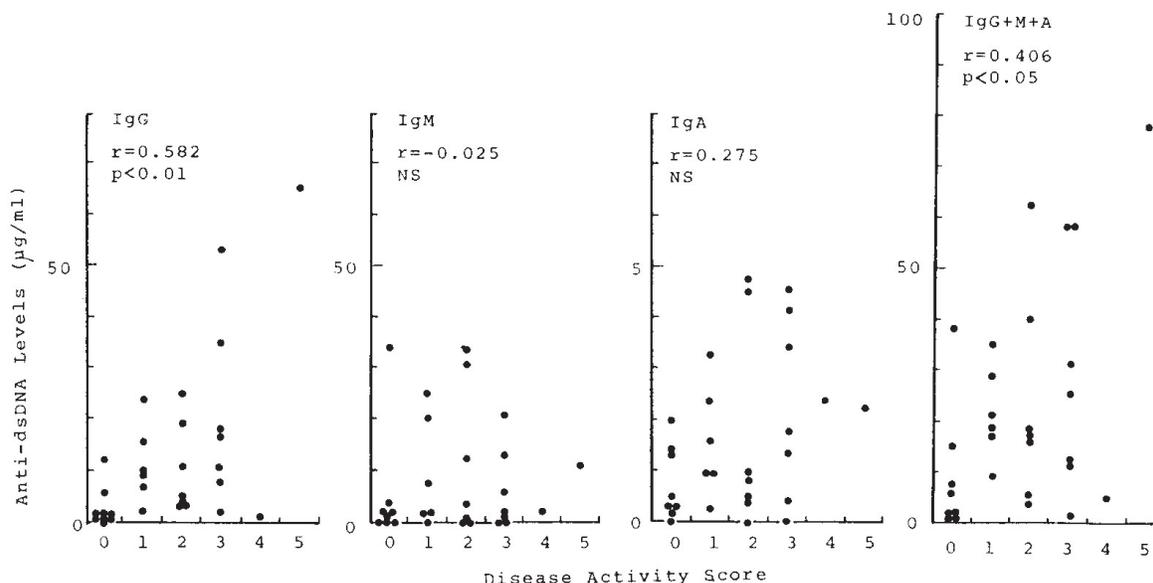


Fig. 9. Anti-dsDNA levels in each of 3 Ig classes and total Ig were examined for relations to disease activity scores ( $n = 30$ ). Statistically significant correlations were revealed for anti-dsDNA levels in IgG and total Ig.

succeeded in coating plastic cups with hydroxyapatite-purified dsDNA, we failed until PLL was found to be a useful reagent for coupling dsDNA to polystyrene tubes. The reason why we could not couple dsDNA to polystyrene tubes was not clear. A material of the plastic used might be different. At any rate, we think it is reasonable to apply PLL, basic amino acid polymer, for coupling strongly charged antigens such as dsDNA to a solid phase. Since the bonding of PLL and dsDNA is ionic, there is a possibility that high ionic strength of buffers and more strongly charged substances interfere with the bonding. However, examination using [ $^{125}\text{I}$ ]dsDNA revealed that dsDNA on a solid phase of polystyrene tubes was quite stable under the conditions used, except for heparin, an anticoagulant, which took dsDNA off from a solid phase. Therefore, heparinized plasma cannot be tested with this method. Another possibility is also conceivable that binding of dsDNA to PLL creates a nucleoprotein linkage which might pick up antibodies other than anti-dsDNA. It was ruled out by performing inhibition studies using dsDNA in solution to eliminate all activities picked up by this method.

Clough (1977) claimed that rheumatoid factors interfere with his test producing either suppression or occasionally enhancement. We also examined RA sera for the effect on our assay. The results were almost the same as Clough's, although none of our RA sera produced enhancement of anti-dsDNA levels in either IgG or IgM. It was not determined whether the substances in RA sera, which interfered with the assay, were really rheumatoid factor. There was lack of correlation between the suppressive effect and titer of rheumatoid factor. Furthermore, among 11 patients with diseases other

than SLE and high dsDNA binding activities on the Millipore filter assay, 7 with rheumatoid factor had rather higher IgM anti-dsDNA levels than 4 without rheumatoid factor on this assay (data not shown). Those findings suggested that rheumatoid factor may not be responsible for the suppression.

In the experiment on the comparison with the Millipore filter assay, it was found that some sera from patients with high dsDNA binding activities on the Millipore filter assay showed total Ig anti-dsDNA levels below the upper limit of normal measured by the method we have developed. The Millipore filter assay might depend less on the Ig nature of the dsDNA binding material detected, although results by both methods correlated significantly. However, the possibility that our method is less sensitive cannot be excluded.

Studies on patients' sera revealed that anti-dsDNA levels of all of the 3 Ig classes were elevated in patients with active SLE. Anti-dsDNA levels of any of 3 Ig classes in patients with inactive SLE were not significantly higher than those in normals, although IgG anti-dsDNA levels in 5 out of 8 patients were over the upper limit of normal. Anti-dsDNA levels in patients with diseases other than SLE, whose dsDNA binding activities were high on the Millipore filter assay, were elevated predominantly in IgM class. Most of the normal sera showed low levels of anti-dsDNA, the majority of which was IgM although IgG and IgA were also detectable. These results seem to indicate that anti-dsDNA, even in IgG class, is not qualitatively but quantitatively characteristic of SLE. The findings are in agreement with the observation by Bankhurst and Williams (1975) that DNA binding B lymphocytes exist in the peripheral blood of both normals and SLE patients. They postulated that anti-dsDNA antibody response may be initiated by the stimulation of pre-existing DNA specific B lymphocytes by several mechanisms. Our data may suggest that the mechanisms of stimulation in active SLE are different from those involved in diseases other than SLE, since the elevation of anti-dsDNA levels in the former was observed in both IgG and IgM, whereas that in the latter was predominantly in IgM.

Studies on the relation of anti-dsDNA levels to the clinical status of patients with SLE revealed that IgG anti-dsDNA levels correlated well with disease activities. The results were consistent with the observation by Pennebaker et al. (1977). They further showed that the occurrence of chiefly IgM-native DNA binding activity in the serum was associated with milder clinical disease and more benign renal histologic findings than observed in the patients with a majority of their native DNA binding in IgG class. Since we had only a few patients with predominantly (86–98%, as they defined) IgM anti-dsDNA in the serum, the same investigation as theirs could not be carried out. However, their concept may be supported by our findings that anti-dsDNA of patients with diseases other than SLE and no renal involvement was predominantly IgM.

Although significantly higher than in normals, IgA anti-dsDNA levels in active SLE were neither remarkably elevated nor associated with the clinical

status of the patients. The measurement of IgA anti-dsDNA seems to be clinically less significant than that of IgG anti-dsDNA levels.

In summary, our results were almost consistent with previous observations that IgG anti-dsDNA is characteristic of SLE and correlates well with the disease activity. However, much remains to be elucidated especially as to the clinical or pathogenetic significance of anti-dsDNA in Ig classes other than IgG. Our method will offer a useful tool for such investigations.

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