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The effect of polyamines on the binding of anti-DNA antibodies from 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.
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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 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 acid 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

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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 OD_{260}/OD_{280} ratio; concentrations were determined from OD_{260} 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 1 mM or buffer only was 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%