Clin. exp. Immunol. (1987) **69**, 394–402

A new assay for anti-DNA antibodies in serum which includes the measurement of anti-Z-DNA

P. D. VAN HELDEN, LURENE VAN LILL, A. J. BESTER, LINDA DE WAAL & EILEEN G. HOAL-VAN HELDEN *MRC Centre for Molecular and Cellular Biology, Department of Medical Biochemistry, University of Stellenbosch Medical School, Tygerberg, South Africa*

(Accepted for publication 25 February 1987)

SUMMARY

A simple, rapid assay for measuring anti-DNA titre of serum which includes anti-Z-DNA is described. The assay involves solution binding of antibody to labelled DNA under conditions such that the DNA is altered to form a left-handed or Z-DNA structure in the presence of cobalt ions. The absence or presence of cobalt determines a B or Z form structure in DNA and antibodies to these forms are detectable. The majority of SLE and RA patients (88%) have a higher anti-DNA titre in the presence of cobalt ions. An additional 25% of SLE patients and 22/23 RA patients who had normal anti-DNA levels according to the Crithidea assay, reacted with abnormal titres in our assay. Patients experiencing a relapse in SLE also showed a large increase in anti-DNA in the presence of antigenic Z-DNA. These results suggest that monitoring anti-DNA levels in SLE and RA to detect anti-Z DNA antibodies, provides significant advantages over methods currently in use to measure anti-DNA antibodies.

Keywords anti-Z-DNA SLE rheumatoid arthritis

INTRODUCTION

The presence of anti-DNA antibodies in the serum of patients with SLE (systemic lupus erythematosus) is characteristic of this autoimmune disease (Casals, Friou & Myers, 1964; Schur & Sandson, 1968; Koffler *et al.*, 1971; Winfield, Koffler & Kunkel, 1975; Aarden, Lakmaker & Feltkamp, 1976; Swaak, Aarden & Feltkamp, 1977; Pennebaker, Gilliam & Ziff, 1977). Serum levels of anti-DNA antibodies are monitored in the clinical situation since they have been shown to correlate with pathogenesis, exacerbations and various manifestations of SLE (Casals *et al.* 1964; Schur & Sandson, 1968; Koffler *et al.*, 1971; Winfield *et al.*, 1975; Aarden *et al.*, 1976; Swaak *et al.*, 1977; Pennebaker *et al.*, 1977). In some cases, the titre of anti-dsDNA may indicate the clinical severity of SLE as well as periods of active disease and remission (Aarden *et al.*, 1976; Swaak *et al.*, 1977).

The antibodies may arise as part of the immune response to other antigens, (perhaps exogenous) as an anti-idiotypic response or a consequence of cross-reactivity. Alternatively, antigenic DNA may be genomic DNA in the serum, which has been shown to have an abnormally high guanine–cytosine (GC) content (Sano & Morimoto, 1982) and an abnormally high occurrence of alternating purine–pyrimidine segments (Van Helden, 1985). This phenomenon may be significant, since it has been shown that segments of alternating Pu/Py residues, particularly poly (dG-dC), can undergo a

Correspondence: P. D. Van Helden, MRC Centre for Molecular and Cellular Biology, Department of Medical Biochemistry, University of Stellenbosch Medical School, PO Box 63, Tygerberg, 7505, South Africa.

transition from a B (or right-handed helical) conformation to a Z (or left-handed helical) conformation (Pohl & Jovin, 1972; Behe & Felsenfeld, 1981; Zacharias *et al.*, 1982; Möller *et al.*, 1984) and since it has also been shown that the Z form of DNA is highly immunogenic (Lafer *et al.*, 1983). If, therefore, Z-DNA segments occur naturally in SLE patients, we may expect a greater production of anti-Z-antibodies than anti-B-DNA antibodies (Van Helden, 1985). That such anti-Z-DNA antibodies do occur in SLE sera has recently been shown (Lafer *et al.*, 1983; Sibley, Lee & Decoteau, 1984).

The aim of the present study was to develop a simple assay for both anti-B and anti-Z DNA antibodies in serum and to compare titres in patients with SLE and rheumatoid arthritis to ascertain whether anti-Z DNA antibody titre could be more relevant than anti-B titre as a diagnostic or investigative aid.

MATERIALS AND METHODS

Preparation of DNA. Poly (dG-dC).poly (dG-dC) was obtained from PL Biochemicals and radiolabelled with ^{3}H-dGTP (Amersham) by nick-translation (Maniatis, Fritsch & Sambrook, 1982). Poly (dG).poly (dC) and poly (dG-Me^{5}dC).poly (dC-Me^{5}dC) were end-labelled by T4 polymerase as described elsewhere (Maniatis *et al.*, 1982).

Estimation of single-strand DNA (ssDNA) in nick-translated poly (dG-dC). poly (dG-dC). Labelled polymer was incubated with the single-strand specific exonuclease S1 as described (Casperson & Voss, 1980). Essentially 100 ng labelled DNA was incubated with 20 units of S1 nuclease for 2 h at 45°C. Every 15 min, samples of the incubation mixture were removed and cold trichloroacetate added to 10% (v/v). They were allowed to stand on ice for 30 min before being filtered through glass fibre filters. The retained radioactivity was measured in a liquid scintillation counter and the percentage of DNA digested from the original labelled DNA was calculated. Some of this DNA was also used in the assay as described below.

Serum preparation. Sera were obtained from Tygerberg Hospital personnel and patients. SLE or rheumatoid arthritis sera were from patients who fulfilled the American Rheumatism Association criteria for disease. Sera were processed according to the following procedure: blood was collected and allowed to stand for 16 h at 4°C to allow coagulation. Samples were subsequently centrifuged for 10 min in a desk-top centrifuge at maximum speed after which the supernatant serum was carefully withdrawn. The sera were all heated at 56°C for 1 h and centrifuged in an Eppendorff centrifuge for 15 min. The supernatant fraction was used directly for assays.

DNA binding assay. Anti-B DNA antibodies were assayed by adding 100 μl of heat treated serum to 0·2 μg of ^{3}H-poly (dG-dC) in 17 μl water. The solution was incubated for 60 min at 21°C after which it was filtered directly through nitrocellulose filters (Schleicher and Schuell BA 85, 0·45 μm) which had been prewetted in 0·2 M sodium hydroxide and then rinsed with 60 mM sodium phosphate pH 7·0, 30 mM EDTA, 200 mM NaCl. After filtration of the samples, the filters were washed with more of this buffer, dried and the retained DNA was determined by liquid scintillation counting.

Anti-Z DNA antibodies were assayed in the same manner with the exception that the ^{3}H-poly (dG-dC) was first incubated for 1 h at 21°C in 17 μl of 25 mM cobalt chloride. The conversion of the DNA into the Z form was monitored spectrophotometrically (Van Helden, 1983) and was complete after 20 min (A_{295}/A_{260} of 0·35). This same increase in the ratio of A_{295}/A_{260} from 0·15 to 0·35 may be observed when poly (dG-dC).poly (dG-dC) is irreversibly converted to the Z form by bromination (B.D. Stollar, pers. comm.) or reversibly in the presence of 3·5 M NaCl or 0·7 M $MgCl_2$. After dilution of the sample with serum, the final concentration of cobalt chloride in the solution was 3·6 mM.

Competition assays. To define the specificity of the assay and the antibodies, the following experiments were done. Unlabelled DNA polymers were incubated in the presence or absence of cobalt for 1 h in assay buffer. Serum was then added, followed by further incubation for 1 h at room temperature. Finally, labelled poly (dG-dC) was added and the assay completed as described.

RESULTS

Antibody-antigen complexes formed between anti-DNA and DNA are retained on filtration through nitrocellulose filters. Free labelled DNA (i.e. DNA not bound to antibodies) was not retained by the nitrocellulose if the procedure of prewetting was followed. The labelled poly (dG-dC) polymer was estimated to contain between 1% and 3% single-stranded regions according to the method described by Casperson & Voss (1980) and was therefore a good substrate for anti-dsDNA antibodies.

In the presence of low concentrations of cobalt normally right-handed poly (dG-dC) is converted to a left-handed or Z-DNA helix (Peck *et al.*, 1982; Gessner *et al.*, 1985). Therefore the absence or presence of cobalt may be the determining element for either B or Z-DNA respectively and so for the assay of anti-B or anti-Z DNA antibodies.

The specificity of the antibody-DNA reactions was tested by competitive immunoassay using both poly (dG).poly (dC) and poly (dG-dC).poly (dG-dC) as competitors. The competitor DNA was added either in the presence or absence of cobalt ions. The results (Fig. 1) show that poly (dG-dC) may compete with either B- or Z-form DNA, but poly (dG).poly (dC) competes only with the B-form substrate. These results suggest that the assay detects distinct antibody specificities and that the cobalt-assay does detect anti-Z DNA antibodies.

The specificity of the assay was tested further using other DNA polymers. End-labelled poly (dG-Me^{5}dC).poly (dG-Me^{5}dC) was used as a substrate, since this polymer assumes a Z-formation in physiological ionic strength solutions. Binding of anti-DNA antibodies was tested in the presence or absence of cobalt and the results of this assay compared with results obtained using poly (dG-dC).poly (dG-dC) on a number of patients. Given that poly (dG-Me^{5}dC).poly (dG-Me^{5}dC) under physiological conditions should exist as a Z-form polymer, one would expect that the results of anti-DNA binding in the presence or absence of cobalt should be the same, i.e. the anti-Z (+Co) to anti-B (−Co) binding ratio should be 1. The results presented in Table 1 show that this was not attained, although an average ratio close to unity was obtained (anti-Z/anti-B; 1·18). This result compares with an average ratio of 4·25 for the same patients using poly (dG-dC).poly (dG-dC) as a test antigen. A similar assay was done using poly (dG).poly (dC) as a test antigen. This polymer does not exist as Z-DNA in the presence of cobalt and once again the average anti-Z/anti-B was lower than that obtained using poly (dG-dC)—1·25 compared to 6·12.

Cross-reactivity with single-stranded DNA (ssDNA) was also tested (Table 2). In this experiment, the competitor was unlabelled single-stranded herring sperm DNA. There is clearly a large amount of cross-reactivity in the antibody population, since approximately 80% of the dsDNA binding in the presence or absence of cobalt may be inhibited. The inhibition in the presence

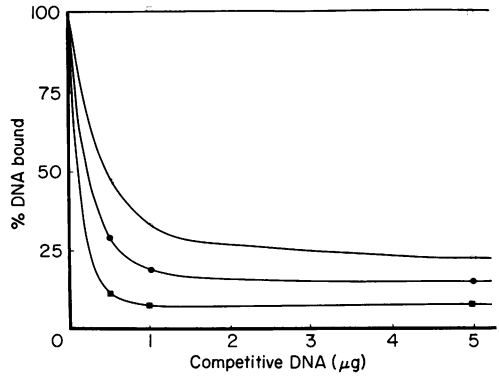

Fig. 1. Competitive radioimmunoassay with ^{3}H-poly (dG-dC).poly (dG-dC) an antigen. Binding to this antigen was competed for by unlabelled poly (dG).poly (dC) (□) with and (○) without cobalt, and by poly (dG-dC).poly (dG-dC) (■) with and (●) without cobalt present as described.

Table 1. Comparison of serum anti-DNA binding in the presence or absence of cobalt using different DNA polymers

	anti-Z/anti-B*	
Patient	Poly (dG-dC)	Poly (dG-Me^{5}dC)
1	3·49	1·16
2	6·06	1·06
3	3·22	1·32
Average	4·25	1·18

* Anti-Z represents binding in the presence of cobalt, anti-B in the absence of cobalt.

Table 2. Competitive radioimmunoassay with sonicated single-stranded herring sperm DNA as competitor and ^{3}H-poly (dG-dC) as substrate

	Competitor DNA (μg/assay)					
	0	0·1	0·5	1	5	10
With cobalt	100*	39	34	24	16	14
Without cobalt	100†	51	41	26	19	21

*† Figures given as a percentage of control; * represents 24432 d/min; † represents 21150 d/min.

of cobalt ions was observed to be marginally less (79%) than that in the absence of cobalt (86%), as measured for three different patients.

A comparison was made of serum DNA binding capacity using poly (dG-dC) in the presence ('anti-Z-DNA') and absence ('anti-B-DNA') of cobalt for normal individuals and patients suffering from SLE and RA. SLE and RA patients were randomly selected from patients attending a clinic and were not selected on the basis of a positive Crithidia assay (Berne, Galland & Welton, 1984). Since the patients attending the clinic were in various stages of the disease, including remission or relapse, the antibody titres were expected to vary considerably. This is shown in Fig. 2, where only 50% of the sera assayed showed an anti-B-DNA titre above the average value for normal sera. The values for anti-Z-DNA titres are higher than anti-B-DNA titres on average. More striking is the observation that 70% of patients had anti-Z-DNA titres above the average value for normal sera in contrast to the 50% that had anti-B-DNA titres above normal average.

The titre of the US National Reference Serum for human antibodies to DNA was measured in our assay for comparative use. The anti-B titre (ng DNA bound/100 μl) was 14 and anti-Z titre was 19. These titres are well above the values found in normal sera and also show a high anti-Z titre compared to anti-B DNA.

Figure 3 shows a comparison of anti-Z against anti-B DNA antibody titres for each of the SLE patients. These results show that in 88% of the patients studied the anti-Z-DNA titres are higher than anti-B-DNA (anti-Z: anti-B > 1).

The average anti-Z: anti-B DNA titre was 2·7:1, but may also be as high as 19:1. Of the SLE patients studied, 25% showed an anti-Z DNA antibody titre above normal average where the anti-

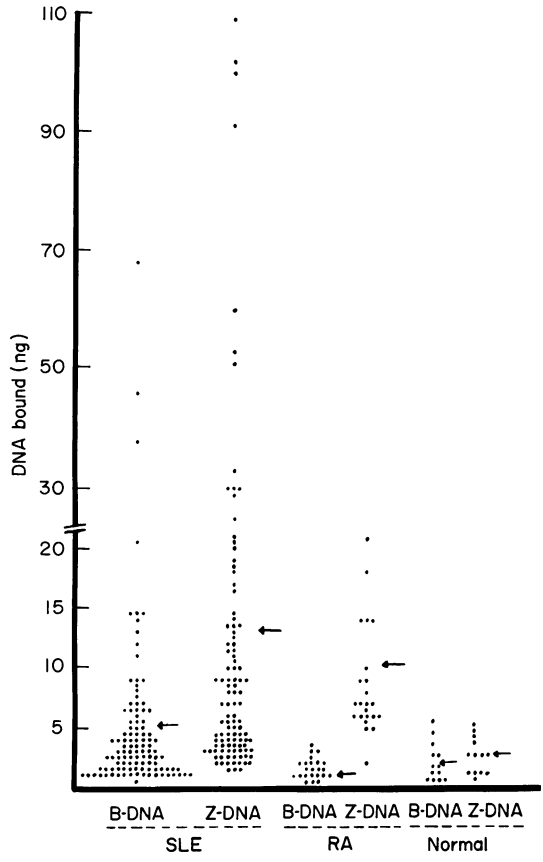

Fig. 2. Radioimmunoassay results of DNA binding capacity of sera. Serum (100 μl) was reacted directly with ^{3}H-poly (dG-dC).poly (dG-dC) and bound DNA measured after filtration of antibody-DNA complexes as described. Arrows indicate average values for patients shown in the figure. Number of sera assayed: SLE (106), rheumatoid arthritis (23), normals (40). Average titres for normals: anti-B DNA 3·0; anti-Z DNA 4·3. (Not all normals have been plotted on figure, but the range of titres is fully represented.)

B-DNA antibody titre was equal to or below normal average. In contrast only 7% showed the presence of anti-B-DNA antibodies in above normal concentrations where anti-Z-DNA antibodies were normal.

The results of the anti-DNA assay of RA patients are also shown in Fig. 1. None of the patients assayed showed an anti-B-DNA titre above normal range, whereas 22/23 showed an anti-Z DNA titre above normal average and 14/23 had a titre above the highest measurement made in the normal sample.

It is known that there may be a correlation between anti-DNA antibodies and disease activity (Casals *et al.*, 1964; Koffler *et al.*, 1971; Swaak *et al.*, 1977; Casperson & Voss, 1980; Lafer *et al.*, 1983) and the results shown in Fig. 4 show that this holds true for both anti-B and anti-Z antibodies for the patient monitored. Similar results were obtained for other patients (results not shown). Periods of active disease were marked by an increase in antibody titre. In May, June and December the anti-Z DNA titre was higher than the anti-B DNA titre. In particular, the May relapse showed a small increase in anti-B titre, but a relatively large (at least 2-fold) increase of anti-Z DNA. For this patient, Crithidia tests in May and June proved negative.

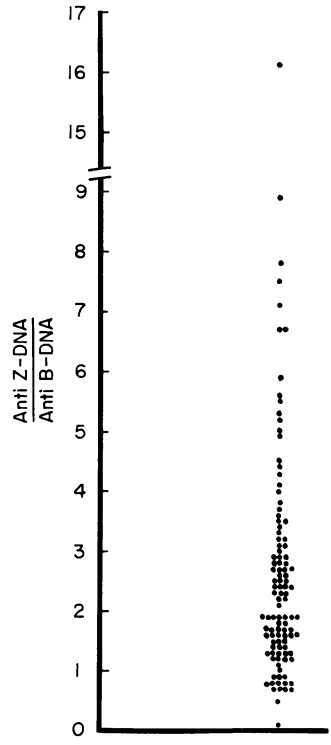

Fig. 3. Relative anti-Z to anti-B DNA titre in individual SLE patients assayed in Fig. 1.

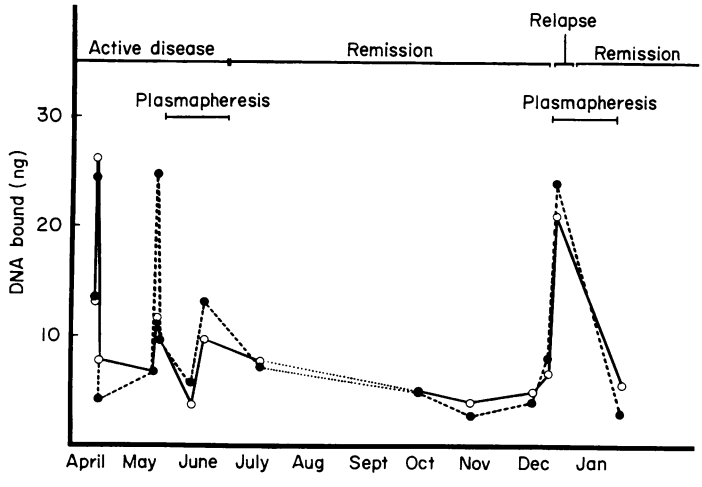

Fig. 4. Radioimmunoassay of DNA binding of serum from one SLE patient assayed over a period of time as described in text. (————) Anti-B-DNA titre; (– – – – – – – – – –) anti-Z-DNA titre; (. . . . . .) no serum available for testing.

DISCUSSION

The poly (dG-dC) used in the assays may form either B-DNA or Z-DNA depending either on base modifications (such as bromination or methylation) (Behe & Felsenfeld, 1981; Zacharias *et al.*, 1982; Möller *et al.*, 1984) or ionic environment (Pohl & Jovin, 1972; Peck *et al.*, 1982; Zacharias *et al.*, 1982). The left-handed or Z form of DNA is strongly immunogenic (Lafer *et al.*, 1983; Möller *et al.*, 1984; Zarlin *et al.*, 1984) and a synthetic form (Br-poly (dG-dC) has been used to raise antibodies in experimental animals and also to detect naturally occurring anti-Z-DNA antibodies such as may be found in SLE patients (Lafer *et al.*, 1983; Sibley *et al.*, 1984). However, it has been shown that antibodies induced by chemically modified DNA, such as Br-poly (dG-dC) differ from those in naturally occurring SLE sera (Lafer *et al.*, 1983; Zarlin *et al.*, 1984) and that the former antibodies exhibit preferences for certain modified forms of DNA (Zarlin *et al.*, 1984). For these reasons we have used an unmodified form of DNA (poly (dG-dC)) as our test antigen. To induce a conformational shift from the B to the Z form we placed the DNA in a cobalt buffer which has been shown to induce the formation of Z-DNA at low ionic strength (see Results and Peck *et al.* (1982)), unlike other salts such as $MgCl_2$ or NaCl where high ionic strengths such as 0·7–3·5 M are needed to ensure conformational change. This high salt concentration interferes with the DNA– anti-DNA reaction (Lafer *et al.*, 1983) and is therefore undesirable for the assay of anti-DNA antibodies.

In an attempt to characterize the nature of the antibodies identifying the different forms of DNA, a number of competition experiments and experiments using different DNA polymers were done. These experiments indicate that much of the antibody titre measured in both the anti-B and anti-Z DNA assays was due to cross-reacting antibody. Possibly only 20% of the antibodies measured in these assays are specific for double-stranded DNA of either B or Z form, since ssDNA inhibited approximately 80% of the antibody binding. However, dsDNA in B form did not appear to inhibit ds specific anti-Z DNA binding, whereas ds in B or Z form did inhibit the binding of anti-B or anti-Z DNA antibodies respectively. Binding to a DNA polymer which exists as Z-DNA under physiological conditions changed little whether cobalt was absent or present (18% increase in presence of cobalt on average) in comparison with assays using a polymer which undergoes a B–Z transition in cobalt, (binding in cobalt was increased 3-fold). We conclude that the presence of cobalt does not significantly affect the antibody–antigen reaction.

We have used this assay to monitor anti-DNA reactivity in hospital personnel and patients, and our results show that the probability of SLE patients having an elevated antibody response to poly (dG-dC) in cobalt buffer (here referred to as anti Z-DNA) is twice that of their showing an abnormal anti-B titre (poly (dG-dC) in serum). We also showed that 88% of SLE patients surveyed had levels of anti-Z-DNA antibodies above those of anti-B-DNA antibodies and that 25% of the patients had a positive titre of anti-Z-DNA (above normal range) although no reactivity above normal range could be detected for anti-B-DNA in these individuals.

The ratio of anti-Z to anti-B reactivity based on total serum reactivity was extremely variable for the individuals monitored. The elevation in anti-Z-DNA antibody levels at a time of relapse of the disease increased dramatically and was not always paralleled or followed by an increase in anti-B-DNA antibodies. These anti-B-DNA antibodies were not elevated above normal average at any time in some of the patients studied, whereas the anti-Z-DNA antibodies were above normal values.

This situation is even more dramatic in RA, where 83% (19/23) of the patients surveyed had anti-Z titres above the highest value found in normal persons, where none of these same patients had an elevated anti-B titre. Although the anti-Z-DNA titre was elevated in RA, the highest titre measured was only one-fifth that of the highest SLE titre measured. The mean anti-Z-DNA titre was higher in SLE than RA but the median values (not shown in Fig. 2) show a higher RA titre. These results support those obtained by Sibley *et al.* (1984) using a different method, although these workers found RA titres to be higher than SLE titres on average. A possible explanation is that these authors used poly (dG-m^5dC) as an antigen and the substitution on DNA of the bulky methyl group has been shown to elicit a different antibody response (Lafer *et al.*, 1983; Zarlin *et al.*, 1984). Lafer *et al.*, (1983), using yet another assay method and brominated poly (dG-dC), also found elevation of anti-Z DNA titres in RA patients, on average, although the majority of their patients fell within the 'normal' range. Again, the explanation for the differences found between their results,

those of Sibley *et al.* (1984) and these could be in the substitution on DNA of the bulky bromine atom, which as Lafer *et al.* point out, produces different antibodies on immunization from natural antibodies to Z-DNA.

Although we have not developed an assay which can detect anti-Z-DNA antibodies exclusively and specifically without further modification to remove cross-reactive antibodies, we feeel that the assay may offer a number of advantages over those currently in use and reported in the literature. These are firstly the relative simplicity and reliability of the assay, which does not involve the use of a second antibody or high energy radioisotopes such as I^{125} or the binding of an antigen to a solid surface and secondly the higher anti-DNA titres found in over 80% of SLE and RA patients when the antibody titre is assayed in the presence of cobalt. This factor alone makes the assay a useful diagnostic aid for the clinician, since the majority of these patients reported were previously regarded as anti-DNA negative.

Financial assistance from the National Cancer Association of South Africa is acknowledged.

REFERENCES

AARDEN, L.A., LAKMAKER, F. & FELTKAMP, T.E.W. (1976) The influence of reaction conditions on the Farr assay as used for the detection of anti-ds DNA. *J. immunol. Meth.* **10,** 39.

BEHE, M. & FELSENFELD, G. (1981) Effects of methylation on a synthetic polynucleotide: the B-Z transition in poly (dG-m^5dC).poly (dG-m^5dC). *Proc. natn Acad. Sci.* **78,** 1619.

BERNE, B.H., GALLAND, K.T. & WELTON, R.C. (1984) Values of the U.S. National Reference Serum for Human Antibodies to native DNA obtained with commercial immunoassays for anti-DNA in systemic lupus erythematosus. *Clin. Chem.* **5,** 757.

CASALS, S.P., FRIOU, G.J. & MYERS, L.L. (1964) Significance of antibody to DNA in systemic lupus erythematosus. *Arthritis Rheum.* **7,** 379.

CASPERSON, G.F. & VOSS, E.W. (1980) Colicin E1 plasmid as a probe for detection and study of anti-DNA activity in SLE sera. *J. immunol. Meth.* **36,** 293.

GESSNER, R.V., QUIGLEY, G.J., WANG, A.H.-J., VAN DER MAREL, G.A., VAN BOOM, J.H. & RICH, A. (1985) Structural basis for stabilization of Z-DNA by cobalt hexaamine and magnesium cations. *Biochemistry* **24,** 237.

KOFFLER, D., CARR, R., AGNELLO, V., THOBURN, R. & KUNKEL, H.G. (1971) Antibodies to polynucleotides in human sera: antigenic specificity and relation to disease. *J. exp. Med.* **134,** 294.

LAFER, E.M., VALLE, R.P.C., MÖLLER, A., NORDHEIM, A., SCHUR, P.H., RIEH A. & STOLLAR, D. (1983) Z-DNA-specific antibodies in human systemic lupus erythematosus. *J. clin. Invest.* **71,** 314.

MANIATIS, T., FRITSCH, E.F. & SAMBROOK, J. (1982) *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratory Publication, MA.

MÖLLER, A., NORDHEIM, A., KOZLOWSKI, S.A., PATEL, D.J. & RICH, A. (1984) Bromination stabilizes poly (dGdC) in the Z-DNA form under low-salt conditions. *Biochemistry* **23,** 54.

PECK, W.J., NORDHEIM, A., RICH, A. & WANG, J.C. (1982) Flipping of cloned p(dCcG)n.p(dCdG)n DNA sequences from right- to left-handed helical structure by salt, Co(111), or negative supercoiling. *Proc. natn Acad. Sci. USA.* **79,** 4560.

PENNEBAKER, J.B., GILLIAM, J.N. & ZIFF, M. (1977) Immunoglobin class of DNA binding activity in serum and skin in systemic lupus erythematosus. *J. clin. Invest.* **60,** 1331.

POHL, F.M. & JOVIN, T.M. (1972) Salt induced co-operative conformational change of a synthetic DNA: equilibrium and kinetic studies with poly (dGdC). *J. mol. Biol.* **67,** 375.

SANO, H. & MORIMOTO, C. (1982) DNA isolated from DNA/anti-DNA-antibody immune complexes in systemic lupus erythematosus is rich in guanosine-cytosine content. *J. Immunol.* **3,** 1341.

SCHUR, P.H. & SANDSON, J. (1968) Immunologic factors and clinical activity in systemic lupus erythematosus. *New Engl. J. Med.* **278,** 533.

SIBLEY, J.T., LEE, J.S. & DECOTEAU, W.E. (1984) Left-handed 'Z' DNA antibodies in rheumatoid arthritis and systemic lupus erythematosus. *J. Rheumatol.* **11,** 633.

SWAAK, A.J.G., AARDEN, L.A. & FELTKAMP, T.E.W. (1977) Immunology of DNA IV: Anti-ds DNA profiles as prognostic and therapeutic guides. Aarden, L.A. Thesis: University of Amsterdam.

VAN HELDEN, P.D. (1983) The effect of adriamycin on Z-DNA formation and DNA synthesis. *Nuc. Acids Res.* **23,** 8415.

VAN HELDEN, P.D. (1985) Potential Z-DNA-forming elements in serum DNA from human systemic lupus erythematosus. *J. Immunol.* **134,** 177.

WINFIELD, J.B., KOFFLER, D. & KUNKEL, H.G. (1975) Role of DNA-anti-DNA complexes in the immu-

nopathogenesis of tissue injury in systemic lupus erythematosus. *Scand. J. Rheumatol.* (suppl) **11,** 59.

ZACHARIAS, W., LARSON, J.E., KLYSIK, J., STIRDIVANT, M., WELLS, R.D. (1982) Conditions which cause the right-handed to left-handed DNA conformation transitions. *J. biol. Chem.* **275,** 2775.

ZARLIN, D.A., ARNDT-JOVIN, D.J., NICOUD, M.R., MCINTOSH, L.P., THOMAE, R. & JOVIN, T.M. (1984) Immunoglobin recognition of synthetic and natural left-handed Z-DNA conformations and sequences. *J. mol. Biol.* **176,** 369.