Published in final edited form as:

Clin Immunol. 2014 July ; 153(1): 94–103. doi:10.1016/j.clim.2014.04.003.

The effect of polyamines on the binding of anti-DNA antibodies for patients with SLE and normal human subjects

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Abstract

Antibodies to DNA (anti-DNA) are the serological hallmark of systemic lupus erythematosus (SLE). To elucidate specificity further, the effect of polyamines on the binding of anti-DNA antibodies from patients with lupus was tested by ELISA to calf thymus (CT) DNA; we also assessed the binding of plasmas of patients and normal human subjects (NHS) to *Micrococcus luteus* (MC) DNA. As these studies showed, spermine can dose-dependently inhibit SLE anti-DNA binding to CT DNA and can promote dissociation of preformed immune complexes. With MC DNA as antigen, spermine failed to inhibit the NHS anti-DNA binding. Studies using plasmas adsorbed to a CT DNA cellulose affinity indicated that SLE plasmas are mixtures of anti-DNA that differ in inhibition by spermine and binding to conserved and non-conserved determinants. Together, these studies demonstrate that spermine can influence the binding of anti-DNA autoantibodies and may contribute to the antigenicity of DNA.

Keywords

SLE; anti-DNA antibody; antigenicity; spermine; polyamines

1. Introduction

Antibodies to DNA (anti-DNA) are the serological hallmark of systemic lupus erythematosus (SLE), a prototypic autoimmune disease characterized by the expression of antibodies to components of the cell nucleus (antinuclear antibodies or ANA) in association with tissue inflammation and injury [1]. A prominent immunological feature of SLE, anti-DNA antibodies serve as markers of diagnostic and prognostic significance and play a direct

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5. Conflict of Interest Statement

The authors declare that there is no conflict of interest.

role in disease pathogenesis via the formation of immune complexes. These complexes can deposit in the kidney to incite nephritis; in addition, complexes can stimulate plasmacytoid dendritic cells to produce type 1 interferon by delivering DNA to internal nucleic acids sensors, including Toll-like receptor (TLR) 9 [2-5]. While long utilized to assess disease activity in the clinic, anti-DNA antibodies have gained renewed interest as a biomarker since their expression may predict the response to immunosuppressive treatments, including the anti-BLyS agent belimumab [6].

Despite the close association of anti-DNA antibodies with clinical events in SLE, only certain antibodies with this specificity appear pathogenic and able to induce nephritis or promote cytokine production. The properties that determine pathogenicity are not well understood although isotype, avidity and charge may all contribute; conventional serological assays, however, do not distinguish pathogenic from non-pathogenic specificities [7]. While delineating pathogenicity would be valuable for clinical assessment and developing novel biomarkers, defining critical interactions responsible for this property has been difficult. In part, this difficulty relates to uncertainty about the actual form of DNA that is exposed to the immune system. In the nucleus, DNA is closely associated with histones to form the nucleosome in which 147 bases of DNA are wrapped around a core octamer of two molecules each of histones H2A, H2B, H3 and H4. To the extent that DNA is part of the nucleosome in its *in vivo* contact with the immune system, it may represent an epitope of a larger antigenic structure [8, 9].

Among other intracellular molecules with DNA binding activity, polyamines display a high intracellular concentration and represent a major source of cations which, along with histones, can shield the anionic charge of the phosphodiester backbone of DNA. The polyamines, spermine (N,N'-Bis(3-aminopropyl)-1,4-diaminobutane); spermidine (N-(3-Aminopropyl)-1,4-diaminobutane); and putrescine (1,4-Butanediamine) are biogenic amines that are found abundantly in eukaryotic and prokaryotic cells and are essential for cell function. These ubiquitous molecules are protonated at physiological pH, allowing interaction with anionic molecules such as DNA, RNA and some DNA-binding proteins [10, 11]. Bound polyamines are in equilibrium with the total free cellular polyamine pool that makes up 7-10% of the cell content. Among the three polyamines, spermine appears the most active because it contains the most charges (four) while putrescine contains the fewest (two) [12, 13].

While studies have extensively analyzed the influence of polyamines on DNA conformation and chromatin structure, few studies have investigated their effect on the binding of antibodies to conventional double-stranded (ds) DNA in the B conformation; polyamines, however, can affect the binding of antibodies to Z-DNA, a rare form of DNA with a zig-zag helix [14]. Because of the close association of polyamines with DNA in the nucleus, we asked whether these compounds, like histones, represent a nuclear component that can interact with DNA to affect its antigenicity. To investigate this possibility, we tested the effect of polyamines on the antigenicity of DNA by enzyme linked immunosorbent assays (ELISA), with a series of plasmas from patients with lupus. For comparison, we also tested the effect of polyamines on the anti-DNA antibodies that bind to bacterial DNA; these antibodies are present in the blood of normal human subjects (NHS) as well SLE patients

and do not have autoantibody activity. These antibodies differ from lupus anti-DNA in their high specificity for DNA from particular bacterial species, indicative of interaction with non-conserved antigenic determinants [15-18].

As the results presented herein show, among polyamines tested, spermine can effectively inhibit the interaction of DNA and anti-DNA from patients with lupus and even displace antibody from pre-formed complexes. Spermine, however, was unable to block the binding of antibodies that are selective for bacterial DNA antigen whether in the plasma of normal human subjects or patients with lupus. Together, these findings identify a molecular interaction that is important for the immune properties of DNA, including its binding to anti-DNA autoantibodies and ability to form immune complexes.

2. Materials and Methods

2.1. Plasmas and antigens

Plasmas of SLE patients were purchased from Plasma Services Group (Southampton, PA, USA) and were selected on the basis of a high binding to calf thymus (CT) DNA. Plasmas of normal healthy subjects (NHS) were purchased from Valley Biomedical Products & Services (Winchester, VA, USA). Double stranded calf thymus (CT) DNA (Worthington Biochemical, Lakewood, NJ, USA) and *Micrococcus luteus* (MC) DNA (Sigma-Aldrich, St. Louis, MO, USA) were first dissolved in TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) at a concentration of 0.5 mg/ml and then gently mixed overnight to assure solubilization. The DNA purity was determined by absorbance measurement of the OD₂₆₀/OD₂₈₀ ratio; concentrations were determined from OD₂₆₀ readings.

2.2. Polyamines

Spermine (N,N'-Bis (3-aminopropyl)-1,4-diaminobutane), spermidine (N-(3-Aminopropyl)-1,4-diaminobutane) and putrescine dihydrochloride (1,4-Butanediamine dihydrochloride) were purchased from Sigma-Aldrich and diluted with distilled water. The pHs of polyamine stock solutions were adjusted to 7.4 with hydrochloric acid.

2.3. Anti-DNA assay

96 well Immulon 2HB (high binding) flat-bottom microtiter plates (Thermo Scientific, Waltham, MA) were coated with CT DNA or MC DNA at 5 µg/ml in SSC buffer (150 mM NaCl, 15 mM sodium citrate, pH 7.0), overnight at 4°C. The plates were then washed 3 times with PBS (phosphate buffered saline, pH 7.4) and blocked with 200 µl/well of PBS pH 7.4 containing 2% bovine serum albumin (BSA) and 0.05% Tween 20 for 2 h. A series of dilutions of polyamines in dilution buffer (PBS containing 0.1% BSA 0.05% and Tween 20) were prepared. Following blocking, plates were washed and 50 µl of diluted polyamine at a concentration from 0 to 1mM or buffer only were added to wells simultaneously with 50 µl of plasma diluted in dilution buffer, and the plates incubated for 1 h. For inhibition experiments, the titer of each plasma was determined by prior titration to produce an OD value of approximately 1.5 when mixed with an equal volume of dilution buffer. After washing, plates were incubated with an anti-human IgG (gamma-chain specific) peroxidase-conjugated antibody (Sigma-Aldrich) at a dilution of 1:1000 for 1 h. The plates were then

washed and incubated with horseradish peroxidase substrate (0.015% 3,3',5,5'-tetramethylbenzidine dihydrochloride, 0.01% H₂O₂ in 0.1M citrate buffer, pH 4.0) for 30 minutes. Finally, 2M H₂SO₄ was added to stop the reaction and the absorbance values were measured at 450 nm with an UVmax spectrophotometer.

In these experiments, dilutions of polyamines, plasmas, and antibody were performed with dilution buffer. For the DNA-antibody complexes displacement assay, 50 µl plasma dilutions were first incubated with DNA coated plates for 1 h to form the DNA-antibody complexes; 50 µl of polyamine solution or dilution buffer were then added for an incubation of 1 h. Plates were washed and the antibody binding was determined as described. Unless otherwise noted, samples had a final volume of 100 µl/well, with incubations at a room temperature. The percent binding remaining in the presence of polyamine (% OD₄₅₀) was calculated as 100% x OD₄₅₀ with polyamine/ OD₄₅₀ without polyamine.

2.4. Anti-tetanus toxoid assay

Immulon 2HB (high binding) flat-bottom microtiter plates were coated with 100 µl/well of 1 µg/ml purified tetanus toxoid (gift from Richard M. Searce, Duke University Medical Center, Durham, NC, USA) in 0.1M sodium phosphate buffer (pH 9.0). Antibody binding of plasmas mixed with polyamine dilutions or with dilution buffer alone was tested by ELISA as described above.

2.5. Adsorption assay

Double-stranded CT DNA cellulose (Sigma-Aldrich) and Sigmacell cellulose (type 50, 50 µm, Sigma-Aldrich) as a control were resuspended at 50 mg/ml with TE buffer (10 mM Tris, 1 mM EDTA, pH 7.9) and rotated overnight at room temperature. After washing, 0.25 ml cellulose was mixed with 1 ml plasma diluted 1:100 in dilution buffer. The mixture was rotated for 2 h and then centrifuged at 1000×g for 4 min at room temperature. The supernatant was collected and diluted for anti-DNA assay as described above. As observed in titration experiments, the Sigmacell cellulose had negligible effect on anti-DNA antibody binding.

2.6. Statistical analysis

One-way ANOVA analysis and paired t test were used to compare data in different treatment conditions. P<0.05 was considered to be statistically significant.

3. Results

3.1. Influence of anti-DNA binding to CT DNA by polyamines

In these studies, three polyamines (spermine, spermidine and putrescine) were tested for their effect on the antigenicity of DNA. Fig. 1A shows the structures of these compounds. To determine whether these polyamines can affect anti-DNA binding, five SLE plasmas with anti-DNA activity were tested by ELISA using CT DNA as a source of mammalian DNA. In these experiments, the titers for the individual plasmas producing an OD value of approximately 1.5 when uninhibited varied from 1:800 to 1:1500. Results of the inhibition studies are presented for the plasmas as a group in Fig. 1B. As these data indicate, both

spermine (range from 50 μ M to 1 mM) and spermidine (range from 100 μ M to 1 mM) inhibited anti-DNA binding in SLE patients ($P<0.01$) while spermidine at 50 μ M and putrescine at all concentrations had no detectable influence ($P>0.05$). Compared to inhibition by spermidine, the inhibitory activity of spermine was significantly higher ($P<0.01$).

3.2. Specificity of spermine inhibition

In this and all subsequent experiments, we focused on spermine since it produced the highest level of inhibition in the ELISA. We first investigated the specificity of this inhibition, using tetanus toxoid as a control antigen and assessing the effect of the same concentrations of spermine as tested in the anti-DNA assay. As data in Fig. 2 indicate, spermine did not affect anti-tetanus binding by SLE plasmas. This finding suggests that inhibition of anti-DNA binding by spermine reflects a particular pattern of interaction with DNA rather than a non-specific binding to antigen on the plate.

3.3. Effect of spermine on anti-DNA binding to CT DNA

3.3.1. Inhibition of antibody binding to CT DNA antigen—To assess the range of spermine action, we tested its effect on antibody binding to CT DNA with the SLE plasmas. Plasmas were tested individually and results are presented for them as a group (Fig. 3A). In all cases, the inhibition was dose-dependent.

3.3.2. Inhibition of spermine on pre-formed immune complexes with anti-DNA
—In the context of SLE, anti-DNA antibody can bind DNA to form immune complexes which can mediate pathogenicity by tissue deposition or cytokine induction. We therefore asked whether spermine can dissociate pre-formed immune complexes. For this purpose, we first incubated SLE plasmas with CT DNA coated plates to create DNA-anti-DNA immune complexes. We then added spermine or dilution buffer to allow dissociation. The ELISA was then performed. As the data in Fig. 3B show, spermine can disassemble the pre-formed DNA-antibody immune complexes. The concentrations required to disassemble pre-formed immune complexes were similar to those used in the direct inhibition assay above. In both instances, inhibition was incomplete at the highest concentration of spermine tested, suggesting possible heterogeneity in the interaction of antibodies to DNA.

3.4. Effect of spermine on anti-DNA binding to bacterial MC DNA

3.4.1. Effect of spermine on antibody binding to MC DNA by NHS plasmas—Anti-DNA antibodies from patients with lupus bind primarily to conserved antigenic determinants that are widely expressed on DNA from various sources and likely correspond to the charge arrays on the phosphate backbone. As such, these non-selective antibodies show extensive cross-reactivity; although designated as autoantibodies, these antibodies bind well to mammalian, viral and bacterial DNA. Anti-DNA autoantibodies, however, represent only one type of anti-DNA specificity [19]. In contrast, normal human subjects (NHS) can express antibodies specific for some bacterial DNA antigens. These findings are consistent with the recognition of backbone determinants by lupus anti-DNA and sequences by NHS anti-DNA [17, 18].

To determine the effect of spermine on the binding of antibodies to selective sites on bacterial DNA, we investigated whether spermine can inhibit the binding to MC DNA by antibodies in NHS plasmas. In these experiments, we tested the hypothesis that, by binding to phosphate groups on the DNA backbone, spermine would preferentially inhibit the binding of lupus anti-DNA whereas its effect on NHS anti-DNA binding to bacterial DNA would be more limited. We therefore first assessed the binding of NHS plasmas to MC DNA. The data presented in Figure 4B. As these data indicate, spermine failed to inhibit anti-DNA binding to the MC DNA. These findings are consistent with the hypothesis that NHS plasma anti-DNA selectively binds to DNA determinants, likely specific sequences, that do not depend on charged phosphate groups of the DNA backbone with which spermine would interact.

3.4.2. Effect of spermine on anti-DNA binding to MC DNA by SLE plasmas—In view of observations that polyamines can interact with phosphates in the DNA backbone, we therefore wondered whether we could use inhibition by spermine as a measure of antibody specificity and the relative content of selective and non-selective anti-DNA antibodies. We next applied this approach to the SLE plasmas, testing binding to MC DNA which displays both conserved and non-conserved determinants. In these experiments, the titers for the individual plasmas producing an OD value around 1.5 when uninhibited varied from 1:1200 to 1:3200. As results of these studies showed, the plasmas could be divided into groups based on the extent of inhibition by spermine. Whereas anti-MC DNA binding in plasmas #1 and 2 (Type 1) was reduced in the presence of spermine, the binding of plasmas #3, 4 and 5 (Type 2) was not affected. Fig. 4B presents the mean % binding values for the two Type 1 plasmas; Fig. 4C presents the mean % binding values for the three Type 2 plasmas.

These findings suggest that the antigenic sites in MC DNA may vary in their binding to spermine such that, for Type 1 plasmas (# 1 and 2), spermine can inhibit antibody reactivity. For these plasmas, inhibition by spermine would be similar to that observed with CT DNA as an antigen. In contrast, for Type 2 plasmas (#3-5), the antigenic sites recognized by lupus anti-DNA would have less interaction with spermine. As a result, spermine would not inhibit antibody binding; in this way, these lupus plasmas would be similar to NHS plasmas. These data indicate that lupus plasmas can be mixtures of anti-DNA antibodies, containing both cross-reactive autoantibodies as well as selective antibodies to bacterial DNA, with the relative amounts of these antibodies influencing the extent of inhibition by spermine.

3.5. Effect of adsorption of plasmas to CT-DNA cellulose affinity matrix on antibody reactivity

As these results suggest, SLE plasmas may be heterogeneous in specificity and may contain a mixture of antibodies that differ in the extent of binding to mammalian and bacterial DNA antigens as well as dependence on charge-charge interactions. To test this possibility further, we used an approach previously applied to analyze the binding of SLE sera to DNA varying in species origin [19, 20]. In these previous experiments, we used a cellulose affinity matrix containing CT DNA to remove antibodies to the mammalian DNA; we then tested the adsorbed sera for binding to MC DNA. These studies showed that SLE sera differ in the

relative content of selective and non-selective binding antibodies; while adsorption removed essentially all reactivity to the bacterial DNA for some sera, for the other sera, antibody reactivity to the bacterial DNA remained, indicating the presence of selective and non-selective antibodies.

We then performed this analysis for the five SLE plasmas, testing for binding to CT and MC DNA following adsorption to the double-stranded CT DNA cellulose affinity matrix. As shown in Fig. 5A, the adsorption removed activity to CT DNA for all SLE plasmas. As shown in Fig. 5B, for the Type 1 plasmas, the affinity matrix removed a significant amount of antibody to MC DNA. This result indicates that the predominant specificity in these plasmas involves cross-reactive binding to DNA. In contrast, the Type 2 plasmas retained significant activity against the MC DNA following adsorption to the CT DNA cellulose. These findings are consistent with results of previous studies and suggest the presence of at least two populations of anti-DNA antibodies, one binding to CT DNA (and cross-reactive with MC DNA) and one specific for determinants on MC DNA [20]. Since we only tested MC DNA, it is possible that these plasmas contain antibodies to other bacterial (or viral) DNA.

We also tested the adsorption efficiency of the CT-DNA cellulose affinity matrix with NHS plasmas. Five NHS plasmas were adsorbed by CT-DNA cellulose affinity matrix and then tested for the binding to MC DNA; the levels of antibodies to CT DNA were low both before and after the adsorption. As shown in Fig. 5C, the level of binding for these plasmas to MC DNA was not affected by CT-DNA cellulose affinity matrix.

3.6. Effect on anti-DNA binding to MC DNA by spermine after adsorption to affinity matrix

Having shown that the lupus plasmas contain anti-DNA antibodies differing in specificity, we next tested the inhibitory activity of spermine on the binding to MC DNA of the plasmas which had been treated with the CT DNA cellulose affinity matrix. As data in Fig. 6 indicate, two patterns of inhibition were observed when testing with MC DNA antigen. While spermine still inhibited the binding to MC DNA by the Type 1 plasma that had been adsorbed with CT-DNA, the inhibition was less than that observed with these two plasmas adsorbed to control DNA cellulose (Fig. 6A). In contrast, spermine failed to inhibit binding to MC DNA of the Type 2 plasmas whether or not antibodies to CT DNA had been removed (Fig. 6B). This pattern resembles that of the NHS plasmas (Fig. 6C). These results indicate that the inhibitory effect of spermine on anti-DNA is closely related to specificity for cross-reactive or conserved antigenic determinants. Thus, inhibition of antibody binding to CT and MC DNA by spermine allows delineation of anti-DNA populations in terms of specificity and provides further evidence that cross-reactive anti-DNA binding reflects interactions with the phosphate backbone based on charge.

4. Discussion

Data presented herein provide new insights into the role of polyamines in determining the immunological properties of DNA, including its binding to anti-DNA antibodies and ability to form immune complexes. Thus, we showed that the polyamine spermine can inhibit the binding of anti-DNA antibodies in SLE plasmas to CT DNA and, furthermore, that spermine

can displace antibody from pre-formed immune complexes. The inhibitory capacity of the other polyamines tested was much less, consistent with the difference in the number of charged amines in these compounds; spermine, spermidine and putrescine have 4, 3 and 2 charged amines, respectively. In view of studies on the molecular interactions of polyamines with DNA [21-23], our findings suggest that the inhibitory activity of spermine reflects binding to the phosphodiester backbone, shielding the negative charge of the phosphate groups and potentially influencing backbone topology and activity as an autoantigen.

As a group, polyamines bind to DNA by electrostatic interactions based on the contact of positively charged amines with the negatively charged phosphate groups [24-26]. This interaction may involve contacts in both the major and minor groove of the double stranded helix [27]. In view of their chemical structure, polyamines serve as important counter-ions to neutralize the charge of DNA in the cell nucleus. In eukaryotic cells, spermine is present at a concentration of 0.9 - 1.6 mM and approximately 4% - 18% of the molecules are bound to DNA [10]. It should be noted that, in rapidly growing cells and G1 and S phases of the cell cycle, the level of polyamines is significantly elevated [28, 29]. In addition to charge neutralization, spermine can interact with nucleic acid bases and can perturb the structure of the deoxyribose sugar moiety [30, 31]. In this regard, while we have analyzed the activity of each polyamine alone, inside the cell, these compounds may interact to generate nuclear aggregates of polyamines (NAPs) [32]. As a result, polyamines, alone or together, have a number of potential binding activities that could affect the antigenicity of DNA.

Studies on the binding properties of lupus anti-DNA antibodies have suggested that interaction with the phosphate groups in the phosphodiester backbone is key to stable antibody binding [33, 34]. Thus, sera from patients with lupus generally react with DNA independent of species origin as well as base composition [15, 35]. This finding suggests predominant binding to a conserved antigenic determinant, with backbone phosphates most likely. Indeed, cross-reactivity with DNA is a distinguishing feature of lupus anti-DNA antibodies. While these antibodies are termed autoantibodies, in fact, they bind widely to other DNA of eukaryotic and prokaryotic origin as well as synthetic DNA antigens [19, 36].

Other evidence for the importance of charge-charge interactions in anti-DNA binding comes from studies on the sequence of monoclonal antibodies from lupus mice. These studies have indicated the striking presence of positively charged amino acid residues in the heavy chain variable region, with arginines in particular an important structural feature associated with high avidity binding [37, 38]. The effect of spermine on the binding of lupus anti-DNA to CT DNA supports the importance of electrostatic interactions in stable DNA binding. Thus, in these experiments, spermine caused dose-dependent inhibition of anti-DNA binding as well as dissociation of DNA-anti-DNA complexes, with 1 mM the highest concentration tested. While this inhibition was partial, it is possible that higher polyamine concentrations would cause more complete inhibition. Indeed, many studies on the effects of polyamines on DNA structure have involved much higher concentrations [39-41]. While the stoichiometry of these interactions in the solid phase is not known, our findings nevertheless indicate that charge interactions are important for lupus antibody binding and that blocking access to phosphates (the target of polyamines) can inhibit antibody binding.

In previous work, we provided evidence that NHS and lupus anti-DNA differ in their interaction with charged groups on DNA [42, 43]. This finding has suggested that NHS anti-DNA recognize non-conserved determinants on bacterial DNA, mostly likely base sequences, whereas lupus anti-DNA recognize a conserved determinant, most likely the phosphodiester backbone. Using MC DNA in an ELISA, we showed that lupus anti-DNA antibody binding is much more sensitive to increasing ionic strength and pH than NHS anti-DNA [16]. As both salt concentration and pH can limit interaction with charged residues, these findings suggest a greater role in antibody interaction for binding to phosphate groups by lupus anti-DNA compared with NHS anti-DNA.

Analysis of the effect of spermine on the binding of lupus anti-DNA to MC DNA herein showed a more complicated situation related to the apparent heterogeneity of antibodies. Along with previous studies [20], these results indicate that lupus plasma can contain mixtures of both cross-reactive anti-DNA (the classic lupus anti-DNA autoantibody) as well as antibodies that bind selectively to bacterial DNA. Importantly, the relative amounts of these types of antibodies can differ among patients. The recognition that plasma of lupus patients can be mixtures allows interpretation of the results of the polyamine inhibition experiments using MC DNA as an antigen. Thus, while all the lupus plasmas were inhibited to at least some extent by spermine with CT DNA as an antigen, only the Type 1 plasmas (#1 and 2) showed significant inhibition by spermine with MC DNA as antigen. In contrast, the Type 2 plasmas (#3-5), despite showing inhibition and complex dissociation by spermine with CT DNA, did not show inhibition by spermine with MC DNA antigen.

Together, these findings suggest that the Type 1 plasmas contain primarily antibodies that bind to the phosphate backbone. In contrast, the Type 2 plasmas most likely have a greater proportion of anti-DNA antibodies that bind selectively to bacterial DNA and do not depend on interactions with the phosphate backbone. In these responses, these patient plasmas resemble those of the NHS whose binding to MC DNA is not affected by spermine. In this regard, although uncertainty in the coating efficiency of different DNA antigens in an ELISA limits direct comparison of antibody titers by ELISA, the titers of antibodies to MC DNA were greater than those for CT DNA for plasmas #3-5 (data not shown).

The experiments on the effects of adsorption with CT DNA cellulose matrix further support both differences in the binding specificity of the two Type 1 SLE plasmas in comparison to the other three Type 2 SLE plasmas as well as differences in the specificity between cross-reactive and selective anti-DNA. Thus, as shown by titration of antibodies on CT and MC DNA, the extent of removal of antibody to MC DNA by the CT DNA affinity matrix appeared greater with the Type 1 than with the Type 2 plasmas. For Type 1 plasmas, the adsorption on CT DNA did not significantly reduce antibody titers on MC DNA, suggesting a robust expression of selective antibodies to MC. In addition, the adsorption with CT DNA cellulose matrix did not affect the binding to MC DNA by NHS plasmas. For these plasmas, spermine did not inhibit their binding to MC DNA. This result is consistent with recognition of a sequence or other antigenic structure whose presentation is not affected by spermine.

The effect of spermine on the binding to MC DNA by Type 1 plasmas following adsorption with CT DNA bears comment. For these plasmas, spermine produced a very limited amount

of inhibition of antibody binding to MC DNA following treatment with the CT-DNA cellulose affinity matrix that removed the cross-reactive or non-selective DNA. It is possible that this inhibition relates to another type of interaction of spermine with DNA since polyamines interact with bases themselves and there is evidence that polyamines can both promote and inhibit interactions of proteins with DNA [11, 27]. In this regard, CT and MC DNA differ markedly in base composition, with CT showing 42% GC residues and MC DNA showing 72% GC residues [44].

Our findings have important implications for understanding the antigenicity of DNA in the setting of lupus as well as prospects for developing specific inhibitors of anti-DNA binding. Clearly, our studies show that polyamines, which exist in both the cell and blood at significant concentrations, can influence the antigenicity of DNA, with the greatest impact on antibodies recognizing the phosphodiester backbone. Since DNA in cells will have bound polyamines, these compounds can influence the antigenicity as well as immunogenicity of DNA. It is of interest in this regard that polyamines have anti-inflammatory and immunosuppressive properties, raising the possibility that polyamines can modulate immune responses *in vivo*, with DNA released from cells bound to molecules that both shield charge, alter topology and modulate immune activity; in this construct, DNA released from cells *in vivo* may have a bound inhibitor that could limit immune activity in normal setting [45-47]. The effect of inflammation on the extent of polyamine binding to DNA during disease pathogenesis is not known although a change in these interactions could affect properties of DNA as an autoantigen, as shown herein, and, potentially, as an immunogen.

The findings may also be relevant to innovations in therapy and the development of small molecules that can block specifically DNA-anti-DNA interaction. Our previous research has explored the use of nucleic acid binding polymers (NABPs) to block both immunostimulatory activities of DNA and RNA as well as DNA-anti-DNA interactions. These compounds vary in chemistry but all bind avidly to DNA by electrostatic interactions [48, 49]. In general, the NABPs we tested are much larger than polyamines and have many more charged residues for phosphate binding. It is therefore interesting that a low molecular compound like spermine can achieve significant levels of inhibition. The use of either small or large molecules to block anti-DNA interactions has obvious relevance to therapy since anti-DNA antibodies play such a crucial role in lupus pathogenesis. Studies are therefore in progress to assess whether polyamines or structurally related compounds can be used *in vivo* to interfere with the formation of immune complexes in lupus or restrain their downstream effects.

Acknowledgments

This work was supported by a VA Merit Review Grant, a grant from Alliance for Lupus Research (ALR), and NIH 5U19-AI056363.

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Highlights

- Spermine can inhibit the binding of lupus anti-DNA to mammalian DNA.
- Spermine does not affect selective anti-DNA binding to bacterial DNA.
- Lupus plasmas contain mixtures of anti-DNA differing in inhibition by spermine.
- The antigenic determinants bound by anti-DNA in normal and lupus plasma differ.
- Spermine may influence the properties of DNA as an autoantigen.

Figure 1.

Influence of polyamines on antibody binding to CT DNA in SLE plasmas. (A) Illustration of chemical structure of the polyamines. The amidogens are protonated at physiological pH. (B) Five SLE plasmas were assayed for anti-DNA binding activity by ELISA in the presence of various concentrations of spermine, spermidine or putrescine. The % binding ($100\% \times \text{OD}_{450} \text{ with polyamine} / \text{OD}_{450} \text{ without polyamine}$) was calculated for each determination. Calculated % binding values at each concentration of polyamine for the five plasmas in three separate experiments were combined. Each point shown is the mean % binding $\pm$ SD.

Figure 2.

Influence of spermine on antibody binding to tetanus toxoid in SLE plasmas. SLE plasmas were assayed for binding to tetanus toxoid by ELISA in the presence of spermine or with dilution buffer alone. The % binding ($100\% \times \text{OD}_{450} \text{ with polyamine} / \text{OD}_{450} \text{ without spermine}$) was calculated for each determination. Calculated % binding values at each concentration of spermine for the five plasmas in three separate experiments were combined. Each point shown is the mean % binding $\pm$ SD.

Figure 3.

Effect of spermine on anti-DNA binding to CT DNA by SLE plasmas. (A) Direct inhibition of antibody binding to CT DNA by spermine. CT DNA binding activity in SLE the five plasmas was determined in the presence of spermine or dilution buffer alone by ELISA. The % binding ($100\% \times \text{OD}_{450} \text{ with polyamine} / \text{OD}_{450} \text{ without spermine}$) was calculated for each determination. Calculated % binding values at each concentration of spermine for the five plasmas in three separate experiments were combined. Each point shown is the mean % binding $\pm$ SD. (B) Displacement of anti-DNA antibody from pre-formed immune complexes by spermine. Fifty microliters of SLE plasma were first incubated with CT DNA in wells for 1 h, and then 50 μl of spermine or dilution buffer were added to wells for an additional hour. Calculated % binding values at each concentration of spermine for the five plasmas in three separate experiments were combined. Each point shown is the mean % binding $\pm$ SD.

Figure 4.

Effect of spermine on anti-DNA binding to bacterial MC DNA. The effect of spermine on anti-DNA binding to MC DNA was assessed for plasmas of normal human subjects (NHS) and patients with SLE. (A) Effect of spermine on antibody binding to MC DNA in NHS plasmas. Five NHS plasmas were assayed by ELISA for anti-DNA activity to MC DNA in the presence of spermine or dilution buffer alone. The % binding ($100\% \times OD_{450}$ with polyamine/ OD_{450} without spermine) was calculated for each determination. Calculated % binding values at each concentration of spermine for the five plasmas in three separate experiments were combined. Each point shown is the mean % binding $\pm$ SD in three experiments. (B) Effect of spermine on antibody binding to MC DNA in type 1 SLE plasmas. SLE plasmas #1 and 2 were assayed in the presence of spermine or dilution buffer alone by ELISA. Calculated % binding values at each concentration of spermine for the two plasmas in three separate experiments were combined. Each point shown is the mean % binding $\pm$ SD. (C) Effect of spermine on antibody binding to MC DNA in type 2 SLE plasmas. SLE plasmas #3 -5 were assayed in the presence of spermine or dilution buffer alone by ELISA. The % binding was calculated for each determination. Calculated % binding values at each concentration of spermine for the three plasmas in three separate experiments were combined. Each point shown is the mean % binding $\pm$ SD.

Figure 5.

Effect of adsorption of plasmas to CT DNA cellulose affinity matrix. SLE plasmas #1 to 5 were incubated with CT DNA cellulose affinity matrix or control cellulose and then were tested for anti-DNA binding to CT DNA and MC DNA by ELISA. The OD₄₅₀ of serial 2-fold dilutions of plasmas starting at 1:100 were measured in three separate experiments. (A) Binding of adsorbed SLE plasmas to CT DNA. The OD₄₅₀ for SLE plasmas #1 to 5 at each dilution of plasma were combined. Solid line shows data for CT DNA cellulose; dotted line shows data for control cellulose. Data shown are mean OD₄₅₀ ± SD. (B) Binding of adsorbed SLE plasmas to MC DNA. The OD₄₅₀ for type 1 SLE plasmas (#1 & 2) and type 2 SLE plasmas (#3-5) at each dilution of plasma were combined. Solid line shows data for CT-DNA cellulose; dotted line shows data for control cellulose. Data shown are mean OD₄₅₀ ± SD. (C) Five NHS plasmas were adsorbed by CT-DNA cellulose affinity matrix or control cellulose and then were tested for anti-DNA binding to MC DNA in the same way as SLE plasmas. Solid line shows data for CT-DNA cellulose; dotted line shows data for control cellulose. Data shown are the mean OD₄₅₀ ± SD in three experiments for one representative NHS plasma.

Figure 6.

Effect of spermine on anti-DNA binding to MC DNA after adsorption to affinity matrix. SLE plasmas #1 to 5 were adsorbed by CT-DNA cellulose or control cellulose and then were assayed for anti-MC DNA antibody by ELISA in the presence of spermine or with dilution buffer alone. The % binding ($100\% \times \text{OD}_{450} \text{ with polyamine} / \text{OD}_{450} \text{ without spermine}$) was calculated for each determination. Calculated % binding values at each concentration of spermine in three experiments were combined in two groups: Type 1 SLE plasmas (#1 & 2) and Type 2 SLE plasmas (#3-5). (A) Each point shown is the mean % binding $\pm$ SD for type 1 SLE plasmas. Solid line shows data for CT DNA cellulose; dotted line shows data for control cellulose. (B) Each point shown is the mean % binding $\pm$ SD for type 2 SLE plasmas. Solid line shows data for CT DNA cellulose; dotted line shows data for control cellulose. (C) Effect of spermine on antibody binding to MC DNA in adsorbed NHS plasmas. Five NHS plasmas were adsorbed by CT DNA cellulose or control cellulose and then were assayed for anti-MC DNA antibody by ELISA in the presence of spermine or with dilution buffer alone. Each point shown is the mean % binding $\pm$ SD in three experiments for one representative NHS plasma. Solid line shows data for CT-DNA cellulose; dotted line shows data for control cellulose.