Utility of a Synthetic Poly dT Band Included in a Commercial Blot Assay to Detect Anti-DNA Antibodies

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Systemic lupus erythematosus (SLE) patients exhibit anti-DNA antibodies with a heterogeneous pattern of individual specificity of binding. Likewise, the methods used for the measurement of anti-DNA antibodies exhibit a wide spectrum of binding avidities detection. We have evaluated the clinical utility of a band of synthetic poly dT included as an antigen in a recently commercialized blot system for antinuclear antibodies to detect anti-DNA antibodies. Sera from 45 individuals (36 patients with SLE, 9 without autoimmune disease negative for anti-nDNA by ELISA) were evaluated by the commercial immunoblot assay, ELISA for anti-nDNA, and conventional immunofluorescence us-

ing *Crithidia lucillae* substrate. Kappa statistics were used to determine the agreement between assays. The results obtained show a better agreement between positive bands for poly dT and anti-nDNA antibodies measured by IIF than by ELISA (Kappa index 0.64 vs. 0.44), although there are discrepant results. Likewise, positive bands for poly dT were obtained in patients with active SLE as assessed by decreased serum C3 concentration. We conclude that anti-poly dT reactivity reveals anti-nDNA antibodies of medium-high avidity, which show best disease activity. J. Clin. Lab. Anal. 13:287–290, 1999. © 1999 Wiley-Liss, Inc.

Key words: poly dT; anti-nDNA antibodies; systemic lupus erythematosus; ELISA; indirect immunofluorescence

INTRODUCTION

Antibodies to DNA and, especially, antibodies to native B-helical DNA (nDNA) occur in patients with systemic lupus erythematosus (SLE) and are considered markers for the disease. However, in patients with SLE there is a heterogeneous pattern of individual anti-DNA specificity. It has been observed that primary antigenic determinants in nDNA are formed by short regions from the sugar-phosphate backbone with negative charges contributed by phosphate groups. Moreover, nDNA antibodies bind primarily to conformational determinants present on all DNA, both self and foreign (1). Thus, antibodies to these epitopes frequently recognize synthetic polynucleotides as antigens (2). The fine antigenic specificity of antibodies against bases has been analyzed using single-stranded homopolymers of purine (poly dA, poly dG) and pyrimidine (poly dC, poly dT) as inhibitors of anti-DNA antibody activity (3,4). It has been found that poly dT and poly dC were the only homopolymers that recognise SLE IgG, leading to the conclusion that antigenic determinants appear to lie on pyrimidines.

It has been pointed out that most of the so-called anti-single-stranded DNA antibodies could be considered as low-avidity anti-DNA antibodies. Binding to double-stranded DNA (dsDNA) should perhaps be considered a more stringent test of DNA self-antigen recognition (5).

The most commonly used methods for antibodies to nDNA are indirect immunofluorescence (IIF) on *Crithidia lucillae*, radiobinding assays (RBAs) (Farr assay and PEG assay), and enzyme-linked immunosorbent assay (ELISAs) (6). The method of IIF on *C. lucillae* detects antibodies to DNA of intermediate-to-high avidity, whereas ELISA assays detect antibodies with a wide range of binding avidities. The objective of this study was to evaluate the clinical utility of a synthetic poly dT band included as an antigen in a recent commercialized blot system to detect anti-DNA antibodies, and to analyze the relationship between anti-poly dT reactivities and low and medium-high avidity anti-nDNA antibodies measured by ELISA and intermediate-high avidity anti-nDNA measured by IIF.

MATERIALS AND METHODS

Patients

We studied 45 serum samples (from 38 females and 7 males) selected from those received in our laboratory for antinuclear

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antibody analysis. Thirty-six samples containing anti-nDNA were from SLE patients who satisfied the American College of Rheumatology revised criteria for the classification of SLE (7). The other nine samples were from individuals of both sexes without autoimmune diseases whose sera gave a negative reaction for antinuclear antibodies and anti-nDNA by ELISA.

Methods

Anti-nDNA antibodies were measured by indirect immunofluorescence (IIF), and enzyme-linked immunosorbent assay (ELISA). For IIF, a 1/20 serum dilution was added to *Crithidia lucillae* slides (Inova Dcs, San Diego, CA). After washing, a 1/200 FITC rabbit antihuman IgG, IgA and IgM (protein concentration 4.1 g/L, F/p fraction = 2.3) (Dako A/S, Denmark) solution was added. Fluorescence was observed in a Leyca microscope (Leitz DMRB) with a I3 blue filter. A qualitative anti-nDNA positive control was included (Inova). Titers greater than 1/20 were considered positive.

Anti-nDNA antibodies were measured by a commercial ELISA according to manufacturer instructions (Inova DCS, San Diego, CA). In brief, diluted calibrator, controls and patient sera were added to separate wells coated with calf thymus dsDNA. After an incubation at 25°C for 30 minutes and washing, a peroxidase labelled antihuman IgG antibody was added to each well. After a second 30-minute incubation at 25°C and washing, the chromogenic substrate TMB was added. After a third 30-minute incubation at 25°C in the dark, the reaction was stopped and the absorbance of each well was read at 450 nm. The results were obtained by comparing the color developed in the patient wells with the color in the calibrator well. Results under 70 IU/mL were considered negative. The whole process was automated in a Gest System (Chemila S.P.A, Guidonia, Italy), an automated microtiter analyzer for immunoenzymatic techniques developed on microplate (96 wells).

Anti-poly dT reactivities were analyzed by the InnoLIA blot system (Innogenetics N.V, Zwijndrecht, Belgium), according to the manufacturer. Recombinant and native nuclear proteins (Sm, RNP, SSA, SSB, Centromere, Scl70, Jo-1, Histones), ribosomal RNP and poly dT were coated as discrete lines on a nylon membrane with plastic backing. In addition to these nuclear antigens, one control line was coated on each strip. The test sample was incubated in a test trough together with the multiple antigen-coated test strip. After washing, a goat antihuman IgG labeled with alkaline phosphatase was added. After incubation and washing, enzyme substrate bromochloroindolyl phosphate/nitro blue tetrazolium in dimethyl formamide (BCIP/NBT) was added. Incubation with enzyme substrate produces a dark brown color in poly dT line in proportion to the amount of specific antibody present in the sample. Color development is stopped with sulphuric acid. Reactivity of poly dT band on the patient strip was com-

pared with that of the cut-off control strip. A donor blood serum as negative control and a nDNA positive control (Inova) were included.

Serum C3 and C4 concentrations were measured by immunonephelometry in the BNA II automated analyzer (Dade Behring, Margburg, Germany).

Statistical Methods

The results were analyzed by SPSS 8.0 program (SPSS Inc., Chicago, IL) on a personal computer. Kappa statistic was applied to evaluate the agreement between test (8). Kappa has a maximum of 1.00 when the agreement is perfect, a value of zero indicates no agreement better than chance. The test does not presume a Gaussian distribution in the analysis of qualitative variables. The relationship between serum C3 and C4 concentrations and both anti-poly dT reactivities and anti-nDNA antibodies measured by IIF was assessed by the Mann Whitney U test. For the relationship between serum C3 and C4 concentrations and anti-nDNA antibodies measured by ELISA, regression analysis was used.

RESULTS

The relationship between anti-poly dT reactivities measured by the InnoLIA system and anti-nDNA antibodies measured by ELISA is shown in Figure 1. There were 12 anti-nDNA positive sera by ELISA that gave negative results for anti-poly dT by InnoLIA, whereas all anti-nDNA negative results by ELISA gave negative results for anti-poly dT by InnoLIA, Kappa index = 0.44 (CI (95%) = 0.22–0.67). The study of concordance between anti-nDNA antibodies measured by IIF and anti-poly dT measured by the InnoLIA system is shown in Figure 2. There were four anti-nDNA positive results by IIF that gave negative results for anti-poly dT by InnoLIA, and four anti-nDNA negative results by IIF that gave anti-poly dT positive results by InnoLIA, Kappa index = 0.64 (CI (95%) = 0.42–0.86). The relationship between the results

Fig. 1. Relationship between anti-nDNA concentration measured by ELISA and anti-poly dT reactivity measured by InnoLIA blot system.

Fig. 2. Relationship between anti-nDNA titer measured by IIF and anti-poly dT reactivity measured by InnoLIA blot system.

obtained for anti-poly dT reactivities and anti-nDNA antibodies measured by IIF and the C3 concentrations are shown in Table 1. A nonsignificant relationship was obtained between C3 concentrations and anti-nDNA antibodies measured by ELISA. As far as serum C4 concentrations were concerned we didn't observe any significant relationship with anti-poly dT reactivities or with anti-nDNA antibodies measured both by IIF and ELISA.

DISCUSSION

In recent years there has been a rapid growth in the use of ELISA assays for the detection of antibodies to nDNA. One problem with the use of ELISA assays for anti-nDNA assessment is the detection of low-avidity antibodies which are thought to be irrelevant to the disease process (9). Thus, when we measure serum anti-nDNA by ELISA we could classify a greater number as anti-nDNA positive SLE patients, in rela-

tion to other techniques. In 12 anti-nDNA positive patients measured by the ELISA technique we did not detect the presence in serum of anti-nDNA by IIF and 12 gave a negative reactivity to poly dT polymer. Both ELISA sensitivity and the broad range of avidity of detected antibodies could explain the greater number of anti-nDNA antibodies detected by ELISA. At the same time, a low concordance with poly dT reactivity has been observed. In our study, antibodies that bind to poly dT polymer agree better with anti-nDNA detected by IIF than by ELISA. Likewise, the agreement between anti-poly dT reactivity and disease activity, assessed by a decreased level of C3, is similar to that of anti-nDNA detected by IIF. These facts suggest that antibodies that react to poly dT would tend to show anti-nDNA reactivities of medium-high avidity. This is interesting because then anti-poly dT reactivities would show a better specificity for SLE with respect to other autoimmune diseases, and theoretically a better relationship with lupus nephritis than ELISA. However, we couldn't find a statistically significant relationship between poly dT reactivities and serum creatinine level, perhaps because of the low number of samples.

It is well known that in SLE patients a heterogeneous population of anti-nDNA antibodies is produced. This fact, together with differences in conformation may explain why, in eight patients, anti-poly dT and anti-nDNA reactivities analyzed by IIF disagreed, although it has been observed that anti-nDNA antibodies correlate better with antisynthetic copoly-

TABLE 1. Relationship between serum C3 complement component concentrations and both poly-dT and IIF anti-nDNA antibodies in SLE patients^a

Antibodies	C3 (mg/dL)		U	P
	Negative/Positive			
Anti poly-dT	113.0 (29.5)/76.9 (19.3)		3.0	0.028
IIF anti-nDNA	115.5 (21.7)/71.8 (18.6)		2.0	0.023

^aData are median (Mid-95%).

mer poly (dA-dT) – poly (dA-dT) than with antidenaturalized DNA (10).

In summary, the antibodies detected with poly dT agree better with anti-nDNA measured by IIF than by ELISA, although there are discrepant results. Consequently, anti-poly dT reactivity tends to reveal anti-nDNA antibodies of medium-high avidity. These antibodies correlate better with disease activity.

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