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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

[^{14}C]DNA was extracted, by the method of Marmur (1961), from nuclei of *E. coli* cultured in the presence of [^{14}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}C]dsDNA, whose molecular weight was about 100×10^4 as judged on CsCl gradient ultracentrifugation. Its specific activity was 1600 cpm/μg. [^{14}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}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 μl of serum inactivated at 56°C for 120 min were incubated at 45°C for 15 min with 195 μl of 0.15 M Tris-HCl buffer, pH 7.6, containing 0.4 μg of [^{14}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}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}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. ^{125}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 ^{125}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 ^{125}I-labeled anti-Ig in 1 ml of incubation buffer, while the other tubes (control tubes) were not exposed to ^{125}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 μg/ml, 1500 cpm/μg) 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 μg/ml of PLL were sufficient for dsDNA coating. For the assay, a concentration of 40 μg/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]-

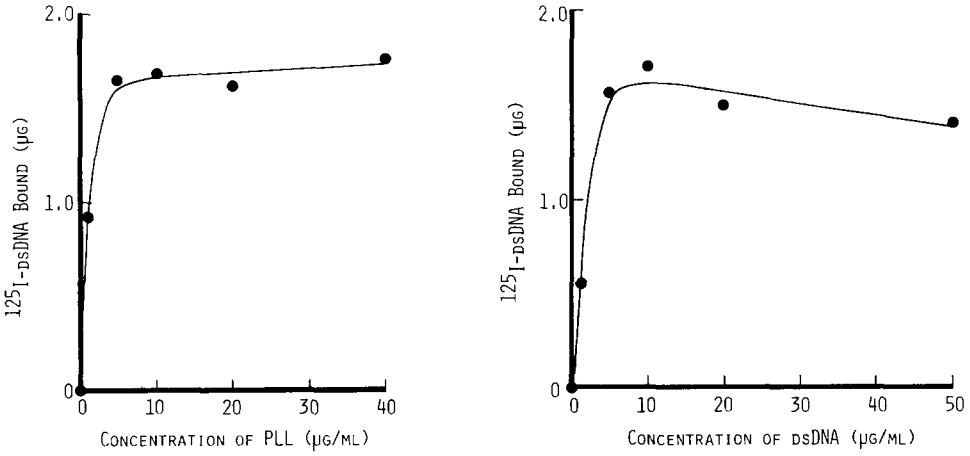

Fig. 1. Concentration of PLL required for coating tubes with [^{125}I]dsDNA was examined. More than 5 μl/ml PLL were sufficient for coating tubes with [^{125}I]dsDNA. Without PLL [^{125}I]dsDNA was not bound to the tubes.

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

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