Anti-DNA Antibodies Bind to DNase I

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Summary

Polyspecificity is a well-known property of the anti-DNA antibodies produced by autoimmune animals. In our search for antigen targets of anti-DNA antibodies within tissue extracts, we identified a 32-kD polypeptide that was recognized by a large panel of anti-DNA antibodies. Direct sequencing of this protein disclosed its identity with DNase I. 22 monoclonal anti-DNA antibodies bound to DNase I in direct and competitive immunoassays; out of 15 autoantibodies that did not bind DNA, none had the ability to bind DNase I. The ability of anti-DNA antibodies to interfere with DNase I enzymatic activity was evaluated in an assay based on the enzyme digestion of phage double strand DNA. Six monoclonal anti-single strand DNA antibodies that did not bind double strand DNA were tested in this assay. Three out of six inhibited DNase I–mediated digestion of phage DNA. The interaction of anti-DNA antibodies with DNase I was further investigated by testing their ability to bind a synthetic peptide that corresponds to the catalytic site of the molecule. 4 out of 22 anti-DNA antibodies bound the active site peptide; two of these had been shown to inhibit DNase I enzymatic activity. This report shows that anti-DNA antibodies recognize both DNA and its natural ligand DNase I. Some anti-DNA antibodies inhibit DNase I enzymatic activity, thus displaying the potential to modulate DNA catabolism. The dual specificity of anti-DNA antibodies offers a clue for understanding the mechanisms that lead to anti-DNA antibody production in autoimmune animals.

Identification of the events leading to the production of anti-DNA antibodies in individuals with systemic lupus erythematosus has been enigmatic. Genetic studies of anti-DNA antibodies from lupus-prone mice suggest that the production of anti-DNA antibodies is antigen driven. However, naturally occurring nucleic acids and, in particular, double strand DNA (dsDNA)[1], are only weakly immunogenic (1). Moreover, antibodies produced after immunization with DNA do not cross-react with multiple autoantigens like their pathogenic counterparts (2–9), nor do they produce lupus lesions (10, 11). In view of this promiscuous reactivity of lupus autoantibodies, we reasoned that self proteins, rather than nucleic acids, could be responsible for eliciting a lupus antibody response in genetically susceptible and/or immunologically impaired individuals. To test this hypothesis, our goal was to identify candidate proteins.

In our search for cross-reactive proteins of lupus autoantibodies, we identified a 32-kD polypeptide that was specifically recognized by a large panel of anti-DNA antibodies. On subsequent analysis, this peptide was determined to be DNase I. Furthermore, a subset of anti-DNA antibodies inhibited the functional activity of this enzyme; epitope mapping of these antibodies localized the binding to a positively charged sequence corresponding to the catalytic site of the molecule. This novel antigenic specificity of anti-DNA antibodies provides clues to understanding the events leading to the production of anti-DNA antibodies in autoimmune individuals, and it raises the possibility that the direct interaction of autoantibodies with constitutive enzymes may influence the autoimmune/inflammatory response.

[1] *Abbreviations used in this paper:* dsDNA, double strand DNA; ssDNA, single strand DNA.

Materials and Methods

Antigens and Buffers. (*a*) Preparation of DNase I. Bovine DNase I, RNase-free, was purchased from Boehringer Mannheim (Mannheim, Germany). (*b*) Preparation of glomerular extract. Human glomerular extract was prepared as described elsewhere in detail (10). Briefly, human glomeruli were lysed in cold hypotonic buffer (20 mM Tris, pH 7.4) containing a cocktail of protease inhibitors (PMSF, leupeptin, aprotinin, pepstatin, and antipain), reduced with 2-mercaptoethanol (1% final vol), and treated with SDS (3% final vol). (*c*) Preparation of polynucleotides. Single strand DNA (ssDNA) and dsDNA were prepared as described elsewhere in detail (10–12). Poly(dT) was purchased from Sigma Chemical Co. (St. Louis, MO). (*d*) Chelex 100 treatment. Chelex 100 resin, a chelating agent for

divalent cations (Ca^{2+}, Mg^{2+}, and Mn^{2+}), was purchased from Sigma Chemical Co. and used according to the manufacturer's instructions. The buffers or the antigen and antibody solutions (100 ml/5 g resin) were absorbed three times on Chelex 100 to ensure complete removal of divalent cations.

Antibodies. Murine monoclonal anti-DNA antibodies (H241, H130, H102, P168, 27, D, 15/9, 110/13, 512, A, and 62/11), as well as anti-DNA–negative autoantibodies (136, A, C, 56, 35, 21/7, 85, 5/5, 100, and 28/12), were prepared from spleen cells of immunized MRL-lpr/lpr mice (3, 5, 13, 14).

Antibodies 21/6, 21/10, 21/12, 21/15, 21/17, and 21/23 were obtained from the fusion of splenocytes of BALB/c mice injected with the ribonucleoprotein-mimicking idiotype light chain G, as elsewhere described in detail (15). The anti-DNA antibodies were all IgM with the following exceptions: monoclonal antibody H241 (IgG_{2a}) and antibody H102 (IgG_{2b}). The anti-DNA–negative autoantibodies were all IgM with a few exceptions: antibody C (IgG_{2a}), antibody 28/12 (IgG_{2b}), and antibody 35 (IgG_3).

Human monoclonal anti-DNA antibodies (Hy2, Hy8, Hy9, Hy10, and Hy12) were derived by EBV transformation of B cells obtained from patients with systemic lupus. Human anti-DNA–negative autoantibodies (A2, A10, A77, and B19.7) were obtained in Dr. R. S. Schwartz's laboratory (New England Medical Center, Boston, MA). All the human monoclonal anti-DNA antibodies, as well as control autoantibodies, were IgM.

The mouse IgM monoclonal anti-DNase I antibody was obtained from the fusion of splenocytes of a BALB/c mouse immunized with bovine DNase I.

Isotype-matched murine Ig was purchased from Cappel Laboratories (Cochranville, PA).

Preparation and characterization of IdssDNA and IddsDNA are described elsewhere in detail (16). Briefly, the two antibody preparations were derived from a patient with active lupus nephritis. IdssDNA was prepared using an oligo(dT)-cellulose column (Pharmacia LKB, Piscataway, NJ); the antibody population had no anti-dsDNA activity.

IddsDNA was prepared using a native dsDNA-cellulose column (Pharmacia LKB); the preparation preferentially reacted with dsDNA.

Polyclonal anti-DNA antibodies were isolated from the serum of eight different patients with active lupus nephritis with an oligo(dT)-cellulose column. The preparations obtained retained both anti-ss and anti-dsDNA activity.

To avoid any interference by DNA–anti-DNA complexes within individual antibody preparations, anti-DNA antibodies were treated with micrococcal nuclease, and both double-stranded and single-stranded exonucleases.

Briefly, 10 μg of individual antibodies 21/6, 21/12, H241, H130, H102, Hy2, Hy9, and Hy12 were first digested with micrococcal nuclease (Sigma Chemical Co.) in 50 mM Tris, 5 mM $MgCl_2$, pH 7.5, and the reaction was stopped by the addition of 10 mM EDTA. Further digestions were obtained with exonuclease III (double-stranded 3′-5′ exonuclease) (Promega Corp., Madison, WI), 20 U in 50 mM Tris, 5 mM $MgCl_2$, 5 mM dithiothreitol, 20 mM KCl, 50 μg/ml BSA, pH 7.5 (1 h, 37°C); and exonuclease VII (5′-3′ and 3′-5′ exonuclease) (Promega Corp.), 0.4 U in 70 mM Tris, 8 mM EDTA, 10 mM β-mercaptoethanol, 50 μg/ml BSA, pH 8. The antibody preparations were then absorbed on Chelex 100 and extensively dialyzed against Chelex 100–treated 20 mM Tris, pH 7.5. Ethidium bromide staining of phenol-extracted digested antibodies (50 μg/lane of an agarose gel) did not reveal any DNA fragment.

Western Blot. The glomerular extracts were separated on a 10% SDS-PAGE in reducing conditions and transferred to nitrocellulose or polyvinyldifluoride membranes (Immobilon P; Millipore Corp., Bedford, MA). Filters were saturated by 1-h incubation at room temperature in 50 mM Tris, 150 mM NaCl, 5% dry nonfat milk. The same buffer was used for antibody dilutions and washing. Purified mAbs at 20 μg/ml or polyclonal Ig at 10 μg/ml were incubated on filters for 4 h at room temperature. After washings, alkaline phosphatase–conjugated goat anti-human IgG, anti-human IgM (Sigma Chemical Co.), or goat anti-mouse IgG-IgA-IgM (Boehringer Mannheim) were added and incubated overnight at 4°C. The immunoreactive bands were visualized using 5-bromo-4-chloro-indoxyl-phosphate and nitroblue tetrazolium as substrate (17). Ethidium bromide staining of phenol-extracted dry nonfat milk did not reveal the presence of DNA.

Microprotein Sequencing. The protein of interest (32 kD) was partially purified from human glomerular extract, using several steps of ion exchange chromatography (on DEAE-Sephadex and carboxymethyl-Sephadex [Sigma Chemical Co.]). The fractions enriched for the 32-kD polypeptide were pooled and concentrated using Centricon microconcentrators (Amicon, Beverly, MA) (10,000 mol wt cut-off). The concentrated material was precipitated with ice-cold 20% TCA for 1 h on ice. The pellet, obtained after centrifugation, was washed twice with cold acetone, dried in a vacuum centrifuge (Univepo 150 H; Uniequip, Martinswed, Germany), and resuspended in a small volume of 50 mM ammonium bicarbonate, 4 M urea, 2% SDS, pH 7.4.

The purified extract was run on a 12.5% SDS-PAGE under reducing conditions and transferred to polyvinyldifluoride membranes (Immobilon, P; Millipore Corp.), using 10 mM 3-cyclohexylamino-1-propane sulfonic acid (CAPS), pH 11. The band of interest was then excised and subjected to microprotein sequence, according to P. Matsudaira (18), using a sequencer (Applied Biosystems, Foster City, CA).

Immunoprecipitation. Purified glomerular extract and DNase I (Boehringer Mannheim) were labeled with I^{125}, using a bead-immobilized preparation of lactoperoxidase and glucose-oxidase (Enzymobead Solid Phase Radioiodination Kit; Bio-Rad Laboratories, Richmond, CA). The labeled material was diluted in cold lysis buffer (20 mM Tris, 150 mM NaCl, 10 mM EDTA, 1% NP-40, 0.2% SDS, pH 7.5, containing a mixture of protease inhibitors) and precleared twice by the addition of one-fifth the volume of Pansorbin (Calbiochem-Novabiochem, San Diego, CA).

Immunoprecipitations were carried out by overnight incubation of the individual antibodies (20 μg of either human or mouse mAbs, 10 μg of human polyclonal Ig) with aliquots of labeled extract or DNase I (10^6 TCA precipitable cpm/experiment). 50 μl of packed Sepharose beads (Pharmacia LKB), previously coupled to either rabbit anti-mouse IgM (Litton Bionetics, Kensington, MD) or goat anti-human IgM (Calbiochem-Novabiochem), or, alternatively, 50 μl of protein A-Sepharose beads, were added to individual tubes, and the samples were rotated for 1 h at 4°C. The beads were extensively washed five times with cold washing buffer (20 mM Tris, 15 mM EDTA, 500 mM NaCl, 1% NP-40, 1% SDS, pH 7.5, containing a mixture of protease inhibitors). For absorption experiments, radiolabeled glomerular extract was repeatedly absorbed on insolubilized antibodies, until no radioactivity could be eluted from the beads. The absorbed glomerular extract was then immunoprecipitated as above described. The immunoprecipitates were eluted in sample buffer and analyzed by SDS-PAGE. Dried gels were autoradiographed at −70°C with an x-ray intensifying screen.

Peptide Synthesis. Synthetic peptides were obtained by solid phase synthesis, using Fmoc-protected amino acids, according to the method of Merrifield as modified by Atherton (19). The peptides

were purified by gel filtration on a Sephadex G-25 column. The peptides, which had as a cysteine the NH_2- and COOH-terminal residue, were oxidized by oxygen bubbling.

ELISA. DNase I and peptides were used at a concentration of 10 μg/ml to coat polystyrene plates (Nunc, Roskilde, Denmark). After blocking for 1 h with 3% BSA in PBS, the antibodies, diluted in 1% BSA, 0.05% Tween in PBS, were added and incubated for 4 h at room temperature. The plates were then washed once with 1% Tween in PBS and twice with PBS. Alkaline phosphatase–conjugated goat anti–human IgG or IgM (Sigma Chemical Co.), goat anti–mouse IgG-IgA-IgM, or anti–mouse IgM (Boehringer Mannheim) in diluting buffer were then added and incubated overnight at 4°C. After washings, the bound enzymic activity was measured with *p*-nitrophenyl-phosphate (Sigma Chemical Co.).

For competitive assays, the amount of antibody that gave 50% of the maximum binding to the antigen on the solid phase was preincubated with different amounts of competitors or buffer for 1 h at 37°C and then transferred to the antigen-coated plates. The assay was then carried on as the direct binding assay. Alternatively, antibody preparations were Chelex 100–treated to remove divalent cations. ELISA plates were coated with DNase I (10 μg/ml in Chelex 100–treated 20 mM Tris, pH 7.5), blocked with 3% BSA in 250 mM EDTA for 1 h at room temperature, and washed with 0.05% Tween in Chelex 100–treated 20 mM Tris, pH 7.5 (buffer A). Buffer A was also used for antibody dilutions and washings. Bound enzymatic activity was revealed as described above. The direct binding and competitive ELISA for antibody against ssDNA, dsDNA, and polynucleotides have been described in detail elsewhere (3, 5, 12, 13). Ethidium bromide staining of phenol-extracted BSA did not reveal the presence of DNA.

DNase I Functional Activity. Hind III-digested λ DNA, (Bio-Rad Laboratories, Inc.) was treated with DNase I (Boehringer Mannheim). Briefly, 3 μg of DNA were incubated with 1 μg of DNase I in 50 mM Tris-HCl, 1 mM $MgCl_2$, 0.1% BSA, pH 7.5 for 20 min on ice. The reaction was then stopped with 10 mM EDTA. In inhibition experiments, 10 μg of anti-DNA antibodies or control Ig were preincubated with 1 μg of DNase I for 20 min on ice. The assay was then carried out as described above. Digested DNA was run on a 1% agarose gel. The gel was then stained with 2 μg/ml of ethidium bromide and photographed under UV light.

Results

Reactivity of Anti-DNA Antibodies with a 32-kD Polypeptide, Highly Homologous to DNase I. To select candidate autoantigen proteins, our overall strategy was to evaluate a large panel of anti-DNA antibodies from MRL-lpr/lpr mice and SLE patients for reactivity with tissue extracts (as a source of potential protein autoantigens). After a brief survey, it became apparent that, although other bands were often observed, reactivity of anti-DNA antibodies with a 32-kD polypeptide was universal (see below). An example of this observation for anti-DNA antibodies derived from human lupus is illustrated in Fig. 1. Our aims were to identify the nature of this/these protein(s) and to further examine the interaction between anti-DNA antibodies and this/these protein(s).

By two-dimensional gel electrophoresis, a single immunoreactive spot was identified. The 32-kD polypeptide was isolated and subjected to partial aminoacid sequence analysis. Its NH_2-terminal sequence was 94% homologous to bovine DNase I (15/16 amino acids).

The putative identity of the 32-kD peptide as DNase I

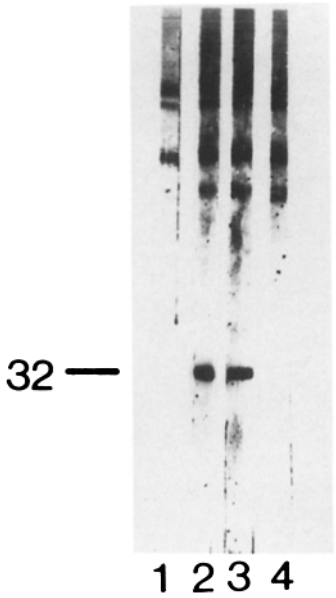

Figure 1. Binding of affinity-purified human anti-DNA antibodies to glomerular extract. Human glomerular extract was run on a 12% SDS gel under reducing conditions and transferred to nitrocellulose. The filters were probed with two anti-DNA polyclonal preparations, anti-ssDNA, IdssDNA, and anti-dsDNA, IddsDNA (lanes *2* and *3*), isolated from a patient with systemic lupus and with normal human polyclonal Ig (lane *4*). Lane *1* is a control lane in which only the second antibody (alkaline phosphatase–conjugated goat anti–human Ig) was used. Anti-DNA antibodies detect a band of 32 kD; no reactivity is detected with normal human Ig.

was confirmed by the following observations: A murine monoclonal anti-DNase I and the anti-DNA antibodies immunoprecipitated a 32-kD band in the radiolabeled glomerular extract. An example is given in Fig. 2 (lanes *5* and *6*). When radiolabeled glomerular extract was absorbed on immobilized anti-DNase antibody before immunoprecipitation, the anti-DNA antibodies did not recognize the 32-kD protein (Fig. 2, lane 7). Conversely, after absorption with anti-DNA antibodies, the monoclonal anti-DNase did not immunoprecipitate the 32-kD protein (Fig. 2, lane *8*). The protein immunoprecipitated by the monoclonal anti-DNase was recognized in Western blot by anti-DNA antibodies (data not shown). The anti-DNA antibodies that reacted with the 32-kD band also recognized labeled DNase I (Fig. 3). Finally, peptide maps, generated from the isolated 32-kD band, and purified DNase I were identical (data not shown). Taken together, these results suggest that the immunoreactive protein in the cellular extract is DNase I.

Anti-DNase I Activity Is Specific and Common among Anti-DNA Antibodies. To examine the specificity of these interactions, an unselected panel of monoclonal anti-DNA antibodies (17 mouse, 5 human) were tested for anti-DNase I activity by direct binding and competition immunoassays (Table 1). All 22 anti-DNA mAbs bound to solid phase DNase I; by comparison, binding was not observed for any of the autoantibodies that did not bind DNA (10 murine and 5 human mAbs), nor by isotype-matched control Ig. The specificity of the results was confirmed by competitive inhibition in each case. The compiled results are given in Table

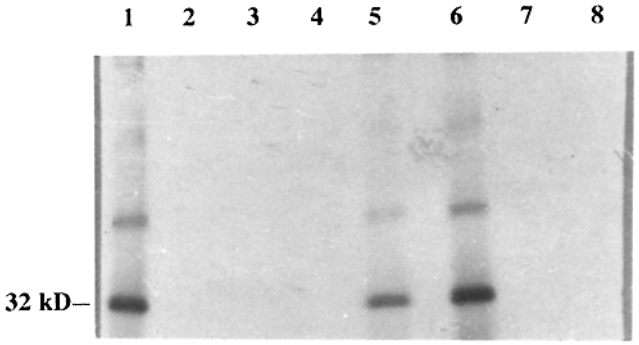

Figure 2. Immunoprecipitation of I^{125}-labeled glomerular extract. Glomerular extract was labeled with I^{125} and incubated with individual antibodies (10^6 TCA precipitable cpm/antibody). The complexes were precipitated by insolubilized anti-mouse Ig or protein A. (Lane *1*) Mouse polyclonal anti-DNase I antibodies. (Lane *2*) Mouse monoclonal anti-DNA negative IgG antibody. (Lane *3*) Mouse monoclonal anti-DNA negative IgM antibody. (Lane *4*) human polyclonal anti-DNA negative IgG antibodies. (Lane *5*) Mouse monoclonal anti-DNase I. (Lane *6*) Human polyclonal anti-ssDNA antibody, IdssDNA. The two anti-DNase I antibodies (lanes *1* and *5*) and the anti-DNA antibody, IdssDNA (lane *6*) detect a band of the same molecular weight (32 kD), which is not recognized by control antibodies (lanes *2–4*). For absorption experiments, radiolabeled glomerular extract was repeatedly absorbed with insolubilized antibodies (mouse monoclonal anti-DNase I antibody (lane 7) or polyclonal anti-DNA antibody, IdssDNA (lane *8*), until no labeled material could be eluted from the beads. The absorbed extract (10^6 TCA precipitable cpm/antibody) was then immunoprecipitated with IdssDNA (lane 7) or mouse monoclonal anti-DNase I antibody (lane *8*). After absorption no 32-kd band could be immunoprecipitated by anti-DNA or anti-DNase I antibodies.

2. The binding of the anti-DNA antibodies to DNAase I was also shown by Western blot and immunoprecipitation techniques (Table 2). Binding to DNase I was also observed among 8/8 human polyclonal anti-DNA antibody preparations derived from the serum of lupus patients; however, it was not present among either Ig that did not bind DNA from the same serum, or Ig isolated from normal human sera (not shown).

We next considered the possibility that DNA was present in the assays and affected the results (i.e., anti-DNA antibody binding to the DNA–DNase I complex). As DNase I binding to DNA is strictly calcium and magnesium dependent (20–23), the assays were performed in Tris buffer treated with chelating agents (Chelex 100). Under these conditions, DNase I cannot bind and digest DNA. However, all the anti-DNA antibodies retained activity against DNase I. Moreover, the DNase I–binding ability of mAbs 21/6, 21/12, H241, H130, H102, Hy2, Hy9, and Hy12 was not significantly reduced by the antibody treatment with DNA-hydrolyzing enzymes, micrococcal nuclease, and double-stranded and single-stranded exonucleases (data not shown).

A Subset of Anti-DNA Antibodies Reacts with the Functional Domain of DNase I and Inhibits Enzymatic Activity. To test whether anti-DNA antibodies interfere with DNase I enzymatic activity, it was necessary to circumvent possible interactions between anti-DNA antibodies and DNA in the assay. For this purpose, six monoclonal anti-ssDNA antibodies without detectable anti-dsDNA antibody activity were selected, and their capacity to inhibit DNase I–mediated digestion of phage dsDNA was evaluated. As illustrated in Fig. 4, three of six mAbs were observed to interact with DNase I and to inhibit the digestion of DNA.

Based on these results, we postulated that some of the anti-DNA antibodies may bind to the functional domain of DNase I and inhibit its enzymatic activity. Alternatively, interactions with other residues could alter the conformation of the enzymatic site to influence enzymatic activity. To address these possibilities, we took advantage of the observations of D. Suck and co-workers (22), who demonstrated that part of the active site of DNase I is formed by a loop corresponding to the sequence 73–78: Arg-Asn-Ser-Tyr-Lys-Glu. Three closely related peptides were synthetized as follows: adding two alanine residues as NH_2- and COOH-terminal amino acids (peptide a); adding two cysteine residues as NH_2- and COOH-terminal amino acids (peptide b); adding two cysteine residues as NH_2- and COOH-terminal amino acids and substituting Arg 73 and Lys 77 with alanine (peptide c). The cysteine-containing peptides were then oxidized so that a cyclic peptide could be formed whenever cysteine was the NH_2- and

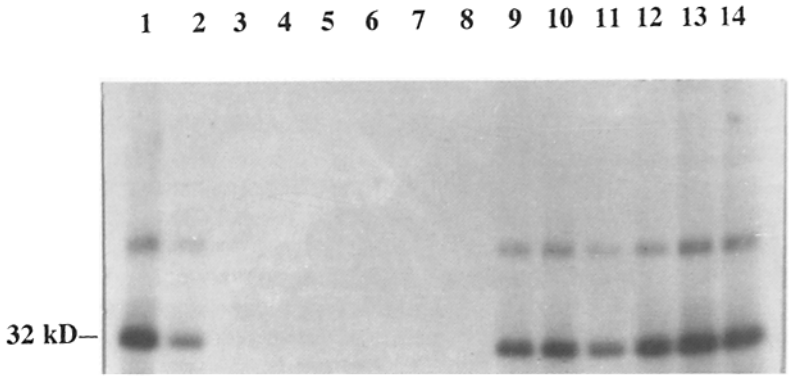

Figure 3. Immunoprecipitation of I^{125}-labeled DNase I. DNase I was labeled with I^{125} and incubated with individual antibodies. The immune complexes were precipitated by the addition of insolubilized anti-human or anti-mouse Ig. (Lane *1*) Mouse polyclonal anti-DNase I serum. (Lane *2*) Mouse monoclonal anti-DNase I antibody. (Lane *3*) Anti-DNA negative mouse monoclonal IgM antibody 21/7. (Lane *4*) Anti-DNA negative mouse monoclonal IgM antibody 136. (Lane *5*) Anti-DNA negative mouse monoclonal IgG antibody (C). (Lane *6*) Anti-DNA negative human monoclonal IgM antibody A2. (Lane *7*) Anti-DNA negative human monoclonal IgM antibody A10. (Lane *8*) Human polyclonal anti-DNA negative IgG antibodies. (Lane *9*) Human monoclonal anti-DNA antibody Hy12. (Lane *10*) Mouse monoclonal anti-DNA antibody H130. (Lane *11*) Mouse monoclonal anti-DNA antibody P168. (Lane *12*) Mouse monoclonal anti-DNA antibody H241. (Lane *13*) Human polyclonal anti-ssDNA antibody, IdssDNA. (Lane *14*) Human polyclonal anti-dsDNA antibody, IddsDNA.

Table 1. *Anti-DNase I Activity of Anti-DNA mAbs*

	ssDNA	dsDNA	DNase I		peptide a	peptide b
	Mouse mAbs					
H241	0.20*	0.16*	2.5*	0.90‡	5.0*	5.0*
H130	0.15	–§	5.0	0.70	–	–
H102	0.20	–	5.0	0.40	–	–
P168	0.60	–	2.5	0.65	5.0	5.0
27	2.00	–	5.0	0.30	±	±‖
D	0.16	–	5.0	0.35	–	–
15/9	1.25	–	2.5	0.50	–	–
110/13	0.40	2.00	3.0	0.55	5.0	5.0
512	1.00	–	2.5	0.48	±	±
62/12	1.25	–	5.0	0.73	±	±
21/6	0.40	2.00	5.0	0.60	–	–
21/10	0.02	–	5.0	0.56	±	±
21/12	0.08	–	8.0	0.34	–	–
21/15	0.02	–	8.0	0.40	–	–
21/17	0.40	–	2.0	0.75	±	±
21/23	0.02	0.40	2.0	0.85	±	±
	Human mAbs					
Hy 2	1.00	–	5.0	0.45	±	±
Hy 8	0.40	–	5.0	0.38	–	–
Hy 9	0.20	–	2.5	0.58	–	–
Hy 10	0.10	–	2.5	0.54	±	±
Hy 12	0.60	–	4.0	1.00	2.5	2.5

* Micrograms of liquid phase inhibitor required for 50% inhibition in competitive homologous assays.
‡ A 405 for 10 μg/ml mAb in direct-binding assay.
§ – indicates no binding to the antigen on the solid phase.
‖ ± indicates direct binding, but >10 μg/ml inhibitor required for 50% inhibition in competitive immunoassays.

COOH-terminal residue. The cyclic peptides should, therefore, closely approximate the tertiary structure of the loop.

The anti-DNA antibodies were then tested for reactivity against the peptides. As shown in Table 1, four of the antibodies bound peptides a and b in direct and competitive ELISA; none of them reacted with peptide c. Two of the peptide-binding antibodies (P168 and Hy12) had been shown to inhibit DNase I functional activity. Moreover, the binding of mAbs 110/13 and Hy12 to solid phase DNase I was competed by peptide a and, to a lesser extent, by peptide b, while peptide c had no effect at all (Fig. 5). These results indicate that a subset of anti-DNA antibodies specifically recognizes a sequence contained in the active site of the enzyme.

Table 2. *Anti-DNase I Activity of mAbs*

	ELISA	Dot blot/ Western blot	Immuno-precipitation
Anti-DNA autoantibodies	22/22	20/20	17/17
Non-anti-DNA autoantibodies	0/15	0/15	0/10
Isotype-matched Ig	0/35	0/25	0/10

Results are given as number of antibodies binding DNase I by ELISA, dot blot, or Western blot, and immunoprecipitation over the number of antibodies tested.

Discussion

This report indicates that autoantibodies, derived from individuals with lupus and initially selected for anti-DNA antibody activity, interact in an antigen-specific manner with DNase I. This appears to be a common property of anti-DNA antibodies, present among both murine and human Ig (as well as in serum from individuals with lupus). The anti-DNase activity is specific, and, in some cases, binding of DNase interferes with its enzymatic activity.

DNase I is a double-stranded specific endonuclease that hydrolyzes DNA to form short oligonucleotides having 5′-phosphate and 3′-hydroxyl termini. It requires the presence of divalent cations (Ca^{2+} and Mg^{2+}) for the cleavage reaction.

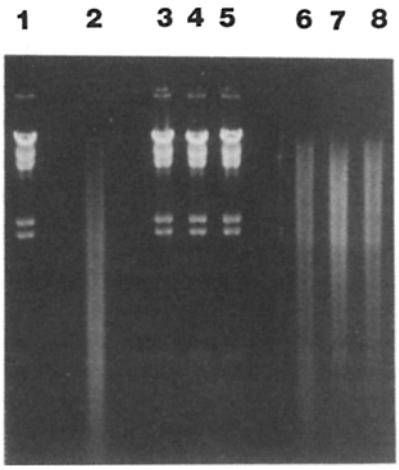

Figure 4. Inhibition of DNase I enzymatic activity. HindIII-digested λ DNA was incubated with DNase I, and the digestion was stopped by the addition of EDTA. Digested DNA was run on a 1% agarose gel, which was then stained with ethidium bromide and photographed under UV light. (Lane *1*) HindIII-digested λ DNA. (Lane *2*) HindIII-digested λ DNA treated with DNase I. (Lane *3–8*) HindIII-digested λ DNA treated with DNase I preincubated with Hy12 (lane *3*), P168 (lane *4*), H130 (lane *5*), H102 (lane *6*), 21/10 (lane *7*) and 21/15 (lane *8*). Preincubation of DNase I with mAbs Hy12, P168, and H130 blocked the ability of the enzyme to digest the substrate.

The crystal structure of the enzyme has recently been solved at high resolution (22). Electrostatic interactions play a prominent role in the DNA binding. In particular, the 2-Å resolution structure of DNase I, complexed to a short oligonucleotide (24), showed that an exposed peptide loop (Arg-Asn-Ser-Tyr-Lys-Glu) fits tightly in the minor groove of DNA. These residues and five additional arginine and lysine residues form salt bridges with phosphate groups of both strands flanking the minor groove. No contacts are formed between DNase I and the major groove. The enzyme interacts with an extensive region of the B-type DNA duplex over a total of 6 bp (34 Å) (25).

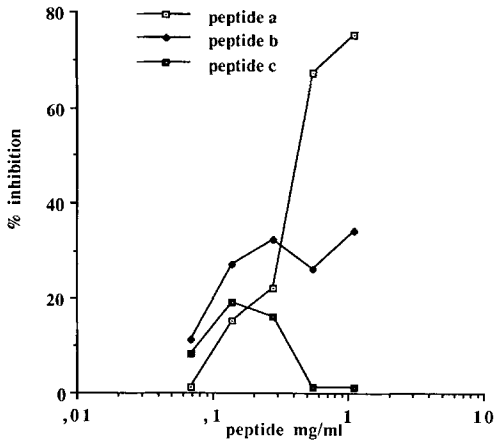

Figure 5. Inhibition of antibody 110-13 binding to DNase I. mAb 110-13 was preincubated with different amounts of peptide a (Ala-Arg-Asn-Ser-Tyr-Lys-Glu-Ala), peptide b (Cys-Arg-Asn-Ser-Tyr-Lys-Glu-Cys), or peptide c (Cys-Ala-Asn-Ser-Tyr-Ala-Cys) for 1 h at 37°C and then transferred to a DNase I–coated plate. Bound antibody was detected by an alkaline phosphatase–labeled anti–mouse antiserum. Results are expressed as percent inhibition: 100 – [100 × absorbance [antibody + peptide]/absorbance [antibody + buffer]].

We took advantage of these features to confirm specificity and map the binding site of a subgroup of anti-DNA antibodies. Using synthetic peptides corresponding to the active site of the enzyme, the epitope recognized by a subgroup of these autoantibodies on DNase I was mapped to the sequence Arg-Asn-Ser-Tyr-Lys-Glu. The substitution of the two positively charged amino acids with alanine abolished the binding, thereby suggesting a prominent role of electrostatic interactions in the antibody recognition. In this regard, clusters of charged residues seem to be a frequent feature of the epitopes bound by auto antibodies. In fact, V. Brendel et al. (26) analyzed the charge properties of rheumatic disease–associated autoantigens and found that clusters and/or runs of charged residues are significantly more frequent in these proteins than in other mammalian proteins.

The potential functional relevance of this interaction is suggested by the observation that three anti-DNA antibodies inhibited the enzyme activity of the molecule. Two of the three inhibitory antibodies (P168 and Hy12) bound to the cyclic peptide corresponding to the active site, suggesting that the inhibition on DNase I was due to a specific reaction with this region of the molecule. The third antibody, H130, did not recognize this peptide, and we conclude that it either binds DNase I in a region close to the active loop and interferes with the activity of the enzyme through steric hindrance, or it induces a conformational change in the molecule that alters enzymatic activity. Nevertheless, the inhibition of DNase I enzymatic activity by either mechanism could have pathological implications by interference with DNase I activity at sites of inflammation.

An immune response to DNase I may also amplify the production of anti-DNA antibodies through immune network interactions. For example, antiidiotypes could mimic DNAase I and bind DNA. In this regard, the DNA-hydrolyzing antibodies described by A. M. Shuster et al. (27) may also represent such an idiotypic cascade. Similar examples have been described for other autoantibodies (13, 14, 16).

DNase I is a ubiquitous enzyme in mammalian tissues and is phylogenetically highly conserved. Distribution studies (28, 29) show that high levels of the enzyme can be detected in digestive tissues, such as the parotid and submaxillary glands and the lining of small intestine. Appreciable levels of DNase I can be found in the kidney, where the enzyme probably plays a scavenging role, and in the lymph node and thymus (30). M. C. Peitsch et al. (30) demonstrated that the apoptosis-specific endonuclease, extractable from both thymocytes and lymph node cells, is functionally and antigenically indistinguishable from DNase I.

The dual specificity of anti-DNA antibodies suggests a role of DNase I in the origin of anti-DNA antibodies. We propose that anti-DNA antibodies arise from an immune response to a complex of DNA with the DNA-binding protein. Peptides from the protein moiety of the complex could elicit a T cell response. Such peptide-specific T cells can preferen-

tially help B cell clones that, through their surface Ig receptors, capture the DNA–protein complex and present DNase I peptides in association with MHC class II molecules. B cells, whose surface Ig bind DNA, the DNA-binding protein, or both, will thus be stimulated. B clones producing Ig with a dual specificity (DNA and DNase I, or Sm and DNA) bind the complex with high avidity and, therefore, are efficient APC. We suggest that this property is a selective advantage that leads to preferential expansion of B cell clones binding both DNA and DNA-binding protein. This hypothesis links several observations that have not so far been connected. Structural studies have shown that lupus anti-DNA antibodies arise from antigen selection of somatically mutated B cells. Such a process is usually T cell driven, and, indeed, helper T cells a process is usually T cell driven, and, indeed, helper T cells that augment the anti-DNA response have been identified (31–33). However, T cells specific for oligonucleotides have never been reported. The relevant T cell epitope may occur on DNA-binding nuclear proteins (34), a possibility strengthened by the observation that nucleoproteins with DNA-binding motifs are a frequent target of anti-DNA antibodies (35, 36) and that anti–native DNA antibodies can be induced by immunization with a protozoan DNA-binding polypeptide (37). Among the DNA-binding proteins, DNase, which is so frequently recognized by anti-DNA antibodies, may play a key role in the induction of the anti-DNA immune response.

Additional studies using autoreactive B and T cell subsets and autoantibodies are planned to further address this issue.

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