

DNA RECEPTOR DYSFUNCTION IN SYSTEMIC LUPUS ERYTHEMATOSUS AND KINDRED DISORDERS

Induction by Anti-DNA Antibodies, Antihistone Antibodies, and Antireceptor Antibodies

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Systemic lupus erythematosus (SLE) is the prototypical systemic autoimmune disease characterized by the production of a variety of autoantibodies and by a heterogeneity of clinical expression. In general, the autoantibody response in SLE is directed toward two major classes of antigens: nuclear constituents and cell surface molecules. There is evidence that surface-reactive antibodies may influence disease expression by modulating lymphocyte subsets or by affecting cell function (1). We have previously described DNA binding to the cell surface of peripheral white blood cells in a manner consistent with a ligand-receptor relationship (2). The binding of λ phage DNA to monocytes, T cells, B cells, and neutrophils, but not erythrocytes, was found to be a saturable phenomenon inhibitable by excess cold DNA, but not RNA or mononucleotides. The apparent K_d was 10^{-9} M with $\sim 10^3$ molecules binding per cell. Electrophoresis of cell membrane proteins followed by blotting onto nitrocellulose revealed a DNA-binding protein migrating at a mol wt of $\sim 30,000$. After internalization, the receptor is reexpressed on the cell surface, a process that is inhibited by cycloheximide. Binding of exogenous DNA to this receptor results in internalization and subsequent degradation of the DNA to oligonucleotides. Other studies have indicated that freshly isolated white blood cells have DNA on their cell surfaces (3-5), and that this can act as the antigen for anti-DNA antibodies (6). We have recently described (7) an apparent defect in DNA receptor function in patients with SLE and kindred disorders. In this paper we amplify these observations and describe experiments that indicate that autoantibodies, found in SLE and similar diseases, markedly affect the expression and function of the DNA receptor.

Materials and Methods

Patients. The sera used in this study were obtained from patients seen in the rheumatology clinics at Oregon Health Sciences University. Patients with SLE fulfilled four or more of the 1982 criteria (8) for SLE. Patients with mixed connective tissue disease

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(MCTD)¹ had high titer antiribonucleoprotein antibodies, and a clinical overlap syndrome as previously described (9). Patients with rheumatoid arthritis were all in the categories of either definite or classical according to the 1958 criteria (10). Patients classified as having Sjogren's syndrome all had the primary form of the disease with keratoconjunctivitis sicca, a minor salivary gland biopsy score of 2 or greater, and at least one serological abnormality in the form of a positive antinuclear antibody test or a positive rheumatoid factor (11). No attempt was made to correlate the severity of disease expression with timing of the serum specimen. The relatives of patients with SLE were either siblings, parents, or adult children; none of them had ever been diagnosed as having SLE and none had symptoms suggestive of SLE.

Reagents. λ phage DNA was purchased from Bethesda Research Laboratories (Gaithersburg, MD) and radiolabeled with ³H by the method of nick translation (2). In cell-binding experiments, the [³H]DNA was used as a tracer by mixing it in a constant proportion to cold λ phage DNA to give $\sim 8 \times 10^4$ cpm/ μ g of total DNA. dNTPs, cycloheximide, BSA, and transfer RNA were purchased from Sigma Chemical Co. (St. Louis, MO); [³H]dTTP was purchased from Amersham Corp. (Arlington Heights, IL). HBSS was purchased from Gibco Laboratories (Grand Island, NY). Cyanogen-activated Sepharose 4B (CNBr-4B) and Ficoll-Paque were purchased from Pharmacia Fine Chemicals (Piscataway, NJ). DNase I was purchased from Worthington Biochemical Corp. (Freehold, NJ).

Cells. Human PBMC were used in all experiments. They were prepared from human buffy coats (40–50 g) obtained from the local Red Cross within 3 h of collection. The buffy coats were collected in acid citrate dextrose solution and centrifuged at 50 g to remove contaminating platelets and diluted in HBSS before separation into PBMC and erythrocytes/neutrophils by centrifugation over Ficoll-Paque. All cell preparations were assessed for viability both after separation and at the conclusion of experiments by the method of Trypan blue exclusion. The PBMC were washed once in HBSS and resuspended in HBSS containing 1% BSA at a final concentration of 10^7 cells/ml. The cell preparation contained >85% mononuclear cells and showed >93% viability at the initiation of experiments with >78% viability at the termination of cell culture, which lasted 24 h.

Monoclonal Antibodies. Monoclonal antinuclear antibodies were produced by fusion of NZB/NZW or MRL-*lpr/lpr* spleen cells (12). The characteristics and specificities of these antibodies have been previously described in detail (12). Antibodies BWD-1 (IgG2a) and BWD-2 (IgG2b) recognize both ssDNA and dsDNA. Antibody BWH-1 (IgG2a) recognizes the histone H2A-H2B complex, but fails to bind to individual histones (i.e., H1, H2A, H2B, H3, and H4). Antibody MH-2 (IgM) binds to individual histones H2A, H3, and H4. mAbs for most experiments were prepared from supernatants of the cloned cell lines, concentrated 10-fold to a concentration of ~ 150 μ g/ml. An irrelevant monoclonal IgG2a antibody (RPC-5; Litton Bionetics, Inc., Charleston, SC) added at the same concentration to supernatant of the nonsecreting parent myeloma line, was used as a control. In other experiments, IgG mAbs were purified from ascites fluid by Sephadex G-200 gel filtration (12). Ascites fluid from an animal injected with the nonsecreting parent myeloma served as a control. Preparations of the anti-DNA or antihistone mAbs demonstrated no cross-reactivity for histone or DNA, respectively, compared with irrelevant control monoclonals (12).

Preparation of Histones from Chromatin. Chromatin was isolated from calf thymus (Pel-Freez Biologicals, Rogers, AR) and histones free of DNA were then extracted with 0.4 M NH₂SO₄ (12). Purified anti-DNA mAbs demonstrated no reactivity in ELISA when these histones were used as antigen and coated to plates at saturation (12). The histone preparation showed no uptake of ethidium bromide when subjected to agarose gel analysis.

DNA Affinity Chromatography. DNA was coupled to cyanogen-activated Sepharose 4B as previously described (6). Briefly, calf thymus DNA (Worthington Biochemical Corp.) was dissolved in 10 mM KPO₄, pH 8.0 (75 m/25 ml buffer). The dissolved DNA was boiled for 10 min and then cooled on ice, to render it predominantly single stranded.

¹ Abbreviation used in this paper: MCTD, mixed connective tissue disease.

CNBr-Sepharose 4B (Pharmacia Fine Chemicals) was washed five times with 0.001 M HCl and then two times with the 10-mM KOP₄ buffer, before incubation with the dissolved DNA. After centrifugation, the OD₂₆₀ of the supernatant dropped to background. Thereafter, it was washed one time with 1 M KCl and once with 0.1 M NaOH. The remaining active groups on the CNBr-Sepharose were then blocked by incubation at 25°C for 30 min with 0.2 M glycine, pH 8.0. Final washes with 0.1 M acetate, 0.5 M NaCl, pH 4.0, and 0.1 M NaHCO₃, 0.5 M NaCl, pH 8.3, preceded storage of the DNA-Sepharose in PBS containing 0.1% sodium azide. Sera were depleted of anti-DNA antibodies by repeated passage over a column of the immobilized DNA. The efficacy of removal was assessed by the measurement of anti-DNA antibodies by an ELISA using ssDNA coated to microtiter wells. In some experiments, the glycine eluate (0.2 M glycine, pH 2.0) was used as a source of affinity-purified anti-DNA antibodies; in other experiments, anti-DNA antibodies were eluted by means of mononucleotides (see below).

IgG and its F(ab)₂ Fragment. Affinity-purified anti-DNA antibodies were isolated from a patient with active SLE whose serum gave 96% DNA binding in a standard Farr assay. The IgG fraction was prepared by DEAE cellulose (Whatman DE-32) ion-exchange chromatography (13), and anti-DNA antibodies were isolated using the procedure described by Manak and Voss (14). Briefly, the IgG fraction was dialyzed against PBS overnight at 4°C and then incubated for 18 h at 37°C with DNA-Sepharose. After three washes with PBS, the anti-DNA antibodies were eluted by incubation with 0.025 M mononucleotides (dAMP, dCMP, dGMP, and dTTP) in 0.005 M PBS, pH 8.0. Igs were precipitated by the addition of ice-cold ammonium sulfate to a final concentration of 50%; the precipitate was redissolved in PBS and chromatographed over Sephacryl S-200 to purify the IgG fraction and remove ammonium sulfate. F(ab)₂ fragments were prepared by pepsin digestion and the F(ab)₂ fragments were isolated by exclusion chromatography using Sephadex G 150. The F(ab)₂ fraction was then passed over a column of protein A-Sepharose (Pharmacia Fine Chemicals) to remove undigested IgG and Fc fragments. The purity of the IgG and F(ab)₂ fragments was verified by SDS-PAGE using nonreducing conditions. The reactivity of the F(ab)₂ with DNA was assessed by incubating 10 µg of ¹²⁵I-labeled F(ab)₂ with 500 µg of salmon sperm DNA and applying the mixture to a Sephacryl S300 column; 97% of the radioactivity eluted with the DNA at the void volume. F(ab)₂ prepared from IgG of a healthy control was not reactive with DNA under similar conditions.

DNA Receptor Assay. Specific [³H]DNA binding to PBMC used modification of the methodology previously described (2). PBMC suspended in HBSS plus 0.5% BSA were plated in V-bottomed 96-well trays at 2 × 10⁶ cells/well. λ phage [³H]DNA (400 µg in 1 ml) was then incubated with the cells for 1 h at 4°C. After washing five times in 400 µl of HBSS, the cells were disrupted with 1 N NaOH and the bound [³H]DNA was counted using 5 ml of 3 A 70 scintillation cocktail (Research Products International Corp., Mt. Prospect, IL). Nonspecific binding was assessed by measuring the binding of [³H]DNA to trypsinized cells (5 mg/ml, 30 min, 37°C). In some experiments, the PBMCs were stripped of their endogenous cell surface DNA by DNase (500 µg/ml, 37°C, 1 h). The effects of sera, Igs, F(ab)₂ fragments, and mAbs were assessed as follows. PBMC (2 × 10⁶ in HBSS + 0.5% BSA) were incubated with 50 µl of the specific humoral modulator, at concentrations described in individual experiments, for 30 min at 37°C. After washing three times with HBSS, the [³H]DNA binding assay was performed as described above.

Internalization Assay. The uptake of exogenously added DNA and subsequent degradation to oligonucleotides (<12 bp) was assessed as previously described (2). PBMC (10⁷/ml) were kept in continuous suspension in HBSS at 37°C. [³H]DNA was added to a final concentration of 200 µg/ml at time 0. Thereafter, timed aliquots of 100 µl of suspension (10⁶ cells) were washed once with PBS, twice with 0.1 M acetic acid, and finally once with PBS; this procedure was previously shown to remove >92% of cell surface-associated DNA from cells incubated at 4°C. To measure intracellular [³H]DNA, the cell pellet was solubilized in 1 N NaOH and counted; TCA (final concentration, 5%) was added to a small aliquot diluted to 100 µl with PBS + 5% BSA and the percentage of precipitated counts was assessed as a measure of DNA degradation. In an otherwise

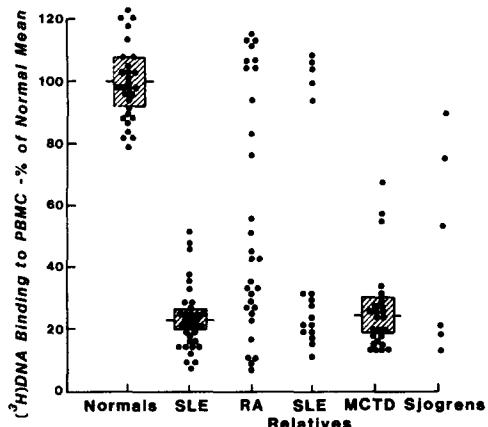


FIGURE 1. Humoral Inhibition of DNA receptor binding of [^3H]DNA. Serum (dilution 1:5) was incubated with the PBMC from a healthy volunteer at 37°C for 30 min; after washing, the binding of [^3H]DNA was measured. The mean [^3H]DNA binding using the sera from 29 healthy controls was arbitrarily designated as 100%. The change in [^3H]DNA binding induced by the patients sera is displayed as a percentage of the mean counts of the normal controls. Where there is a discrete clustering of points (normals, SLE, and MCTD), the mean (horizontal line) and SEM (hatched box) are shown.

identical experiment, 100 $\mu\text{g}/\text{ml}$ of affinity-purified anti-DNA $\text{F}(\text{ab})_2$ was incubated with the PBMC for 30 min at 37°C, and after washing three times with PBSS, [^3H]DNA was added at time 0 and its interiorization and degradation were assessed as described.

Detection of Antireceptor Antibodies by Immunoblotting. Cell membranes from PBMC were prepared as previously described (2). Briefly, 10^8 DNase-treated cells were homogenized in 10 mM phosphate (pH 7.4), 0.005 M PMSF, 1 mM dithiothreitol, 10 mM EDTA sodium azide, 10 $\mu\text{g}/\text{ml}$ DNase, 1 mM MgCl_2 , and 30 mM NaCl. An ice-cooled cell suspension was disrupted by 2–3 s bursts of a Polytron homogenizer (Brinkmann Instruments Co., Westbury, NY) using a power setting of seven. The homogenate was layered over 4% sucrose and centrifuged at 95,000 g for 1 h. The purified cell membranes were collected at the buffer/sucrose interface and were aspirated and washed three times in homogenization buffer. The membrane pellet was boiled for 2 min in a 0.06-M Tris-HCl buffer (pH 6.8) containing 2% SDS. The samples were diluted to a protein concentration of 50 $\mu\text{g}/\text{ml}$ and mixed with 20% glycerol and 0.001% bromophenol blue before SDS-PAGE (9% polyacrylamide in 3.75 M Tris HCl buffer, 0.1% SDS, pH 8.8; stacking gel 3% polyacrylamide in 0.125 M Tris HCl buffer, 0.1% SDS, pH 6.8). The separated proteins were electrophoretically transferred to nitrocellulose sheets (Bio-Rad Laboratories, Richmond, CA) using a Trans Blot apparatus (Bio-Rad Laboratories). The nitrocellulose sheet was cut into strips for further processing: (a) the position of the DNA receptor protein was demonstrated using a probe of biotin-labeled DNA as previously described (2, 15); (b) one strip was incubated with the IgG of an asymptomatic SLE relative (this serum had been extensively adsorbed over DNA-Sepharose and was still found to induce receptor dysfunction in DNase-treated cells); and (c) in control experiments, strips were incubated with normal human serum in one instance and preincubated with DNA (10 $\mu\text{g}/\text{ml}$) before incubation with the test serum in another experiment. Bands of fixed Igs were visualized by means of a peroxidase-conjugated goat anti-human IgG developed with diaminobenzidine tetrahydrochloride.

Results

Induction of a DNA Receptor Defect by Humoral Factors. Normal human PBMC were incubated at 37°C for 30 min with sera (1:5 dilution) obtained from normal individuals or patients with SLE and related diseases, and then tested for their ability to bind [^3H]DNA (Fig. 1). Incubation of cells with normal sera did not materially affect the binding of [^3H]DNA when compared with untreated cells. The mean [^3H]DNA binding of cells incubated with 20 normal sera was arbitrarily assigned as 100% and the effect of patient's sera was compared with this figure. All SLE sera tested inhibited DNA binding (mean \pm SE, $77 \pm 8\%$; range,

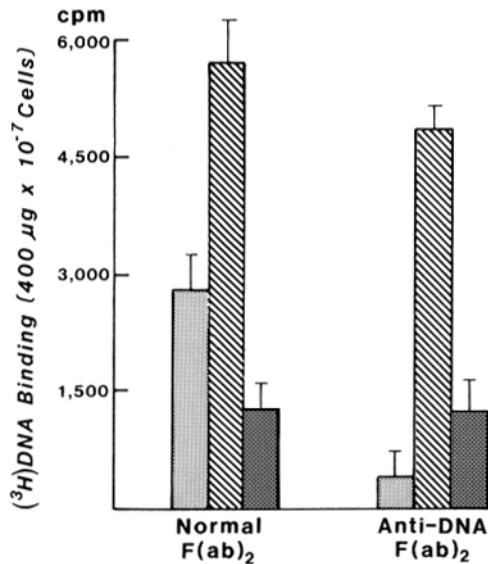


FIGURE 2. Inhibitory effect of anti-DNA F(ab)₂ on [³H]DNA binding; reversal by culture requires active receptor regeneration. PBMC from a healthy volunteer were incubated with the F(ab)₂ fraction of IgG from a normal control and with anti-DNA F(ab)₂ from a patient with SLE at a concentration of 660 µg/ml, at 37°C for 30 min; [³H]DNA binding was measured at (□) time 0 (i.e., immediately after washing F(ab)₂-treated cells) and at (▨) 24 h after the end of the incubation with F(ab)₂. For each F(ab)₂ incubation a similar experiment was performed on PBMC that had been treated with cycloheximide (■). The results shown are the mean of three experiments, the bars represent the SEM. The mean base-line binding at time 0, in the absence of antibodies, was 3,200 cpm.

53–94% inhibition). No overlap was observed between the values obtained with normal sera and SLE sera. All sera obtained from patients with MCTD induced marked inhibition of DNA binding (mean inhibition ± SE, 76 ± 12%; range, 36–84% inhibition). Sera from some patients with rheumatoid arthritis and primary Sjogren's syndrome showed inhibition similar to that seen in SLE, while other sera demonstrated minimal or no inhibition. Particularly interesting is the observation that the sera from many asymptomatic first degree relatives of patients with SLE also caused a profound inhibition of DNA binding.

Anti-DNA Antibodies in SLE Sera Induce the DNA Receptor Defect. We considered the possibility that anti-DNA antibodies in SLE sera might mediate the DNA receptor defect, perhaps by interacting with DNA already bound to the receptor on freshly isolated PBMC (6). We correspondingly prepared affinity-purified anti-DNA antibodies from SLE sera. To ensure that Fc-receptor binding was not participating, F(ab)₂ fragments of the affinity-purified Igs were prepared, and their ability to affect DNA binding was compared with that of IgG F(ab)₂ prepared from a pool of normal sera. These F(ab)₂ preparations were incubated with normal PBMC for 30 min at 37°C and the binding of [³H]DNA was studied immediately after incubation with antibody or after 24 h of culture (Fig. 2). Affinity-purified anti-DNA F(ab)₂ resulted in a profound reduction in [³H]DNA binding when cells were tested immediately after washing (Fig. 2). However, this inhibition was abrogated if cells were incubated for an additional 24 h (without antibody) after the initial exposure to the anti-DNA antibodies. It should be noted that DNA binding is nearly doubled when freshly isolated PBMC are incubated in culture for 24 h, whether or not PBMC had been preincubated with normal F(ab)₂. When cells were incubated with cycloheximide for 15 min and washed once before culture, no increase in DNA binding was observed over a 24-h period, whether cells had been pretreated with normal F(ab)₂, anti-DNA F(ab)₂, or without any antibodies.

TABLE I
Induction of DNA Receptor Dysfunction by mAbs

Antibody	Source	[³ H]DNA binding (cpm, mean ± SD)	Effect of DNAse (cpm, mean ± SD)
Controls	BALB/c serum	3,729 ± 373	4,265 ± 283
	IgG2a supernatant	4,612 ± 475	4,732 ± 356
	Control ascites fluid	5,741 ± 46	5,716 ± 205
	Human lupus serum	291 ± 58	4,683 ± 156
Anti-DNA mAbs	BWD-1 supernatant	902 ± 67	4,326 ± 216
	BWD-2 supernatant	821 ± 74	4,653 ± 274
	BWD-1-ascitic fluid	432 ± 81	3,991 ± 183
Antihistone mAbs	BWH-1 supernatant	409 ± 23	3,986 ± 361
	MH-2 supernatant	907 ± 101	4,743 ± 403
	BWH-1 ascitic fluid	378 ± 83	3,867 ± 312

Freshly isolated PBMC (10^7 /ml) were incubated with each mAb or serum (dilution 1:5) for 30 min at 37°C. After washing, DNA receptor function was assessed by measuring the binding of [³H]DNA. IgG2a refers to the Ig subclass of an irrelevant mAb used as a control. Pretreatment of the PBMC with DNAase is seen to abrogate the receptor loss induced by anti-DNA and antihistone antibodies. Each value represents the results triplicate experiment. Similar results were obtained on repeating this experiment on four different occasions.

Monoclonal Anti-DNA and Antihistone Antibodies Induce a DNA Receptor Defect. To further determine whether anti-DNA antibodies mediated the DNA receptor defect, we studied the effect of murine mAbs specific for DNA. Monoclonal antihistone antibodies and irrelevant mAb of the same IgG subclasses were used as controls.

As is shown in Table I, the two anti-DNA mAbs markedly reduced the binding of [³H]DNA to freshly isolated PBMC. To further determine whether these antibodies were functioning via their DNA specificity, we also tested their ability to inhibit DNA binding to cells pretreated with DNAase. Prior DNAase treatment prevented the anti-DNA antibody-induced receptor defect (Table I). The serum from a BALB/c mouse, the IgG2a control supernatant, and control ascites fluid did not influence the expression of the DNA receptor. Somewhat surprisingly, the two antihistone mAbs had a profoundly depressive effect on DNA binding, which was quantitatively equal to that observed with the anti-DNA mAbs (Table I). This inhibition by antihistone antibodies was also abrogated by prior treatment of the cells with DNAase (Table I). These results confirmed the involvement of DNA and anti-DNA antibodies in modulating a DNA-receptor function, but also suggested an unexpected role for histone and antihistone antibodies. Interestingly, the latter reactivity depended upon the presence of cell-surface DNA.

Effect of DNAase and Reconstitution with DNA and Histones on Antibody-mediated Receptor Dysfunction. As demonstrated above, the exposure of PBMC to DNAase I before incubation with either anti-DNA or antihistone antibodies, abrogates the loss of the DNA-receptor. This result was expected in the case of anti-DNA antibodies, but it remained unclear as to why the removal of cell-surface DNA abrogates the effect of antihistone antibodies. In an attempt to elucidate this observation, reconstitution experiments with histones and DNA

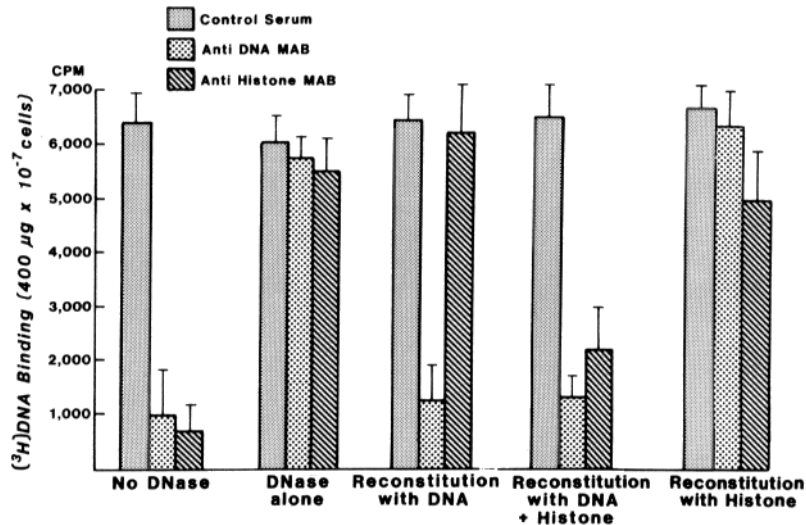


FIGURE 3. Anti-DNA and antihistone mAbs inhibit ^3H]DNA binding; abrogation by DNase treatment of cells and restoration by reconstitution with DNA and histones. PBMCs from a healthy volunteer were incubated with mouse serum (BALB/c) and mAbs to both DNA (BWD-1) and histone (BWH-1) at 37°C for 30 min. After washing, ^3H]DNA binding was measured. In some instances cells were treated with DNase before incubation with mAbs. A subset of cells pretreated with DNase was extensively washed and then incubated (RT, 30 min) with DNA ($100\ \mu\text{g}/\text{ml}$), or a mixture of total histones ($15\ \mu\text{g}/\text{ml}$), or DNA followed by histones, before measurement of ^3H]DNA binding. The results shown are the mean of four experiments done at different times, the bars represent the SEM. The mean base-line binding in the absence of antibody was 6,480 cpm.

were performed on DNase 1-treated PBMC (Fig. 3). If DNase-treated cells were reconstituted with purified DNA, then subsequent treatment with an mAb anti-DNA but not a monoclonal antihistone antibody inhibited DNA binding. When purified histones were reacted with the DNA-reconstituted cells, both anti-DNA and antihistone antibodies induced a defect in DNA receptor function. When purified histones were reacted with DNase-treated cells, without prior reconstitution with DNA, a moderate inhibition ($\sim 28\%$) of DNA binding was observed after treatment with antihistone antibodies; no inhibition was observed after treatment with anti-DNA antibodies. These results suggest that antihistone antibodies mediate DNA receptor dysfunction by binding to histones complexed with DNA, which in turn is bound to the DNA receptor.

Kinetics of Receptor Dysfunction Induced by Autoantibodies. In all the previous experiments cells had been routinely incubated with antibody for 30 min at 37°C before assessing ^3H]DNA binding. To determine the kinetics of the loss of DNA receptor function, freshly isolated PBMC were incubated with different antibody preparations for increasing periods of time. The results of a representative experiment are shown in Fig. 4; it is seen that compared with a normal $\text{F}(\text{ab})_2$ preparation, anti-DNA $\text{F}(\text{ab})_2$ and mAbs to DNA and histones cause an accelerated loss of DNA-receptor function with a $t_{1/2}$ of ~ 3 min. A markedly attenuated receptor loss ($\sim 12\%$ at 5 min) occurred if cells were incubated with anti-DNA antibodies at 4°C in the presence of 1 mM sodium azide (data not shown). Previous experiments have demonstrated the $t_{1/2}$ of the DNA-receptor internali-

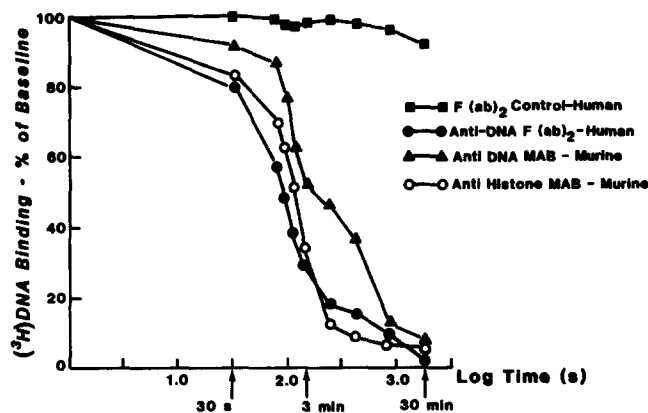


FIGURE 4. Kinetics of DNA receptor loss after incubating cells with anti-DNA and anti-histone antibodies. Freshly isolated normal PBMCs were incubated with different antibody preparations for specific time periods. At each time point the cell suspension (100 μ l) was removed and immediately diluted with 1 ml of ice-cold HBSS containing 0.1% sodium azide. After a further two washes with ice-cold HBSS, the [3 H]DNA binding was measured. The results are expressed as a percentage of [3 H]DNA binding in the absence of any antibodies (22,000 cpm).

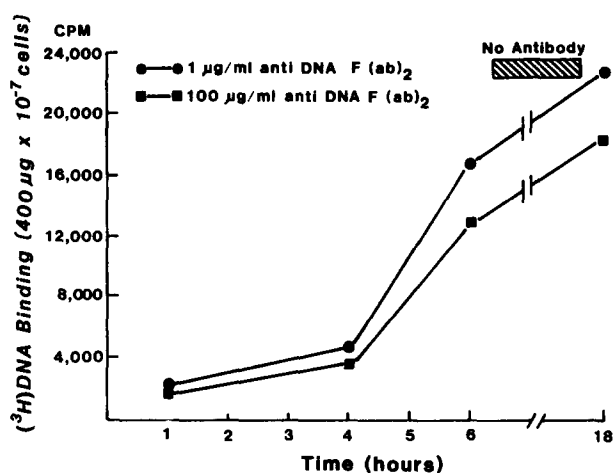


FIGURE 5. Restoration of DNA receptor function during culture, after antibody-mediated receptor loss. Normal PBMC were incubated with affinity-purified anti-DNA F(ab)₂ at concentrations of 1 μ g/ml and 100 μ g/ml for 30 min at 37°C. After extensive washing the cells were placed in culture and [3 H]DNA binding was measured at timed intervals. The mean base-line binding in the absence of antibody was 23,900 cpm (hatched bar).

zation is \sim 90 min after λ phage DNA is bound to the cell surface (Bennett, R. M., and M. J. Merritt, unpublished observations).

We also studied the time course of the reexpression of DNA binding. PBMC were incubated with increasing concentrations of antibody for 30 min, washed extensively, placed back into culture, and then tested for DNA binding at various times thereafter. Consistent with Fig. 2 above, marked reexpression occurred between 4 and 18 h of culture with 1–100 μ g/ml of antibody (Fig. 5). These data should be compared with a reappearance time of 15 min if the surface receptors are removed by trypsinization (data not shown).

Paralysis of Receptor Function. Previous experiments (2) have indicated that binding of DNA to the DNA receptor allows for subsequent internalization and degradation of DNA to oligonucleotides. We therefore studied whether incubation of PBMC with anti-DNA antibodies also prevented DNA internalization and degradation. Under normal circumstances, exogenous DNA gains access to the interior of the cells, as assessed by its resistance to acid washes, and it is degraded to oligonucleotides as assessed by its inability to be precipitated by TCA (Fig. 6).

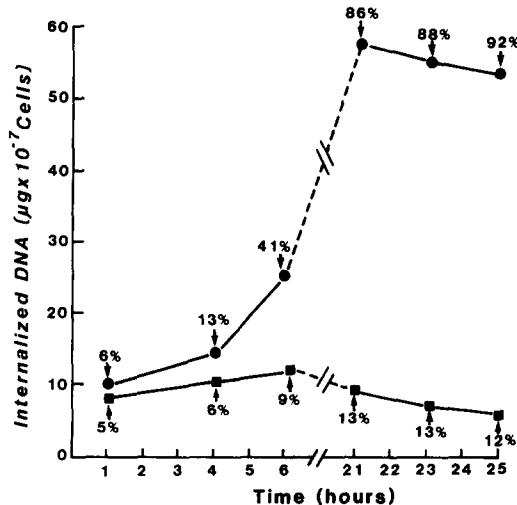


FIGURE 6. Anti-DNA antibodies inhibit the internalization and degradation of exogenous [³H]DNA. Normal PBMC were kept in continuous suspension in HBSS while incubating with [³H]DNA at 37°C. At timed intervals the acid-resistant [³H]DNA (internalized DNA) was measured, and an aliquot of 1 N NaOH-solubilized cells was subjected to precipitation with 5% TCA; the percentages refer to TCA soluble counts. In an identical experiment, the effect of a prior incubation (37°C, 30 min) of PBMC with affinity-purified anti-DNA F(ab)₂ was assessed as regards internalization and degradation of DNA. The dashed line is a truncated representation of the period between 6 and 21 h during which no experiments were performed.

When affinity-purified anti-DNA F(ab)₂ was incubated with the cells for 30 min before the addition of ¹²⁵I-DNA, the subsequent internalization and degradation of the DNA was inhibited (Fig. 6). This effect of preincubation with anti-DNA antibodies mimicked the previously observed action of cycloheximide (2), suggesting that anti-DNA antibodies influence the regeneration and surface repopulation of DNA receptors.

Evidence for Antibodies Directed against the DNA Receptor. During the course of experiments to verify the involvement of anti-DNA antibodies in inducing DNA receptor dysfunction, multiple sera from patients with SLE and their asymptomatic relatives were passed over immobilized DNA (Sephacrose 4B-CNBr-DNA). As described above, in nearly all SLE sera tested the initial neutral wash did not induce the receptor defect, whereas a glycine eluate (pH 2.3), containing anti-DNA antibodies did induce the receptor defect. In one serum (serum BI, a 31-yr-old woman whose mother had well-documented SLE), extensive adsorption over Sepharose-DNA, resulted in a neutral eluate as well as a glycine eluate that repeatedly induced the receptor defect. Furthermore, unlike the glycine eluate, the neutral eluate abrogated [³H]DNA binding to cells that had been treated with DNAase I (Table II). Ion-exchange chromatography with DEAE (Whatman Inc.) indicated that the reactive fraction of both eluates was IgG. The inhibitory effect of the glycine eluate in these experiments appeared to be due to the presence of antibodies to ssDNA in serum BI. However, the neutral eluate contained undetectable levels of antibodies to ssDNA and dsDNA or to total histones, as determined by ELISA. Thus, serum BI appeared to contain an antibody activity that inhibited cell-surface DNA binding, but did not contain anti-DNA or antihistone specificities.

The ability to react with DNAase treated cells suggested that serum BI could contain an antireceptor antibody, and this notion was explored further by immunoblotting. Solubilized membrane proteins from PBMC were electrophoretically separated, transferred to nitrocellulose, and then reacted with the IgG from patient BI or IgG from a healthy donor. Antibody binding to the blotted proteins was detected with peroxidase-conjugated goat anti-human IgG. As

TABLE II
Humoral Modulation of ^3H -DNA Binding of SLE Sera Compared with Serum BI

Serum	^3H -DNA Binding assay (cpm, mean \pm SD)			
	Neutral eluate	Glycine eluate	Neutral eluate with DNase/cell	Glycine eluate with DNase/cell
SLE serum	3,649 \pm 401	841 \pm 68	3,324 \pm 203	3,596 \pm 326
Serum BI*	515 \pm 63	861 \pm 74	535 \pm 62	483 \pm 54
Anti-DNA F(ab) ₂	3,486 \pm 314	936 \pm 83	4,426 \pm 521	3,857 \pm 391
Normal serum	5,034 \pm 441	4,135 \pm 279	4,286 \pm 445	4,326 \pm 426

The serum from patient BI, an SLE serum, affinity-purified anti-DNA F(ab)₂, and a normal serum were passed over a column (5 ml) of Sepharose-DNA. The neutral eluate and the glycine eluate were incubated with PBMC for 30 min at 37°C. After extensive washing, the binding of [^3H]DNA was assessed. An identical experiment was performed with cells that had been treated with DNAase 1. Each value is the mean of triplicate experiments.

* It should be noted that serum BI contained antibodies to ssDNA but not dsDNA.

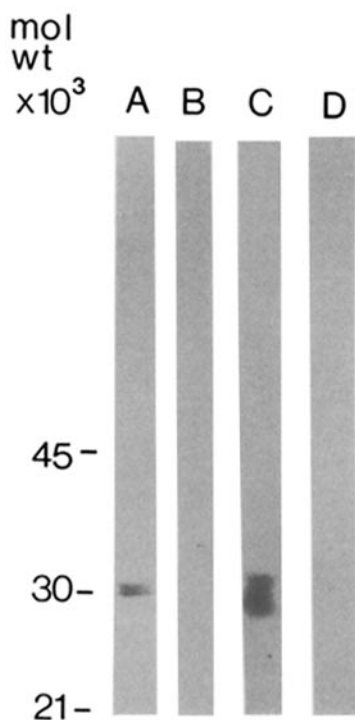


FIGURE 7. Immunoblotting shows that the IgG from patient BI has a reactivity with the 30,000 mol wt DNA-binding protein on cell membranes. Solubilized cell membrane proteins from normal PBMC were electrophoretically separated and transferred to nitrocellulose. Individual nitrocellulose strips were incubated with the IgG from BI (600 $\mu\text{g}/\text{ml}$) or the IgG from a healthy volunteer. In one instance the strip was preincubated with DNA (10 $\mu\text{g}/\text{ml}$) before combination with BI's IgG. Bands of fixed IgG were visualized by means of a peroxidase-conjugated second antibody. The position of IgG bands was compared with that of a DNA-binding protein (visualized by a probe of biotin-DNA) that migrated in a 30,000 mol wt position. (A) Biotin-DNA probe; (B) normal IgG; (C) BI's IgG; (D) blocking DNA.

shown in Fig. 7, one band of staining occurred at a molecular weight of 30,000, an identical position to that obtained with a biotin-labeled DNA probe. When the nitrocellulose strip was first incubated with DNA (10 $\mu\text{g}/\text{ml}$) before application of BI's IgG, or when normal IgG was substituted, no Ig binding was observed (Fig. 7). Thus, it appeared as if serum BI contained antibodies with specificity for the DNA receptor. However, it was also conceivable that this technique was measuring anti-DNA antibodies that were combined with short strands of DNA and that binding to the receptor was mediated via this bound DNA. To test this

possibility, the IgG fraction of BI's serum was depleted of anti-DNA antibodies as previously described above. On gel chromatography (Sephacryl G300) the IgG gave three peaks: one small peak at the void volume (consistent with aggregated IgG or DNA-anti DNA complexes), a moderate peak at ~300,000 (consistent with dimeric IgG), and a large peak at 160,000 (consistent with monomeric IgG). Samples were taken from the 160,000 peak in positions corresponding to the leading edge, the apex, and the trailing edge. These three samples plus samples from the apices of the two other peaks were tested for inhibitory activity for [³H]DNA binding to DNase-treated cells. In all five samples a comparable degree of inhibition was observed (data not shown). Immunoblots using the leading edge and the trailing edge of the monomeric IgG peak gave identical bands of IgG binding to the unprocessed serum. Thus, these results indicate that the DNA receptor binding and inhibition did not require the presence of anti-DNA-DNA complexes in the sera. In other experiments, inhibitory IgG from patient BI was subjected to extensive DNAase treatment before assay; this treatment did not decrease the ability of the patients' IgG to inhibit binding of [³H]DNA to PBMC or its ability to bind to the 30,000 mol wt protein by immunoblotting.

Discussion

These experiments indicate that sera from patients with SLE and MCTD contain antibodies that interact with the cell-surface DNA or DNA receptors found on normal PBMC. This interaction subsequently prevents the binding of exogenous DNA to these cells. Antibodies with at least three different specificities appear to be capable of resulting in DNA receptor dysfunction, namely anti-DNA, antihistone, and anti-DNA receptor. The conclusion that the anti-DNA antibodies can inhibit the binding of [³H]DNA to PBMC is based upon the fact that: (a) the F(ab)₂ portion of affinity-purified IgG anti-DNA antibodies induced the receptor defect; (b) two mAbs directed against DNA induced the defect; (c) the humorally induced inhibition of [³H]DNA binding was abrogated if cells were first subjected to DNase treatment; and (d) DNase-treated cells could be reconstituted by incubation with exogenous DNA with a resulting restoration of their ability to be negatively affected by anti-DNA antibodies.

It would seem likely that the interaction of the anti-DNA antibody with DNA already present on the cell surface (2, 4-6, 16) directly affects receptor function and the subsequent binding of [³H]DNA. Kinetic experiments indicated that the interaction of anti-DNA antibody with the cell-surface ligand causes an accelerated loss of receptor function. In previous experiments, the *t*_{1/2} of receptor turnover was ~90 min measured after the interaction with DNA. After exposure to anti-DNA antibodies, functional receptor loss occurred within 3 min, and the effect on receptor reexpression was prolonged. Cells rendered unable to bind [³H]DNA by the action of anti-DNA antibodies gradually exhibited a return of functionally active receptor over a 6-18 h time period in the absence of any further exposure to anti-DNA antibodies. The receptor repopulation was also completely abrogated if the cells were cultured in the presence of cycloheximide (2). These observations are consistent with the contention that after interaction with antibodies, the DNA receptor undergoes a cycle of internalization, destruc-

tion, and active regeneration (2). The synthesis of another protein required for cell surface expression is also possible. Previous data have indicated that ~20% of DNA receptors are occupied by cell-surface DNA (2), this makes it very unlikely that the observed results are simply due to a blocking phenomenon. It is therefore necessary to infer a linkage of cell-surface receptor molecules in such a way that the accelerated internalization of a critical number of occupied receptors causes a comigration of unoccupied receptors.

Unexpectedly, these experiments also demonstrated that antihistone antibodies also affect DNA receptor function to a similar degree compared with anti-DNA antibodies. Normal IgG, IgG depleted of anti-DNA antibodies, and unrelated mouse mAbs of the same subclass and at the same concentration did not affect DNA receptor function. A clue to the nature of the antihistone reactivity was provided by reconstitution experiments of DNase-treated cells with DNA and histones. These experiments showed a loss of antihistone-mediated inhibition if cells were DNase treated and reconstituted with purified DNA. A moderate but persistent inhibition (~28%) was noted when cells were reconstituted with histones alone followed by antihistone antibodies; this inhibition is unexplained, but may be due to minor amounts of DNA contamination in the histone preparation or in the antihistone mAb. Thus, the antihistone antibodies appear to be reactive with histones complexed to cell-surface DNA, which in turn is bound to the DNA receptor. This explanation of the present findings would be in accord with recent studies (16) documenting the occurrence of chromatin on cell surfaces. The result with antihistone antibodies raises the question as to whether any DNA-binding molecule can modulate DNA receptor expression. We have previously described the binding of lactoferrin to cell-surface DNA (3); in our present system, lactoferrin itself or antilactoferrin bound to the lactoferrin/DNA complex did not affect DNA receptor expression (our unpublished results).

As the apparent function of the DNA receptor is to internalize and degrade exogenous DNA (2), its paralysis by autoantibodies may be of relevance to the elevated levels of circulating DNA that have been described in SLE (17). It is conceivable that the production of anti-DNA antibodies causes the elevated levels of free DNA in SLE serum by means of the receptor dysfunction described in this paper. Such a scenario would link increasing levels of anti-DNA antibodies to increasing amounts of circulating DNA, and thus set in motion an accelerating cascade of immune-complex formation.

Although the presence of anti-DNA antibodies (both to dsDNA and ssDNA) and antihistone antibodies probably account for the modulation of DNA receptor expression by most of the sera from patients with active SLE and kindred disorders, it is unlikely that they account for the profound effect seen with ~70% of the sera from asymptomatic relatives of SLE patients. Many of these sera do not have detectable levels of antinuclear antibodies although the sera from some asymptomatic SLE relatives are documented to contain antibodies to ssDNA (18) and antibodies to anti-DNA antibody idiotypes (19). In one serum that we have studied in detail, extensive absorption of antibodies to ssDNA did not affect its ability to cause receptor dysfunction. Furthermore, the adsorbed serum did not contain antibodies to histones and was reactive with DNase-treated cells. This

third antibody had several of the characteristics expected of an antireceptor antibody, including direct binding to a DNA-binding cell-surface protein by immunoblotting. Furthermore, receptor dysfunction and binding on immunoblots was not dependent on the presence of DNA-anti-DNA complexes.

If antibodies to the DNA receptor turn out to be a common occurrence in the relatives of SLE patients, it would provide an important new avenue for research endeavors. There is increasing evidence that antibodies to ligand may be accompanied by an antiidiotypic response that has reactivity with the ligand's receptor (20-22). This may also be true for anti-DNA antibodies where corresponding antiidiotypic antibodies could possess anti-DNA receptor activity. Antibodies to anti-DNA idiotypes have recently been described (19, 23) in the relatives of patients with SLE as well as in inactive SLE patients. It will be of great interest to determine whether such antibodies are also reactive with the DNA receptor.

Summary

The ability of sera from patients with SLE and similar connective tissue diseases to induce dysfunction of the receptor for DNA was studied. All SLE and MCTD sera studied resulted in marked inhibition of DNA receptor binding. Furthermore, the sera from a subgroup of patients with other rheumatic diseases and a surprisingly high percentage of asymptomatic relatives of SLE patients exhibited a similar effect. The humoral factors causing this defect were shown to be of at least three reactivities: (a) antibodies to DNA, (b) antibodies to histones, and (c) antibodies to the DNA receptor itself. The reactivity of anti-DNA and antihistone antibodies is dependent upon intact cell-surface DNA, and reconstitution experiments suggest that antihistone antibodies are reactive with histones complexed to this DNA, which in turn is bound to the DNA receptor. Cells with an antibody-induced DNA receptor defect are unable to bind DNA; the subsequent inability to degrade DNA may have important consequences in diseases such as SLE in which DNA-anti-DNA immune complexes are of pathogenetic significance.

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