Nucleic acid specificity was tested for two monoclonal anti-double-stranded DNA autoantibodies, 2C10 and H241, derived from two lupus-prone MRL/Mp-1pr/1pr mice. Antibody 2C10 bound double-stranded oligonucleotides with a preference for dA-dT over dG-dC base pairs and did not bind single-stranded oligonucleotides. Distamycin A, an antibiotic that binds to the minor groove, inhibited 2C10 binding of double-stranded DNA, suggesting that this antibody interacts with dA-dT base pairs in the minor groove. Antibody H241 binding was previously shown to have a dG-dC preference and to involve both major and minor grooves. In attempted footprinting assays, both 2C10 and H241 markedly enhanced rather than protected against cleavage of DNA by hydroxyl radical-generating systems. With 2C10, this enhancement effect was observed only when hydroxyl radical generation was associated with oxidation of Fe(II). In contrast, H241 enhancement occurred in the presence of H2O2 and ascorbate or UV light irradiation and did not depend on added metal ion. The enhancement sites were related to the antibody binding specificities. The oligonucleotide 5'-AAAAATATATATT-3' was a much more effective inhibitor of the 2C10 enhancement than of the H241 effect, whereas the oligonucleotide 5'-GGGCAGCGCC-3' was a much more effective inhibitor of the H241 enhancement. In addition, the enhanced cleavage occurred preferentially at dA-dT-rich regions with 2C10 and at dG-dC-rich regions with H241. These findings raise the possibility that anti-DNA autoantibodies could enhance DNA damage in inflammatory lesions in which hydroxyl radicals are generated.

Autoantibodies to dsDNA are characteristic of the autoimmune disease systemic lupus erythematosus. Increased production of anti-dsDNA antibodies reflects or predicts periods of active clinical disease, and anti-DNA antibodies can contribute to pathogenesis of lesions. There are several possible bases for their contribution to tissue damage. Anti-DNA antibodies are concentrated in immune complexes in glomerular lesions of lupus nephritis, where they may initiate inflammatory reactions. It was originally proposed that glomerular lesions resulted from deposition of DNA-anti-DNA complexes that formed in the circulation, but many anti-DNA antibodies cross-react with other negatively charged polymers or membrane proteins and may bind directly to structures present in the glomerular basement membrane. As well as initiating inflammation, anti-DNA antibodies may cause cell damage more directly. Some anti-DNA antibodies bind to cell surfaces and initiate complement-dependent cytotoxicity, whereas others induce the membrane of living cells and may reach the nucleus. It has also been reported that some anti-DNA autoantibodies present in sera of systemic lupus erythematosus patients can catalyze the hydrolytic cleavage of DNA.

Because some, but not all, anti-DNA antibodies are pathogenic, there has been much interest in whether the pathogenicity is related to binding specificity, either for DNA epitopes or for cross-reactive structures. Thus, many anti-DNA autoantibodies have been analyzed extensively at the level of primary structure and modeling of their binding sites or by x-ray crystallography. In addition, DNA-binding sites of monoclonal anti-DNA antibodies and epitopes have been mapped by competitive immunosassay with polynucleotides or oligonucleotides. High-resolution footprinting, measuring protection against chemical cleavage of DNA, has been useful in identifying the epitopes for autoantibodies to single-stranded DNA and experimentally induced antibodies to Z-DNA.

In a previous study, we had examined the specificity of a pathogenic monoclonal IgG anti-dsDNA antibody, H241, derived from an MRL/Mp-1pr/1pr lupus mouse. In competitive immunoassays with a series of synthetic double-stranded oligonucleotides, H241 bound to a (dG-dC)n or (dG-dC)n core in the center of a base-paired octadecanucleotide. This selectivity was also reflected in the very marked preference of this antibody for poly(dG-dC) over poly(dA-dT). A second IgG antibody, 2C10, isolated from a different MRL/Mp-1pr/1pr mouse, had a very different selectivity, markedly favoring poly(dA-dT) over poly(dG-dC). Competitive assays of antibody 2C10 with synthetic oligonucleotides indicated that it could bind well to alternating dA-dT, to stretches of (dA)n-(dT)n, or to (dA-dG)-(dC-dT) sequences, so it was more difficult to assign a single kind of epitope to it. Recently, we tried to apply a DNA footprinting assay to observe the DNA sites recognized by these two monoclonal anti-dsDNA antibodies more directly. Unexpectedly, we found that these two anti-dsDNA antibodies enhanced rather than protected against the cleavage of DNA by hydroxyl radical-generating systems. The two antibodies differed in the conditions under which they were effective and in the sites of DNA cleavage they supported.

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1The abbreviations used are: dsDNA, double-stranded DNA; ELISA, enzyme-linked immunosorbent assay; dMC, deoxymethylcytidylylate; bp, base pair.

2T. Kubota, Y. Kanai, and B. D. Stollar, unpublished data.
MATERIALS AND METHODS

Purification of Monodonal Anti-DNA Antibodies—Monodonal anti-dsDNA antibodies 2C10 and H241 (both IgG2b, k) are hybridoma products derived from two lupus-prone MRL/Mp-lpr/lpr mice (21, 22). Hybridoma cells were cultured in serum-free medium (HyClone Medium 606, KojinBio, Sakata, J pan), and the culture supernatants were loaded on a matrix bearing immobilized staphylococcal protein A (Chromatotop Super Protein A, Nihon Gaishi, Handa, J pan). The matrix was washed with 25 mM Tris-HCl, 140 mM NaCl, pH 7.4, followed by 5 mM NaCl, 25 mM Tris-HCl, pH 7.4, in order to remove DNA and DNA-binding proteins that may have attached to antibodies during culture and isolation; the antibodies remained bound to the protein A. Then the antibodies were recovered with elution buffer (250 mM glycine HCl, pH 3.5) and dialyzed immediately against PBS (8.1 mM Na2HPO4, 1.5 mM KH2PO4, 2.7 mM KCl, 137 mM NaCl, pH 7.3). Purity of the eluates was confirmed by SDS-polyacrylamide gel electrophoresis, agarose gel electrophoresis, and electron microscopy, which demonstrated that washing the column with a solution of high ionic strength did remove bound antigens; no contamination of DNA or DNA-binding proteins was detectable.1 A mouse myeloma protein, MPC11 (IgG2b, k), which does not bind to DNA, was similarly purified with the protein A matrix and served as an isotype-matched control IgG.

ELISA—UV-irradiated polystyrene microtiter plates (Immulon 1, Dynatech Laboratories Inc., Chantilly, VA) (23) were coated with calf thymus native DNA, and competitive assays using a series of synthetic oligonucleotides were performed as described previously (21).

Preparation of a DNA Probe for Hydroxyl Radical Footprinting—A self-complementary oligonucleotide that contains alternating dA-dT and dG-dC segments (5'-CGCGCGATATATATATCGCGCGATATATATCGCGCGG-3') was inserted into the BamHI site of a pUC19 plasmid vector (Takara, Kyoto, J pan). After amplification in Escherichia coli DH5a (Life Technologies, Inc.), the plasmid was recovered by an alkaline lysis miniprep procedure using a QIA Quick_plasmid kit and cleaved with EcoRI (Takara). The 5'-end of the EcoRI digestion product was dephosphorylated with calf intestine alkaline phosphatase (Boehringer Mannheim, Tokyo) following a standard protocol (24), and the oligonucleotide was purified with a spin column (QiA Quick spin polymerase chain reaction purification kit, QiA Quick Inc., Chatsworth, CA) following the manufacturer’s protocol. This product was treated with PvuI (Takara), and a 274-bp fragment containing a 4-base protruding end was purified by 2% agarose gel electrophoresis. This procedure allowed several 32P labeling by T4 polynucleotide kinase (Stratagene, La Jolla, CA) (24) at the end of just one strand.

Gel Retardation Assay—Mixtures of 7.2 ng of the 274-bp DNA probe and various quantities of antibodies in PBS were incubated for 30 min at room temperature and analyzed by electrophoresis in a 10% polyacrylamide gel with a 4% stacking gel as described previously (25). In an experiment to test involvement of the DNA minor groove in binding of anti-DNA antibodies, 7.2 ng of the DNA probe was preincubated with 310 ng of distamycin A (Sigma) for 20 min at room temperature before addition of antibodies or a control IgG in 8 µl of PBS and incubated for 30 min at room temperature. In the experiments shown in Fig. 4 (A and B), 1 µl of freshly prepared aqueous solution of ferrous ammonium sulfate, zinc sulfate, magnesium chloride, calcium chloride, or copper sulfate (100 µM final concentration) was added to the DNA/antibody mixture and allowed to react for 4 min at 25 °C. In the experiments shown in Fig. 4 (C and D) and in Figs. 5–8, one of the metal ions indicated in the legends was mixed with Na2EDTA; then the metal ion-EDTA complex was mixed with sodium ascorbate and H2O2, and added immediately to the DNA/antibody mixture according to the protocol of Tullius et al. (26) with modifications. Final concentrations of metal ion, EDTA, ascorbate, and H2O2 in the standard reaction mixture were 10 µM, 20 µM, 1 mM, and 9 mM, respectively. Concentrations were changed as indicated for some experiments. The reaction time was 4 min at 25 °C unless otherwise indicated. In all cases, the reactions were quenched by addition of thiourea (to 0.1 M) and Na2EDTA (to 0.2 M). After addition of 0.3 M sodium acetate and 2.75 µM yeast tRNA (Life Technologies, Inc.), DNA was extracted with phenol/chloroform/isooamyl alcohol (25:24:1) and precipitated with cold ethanol. The pellets were washed with 70% ethanol, resuspended in loading buffer containing 85% formamide and 1 mM Na2EDTA, heated at 98 °C for 3 min, chilled on ice, and applied to an 8% polyacrylamide sequencing gel. Autoradiographs were analyzed with a densitometer (CS-900, Shimadzu, Kyoto, J pan). In the experiment shown in Fig. 9, DNA was cleaved by hydroxyl radical produced by a 4-min incubation in a mixture of 1 µM copper sulfate, 0.1 mM sodium ascorbate, and 0.9 mM H2O2.

RESULTS

Specificity of Anti-dsDNA Antibodies—We studied two monoclonal anti-dsDNA antibodies, 2C10 and H241, both derived from MRL/Mp-lpr/lpr mice. In a competitive ELISA with a series of synthetic oligonucleotides, antibody 2C10 bound to double-stranded oligonucleotides, but did not react with single-stranded ligands in the tested concentration range (Fig. 1A). These competitive assays with various double-stranded polynucleotides revealed a striking preference of 2C10 for poly(dA-dT) sequences over poly(dG-dC) or poly(dG-dmC) sequences (Fig. 1B). In separate experiments with other base-
paired oligonucleotides, substitutions of thymine with uracil or fluourouracil did not significantly affect the affinity of binding to 2C10 (data not shown). Previous competitive radioimmunoassay studies had indicated that H241 recognized a G-dC core in AGCGCGCGCTATAT-3’ (21).

For further experiments, we used a 274-bp dsDNA probe purified from a modified plasmid as described under “Materials and Methods.” First we confirmed the binding of the antibodies to this DNA probe with an assay for retardation of migration in gel electrophoresis. Antibody 2C10 shifted the mobility of the 32P-labeled DNA almost completely at a DNA/antibody molar ratio of 1:40 (lanes 2 and 7) and 1:80 (lanes 4 and 9) or with a DNA/distamycin A mixture at DNA/antibody ratios of 1:40 (lanes 3 and 8) and 1:80 (lanes 5 and 10). The mixtures were then loaded on a 10% polyacrylamide gel with a 4% stacking gel. Molar ratios of DNA to antibody are indicated at the top of each lane.

FIG. 2. Gel retardation assay for the binding of anti-dsDNA antibodies to the 274-bp dsDNA probe. DNA was incubated with anti-dsDNA antibody 2C10 (A) or H241 (B) or with a control myeloma protein, MPC11, and loaded on a 10% polyacrylamide gel with a 4% stacking gel. Molar ratios of DNA to antibody are indicated at the top of each lane.

FIG. 3. Effect of distamycin A on the binding of 2C10 and H241 to dsDNA. Antibodies 2C10 (lanes 2-5) and H241 (lanes 7-10) were incubated with the 274-bp dsDNA probe alone at DNA/antibody ratios of 1:40 (lanes 2 and 7) and 1:80 (lanes 4 and 9) or with a DNA/distamycin A mixture at DNA/antibody ratios of 1:40 (lanes 3 and 8) and 1:80 (lanes 5 and 10). The mixtures were then loaded on a 10% polyacrylamide gel with a 4% stacking gel. Lanes 1 and 6 are the DNA without antibodies.

Preincubation of the 274-bp DNA probe with distamycin A, an antibiotic that binds in the minor groove with preference for dA-dT-rich sequences (27), caused marked inhibition of antibody 2C10 binding, but did not affect H241 binding (Fig. 3). Together with ELISA data described above, this result suggests that 2C10 recognizes double-helical structure of DNA and makes contacts preferentially with regions of dA-dT base pairs in or over the minor groove.

Enhancement Effect of Anti-DNA Antibodies on Hydroxyl Radical DNA Cleavage—When we began to apply chemical footprinting assay to identification of DNA regions covered by anti-DNA antibodies, we found, unexpectedly, a significant enhancement of DNA cleavage by hydroxyl radical in the presence of 2C10 or H241. In these experiments, the DNA probe in aerobic solution was preincubated with 2C10 or with the control IgG MPC11 for 30 min in PBS. Then a 100 μM concentration of the divalent metal ion indicated at the top of each lane was added. Four min later, an excess of Na4EDTA and thiourea was added to quench the reaction. In C, the dsDNA probe was incubated with H241 for 30 min, and the complexes were then incubated further with no additions (lane 1) or with 1 mM sodium ascorbate plus 9 mM H2O2 (lane 2); ascorbate alone (lane 3); H2O2 alone (lane 4); or ascorbate plus H2O2 plus 10 μM EDTA-chelated Fe(II) (lane 5), Mg(II) (lane 6), Zn(II) (lane 7), or Ca(II) (lane 8). Four min later, the reaction was quenched by EDTA and thiourea. In D, the dsDNA probe was incubated with MPC11 or 2C10 for 30 min and then for 4 min with no additions (lane 1) or with ascorbate and H2O2 (lanes 2 and 4) or ascorbate plus H2O2 plus EDTA-chelated Fe(II) (lanes 3 and 5).

Enhancement of hydroxyl radical cleavage of DNA by anti-dsDNA antibodies. The 274-bp dsDNA probe was incubated with anti-dsDNA antibody 2C10 (A) or H241 (B) or with a control myeloma protein, MPC11, for 30 min in PBS. Then a 100 μM concentration of the divalent metal ion indicated at the top of each lane was added. Four min later, an excess of Na4EDTA and thiourea was added to quench the reaction. In C, the dsDNA probe was incubated with H241 for 30 min, and the complexes were then incubated further with no additions (lane 1) or with 1 mM sodium ascorbate plus 9 mM H2O2 (lane 2); ascorbate alone (lane 3); H2O2 alone (lane 4); or ascorbate plus H2O2 plus 10 μM EDTA-chelated Fe(II) (lane 5), Mg(II) (lane 6), Zn(II) (lane 7), or Ca(II) (lane 8). Four min later, the reaction was quenched by EDTA and thiourea. In D, the dsDNA probe was incubated with MPC11 or 2C10 for 30 min and then for 4 min with no additions (lane 1) or with ascorbate and H2O2 (lanes 2 and 4) or ascorbate plus H2O2 plus EDTA-chelated Fe(II) (lanes 3 and 5).

A mixture of Fe(II) chelated with Na4EDTA, H2O2, and...
Enhanced the cleavage of DNA by H$_2$O$_2$ and ascorbate, and this enhancement by H$_2$O$_2$ and ascorbate, known as Udenfriend’s system (28), is a convenient reagent for continuous production of hydroxyl radical. Thus, we tested the effect of anti-DNA antibodies in the mixture of H$_2$O$_2$ and ascorbate with or without divalent metal ions and Na$_4$EDTA (Fig. 4, A and D). Antibody H241 markedly enhanced the cleavage of DNA by H$_2$O$_2$ and ascorbate, and this effect was not dependent on the presence of any metal ions (Fig. 4C). In fact, the presence of Fe(II) partially inhibited the enhanced cleavage by H241 (Fig. 4C). In contrast, enhanced DNA cleavage by 2C10 was observed in the presence of Fe(II), ascorbate, and H$_2$O$_2$, but not with ascorbate and H$_2$O$_2$ alone (Fig. 4D) or ascorbate, H$_2$O$_2$, and other metals (data not shown). The control antibody MPC11 did not enhance cleavage under any of these conditions (Fig. 4D).

Kinetics of DNA Cleavage with Anti-DNA Antibodies—The standard reaction mixture for the following experiments was 10 $\mu$M Fe(II), 20 $\mu$M Na$_4$EDTA, 9 mM H$_2$O$_2$, and 1 mM ascorbate. To investigate the dependence of DNA cleavage enhancement on extent of hydroxyl radical production, the concentrations of these four components were changed simultaneously to give mixtures with 0.25–4.0 $\times$ the standard concentrations (Fig. 5). Antibody H241 strikingly enhanced the DNA cleavage with the standard reagent concentration, reproducibly yielding ~90% cleavage of the DNA in 4 min, as estimated from densitometric measurement of the starting material. Antibody 2C10 also enhanced DNA degradation, but it required higher concentrations of the reaction mixture. At 4 $\times$ the standard concentrations, most of the starting material was degraded in 4 min even without anti-DNA antibodies.

The next experiment measured the time dependence of this effect of anti-DNA antibodies in the standard reaction mixture (Fig. 6). The reaction was rapid in the presence of H241; >50% of the starting DNA was degraded within 15 s. The reaction with 2C10 was slower under this assay condition, but there was still significant enhancement of cleavage over a 2–8-min period.

Specificity of the DNA Cleavage Enhancement Effect—To test whether the enhanced cleavage was caused by specific binding of the anti-DNA antibodies, self-complementary 13-mer oligonucleotides were added to the reaction mixture to determine whether they could inhibit the effects of 2C10 and H241. This experiment was done at 15°C to ensure that the complementary oligonucleotides remained base-paired. With antibody 2C10, 0.2 and 2.0 $\mu$g of 5'-AAAAATATATTT-3' (K5) inhibited the enhancement effect of 2C10 by 49 and 83%, respectively (Fig. 7), whereas 0.2 $\mu$g of 5'-GGGGCCCGGGCC-3' (K6) did not show significant inhibitory effect, and 2.0 $\mu$g caused 33% inhibition. In contrast, the GC oligonucleotide K6 was a more effective inhibitor than K5 for the effects of 2C10; 0.2 and 2.0 $\mu$g of K6 caused 52 and 100% inhibition, respectively, whereas the same concentrations of K5 caused only 6 and 32% inhibition. These results are consistent with the specificity of 2C10 and 2C10 described above. Thus, it is likely that the enhancement of hydroxyl radical DNA cleavage by anti-DNA antibodies did involve the DNA-binding sites of the two antibodies.

As a further test of this interpretation, the extents of cleavage at specific sites in the DNA sequence were compared in the analytical sequencing gels (Fig. 8). The susceptibility for hydroxyl radical cleavage in the presence of 2C10 and H241 showed a reciprocal pattern. The enhancement with antibody 2C10, relative to the reaction in the absence of antibody, was greatest in dA-dT-rich sequences, whereas the enhancement with antibody H241 was greatest in dG-dC-rich sequences, again indicating that increased susceptibility for cleavage is related to the specific binding sites of each antibody. In this experiment, >95% of the DNA was uncut following the hydroxyl radical reaction with a control antibody, MPC11. This condition is consistent with single-hit kinetics (29), supporting the conclusion that the result truly reflects substrate specificity.

Footprinting Protection Assay for Cro Protein—To test whether any DNA-binding protein would enhance cleavage...
under the conditions we used, we incubated the Cro repressor protein (provided by J. Bakja, Tufts University) with a plasmid containing its recognition sequence. The sequence was cloned into the BamHI site of pGEM-3, the same site used for the 274-bp probe described above. The standard hydroxyl radical-generating concentration was used. The Cro protein protected its specific binding site from cleavage, as expected in a footprinting assay (data not shown), consistent with previously reported studies (30).

DNA Cleavage by Cu(I) or UV Light—To explore the effect of other sources of hydroxyl radical on our assay system, we tested oxidative reactions of Cu(I) and UV irradiation. For the former reaction, Cu(II) was used in place of the very unstable Cu(I) and was combined with H2O2 and ascorbate. Antibody 2C10 did not enhance cleavage by this reagent; relative cleavage sensitivities of individual sequence sites in the presence of 2C10, in comparison with cleavage with MPC11, ranged from 0.8 and 2.2 (Fig. 9). Indeed, in the reaction with copper, the dA-dT sequences were relatively protected by 2C10, giving a pattern opposite to that seen in the reaction with iron. Under this experimental condition, the uncut fraction of DNA with MPC11 was >95%. The copper system was not tested with H241 because H2O2 and ascorbate alone caused marked degradation of DNA (Fig. 4C). To test the effect of UV irradiation, we added 9 mM H2O2 to the DNA-antibody complex and exposed the solution to 254 nm UV light for 10 min. H241 enhanced the DNA cleavage in this reaction, but 2C10 did not (data not shown). Thus, enhancement of DNA cleavage by 2C10 was observed only in association with the oxidation reaction of Fe(II), whereas H241 enhanced cleavage irrespective of metal ions, and it also exhibited the effect on UV irradiation.

**DISCUSSION**

These experiments indicate that two anti-DNA autoantibodies from lupus mice enhance the degradation of DNA by hydroxyl radical-generating systems. Steps were taken to test whether the effect was truly due to the antibody rather than a contaminant that might have been present in an immune complex. The antibodies were highly purified, as described under "Materials and Methods." Their purity was confirmed by SDS-polyacrylamide gel electrophoresis, which detected no contamination by other DNA-binding proteins. Further verification was obtained by counting the number of IgG-nucleosome complexes or free nucleosomal particles among IgG particles in a fixed area in an electron micrograph of a preparation that included mica flakes for quick-freezing and deep-etching. In the case of monoclonal anti-DNA antibodies obtained after a wash with a high salt concentration, neither IgG-nucleosome complexes nor free nucleosomes were found among 80 or more IgG particles, whereas 42 out of 80 IgG particles were associated with nucleosomes in the case of monoclonal antibodies obtained by the conventional affinity chromatography without a high salt wash. The effectiveness of this washing is consistent with the previous demonstration of salt sensitivity of DNA binding by antibody 2C10 (31) and other anti-DNA autoantibodies (32). Removal of the DNA also removes proteins that may be associated with it in DNA-antibody complexes.

Specificity of the effects provided more direct evidence for the role of the antibody-binding sites in this phenomenon. The relative effectiveness of oligonucleotides as inhibitors of the enhancement corresponded with their effectiveness as inhibitors of DNA binding. Both the cleavage enhancement and DNA binding by antibody 2C10 were inhibited preferentially by the AT oligonucleotide, and both activities of H241 were inhibited preferentially by the GC oligonucleotide. Furthermore, enhancement occurred preferentially at dA-dT segments of the test DNA with antibody 2C10, but at dG-dC segments with H241. The sequence specificity was not absolute, and neither were the binding differences (21). The effect was not due to uncontrolled conditions of the hydroxyl-generating system; unlike the antibodies, the Cro protein protected its specific target under the same conditions, just as expected in a footprinting assay.

Extensive studies have demonstrated that hydroxyl radical generated by the Fenton reaction modifies bases and cleaves DNA (33–36). The ability of bound protein to protect against this cleavage is the basis of high resolution footprinting assays

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**Fig. 7. Inhibition of the DNA cleavage enhancement effect of anti-dsDNA antibodies by oligonucleotides.** Oligonucleotide K5 (5'-AAATAATATATATT-3') or K6 (5'-GGGGCGCGCGCCC-3') was added to the DNA-antibody complex described using Udenfriend's system (28) in the presence of anti-dsDNA antibodies 2C10 and H241 or a control myeloma protein, MPC11. Based on the densitometric estimation of the amounts of the starting materials, percent inhibition was calculated as follows: inhibition = (1 – (cleavage with anti-DNA and inhibitor) / (cleavage with anti-DNA)) × 100.

**Fig. 8. DNA cleavage pattern enhanced by anti-DNA antibodies.** A, the DNA probe was cleaved by hydroxyl radical generated by reduction of H2O2 in Udenfriend's system (28) in the presence of anti-DNA antibodies 2C10 and H241 or a control myeloma protein, MPC11. G, Maxam-Gilbert's G-reaction; G-control, Maxam-Gilbert's G-reaction without alkaline cleavage. B, densitometric analysis corresponds to the position of the oligonucleotide insert described under "Materials and Methods." The ordinate represents the relative sensitivity to the cleavage calculated as follows: relative cleavage sensitivity = (density of cleaved ladder in the presence of anti-DNA)/(density of cleaved ladder in the presence of MPC11). Hatched columns, H241; closed columns, 2C10.

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in vitro (19, 26), and protection against oxidative species is afforded within cells by histones in condensed chromatin (37). On the other hand, DNA-nuclear matrix protein association presents hypersensitive sites for oxidative damage, possibly related to the binding of copper ions (37, 38). Metal-binding proteins such as ferritin (39) also mediate increased DNA cleavage, as can lactoferrin with copper bound to its surface (40), an inorganic polymer that binds both iron and DNA (41) or small DNA-binding molecules such as adriamycin (42). These proteins, polymers, and small molecules may focus the Fenton reaction on simultaneously bound DNA. In addition, binding of iron to DNA may determine cleavage sites (34).

It is not known how antibodies 2C10 and H241 enhanced DNA cleavage, but there were differences in the mechanisms operating with the two antibodies. Because the enhancement with antibody 2C10 occurred either with simple addition of Fe(II) to the DNA-antibody complexes or with Fe(II) and the H$_2$O$_2$/ascorbate mixture, but not with the copper/periodate/ascorbate reagent or periodate/ascorbate alone, it is possible that antibody 2C10 selectively bound both DNA and Fe(II), concentrating hydroxyl radical generation near the target sites. In analogy, deliberate incorporation of a metal-binding site into an antibody Fv domain has been proposed as a means of generating a designed metalloenzyme (43).

Antibody H241 acted differently, as it was effective with H$_2$O$_2$ and ascorbate without added metal ion. The presence of iron actually reduced the antibody-mediated enhancement, whereas other ions had no effect. Antibody H241 (and perhaps 2C10 as well) may have facilitated cleavage by distorting the DNA structure, making target sites more accessible. X-ray crystallographic analysis of an autoantibody to single-stranded DNA, for example, revealed antibody stabilization of an unpredicted conformation of oligo(dT) in the immune complex (16). Comparable structural data are not available for antibodies 2C10 and H241 and their complexes with DNA. The effect is not a property of all anti-DNA antibodies because hydroxyl radical generation has been used effectively for protection-based footprinting of anti-single-stranded DNA autoantibody epitopes (19).

Because an excess of antibody was present in the solution and the precise number of cleavage events was not known, it is not clear whether the rate enhancement involved turnover, as in enzyme catalysis, or whether it was stoichiometric. The antibodies alone did not hydrolyze DNA in the time course of these reactions, up to 30 min. Hydrolysis of DNA by purified IgG and Fab fragments from systemic lupus erythematosus patients' sera has been detected by sensitive assays (11, 12). In addition, Paul and co-workers have reported hydrolysis of vaso-intestinal peptide by IgG autoantibodies and their Fab fragments (44, 45) or isolated light chains (46) and by the purified (47) or recombinant (48) light chain of a monoclonal antibody from a mouse immunized with this peptide. Additional study will determine the extent to which autoantibodies provide new approaches to isolation of catalytic antibodies, adding to the large number that have been induced with haptens based on transition state analogues (49) or obtained by selection from combinatorial libraries (50).

Antibody that increases the sensitivity of DNA to cleavage by hydroxyl radical or other reactive oxygen species could have an effect in vivo in sites of inflammation, where such species are generated. For example, higher than normal oxygen radical production has been detected in both bronchial alveolar cells and polymorphonuclear cells from patients with systemic lupus erythematosus (51). Increased numbers of chromosomal breaks and increased sensitivity to UV light, with involvement of reactive species, have also been detected and suggested as a basis for the well known sensitivity of these patients to UV irradiation (52, 53). Protection by superoxide dismutase provided evidence that reactive oxygen species were involved in the damage by UV radiation (52, 54). The presence of anti-DNA antibodies such as 2C10 or H241 could enhance the sensitivity of DNA to such damage. In turn, hydroxyl radical-mediated damage to DNA may increase the binding by anti-DNA antibodies (55, 56) and increase the immunogenicity of DNA (56).

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REFERENCES

1. Swaak, T., and Smeenk, R. (1985) Ann. Rheum. Dis. 44, 245–251
2. Koffler, D., Schur, P. H., and Kunkel, H. G. (1967) J. Exp. Med. 126, 607–624
3. Koffler, D. (1974) Annu. Rev. Med. 25, 149–164
4. Termaat, R. M., Assmann, K. J. M., Dijkman, H. B. P. M., Van Gompel, F., Smeenk, R. J. T., and Berden, J. H. M. (1992) Kidney Int. 42, 1363–1371
5. Raz, E., Ben-Bassat, H., David, T., Shlomai, Z., and Eliat, D. (1993) Eur. J. Immunol. 23, 393–390
6. Reichlin, M., Martin, A., Taylor-Albert, E., Tsuchida, K., Wang, Z., Reichlin, M. W., Koren, E., Ebling, F. M., Tsao, B., and Hahn, B. H. (1994) J. Clin. Invest. 93, 443–449
7. Raz, E., Brezis, M., Rosenmann, E., and Eliat, D. (1989) J. Immunol. 142, 3076–3082
8. Koren, E., Koscek, M., Wolfof-Reichlin, M., Ebling, F. M., Tsao, B., Hahn, B. H., and Reichlin, M. (1995) J. Immunol. 154, 4873–4884
9. Okudaira, K., Yoshizawa, H., and Williams, R. C., J. (1987) Arthritis Rheum. 30, 669–678
10. Yara, S., Smith, R. M., Cziman, B., Foster, M. H., Peache, L. D. J, Arell, and Mado, M. P. (1994) Lab. Invest. 71, 52–60
11. Shuster, A. M., Gollubov, G. V., Krasovskii, V. A., Bogomolova, A. E., Smirnov, D. V., and Gabbov, A. G. (1992) Science 256, 655–667
12. Gollubov, G. V., Chernova, E. A., Shchurov, D. V., Smirnov, I. V., Kudelina, I. A., and Gabbov, A. G. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 254–257
13. Hakoshima, T., Teranishi, Y., Oshino, T., Suzuki, K., Shimizu, M., Shirakawa, M., Koyaguchi, Y., Ogawa, N., and Oshima, Y. (1993) J. Biol. Chem. 268, 528–533
14. Radic, M. Z., and Weigert, M. (1994) Annu. Rev. Immunol. 12, 487–520
15. Cullen, M., Boodhoo, A., Lee, J. S., and Anderson, W. F. (1987) J. Biol. Chem. 262, 643–648
16. Herron, J., He, X. M., Ballard, D. W., Blier, P. R., Pace, P. E., Bothwell, A. L. M., Voss, E. W., Jr., and Edmundson, A. B. (1993) Proteins 11, 159–175
17. Stollar, B. D. (1973) In The Antigens (Sela, M. ed.) pp. 1–89, Academic Press, New York
18. Stollar, B. D. (1986) CRC Crit. Rev. Biochem. 20, 1–36
19. Swanston, P. C., Cooper, B. C., and Blick, G. D. (1994) J. Immunol. 152, 2601–2612
20. Runkel, L., and nordheim, A. (1986) J. Mol. Biol. 198, 487–501
21. Stollar, B. D., von, G., and Pastor, R. W. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 4469–4473
22. Kobata, T., Akatsuka, T., and Kanai, Y. (1986) Immunol. Lett. 14, 53–58
23. Zouali, M., and Stollar, B. D. (1986) J. Immunol. Methods 90, 105–110
24. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (eds) (1995) Current Protocols in Molecular Biology, Vol. 2, pp. 10.1–10.15, John Wiley & Sons, Inc., New York
25. Jang, Y. J., and Stollar, B. D. (1990) J. Immunol. 145, 3533–3539
26. Tullius, T. D., Dombroski, B. A., Churchill, M. E., and Kam, L. (1987) Methods Enzymol. 155, 537–558
27. Pelton, J. G., and Wemmer, D. E. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 5723–5727
28. Ullman, I., Clark, C. T., Axelrod, J., and Brodie, B. B. (1954) J. Biol. Chem. 208, 731–739
29. Brenowitz, M., Sene, D. F., Shea, M. A., and Ackers, G. K. (1986) Methods Enzymol. 130, 132–181
30. Tullius, T. D., and Dombroski, B. A. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 5469–5473
31. Kanai, Y., and Kubota, T. (1989) Immunol. Lett. 22, 293–300
32. Braun, R. P., and Lee, J. S. (1987) J. Immunol. 139, 175–179
33. Stohs, S. J., and Bagchi, D. (1995) Free Radical Biol. & Med. 18, 321–336
34. Luo, Y., Han, Z., Chin, S. M., and Linn, S. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 12438–12442
35. Mello-Filho, A. C., and Meneghini, R. (1991) Mutat. Res. 251, 109–113
36. Halliwell, B., and Aruoma, O. I. (1991) FEBS Lett. 281, 9–19
37. Chiu, S., Xue, L., Friedman, L. R., and Oleinick, N. L. (1995) Biochemistry 34, 2653–2661.
38. Oleinick, N. L., Balasubramaniam, U., Xue, L., and Chiu, S. (1994) Int. J. Radiat. Biol. 66, 523–529
39. Sakurai, K., and Ogiso, T. (1995) Biol. Pharmaceut. Bull. 18, 262–266
40. Zhao, X. Y., and Hutchers, T. W. (1994) Adv. Exp. Med. Biol. 357, 271–278
41. Hardy, J. A., and Aust, A. E. (1995) Carcinogenesis (Lond.) 16, 319–325
42. Feinstein, E., Canaani, E., and Weiner, L. M. (1993) Biochemistry 32, 13156–13161
43. Roberts, V. A., and Getzoff, E. D. (1995) FASEB J. 9, 94–100
44. Paul, S., Volle, D. J., Beach, C. M., Johnson, D. R., Powell, M. J., and Massey, R. J. (1989) Science 244, 1158–1162.
45. Paul, S., Volle, D. J., and Mei, S. (1990) J. Immunol. 145, 1196–1199
46. Mei, S., Mody, B., Eklund, S. H., and Paul, S. (1991) J. Biol. Chem. 266, 15571–15574
47. Sun, M., Gao, Q. S., Li, L., and Paul, S. (1994) J. Immunol. 153, 5121–5126
48. Gao, Q. S., Sun, M., Tyutyulkova, S., Webster, D., Rees, A., and Lerner, R. A. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 2532–2536
49. Groen, H., Aslander, M., Bootsma, H., and van der Mark, T. W., and Kallenberg, C. G. (1993) Clin. Exp. Immunol. 94, 127–133
50. Emerit, I., and Michelson, A. M. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 2537–2540
51. Golan, T. D., Sigal, D., Hacham, H., Gottfried, V., and Kimmel, S. (1994) Lupus 3, 103–106
52. Emerit, I., and Michelson, A. M. (1980) Acta Physiol. Scand. 492, 59–65
53. Blount, S., Griffiths, H., Emery, P., and Lunec, J. (1990) Clin. Exp. Immunol. 81, 384–389
54. Ara, J., and Ali, R. (1992) Immunol. Lett. 34, 195–200

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31. Kanai, Y., and Kubota, T. (1989) Immunol. Lett. 22, 293–300
32. Braun, R. P., and Lee, J. S. (1987) J. Immunol. 139, 175–179
33. Stohs, S. J., and Bagchi, D. (1995) Free Radical Biol. & Med. 18, 321–336
34. Luo, Y., Han, Z., Chin, S. M., and Linn, S. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 12438–12442
35. Mello-Filho, A. C., and Meneghini, R. (1991) Mutat. Res. 251, 109–113
36. Halliwell, B., and Aruoma, O. I. (1991) FEBS Lett. 281, 9–19
37. Chiu, S., Xue, L., Friedman, L. R., and Oleinick, N. L. (1995) Biochemistry 34, 2653–2661.
38. Oleinick, N. L., Balasubramaniam, U., Xue, L., and Chiu, S. (1994) Int. J. Radiat. Biol. 66, 523–529
39. Sakurai, K., and Ogiso, T. (1995) Biol. Pharmaceut. Bull. 18, 262–266
40. Zhao, X. Y., and Hutchers, T. W. (1994) Adv. Exp. Med. Biol. 357, 271–278
41. Hardy, J. A., and Aust, A. E. (1995) Carcinogenesis (Lond.) 16, 319–325
42. Feinstein, E., Canaani, E., and Weiner, L. M. (1993) Biochemistry 32, 13156–13161
43. Roberts, V. A., and Getzoff, E. D. (1995) FASEB J. 9, 94–100
44. Paul, S., Volle, D. J., Beach, C. M., Johnson, D. R., Powell, M. J., and Massey, R. J. (1989) Science 244, 1158–1162.
45. Paul, S., Volle, D. J., and Mei, S. (1990) J. Immunol. 145, 1196–1199
46. Mei, S., Mody, B., Eklund, S. H., and Paul, S. (1991) J. Biol. Chem. 266, 15571–15574
47. Sun, M., Gao, Q. S., Li, L., and Paul, S. (1994) J. Immunol. 153, 5121–5126
48. Gao, Q. S., Sun, M., Tyutyulkova, S., Webster, D., Rees, A., and Lerner, R. A. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 2532–2536
49. Groen, H., Aslander, M., Bootsma, H., and van der Mark, T. W., and Kallenberg, C. G. (1993) Clin. Exp. Immunol. 94, 127–133
50. Emerit, I., and Michelson, A. M. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 2537–2540
51. Golan, T. D., Sigal, D., Hacham, H., and Kimmel, S. (1994) Lupus 3, 103–106
52. Emerit, I., and Michelson, A. M. (1980) Acta Physiol. Scand. 492, 59–65
53. Blount, S., Griffiths, H., Emery, P., and Lunec, J. (1990) Clin. Exp. Immunol. 81, 384–389
54. Ara, J., and Ali, R. (1992) Immunol. Lett. 34, 195–200

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