Clin Exp Immunol 1993; **92**:51–57

Interactions of anti-DNA antibodies with Z-DNA

P. KRISHNA, M. J. FRITZLER & J. H. VAN DE SANDE *Department of Medical Biochemistry, University of Calgary, Calgary, Alberta, Canada*

(Accepted for publication 11 December 1992)

SUMMARY

Systemic lupus erythematosus (SLE) sera, two classes of serum lipoproteins, and IgG antibodies from SLE and normal sera were tested for their reactivity with a Z-DNA polymer, Br-poly (dG-dC). In all cases preferential binding to Z-DNA over B-DNA was observed. This interaction, for the most part, could be inhibited by the negatively charged phospholipid, cardiolipin, which suggests that most of the anti-Z-DNA activity associated with sera arises from relatively non-specific ionic interactions between proteins and polyanionic molecules. An assay has been described that can eliminate proteins cross-reactive with negatively charged phospholipids.

Keywords Z-DNA systemic lupus erythematosus high density lipoproteins low density lipoproteins phospholipids

INTRODUCTION

Autoimmune diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) are characterized by the presence of autoantibodies capable of reacting with a variety of nuclear, cytoplasmic and cell surface antigens [1,2]. Antibodies to double-stranded DNA (dsDNA) are highly specific for SLE and the level of their production often correlates with disease severity [1]. Such findings have led to the proposition that anti-DNA antibodies are involved in the pathogenesis of SLE, but factors leading to their production and the exact role of antibodies in the development of the disease remain largely unresolved.

Spontaneously occurring antibodies reactive with left-handed Z-DNA have also been identified in sera of patients with SLE and RA [3,4] and in autoimmune MRL mice [5]. Certain physiologically relevant factors (e.g. polyamines spermidine and spermine) have been shown to promote the Z-conformation of polynucleotides [6]. Enhanced binding of SLE sera to DNA conformations altered by these factors has led to the suggestion that these factors may play a pathogenic role in SLE [6–8]. Since Z-DNA is highly immunogenic [9,10], it is possible that the conversion of a segment of B-DNA to Z-DNA in the presence of such factors may trigger the production of anti-Z-DNA antibodies in SLE patients. The validity of this hypothesis remains a matter of debate. For example, information on the binding specificity of anti-Z-DNA autoantibodies, particularly defined by the ability to interact with supercoil induced Z-DNA in plasmid molecules, is scant [5,11]. Further, the binding characteristics of anti-Z-DNA antibodies induced by immunization are found to differ from spontaneously arising autoantibodies [3,12]. These observations suggest that molecules antigenically similar to Z-DNA, rather than Z-DNA *per se*, may trigger the production of autoantibodies. The apparent binding to Z-DNA may be the result of a cross-reacting anti-Z-DNA antibody.

We have previously shown that several phospholipid-binding proteins strongly and preferentially interact with Z-DNA polymers [13,14]. This binding was inhibited by negatively charged phospholipids like cardiolipin and phosphatidic acid. These and other studies [15,16] have clearly demonstrated that the ability of proteins to bind Z-DNA polymers does not necessarily imply a biological relevance. Since in most cases anti-Z-DNA autoantibodies have been characterized on the basis of their ability to bind Z-DNA polymers [6–8], we were interested in determining if there was a correlation between Z-DNA binding and phospholipid-binding activities in SLE sera. The results obtained in such studies would be important in understanding the antigenicity of Z-DNA and the factors that are responsible for the measurement of anti Z-DNA antibody levels in SLE [8]. The results of the present study show that several serum proteins and IgGs isolated from both normal and SLE sera have the ability to bind to Z-DNA. This binding, unlike that of anti-Z-DNA antibodies raised in experimental animals, could be inhibited by the negatively charged phospholipid, cardiolipin. Thus, as previously observed for other proteins [13–15], proteins present in normal and SLE sera interact with Z-DNA. Since a consistent relationship between anti-Z-DNA activity and disease activity cannot be established, the use of Z-DNA polymers in diagnostic tests [8] could lead to incorrect conclusions.

Correspondence: P. Krishna, Department of Plant Sciences, University of Western Ontario, Biological & Geological Sciences Bldg., London, Ontario, Canada N6A 5B7.

MATERIALS AND METHODS

Proteins

Sera were obtained from patients with SLE who fulfilled the American Rheumatism Association criteria for that disease [17]. Antibodies to DNA were measured by an ELISA and the *Crithidia lucilliae* assay [18]. Antibodies to cardiolipin [19] and saline soluble nuclear antigens [20] were determined as previously published. Three out of eight sera were characterized to have high anti-DNA activity, three to have high anti-cardiolipin activity, and one to have high anti-ribonucleoprotein (RNP) activity. Four normal sera were included as controls. The sera were heated at 56°C for 1 h to inactivate complement and centrifuged at 11 750 *g* in an Eppendorf centrifuge. The supernatant was used in the binding assays.

IgG antibodies were purified by passing sera over a protein A-Sepharose column. Bound IgGs were eluted with 100 mM glycine (pH 3·0) and the solution was neutralized by addition of 1/10 the volume of 1 M Tris hydrochloride (pH 8·0). Purified fractions were dialysed against 10 mM Tris (pH 8·0), 1 mM EDTA and 50 mM NaCl, and protein concentrations were determined by measuring absorbance at 280 nm, using an extinction coefficient of $\Sigma_{280} = 1{\cdot}43\ A_{280}$ mg/ml.

Plasma lipoproteins were obtained from Sigma (catalogue nos L-2014 and L-2139). The polyclonal anti-Z-DNA antibody AZ-6, kindly provided by T. Hutcheon (University of Calgary, Canada), was produced by repeated injection of left-handed Br-poly (dG-dC) into rabbits [13]. The anti-Z-DNA MoAbs Jel 150 and Jel 131 were derived from mice immunized with Br-poly (dG-dC) and poly (dG-m^{5}dC), respectively [21], and were kindly provided by Dr J. S. Lee (University of Saskatchewan, Canada).

Nucleic acids

The synthesis of labelled poly (dG-dC), its chemical conversion by bromination into the Z-DNA, Br-poly (dG-dC), and verification of the latter structure were carried out as described previously [13]. Calf thymus DNA was obtained from Sigma. Single-stranded M13 phage DNA was prepared as described [15]. DNA concentrations were calculated using $A_{260} = 1$ as equivalent to 50 μg of dsDNA/ml, 36 μg of ssDNA/ml, 55 μg of poly (dG-dC)/ml and 69 μg of Br-poly (dG-dC)/ml.

Preparation of phospholipid micelles

Phospholipid solutions were obtained from Sigma. Organic solvents were evaporated from aliquots and liposomes were prepared by sonication in 10 mM Tris (pH 8·0). All phospholipid solutions were used in competition experiments within 3–4 h of preparation.

Filter binding assay

An anti-DNA assay based on Millipore filter binding was adapted after the original protocol of Ginsberg & Keiser [22]. A typical binding reaction was performed in a total volume of 100 μl containing 10 mM Tris hydrochloride (pH 8·0), 50 mM NaCl, 1 mM EDTA and 0·25 μg of the ^{3}H-DNA probe and varying amounts of protein. After 30 min at room temperature, a portion of the reaction (95 μl) was passed through a nitrocellulose filter (2·5 cm diameter Millipore type HA, 0·45 μm, prewashed in 0·3 M NaOH). Filters were washed with 2 ml of the binding buffer and dried; retained radioactivity was measured by liquid scintillation counting.

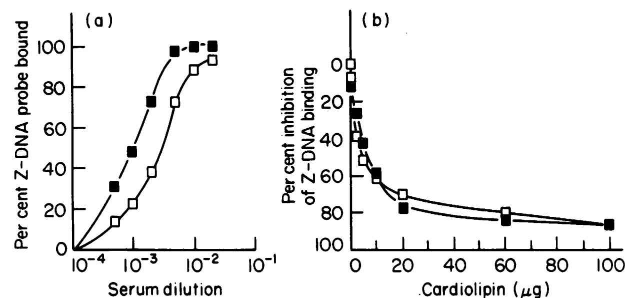

Fig. 1. Binding of systemic lupus erythematosus (SLE) sera with high anti-cardiolipin activity to Br-poly (dG-dC) (Z-DNA), and inhibition of the binding activity by cardiolipin. (a) Binding of serum 1 (■) and serum 2 (□) to the Z-DNA probe was carried out in the presence of 10 mM Tris-HCl (pH 8·0), 50 mM NaCl, 1 mM EDTA and 200-fold excess of competitor calf thymus DNA. (b) Inhibition of the Z-DNA binding activity of serum 1 (■) and serum 2 (□) by increasing amounts of cardiolipin.

In competition experiments, proteins were preincubated for 10 min with phospholipids; the Z-DNA probe was then added and the reaction was incubated for a further 30 min before filtration.

Blotting procedure to detect Z-DNA binding proteins

A portion of the high density lipoprotein (HDL) and low density lipoprotein (LDL) samples were fractionated on a 7·5-15% polyacrylamide–SDS gradient gel and electrophoretically transferred to nitrocellulose. Binding of ^{32}P-labelled Z-DNA (Br-poly (dG-dC)) or B-DNA (poly (dG-dC)) was carried out by the procedure of Bowen *et al.* [23] in the presence of the binding buffer (10 mM Tris hydrochloride (pH 8·0), 100 mM NaCl, 0·5 mM EDTA, 0·02% bovine serum albumin (BSA), 0·02% Ficoll and 0·02% polyvinylpyrollidone (PVP)). Blots were washed in binding buffer (as above but containing 200 mM NaCl), dried and autoradiographed.

In competition experiments, nitrocellulose blots were preincubated with phospholipids in the binding buffer for 10 min. Subsequently labelled DNA was added.

Detection of Z-DNA binding antibodies by dot blot assay

Protein (5–10 μg) was directly applied to the nitrocellulose membrane prewetted in transfer buffer (25 mM Trizma base, 192 mM glycine, 20% v/v methanol). The membrane was placed in a plastic pouch and treated as described above. A dot blot apparatus was used when several samples had to be applied to the nitrocellulose membrane.

RESULTS

We have previously shown that several Z-DNA binding proteins are phospholipid-binding proteins and *vice versa* [13,14,24]. In order to know if anti-cardiolipin antibodies had the property of preferentially interacting with Z-DNA over B-DNA, we used a filter binding assay to measure the amount of Z-DNA bound by SLE sera with elevated anti-cardiolipin activity. Figure 1a shows that the two samples of SLE sera used in our studies exhibited binding to ^{32}P-labelled poly (dG-dC) that had been stabilized in the Z-conformation by bromination. Binding was also observed with single-stranded DNA, but only

Table 1. Binding to B-DNA, ssDNA and Z-DNA* by two systemic lupus erythematosus (SLE) sera† with high affinity for cardiolipin

DNA probe	Per cent DNA bound	
	Serum 1	Serum 2
B-DNA (0·25 μg)	15·5	7·9
ssDNA (1·0 μg)	41·1	21·1
Z-DNA (0·25 μg)	100·0	93·4

* Binding to Z-DNA was measured in the presence of 200-fold excess of calf thymus DNA as competitor.

†1:50 dilution.

an insignificant interaction was observed with B-DNA (Table 1). The Z-DNA binding activity of SLE sera represented in Fig. 1a was unaffected by the presence of 200-fold excess B-DNA in the binding reaction, but was largely inhibited in the presence of modest amounts of cardiolipin (Fig. 1b). Preferential and high binding to Z-DNA was also obtained with normal sera (not shown), which suggested that proteins commonly present in both normal and SLE sera were responsible for the apparent Z-DNA binding.

We have noted before that two classes of lipoproteins found in human serum, HDL and LDL, have the ability to bind preferentially to Z-DNA [14]. As an extension of the results previously obtained in a filter binding assay, and to gain further information on the number and nature of serum proteins contributing to the binding of Z-DNA, we used a protein blotting assay in the present study. The plasma proteins belonging to the HDL and LDL classes of lipoproteins were electrophoresed on SDS–PAGE and transferred to nitrocellulose. The filter was then probed with ^{32}P-labelled poly (dG-dC) (B-DNA) or with ^{32}P-labelled Br-poly (dG-dC) (Z-DNA). Results of the blotting assay shown in Fig. 2 were found to correlate with the results obtained in a filter binding assay [14]. Several proteins interacting with Z-DNA were observed in the HDL fraction. A protein corresponding to an apparent molecular weight of 33 kD bound both to Z-DNA and to B-DNA and was only partially inhibited by cardiolipin for its binding to Z-DNA. Taken together, these data suggest that the 33-kD protein is a specific DNA-binding protein. Since LDL consists of one predominant protein apoB-100 with a molecular weight of 250 kD that constitutes 95% of the protein mass and is extremely prone to proteolysis [25], it is likely that some of the bands observed in the LDL fraction represent apoB-related proteolytic fragments. A second possibility is that some of these proteins represent contaminating plasma proteins. Proteins that bound to the Z-DNA polymer, but not to B-DNA, were also observed in the HDL fraction. Based on the apparent molecular weights, two of these proteins can be correlated to apolipoprotein A-I (mol. wt 28 000) and apolipoprotein H (mol. wt 54 000) normally found in the HDL fraction of human serum. The Z-DNA binding of proteins belonging to the HDL class of lipoproteins was totally inhibited by cardiolipin (blot 3 of Fig. 2). These results are consistent with the simple notion that the Z-DNA binding activity of most proteins demonstrates a non-specific ionic characteristic in being inhibited by other polyanions. However, there are exceptions to this generally observed phenomenon. Examples of this are the antibodies raised against Z-DNA in experimental animals. The antibodies used in our studies consisted of a polyclonal preparation AZ-6 [13], and MoAbs Jel 150 and Jel 131 [21]. AZ-6 and Jel 150 were raised against Br-poly (dG-dC) and possess the ability to bind strongly to this Z-DNA polymer (Figs 3a and 4). Jel 131 was raised against poly (dG-m^5 dC) and is known to interact weakly with Br-poly (dG-dC) [21]. AZ-6 can also bind specifically to a small segment of plasmid DNA that has been stabilized in the Z-conformation causing electrophoretic retardation of the resulting DNA:IgG complexes in polyacrylamide gels [15]. On the other hand, Jel 150 and Jel 131 do not interact with the chemically unmodified Z-insert of the plasmid. We used both the filter binding assay and the dot blot assay to assess the effect of phospholipids on the binding of these antibodies to Br-poly (dG-dC). Figures 3b and 4 show that antibodies specific for Z-DNA remain unaffected in their binding to Z-DNA even in the presence of large excesses of phospholipids. The inhibition of Jel 131 binding to Z-DNA by cardiolipin is not surprising given that this antibody interacts only weakly with Br-poly (dG-dC) [21].

We next determined the prevalence of anti-Z-DNA activity in purified IgG fractions of SLE and normal sera using the dot blot assay. IgGs were prepared from four normal sera, three SLE sera with enhanced anti-DNA activity, one SLE sera with high anti-RNP activity, and three SLE sera with elevated anti-cardiolipin activity.

Purified IgG preparations from most sera showed some interaction with Z-DNA (Fig. 5). The high level of interaction observed with IgGs isolated from normal sera was somewhat unexpected. However, it has long been known that detection of anti-DNA antibodies depends, in part, on the assay used [26]. When a subset of purified IgG samples were tested for their ability to bind plasmid DNA (B-DNA) in a gel retardation assay, IgGs isolated from normal sera produced much less retardation of DNA compared with the IgGs isolated from SLE sera with enhanced anti-DNA activity (not shown). Thus, enhanced binding of Z-DNA to IgGs from normal sera in a dot blot assay may be inherent to the assay method. A second and more likely possibility is that the interaction of IgGs with Z-DNA is an ionic phenomenon which is relatively non-specific and dependent on charge distribution in the IgG molecules. The fact that most Z-DNA binding activity of IgGs, regardless of the source from which they were purified, could be inhibited by cardiolipin (Fig. 5) supports this idea.

DISCUSSION

Several reports have indicated enhanced binding of SLE sera to left-handed Z-form of DNA polymers [6–8]. This binding has been considered specific as dsDNA in the B-form has been found to have little or no effect on the Z-DNA binding ability of sera or purified antibodies. Proteins from several other sources have also been observed to display a similar characteristic in their binding to Z-DNA [13–16]. We have shown that the binding of proteins to Z-DNA, although poorly inhibited by a wide variety of nucleic acids, is strongly inhibited by negatively charged phospholipids like cardiolipin and phosphatidic acid [13,14,24]. In addition, we ([14] unpublished results) and others [16] have found that proteins appearing to interact specifically

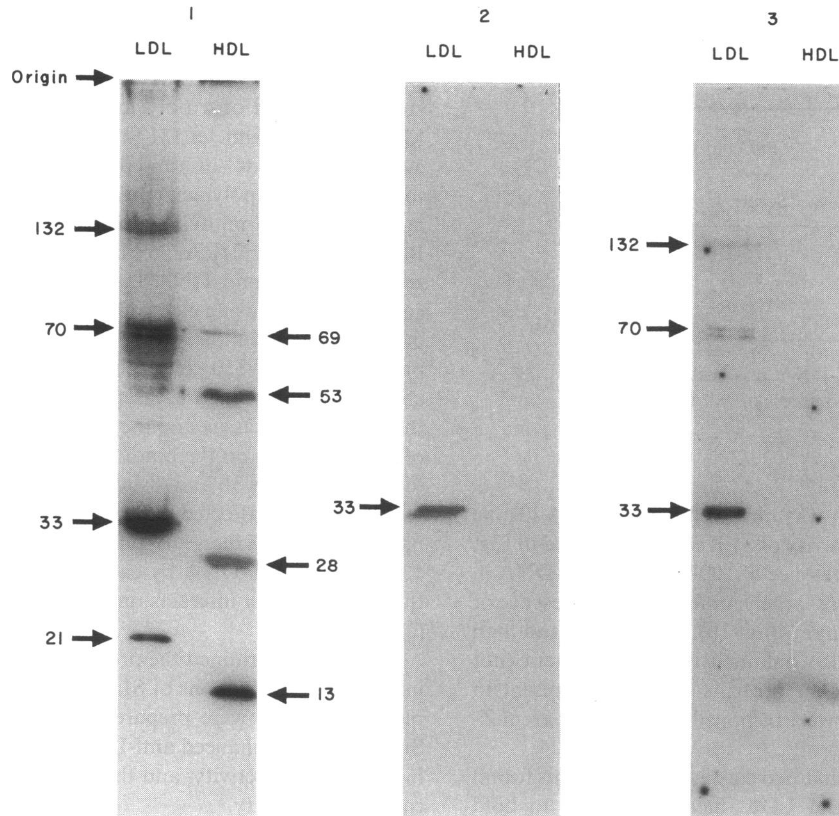

Fig. 2. Detection of Z-DNA and B-DNA binding of high density lipoproteins (HDL) and low density lipoproteins (LDL) by a protein blotting assay, and inhibition of the binding activity by cardiolipin. Binding of ^{32}P-labelled Z-DNA (Br-poly (dG-dC)) and B-DNA (poly (dG-dC)) was carried out as described in Materials and Methods, in the presence of 100 mM NaCl. Blot 1 represents proteins bound to the Z-DNA probe in the presence of competitor B-DNA (100 μg/ml); blot 2, proteins bound to the B-DNA probe in the absence of competitor; blot 3, proteins bound to the Z-DNA probe in the presence of cardiolipin (250 μg/ml). Molecular weights (in 10^{-3}) of the major DNA-binding proteins are indicated.

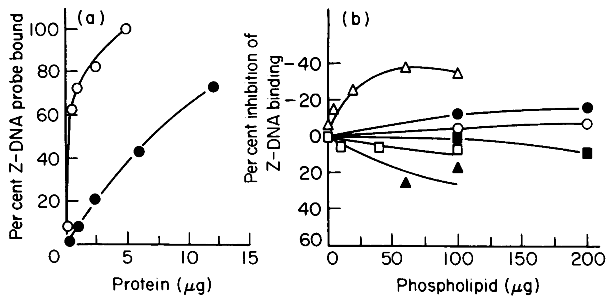

Fig. 3. Binding of polyclonal and monoclonal anti-Z-DNA antibodies to Br-poly (dG-dC) (Z-DNA) in a filter binding assay, and inhibition of the binding activity by phospholipids. (a) Binding of polyclonal antibody AZ-6 (●) to Z-DNA was measured in a buffer containing 10 mM Tris-HCl (pH 7·5), 50 mM NaCl and 1 mM EDTA. Binding of MoAb Jel 150 (○) to Z-DNA was carried out in the presence of 10 mM Tris-HCl (pH 8·0), 100 mM NaCl and 1 mM EDTA. (b) Binding of AZ-6 to Z-DNA was measured in the presence of increasing amounts of phosphatidylcholine (●), glycerophosphorylcholine (○), phosphatidic acid (■) and cardiolipin (□). Binding of Jel 150 to Z-DNA was measured in the presence of increasing amounts of phosphatidic acid (▲) and cardiolipin (△).

with Z-DNA polymers are incapable of recognizing the supercoil induced Z-conformation in DNA plasmids. Thus methods currently used in studying Z-DNA specific proteins are not sufficient to eliminate proteins cross-reacting with phospholipids.

There are several reasons to believe that an even more complex situation may arise when studying anti-Z-DNA activity of autoantibodies. Anti-DNA antibodies from SLE sera are heterogeneous, showing different degrees of binding specificity towards different DNA sequences and conformations as well as cross-reactivity with polyanionic structures such as phospholipids [26], proteoglycans, dextran sulphate and membrane-associated proteins (see [27] and references therein). Thus in view of the broad range of cross-reactions expressed by autoantibodies, the possibility of their interacting non-specifically with Z-DNA is even higher than that of other proteins.

There is no report that convincingly establishes that autoantibodies from any source bind exclusively to Z-DNA. Also, no rigorous correlation has been made between antibodies binding to Z-DNA polymers and their ability to recognize Z-determinants in supercoiled DNA molecules. Limited efforts in this direction have led to differing conclusions [11,28]. Bergen *et al.* [5] have been successful in demonstrating that an anti-Z-DNA MoAb from unimmunized MRL mice is capable of interacting with the supercoiled form of a plasmid containing a Z-insert. However, further substantiation such as testing the interaction of the antibody with non-nucleic acid molecules and mapping the precise site of interaction on the plasmid would be

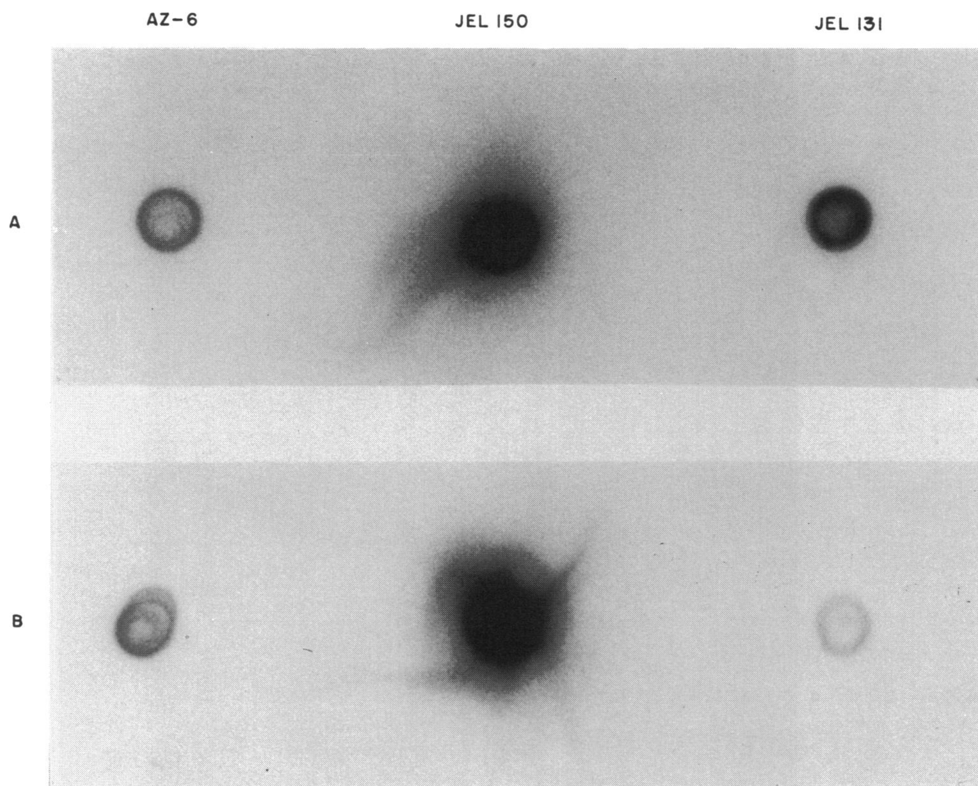

Fig. 4. Effect of cardiolipin on the Z-DNA binding activity of anti-Z-DNA antibodies in a dot blot assay. Binding to Z-DNA probe (Br-poly (dG-dC)) was carried out as described in Materials and Methods, in the presence of 100 mM NaCl. Blot A and blot B represent antibody binding to the Z-DNA probe in the absence of any competitor, and in the presence of cardiolipin (500 μg/ml), respectively.

desirable in defining the specificity of this antibody for Z-DNA. Despite the lack of evidence to establish unequivocally the presence of a specific anti-Z-DNA autoantibody, the pathological importance of anti-Z-DNA antibodies in SLE has continued to be stressed [6–8].

The studies reported here were initiated with the aim of determining if Z-DNA could be used as a ligand to measure the levels of anti-DNA antibodies in SLE serum as suggested by others [8]. The results of the present study indicate that current assays used to measure anti-Z-DNA activity could easily lead to incorrect and misleading conclusions. The reasons are: (i) since several serum proteins have the ability to bind strongly and preferentially to Z-DNA, a clear idea of the antibody levels will not be possible when SLE sera are used for studies; and (ii) since most Z-DNA binding activity of purified IgGs can be inhibited by cardiolipin, measuring anti-Z-DNA levels appears to be the equivalent of measuring non-specific ionic interaction of IgGs, which may not always correlate with the disease. Although antibody cross-reactivity with DNA and cardiolipin has been observed [26], our results show that the cross-reactivity between Z-DNA binding proteins and cardiolipin is also high. In the case of antibodies that react with both DNA and phospholipids, the diversity is explained by their reactivity to a phosphodiester epitope. However, if the phosphate backbone of nucleic acids and phospholipids is the cause for the cross-reactivity of proteins, the quantitative differences among the polynucleotide reactions (B-DNA and Z-DNA) indicate that the proteins are sensitive to different geometries of the backbone structure and charge distribution on the two forms of DNA. Z-DNA polymers have the ability to aggregate in solution [29]. It is possible that a high charge density on the surface of aggregated molecules is responsible for the increased interaction with proteins. The blotting assay described here can eliminate most of the proteins cross-reactive with negatively charged phospholipids and therefore offers advantages over other methods. However, proteins that bind Z-DNA by this method will require further testing with other Z-DNA ligands before a specific interaction with the left-handed form of DNA can be established.

Attention has recently focused on the importance of β_2-glycoprotein I, also known as apolipoprotein H, as an autoantigen in SLE and the primary anti-phospholipid syndrome [30–33]. Anti-cardiolipin (aCL) and other phospholipid (aPL) antibodies have been implicated in a number of clinical conditions, including recurrent spontaneous abortion and recurrent thromboses [32,33]. The binding of aCL and aPL to phospholipids has been shown to require β_2-glycoprotein I [30]. Furthermore, β_2-glycoprotein I itself has been suggested as the target of certain aCL [30,31,34], although this remains controversial [30]. The interaction of β_2-glycoprotein I and Z-DNA demonstrated in the present study is of interest because β_2-glycoprotein has been shown to bind lipoproteins and anionic phospholipids ([35] and references therein). This raises the possibility that the apparent anti-Z-DNA antibody binding is an artefact related to the presence of β_2-glycoprotein I–antibody complexes in the serum. Others have shown that lipoproteins

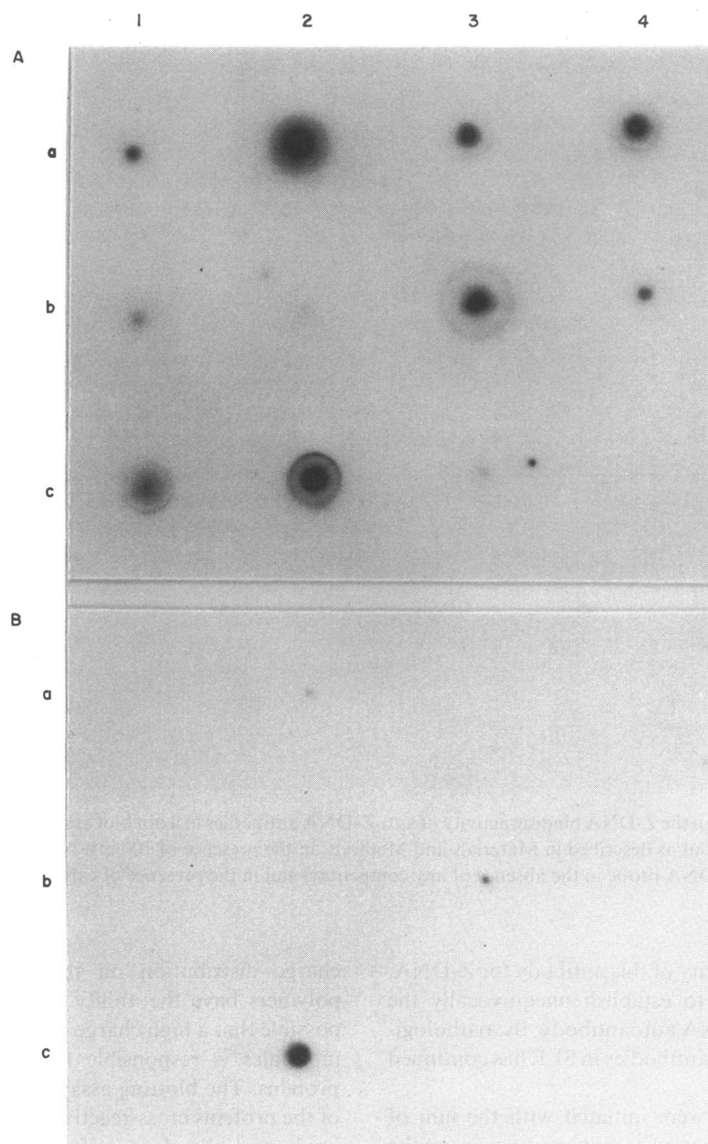

Fig. 5. Effect of cardiolipin on the Z-DNA binding ability of IgGs purified from normal and systemic lupus erythematosus (SLE) sera in a dot blot assay. Blot A and blot B represent IgG binding to the Z-DNA probe in the absence of any competitor, and in the presence of cardiolipin (500 μg/ml). Row a (left to right) represents IgGs from normal sera; row b, IgGs from SLE sera with high anti-DNA activity (1–3) and with high anti-RNP activity (4); row c, IgGs from SLE sera with high anti-cardiolipin activity.

can produce false positive binding in the *Crithidia luciliae* assay for anti-DNA antibodies [36]. Although β_2-glycoprotein I levels are elevated in patients with lupus anticoagulants [37], they are lower in SLE than normal controls and do not appear to be influenced by antiphospholipid antibodies [38]. Furthermore, it has not been established if respective antigen–antibody complexes are elevated.

The results of the present study suggest that an analysis of Z-DNA antibodies should be carried out with care. Autoantibodies with characteristics of AZ-6 may exist in serum; however, unless the existence of such antibodies has been clearly demonstrated, the functional relevance of anti-Z-DNA activity in SLE should be questioned.

ACKNOWLEDGMENTS

M.J.F. is a Scientist of the Alberta Heritage Foundation for Medical Research. This research was funded by the Arthritis Society and the Medical Research Council of Canada.

REFERENCES

1 Tan EM. Antinuclear antibodies: diagnostic markers for autoimmune diseases and probes for cell biology. Adv Immunol 1989; **44**:93–151.

2 Sinha AA, Lopez MT, McDevitt MO. Autoimmune diseases: the failure of self tolerance. Science 1990; **248**:1380–8.

3 Lafer EM, Valle RPC, Moller A, Nordheim A, Schur PH, Rich A,

Stollar BD. Z-DNA-specific antibodies in human systemic lupus erythematosus. J Clin Invest 1983; **71**:314–21.

4 Sibley JT, Lee JS, Decoteau WE. Left-handed "Z" DNA antibodies in rheumatoid arthritis and systemic lupus erythematosus. J Rheumatol 1984; **11**:633–7.

5 Bergen HR, Losman MJ, O'Connor T *et al.* Specificity of monoclonal anti-Z-DNA antibodies from unimmunized MRL/M_p-*lpr*/*lpr* mice. J Immunol 1987; **139**:743–8.

6 Thomas TJ, Meryhew NL, Messner RP. Enhanced binding of lupus sera to the polyamine-induced left-handed Z-DNA form of polynucleotides. Arthritis Rheum 1990; **33**:356–65.

7 Thomas TJ, Meryhew NL, Messner RP. DNA sequence and conformation specificity of lupus autoantibodies. Arthritis Rheum 1988; **31**:367–77.

8 Van Helden PD, van Lill L, Bester AJ, de Wall L, Helden EGH-V. A new assay for anti-DNA antibodies in serum which includes the measurement of anti-Z-DNA. Clin Exp Immunol 1987; **69**:394–402.

9 Lafer EM, Moller A, Nordheim A, Stollar BD, Rich A. Antibodies specific for left-handed Z-DNA. Proc Natl Acad Sci USA 1981; **78**:3546–50.

10 Stollar BD. Antibodies to DNA. CRC Crit Rev Biochem 1986; **20**:1–36.

11 Krippner H, Boehm BO, Pirlet K. Antibodies to dsDNA: failure to detect antigenic determinants specific for superhelical B-DNA. Mol Immunol 1985; **22**:623–7.

12 Zouali M, Migliorini P, Stollar BD. Murine lupus anti-DNA antibodies cross-react with the hapten (4-hydroxy-5-iodo-3-nitrophenyl) acetyl, but immunization-induced anti-DNA antibodies do not. Eur J Immunol 1987; **17**:509–13.

13 Krishna P, Kennedy BP, van de Sande JH, McGhee JD. Yolk proteins from nematodes, chickens, and frogs bind strongly and preferentially to left-handed Z-DNA. J Biol Chem 1988; **263**:19066–70.

14 Krishna P, Kennedy BP, Waisman DM, van de Sande JH, McGhee JD. Are many Z-DNA binding proteins actually phospholipid-binding proteins? Proc Natl Acad Sci USA 1990; **87**:1292–5.

15 Krishna P, Morgan AR, van de Sande JH. Interaction of recA protein with left-handed Z-DNA. Biochem J 1991; **275**:711–9.

16 Rohner KJ, Hobi R, Kuenzle CC. Z-DNA-binding proteins: identification critically depends on the proper choice of ligands. J Biol Chem 1990; **265**:19112–5.

17 Tan EM, Cohen AS, Fries JF *et al.* The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982; **25**:1271.

18 Ballou SP. *Crithidia luciliae* immunofluorescence test for antibodies to DNA. In: Rose NR, de Macario EC, Fahey JL, Friedman H, Penn GM, eds. Manual of clinical laboratory immunology. Washington: American Society for Microbiology, 1992:730–4.

19 Lockshin MD, Gharavi AE. Antiphospholipid antibody. In: Rose NR, de Macario EC, Fahey JL, Friedman H, Penn GM, eds. Manual of clinical laboratory immunology. Washington: American Society for Microbiology, 1992:785–8.

20 Fritzler MJ. Autoantibody testing: procedures and significance in systemic rheumatic diseases. In: Jasmin G, Simard R, eds. Methods and achievements in experimental pathology. New York: S Karger, 1986:225–60.

21 Lee JS, Woodsworth ML, Latimer LJP. Functional groups on 'Z' DNA recognized by monoclonal antibodies. FEBS Lett 1984; **168**:303–6.

22 Ginsberg B, Keiser H. A millipore filter assay for antibodies to native DNA in sera of patients with systemic lupus erythematosus. Arthritis Rheum 1973; **16**:199–205.

23 Bowen B, Steinberg J, Laemmli UK, Weintraub H. The detection of DNA-binding proteins by protein blotting. Nucleic Acids Res 1980; **8**:1–20.

24 Krishna P, van de Sande JH. Interaction of recA protein with acidic phospholipids inhibits DNA-binding activity of recA. J Bacteriol 1990; **172**:6452–8.

25 Fisher WR, Schumaker VN. Isolation and characterization of Apolipoprotein B-100. Methods Enzymol 1986; **128**:247–62.

26 Lafer EM, Rauch J, Andrzejewski C *et al.* Polyspecific monoclonal lupus autoantibodies reactive with both polynucleotides and phospholipids. J Exp Med 1981; **153**:897–909.

27 Brinkman K, Termaat R, Berden JHM, Smeenk RJT. Anti-DNA antibodies and lupus nephritis: the complexity of crossreactivity. Immunol Today 1990; **11**:232–4.

28 van Helden PD, Bester AJ. Naturally occurring anti-DNA antibodies from systemic lupus erythematosus patients may be used to study gene expression *in vitro*. Biochim Biophys Acta 1988; **949**:273–8.

29 van de Sande JH, Jovin TM. Z*DNA, the left-handed helical form of poly [d(G-C)] in $MgCl_2$-ethanol, is biologically active. EMBO J 1982; **1**:115–20.

30 McNeil HP, Simpson RJ, Chesterman CN, Krilis SA. Antiphospholipid antibodies are directed against a complex antigen that induces a lipid-binding inhibitor of coagulation: β_2-glycoprotein I (apolipoprotein H). Proc Natl Acad Sci USA 1990; **87**:4120–4.

31 Galli M, Comfurius P, Maassen C *et al.* Anticardiolipin antibodies (ACA) directed not to cardiolipin but to plasma protein cofactor. Lancet 1990; **335**:1544–7.

32 Harris EN, Gharavi AE, Hughes GRV. Antiphospholipid antibodies. Clin Rheum Dis 1985; **11**:591–609.

33 Petri M. The clinical syndrome associated with antiphospholipid antibodies. J Rheum 1992; **19**:505.

34 Oosting JD, Derksen RHWM, Entjes HTI, Bouma BN, de Groot PG. Lupus anticoagulant activity is frequently dependent on the presence of β_2-glycoprotein I. Thromb Haemostas 1992; **67**:499–502.

35 Nimpf J, Wurm H, Kostner GM Interaction of β_2-glycoprotein I with human blood platelets: influence upon the ADP-induced aggregation. Thromb Haemostas 1985; **54**:397–401.

36 Kumar V, Krasny S, Beutner EH. Specificity of the *Crithidia luciliae* method for detecting anti-DNA antibodies. Effect of absorption for lipoproteins. Immunol Invest 1985; **14**:199–210.

37 Galli M, Cortelazzo S, Daldossi M, Barbui T. Increased levels of β_2-glycoprotein I (aca-cofactor) in patients with lupus anticoagulant. Thromb Haemostas 1992; **67**:386.

38 Ichikawa Y, Takamatsu K, Shimizu H *et al.* Serum apolipoprotein H levels in systemic lupus erythematosus are not influenced by antiphospholipid antibodies. Lupus 1992; **1**:145–9.