Identification of a Cell-Surface DNA Receptor and Its Association with Systemic Lupus Erythematosus

Steven H. Hefeneider, Ph.D., Robert M. Bennett, M.D., Tuan Q. Pham, B.A., Kenneth Cornell, M.S., Sharon L. McCoy, M.S., and Michael C. Heinrich, M.D.

Department of Immunology, Portland Veterans Medical Center and The Division of Arthritis and Rheumatic Diseases, The Oregon Health Sciences University, Portland, Oregon, U.S.A.

We have previously reported the existence of a cell-membrane-associated molecule on human PBMC, which binds DNA and has the characteristics of a receptor. Monoclonal antibodies have been made to this receptor and have been used successfully for the purification of this cell-surface molecule. Preliminary studies have indicated a receptor for DNA on murine kidney and spleen cells which is similar in molecular weight to the human DNA receptor (30 kD). The occurrence of autoantibodies to cell-surface receptors has been described in several autoimmune diseases and we have noted that the serum of patients with lupus and similar disorders inhibit the binding of labeled DNA to human leukocytes. Using a "dot-blot" assay with affinity-purified human DNA receptor, sera from patients with various CTD and from healthy volunteers were screened for anti-receptor antibodies; anti-receptor antibodies were found in many patients with CTD and some of their first-degree relatives. The prevalence of anti-receptor antibodies in normal blood donors was $<2\%$. It is hypothesized that anti-receptor antibodies represent an early immune response in lupus and kindred disorders and that anti-DNA antibodies may arise from the corresponding anti-idiotypic response. *J Invest Dermatol, 94:79S–84S, 1990*

Systemic lupus erythematosus (SLE) is a multi-systemic autoimmune disease of unknown etiology. There is considerable evidence that immunologic mediated mechanisms of tissue injury play an important role in its pathogenesis. In general, the autoantibody response in patients with SLE is directed toward two major classes of antigens: nuclear constituents and cell-surface molecules. There is evidence that cell-surface reactive antibodies may influence disease expression by modulating lymphocyte subsets or by affecting cell function [1]. DNA anti-DNA immune complexes are postulated to play a principal role in the pathogenesis of tissue injury [2,3]. Increasing evidence has demonstrated that human peripheral blood cells can both excrete DNA [4,5] and have DNA associated with their cell surface [6]. The source of DNA associated with these cells may originate from either dying cells or be produced by activated lymphocytes. Bennett and his colleagues [7] have demonstrated that the association of DNA with the cell-surface of human leukocytes occurs in a manner consistent with a ligand-receptor relationship. The apparent function of this receptor is to scavenge and degrade exogenous DNA; thus possibly constituting a nucleotide salvage pathway. Recent studies have demonstrated defective binding of labeled DNA to human peripheral blood mononuclear cells (PBMC) from patients with SLE. Herein we review the evidence supporting the existence of a DNA receptor and describe the production of autoantibodies to this receptor in patients with SLE and similar connective tissue diseases.

This work was supported by a Merit Review Grant from the Veterans Administration and by Research Grant AM 34295 from the United States Public Health Service.

Reprint requests to: Steven H. Hefeneider, Ph.D., Department of Immunology 151-R, Portland Veterans Medical Center, Portland, OR 97201.

Abbreviations:

- ADMC: adherent mononuclear cells
- CTD: connective tissue disease
- E+ cells: rosetting cells
- E− cells: nonrosetting cells
- MoAb: monoclonal antibody
- MCTD: mixed connective tissue disease
- PAGE: polyacrylamide gel electrophoresis
- PBMC: peripheral blood mononuclear cells
- PMN: polymorphonuclear leukocytes
- SLE: systemic lupus erythmatosus

MATERIALS AND METHODS

Agents Used Lambda phage DNA and DNA polymerase I were purchased from Bethesda Research Laboratories (Gaithersburg, MD). DNA was dissolved in 10 mM Tris HCl, pH 7.5, 120 mM NaCl, and 0.1 mM EDTA. Just before use, the solution was heated to 60°C for 10 min and then quickly cooled on ice, to restore the DNA to its linear form. (In storage it tends to form circles due to "sticky" ends.) Biotinylated DNA (B-DNA) was prepared from lambda phage DNA using nick translation to incorporate biotinylated d-uridine triphosphate (UTP) [8]; the reagents for this procedure (d-UTP), streptavidin-biotinylated horseradish peroxidase complex, DNA polymerase, and DNase 1 were purchased in kit form from Enzo-Biochem, Inc. (New York, NY) and processed as per the supplier's instructions. Goat anti-biotin and rabbit fluoresceinated anti-goat were purchased in kit form from Enzo-Biochem, Inc.

Radiolabeling of DNA DNA was radiolabeled by nick translation [9]. In brief, the reaction was performed on 12.5 μg of DNA in a final volume of 250 μl. The incubation buffer was 50 mM Tris HCl, pH 7.9, 10 mM $MgCl_2$, 10 mM dithiothreitol, 50 μg/ml BSA, and all four deoxynucleoside 5′-triphosphates. The concentration of unlabeled triphosphates (dATP, dGTP, dCTP) was 300 μM; the concentration of [^{3}H]deoxy-thymidine triphosphate was 1.33 mM. The reaction was initiated by adding *Escherichia coli* DNA polymerase (5 U) and DNase l (100 pg); incubation was for 30 min at 16°C. The reaction was terminated by adding EDTA

0022-202X/90/$03.50 Copyright © 1990 by The Society for Investigative Dermatology, Inc.

(final concentration, 20 mM). After addition of carrier DNA and sodium acetate (0.1 M, final concentration), the DNA was precipitated by the addition of 3 times volume of ice-cold absolute ethanol (−70°C, 10 min). After being washed 2 times in absolute ethanol, the dried pellet was gently dissolved in 1 ml Hank's bufferred salt solution (HBSS); this process took ~48 h.

[³H]DNA Binding to Cells All binding studies were performed at 4°C. Each experiment contained two controls, namely, trypsinized cells and a 50-times excess of cold lambda phage DNA (this was added after boiling for 10 min to produce single-strand DNA; at the concentrations used, double-strand DNA [dsDNA] was too viscous). Bound DNA was calculated from the percentage of counts bound after subtracting the counts of non-specific binding. The input DNA was plotted against the bound DNA using the CURFIT program (Hewlett-Packard Co., Palo Alto, CA); experiments with a closeness of fit (R^2) less than 0.85 were rejected. From the idealized curve generated by CURFIT, a standard Scatchard plot analysis [10] was used to estimate the binding affinity (K_d) and the number of molecules bound. To further characterize the binding of DNA to cell surfaces, we modified the basic binding assay: the cells were subjected to enzymatic treatment as previously described [11], cells were preincubated with ligands that might exhibit a competitive inhibition, and cells were pulsed with cold DNA to determine if this modulated ligand binding.

Detection of DNA Binding Proteins A search for a putative DNA receptor was initiated using a modification of the method of Bowen et al [12] to detect DNA binding proteins as we have previously described [13].

Isolation of Partially Purified Receptor The antigen used in the production of murine hybridomas was a partially purified extract of solubilized cell membrane proteins obtained from human PBMC. Cell membranes from 2×10^{10} PBMC were isolated as previously described [11] and electrophoretically separated by SDS-PAGE using two gels without individual troughs. A strip of gel, 1 inch in width, which corresponded to proteins migrating to the 30,000 region, was excised and crushed up in elution buffer (0.05 M Tris-HCl, pH 7.9, 0.1% SDS, 5.0 mM dithiothreitol, 0.1 mg/ml BSA, 0.2 M NaCl). Proteins were extracted by rocking the suspension for 48 h at 4°C. After centrifugation, the cleared supernatant was transferred to a 30 ml pre-siliconized tube, and 3 times the volume of acetone at −20°C was added; after incubation on ice-ethanol for 30 min the precipitate was collected by centrifugation. The precipitate was washed once with 80% acetone and 20% dilution buffer (0.05 M Tris-HCl, pH 7.9, 20% glycerol, 0.1 mg/ml BSA, 0.15 M NaCl, 1 mM dithiothreitol, 0.1 mM EDTA) and then re-solubilized in 2 ml of 6 M guanidine hydrochloride dissolved in dilution buffer. After incubation at room temperature for 20 min the mixture was made up to 10 ml with dilution buffer and incubated for 60 min at room temperature. Thereafter gradual renaturation of the proteins was achieved by dialysis for 24 h against PBS containing 0.1% SDS. Verification that the solubilized proteins contained the DNA receptor was achieved by immobilizing the antigen on nitrocellulose (Bio-Rad Laboratories, Richmond, CA) and using a dot-blot assay using a probe of biotinylated DNA [13].

Immunization and Cell Fusion Female BALB/c mice (The Jackson Laboratory, Bar Harbor, ME) 8 to 12 weeks of age were immunized with the partially purified DNA receptor preparation. Each of five animals received 20 to 50 μg of material in 0.4 ml of CFA intradermally to the hind legs. A series of five additional weekly injections was given to each animal using 20 to 50 μg of material in IFA. Sera were collected and tested individually for binding to the immunizing antigen (200 μl, 60 μg/ml) immobilized on nitrocellulose in a dot-blot assay employing goat-anti-mouse IgG conjugated to horseradish peroxidase (Bio-Rad) as the second antibody; sera collected before immunization served as a negative control. Each of the animals demonstrated significant binding in this assay. The animal found to have the highest reactivity on the dot-blot assay received an additional 40 μg of immunogen in PBS, administered intravenously. The animal was killed 4 d later and the spleen was removed and used for fusion with the murine SP/2 myeloma cell line [14]. Selected hybridomas were inoculated interperitoneally (1 to 2×10^6 cells/animal) into BALB/c mice with pristane-induced ascites; IgG was isolated by ion-exchange chromatography on DEAE cellulose (Whatman DE-52) followed by elution from a column of protein A Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, NJ).

Inhibition of the Binding of DNA to PBMC by MoAb Ascites-derived MoAb, at various concentrations, were incubated with PBMC (2×10^6 in HBSS + 0.5% BSA) for 30 min at 4°C. After washing 3 times with HBSS, the ability of cells to bind [³H]DNA was assessed as previously described [7]. In each experiment, controls included cells incubated with an irrelevant MoAb of the same isotype (Litton Bionetics, Inc., Charleston, SC) and cells not exposed to any IgG.

Isolation of Cell Surface Protein with DNA Binding Properties A purified preparation of cell membranes from PBMC was prepared as previously described [7]. Membrane proteins were solubilized by incubation with 10 mM deoxycholate in 0.05 M NaCl (4°C, 2 h). The solubilized proteins were cleared by centrifugation at 100,000 × g for 1 h; the supernatant was dialyzed against 0.05 M PBS 2 times and against HBSS one time. The dialyzed proteins were concentrated with polyethylene glycol to a final concentration of 0.38 mg/ml. Two immunoaffinity matrices were constructed by binding MoAb 12A and 24T to protein A Sepharose CL-4B followed by cross-linking with dimethyl pimelimidate [15]; as a control, a third immunomatrix was constructed with an irrelevant MoAb. As the amount of receptor likely to be eluted from the immunomatrix would be at the limit of detection by OD_{260} measurements, a 1-μg fraction of the membrane protein preparation was labeled with ^{125}I, by the chloramine T method, for use as a tracer in monitoring the affinity column eluates. A total of 25.8 mg of membrane proteins, plus added tracer was incubated (37°C, 1 h) with 1 g of each immunomatrix suspended in HBSS containing 0.1% SDS. The immunomatrix was then washed extensively with HBSS + 1 M NaCl, pH 7.4, and packed into a small column. Successive buffers of HBSS + 1 M NaCl, each decreasing by 1 pH unit, were passed through the column and the eluate was monitored for radioactivity. The peak counts were pooled and concentrated 10 times. The eluted proteins were then boiled in a Tris buffer, pH 6.8, containing 3% SDS, 20 μl 5 mM dithiothreital, 15% glycerol, and 0.01% bromphenol blue. Proteins were electrophoretically separated by SDS-PAGE; one gel was stained with Bio-Rad silver stain (according to the manufacturers instruction) and another gel was used to transblot the separated proteins onto a nitrocellulose sheet. The nitrocellulose was developed for DNA binding proteins using a probe of biotin-DNA, as previously described [7].

RESULTS

DNA Binding to Whole Cells Using ³H-labeled lambda phage DNA, initial studies demonstrated binding of labeled DNA to human PBMC. The binding was maximal after incubation for 10 min at 4°C, and was dependent upon the presence of Ca, Mg, and SO_4 ions [7]. DNA binding to isolated cell populations (PMN, E−, E+, ADMC) was abolished by proteolytic digestion of the cell surface but was not affected by treatment with neuraminidase, phospholipase, or RNase. Moreover, the binding of labeled DNA was saturable and specifically inhibited by excess unlabeled DNA (Fig 1). Scatchard plot analysis of the binding curves indicated a high affinity (approximately 10^{-9} M) for DNA binding and a single population of binding sites with approximately 10^3 receptors/cell (Fig 2).

Preliminary Characterization of the DNA-Binding Molecule Initial attempts to characterize the human DNA-binding receptor were done by solubilizing cell membrane proteins from human PBMC [7,13]. The solubilized proteins were separated by SDS-PAGE and electrophoretically transferred to nitrocellulose.

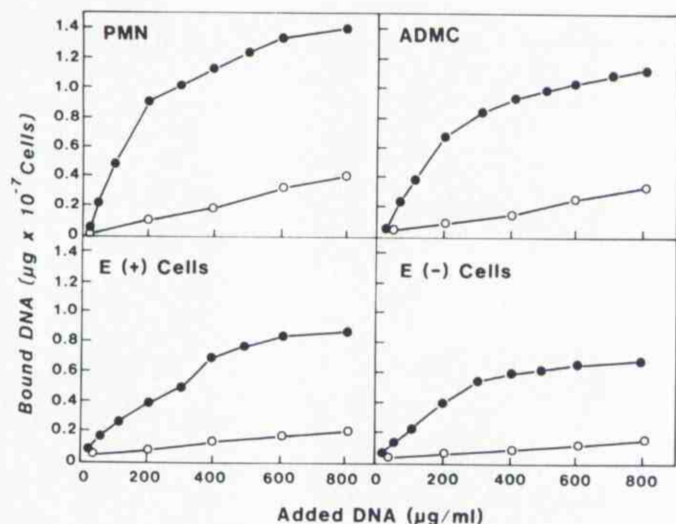

Figure 1. It is seen that for each cell type a saturability of [^{3}H]DNA binding is demonstrated. Untreated cells, *solid circles*; excess cold DNA, *open circles*. Trypsinized cells: points not shown, exhibited a parallel curve to the "excess cold DNA" curve. Reproduced from J Clin Invest 76:2182–2190, 1985.

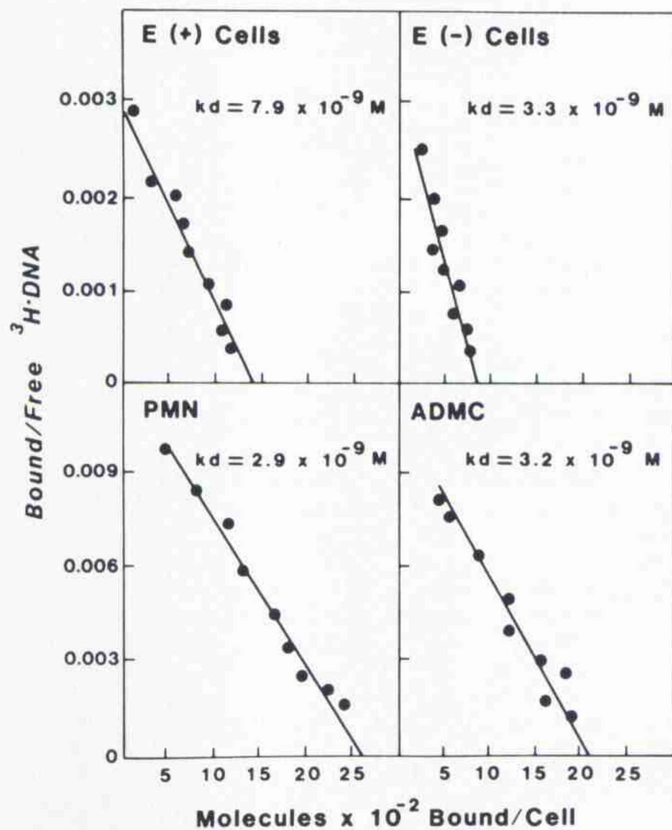

Figure 2. A linear transformation of the data shown in Fig 1 to yield Scatchard plots. For each cell type a straight line relationship was observed ($r^2 > 0.89$), indicating a single species of binding molecule. The dissociation constants (K_d) were $\sim 10^{-9}$ M, and the DNA binding capacity varied from 0.81×10^3 to 2.6×10^3 molecules per cell, depending upon the cell type. Reproduced from J Clin Invest 76:2182–2190, 1985.

DNA-binding proteins were probed for with biotin-DNA. Development was accomplished with a streptavidin-biotinolyted horseradish peroxidase complex plus substrate. These studies demonstrated a single DNA binding protein with a molecular weight of 30,000 for each cell population examined, except red blood cells (Fig 3). In addition, recent studies from our laboratory have identified a DNA binding molecule on murine spleen and kidney cells, which has a molecular weight of 30 kD (data not shown).

Production of Monoclonal Antibodies to the DNA Receptor In order to further characterize the DNA receptor from the PBMC, we made monoclonal antibodies to this molecule. Repeated immunization of Balb/c mice with a partially purified DNA receptor preparation resulted in the production of antibodies reactive with the immunizing antigens. One immune animal was sacrificed and its spleen cells fused with the murine SP/2 myeloma cell line. After 10 d in culture, greater than 85% of the microcultures exhibited hybrid cell growth. Using a dot-blot screening test, 90 out of 350 supernatants tested demonstrated reactivity. These hybrids were transferred to 1 ml cultures and allowed to reach 75–90% confluence and the supernatants tested for their ability to inhibit the binding of ^{3}H-DNA to human PBMC. Of these 90 hybrids tested, 12 demonstrated significant inhibition of DNA binding. Two hybrids which demonstrated the highest level of inhibition were weaned to HAT-free medium and subcloned twice at 1 cell/well. These two hybridomas were designated as 12A and 24T, and were isotyped as IgG1/k and IgG2b/k, respectively [16].

Reactivity of Monoclonals to the DNA Receptor Monoclonal antibodies 12A and 24T, derived from inoculated ascites fluid, demonstrated inhibition of ^{3}H-DNA binding to PBMC, as determined by the initial screening of the hybridomas. As seen in

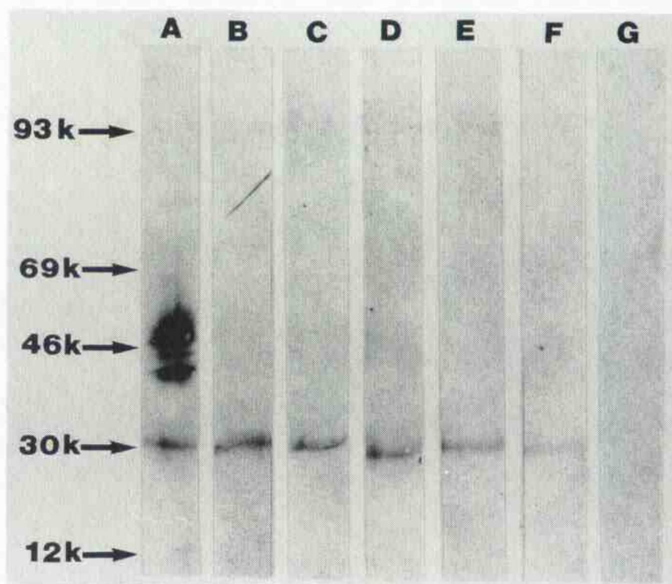

Figure 3. Nonhistone nuclear proteins *(A)*, membrane preparation of neutrophils *(B)*, monocytes *(C)*, T lymphocytes *(D)*, B lymphocytes *(E)*, a cytosol preparation *(F)*, and erythrocyte membranes *(G)* were subjected to SDS-PAGE. The separated proteins were electrophoretically blotted onto nitrocellulose sheets: DNA binding proteins were probed for by biotin-DNA. Development was accomplished with a streptavidin-biotinylated horseradish peroxidase complex using the substrate diamino-benzidine tetrahydrochloride. All cells, except erythrocytes, possessed a single DNA binding protein, migrating in a position consistent with a molecular weight of 30,000. Cytosol exhibited a weak binding band. Nonhistone nuclear proteins are included as a positive control and also showed a DNA binding protein in an identical position to the cell membrane preparations. Reproduced from J Clin Invest 76:2182–2190, 1985.

Fig 4, when increasing dilutions of the antibodies were incubated with PBMC, significant inhibition of labeled DNA binding was noted down to a dilution of 3×10^{-4}. When PBMC were incubated with either monoclonal antibody for 10 min at 4°C, subsequent labeled DNA binding was inhibited for 18 h but cell viability was not affected. A control, irrelevant murine ascites fluid did not influence labeled DNA binding.

Isolation of the DNA Binding Protein To determine whether the monoclonal antibodies reacted specifically with the DNA binding protein, immunomatrices of 12A and 24T were constructed and the reactive molecule from solubilized cell-membrane preparations isolated by the method of affinity chromatography. After washing the immunomatrix extensively with a starting buffer of pH 7.0, the pH was successively decreased by 1 pH unit, and the proteins which had specifically bound to the immunomatrix were eluted at a pH of 4.0. The proteins obtained from the acid wash of the immunomatrix were electrophoretically separated under reducing conditions on SDS-PAGE. A single band was visualized after silver staining of the gels and both MoAb 12A and 24T affinity-purified proteins exhibited a band at the 30-kD position (which is identical to the size of the DNA receptor). To determine whether this protein was the DNA receptor, the proteins from unstained SDS-PAGE gels were electrophoretically transferred to nitrocellulose, and reacted with a probe of biotin-labeled DNA [16]. It was seen that the proteins which reacted specifically with MoAb 12A and 24T also reacted with a biotin-DNA probe (Fig 5). Moreover, high-performance liquid chromatography (HPLC) analysis of the isolated protein from the immunomatrix column demonstrated a 97% purification of the DNA-binding protein (data not shown). These results support the conclusion the MoAb 12A and 24T are specific for the DNA receptor.

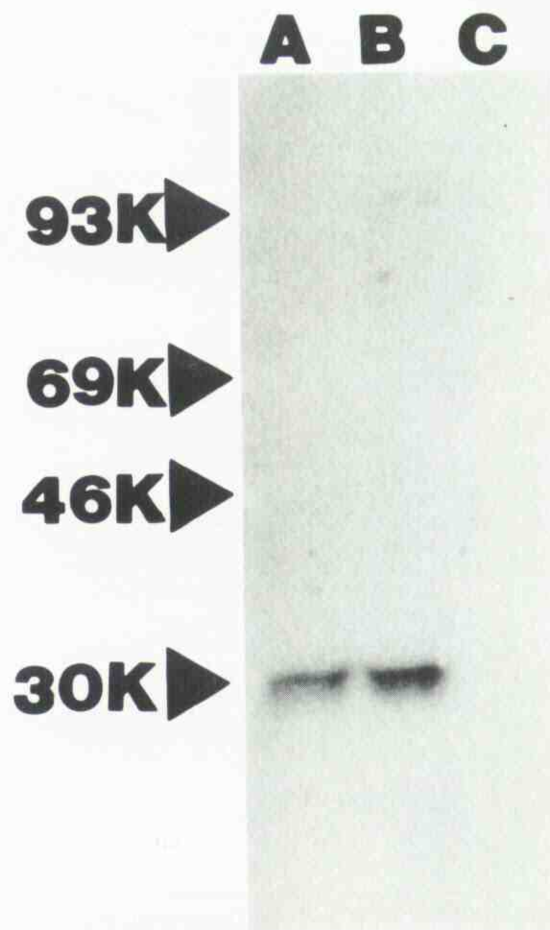

Figure 5. The purified receptor preparations isolated from the 12A and 24T immunomatrices were resolved on SDS-PAGE and electrophoretically blotted onto a nitrocellulose sheet. To determine whether the proteins migrating at M_r 30,000 were the DNA receptor, the blots were exposed to a probe of biotin-labeled DNA. It is seen that the proteins isolated from immunomatrix 12A *(lane A)* and immunomatrix 24B *(lane B)* both bound DNA, whereas proteins eluted from X, an immunomatrix of an irrelevant MoAb, showed no DNA binding *(lane C)*. Reproduced from J Immunol 140:2937–2942, 1988.

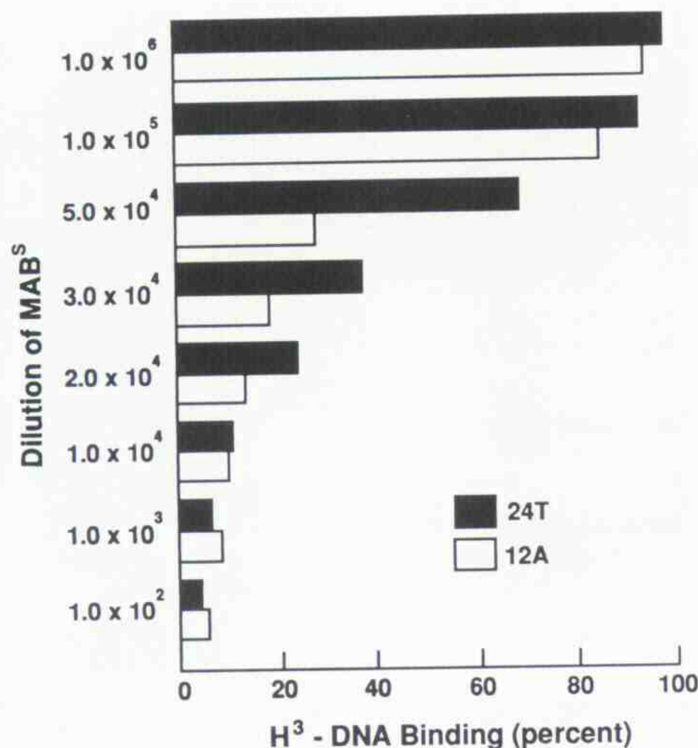

Figure 4. Inhibition of DNA binding to human PBMC by MoAb 12A and 24T at different dilutions. MoAb were incubated with 2×10^6 PBMC, 4°C, 30 min; after washing 3 times with HBSS the ability to bind [^{3}H]DNA was assessed. Results are expressed as the mean binding of three replicate wells. Maximal binding of [^{3}H]DNA was assessed without the addition of MoAb and with the addition of an irrelevant MoAb, giving counts of 22,705 ± 1,033 and 20,614 ± 983/min, respectively. The original concentrations of the MoAb were 3 mg/ml. This experiment is representative of six similar experiments done over a 2-month period. Reproduced from J Immunol 140:2937–2942, 1988.

Induction of a DNA Receptor Defect by Humoral Factors With the development of monoclonal antibodies to the DNA receptor and the ability to obtain purified receptor protein, it was now possible to explore the relevance of the DNA receptor as a target antigen for the production of autoantibodies in patients with SLE. Our early studies had demonstrated that sera from patients with SLE significantly inhibited the binding of labeled DNA to normal human PBMC [17]. Incubation of the PBMC with sera from healthy normal individuals did not affect the binding of labeled DNA when compared with untreated cells. The mean ^{3}H-DNA binding of cells incubated with 29 normal sera was arbitrarily assigned as 100% and the effect of patient's sera was compared with this value [18]. As seen in Fig 6, all SLE sera tested inhibited DNA binding (mean ± SE, 77 ± 8%; range, 53–94%). No overlap was observed between the values obtained with normal sera and SLE sera. Moreover, all sera obtained from patients with MCTD induced marked inhibition of DNA binding (mean inhibition ± SE, 76 ± 12%; range, 36–84%). Sera from some patients with rheumatoid arthritis and primary Sjögren's syndrome showed inhibition similar to that noted in patients with SLE, whereas other sera dem-

onstrated minimal or no inhibition [18]. Of interest, sera from many asymptomatic first-degree relatives of patients with SLE also showed marked inhibition of DNA binding. It was shown that the IgG component of SLE sera was responsible for the inhibition of labeled DNA binding to human PBMC [18]. Moreover, we have recently completed similar studies demonstrating that serum from lupus-prone mice can significantly inhibit the binding of labeled DNA to normal mouse spleen cells. Sera collected from normal animals, or lupus-prone animals prior to disease expression, failed to significantly affect the binding of labeled DNA (data not shown).

Cell-Surface Molecules Reactive with SLE Sera To further examine the reactivity of SLE sera with the DNA receptor protein, PBMC membrane proteins were resolved on SDS-PAGE followed by Western blotting using sera that were known to contain anti-receptor antibodies. A control serum from a normal healthy individual was appropriately negative. Of interest, when we examined sera from patients with connective tissue diseases, a band at 30 kD represented the major site of reactivity. Patients with both SLE and mixed connective tissue disease demonstrated significant immune reactivity at the 30-kD site corresponding to the DNA receptor. Bands of weaker intensity at other positions were also noted with sera from patients with connective tissue diseases (data not shown).

Detection of Anti-DNA Receptor Antibodies The availability of highly purified DNA receptor, isolated from immunomatrices constructed from the anti-receptor monoclonals, has permitted the development of a dot-blot assay procedure for the detection of anti-DNA receptor antibodies in human sera. In an initial study of 256 sera samples obtained from normal healthy individuals, only six (2%) were positive for anti-receptor antibodies. In comparison, approximately 60–70% of sera obtained from patients with rheumatoid arthritis, SLE, mixed connective tissue disease, and primary Sjögren's syndrome were positive for anti-receptor antibodies (data not shown). This data lends additional support to the notion that the DNA receptor is a common target antigen for autoimmunity in patients with connective tissue diseases.

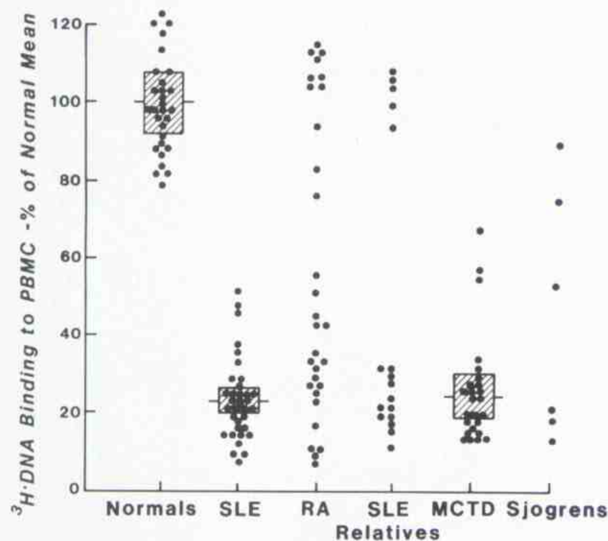

Figure 6. Humoral inhibition of DNA receptor binding of [^{3}H]DNA. Serum (dilution 1 : 5) was incubated with the PBMC from a healthy volunteer at 37°C for 30 min; after washing, the binding of [^{3}H]DNA was measured. The mean [^{3}H]DNA binding using the sera from 29 healthy controls was arbitrarily designated as 100%. The change in [^{3}H]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. Reproduced from J Exp Med 166:850–863, 1987.

DISCUSSION

There is a large literature that supports the concept that nucleic acids are found in association with cell surfaces [6,19–22]. The source of DNA associated with these cells may originate from either dying cells or be produced by activated lymphocytes [4,23]. The notion that the association of DNA with cell surfaces is anything more than a technical artifact or a non-specific interaction is an area of some controversy. Of interest are reports that cell-surface DNA can be taken up by cells and the information integrated into the cell to become a stable characteristic. If exogenous DNA can be taken up by cells it raises the question as to whether a specific cell-surface receptor exists for DNA. In this paper we have presented studies supporting the existence of a specific receptor for DNA and described the initial characterization of this molecule. In addition, the involvement of the DNA receptor as a target antigen for autoantibodies in patients with SLE has been reviewed.

Our data supports the conclusion that the interaction of DNA with the cell surface occurs in a manner consistent with a receptor-ligand interaction. This interpretation is supported by studies demonstrating the saturability and specificity of this interaction. Moreover, using biotin-labeled DNA, a single DNA binding molecule of 30 kD was identified. Further evidence for the existence of a specific cell-surface receptor for DNA is provided by the production of two monoclonal antibodies that exhibit the properties predicted for a specific interaction with the DNA receptor. These antibodies inhibit the binding of labeled DNA to PBMC, suggesting they are reactive with the DNA binding domain of the receptor or an adjacent area. Moreover, construction of an immunomatrix incorporating the monoclonals allows the DNA receptor to be purified to near homogeneity (as assessed by SDS-PAGE and HPLC analyses). The affinity-purified protein migrates as a single band on SDS-PAGE and is easily visualized by silver staining. Most importantly, the affinity-purified protein after SDS-PAGE and Western blotting reveals a single DNA binding molecule of 30 kD, as assessed by a biotin-DNA probe. These results are consistent with the conclusion that a specific DNA-binding receptor is present on human PBMC. In addition, flow-cytometry studies using the two anti-receptor monoclonals support our previous observations indicating that the DNA receptor is expressed on monocytes, B cells and T cells (data not shown).

There are several other reports that either directly support or are consistent with the existence of a specific receptor for DNA. Bankhurst and Williams [24] described DNA binding to B cells in patients with SLE and some normals. Okudaira and colleagues [25] have recently reported a murine monoclonal antibody to DNA which binds to cell-membrane-associated DNA on mouse thymocytes and human T cells and becomes internalized in a manner consistent with receptor-mediated endocytosis of DNA. Abdou and co-workers [26] have demonstrated that sera from a patient with SLE suppresses the binding of labeled DNA to B cells. They hypothesized this inhibition was due to anti-idiotypic antibodies blocking the paratope of cell-surface anti-DNA IgG; however, these results could also be interpreted as evidence for anti-DNA receptor antibodies. Sudar et al [27], have presented direct evidence for DNA receptors on macrophages. Emlen and colleagues [28] have recently reported a DNA receptor on murine Kupffer cells. This result is of particular interest as DNA administered intravenously is preferentially cleared by the liver.

The apparent function of the DNA receptor is to scavenge and degrade exogenous DNA; whether the resulting nucleotides can be reduced as part of a "nucleotide salvage pathway" remains to be investigated. Experiments testing DNA binding in the presence of serum from patients with lupus have demonstrated defective binding to human PBMC. This dysfunction of DNA receptor activity was found to be due, in part, to the presence of anti-receptor antibodies in the serum of these patients. Blocking and/or modulation of the DNA receptor may interfere with the function of the receptor and may result in the elevated levels of circulating DNA which has been described in SLE. Receptor blockade might therefore be of

pathogenetic relevance as the interaction of circulating DNA and anti-DNA antibodies is considered to be a major determinant of immune-complex-mediated vasculitis and glomerulonephritis.

The origin of anti-DNA antibodies remains an engima. DNA itself is not immunogenic, yet there is mounting evidence that autoimmunity in SLE is "antigen-driven" rather than a non-specific polyclonal activation. The demonstration of a DNA receptor on human cells and the observation that this receptor can be a target antigen for autoimmunity has led us to an alternative theory for the generation of anti-DNA antibodies. This hypothesis is based on an immunoregulatory network of idiotypic pairs as originally proposed by Jerne [29]. An antibody response to the binding site of the DNA receptor would serve as an antigen for development of an anti-idiotypic antibody. This anti-idiotypic antibody would mimic the original ligand (the binding site of the DNA receptor) and thus have anti-DNA reactivity. These ideas form the basis for our ongoing investigations.

REFERENCES

1. Morimoto C, Reinherz EL, Distaso JA, Steinberg AP, Schlossman SF: Relationship between SLE and T cell subsets: anti-T cell antibodies and T cell function. J Clin Invest 73:689–700, 1984
2. Agnello V: The immunopathogenesis of lupus nephritis. Adv Nephrol 6:119, 1976
3. Emlen W, Pisetsky DS, Taylor RP: Antibodies to DNA: a perspective. Arthritis Rheum 29:1417–1426, 1986
4. Distelhorst CW, Cramer K, Roger JC: Selective release of excreted DNA sequences from phytohemagglutinin-stimulated human peripheral blood lymphocytes. J Clin Invest 61:1204–1217, 1978
5. Boldt DH, MacDermott RP, Speckart SF, Nash GS: Excretion of DNA from purified human lymphocyte populations. J Immunol 118:1495–1498, 1977
6. Moyer MP: The association of DNA and RNA with membranes. Int Rev Cytol 61:1–61, 1979
7. Bennett RM, Gabor GT, Merritt MJ: DNA binding to human leukocytes: evidence for a receptor-mediated association, internalization, and degradation of DNA. J Clin Invest 76:2182–2189, 1985
8. Langer PR, Waldrop AA, Ward DC: Enzymatic synthesis of biotin labelled polynucleotides: novel nucleic acid affinity probes. Proc Natl Acad Sci USA 78:6633–6637, 1981
9. Rigby DWJ, Dieckman M, Rhodes C, Berg P: Labelling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase 1. J Mol Biol 113:237–251, 1977
10. Scatchard G: The attraction of proteins for small molecules and ions. Ann NY Acad Sci 51:660–672, 1949
11. Bennett RM, Davis J, Campbell S, Portnoff S: Lactoferrin binds to cell membrane DNA: association of surface DNA with an enriched population of B cells and monocytes. J Clin Invest 71:611–618, 1983
12. Bowen B, Steinberg J, Laemmli UK, Weintraub H: The detection of DNA binding proteins by protein blotting. Nucl Acid Res 8:1–20, 1980
13. Gabor G, Bennett RM: Biotin-labeled DNA: a novel approach for the recognition of a DNA binding site on cell membranes. Biochem Biophys Res Commun 122:1034–1039, 1984
14. Nowinski RC, Lostrom ME, Tam NR, Stone MR, Burnette WN: The isolation of hybrid lines producing monoclonal antibodies against p15(E) protein of ectopic murine leukemia viruses. J Virol 93:111–126, 1979
15. Schreider C, Newman RA, Sutherland DR, Asser U, Greaves MF: A one-step purification of membrane proteins using a high efficacy immunomatrix. J Biol Chem 257:10766–10769, 1982
16. Bennett RM, Hefeneider SH, Bakke A, Merritt M, Smith CA, Mourich D, Heinrich MC: The production and characterization of murine monoclonal antibodies to a DNA receptor on human leukocytes. J Immunol 140:2937–2942, 1988
17. Bennett RM, Peller JS, Merritt MJ: Defective DNA-receptor function in systemic lupus erythematosus and related diseases: evidence for an autoantibody influencing cell physiology. Lancet I:186–188, 1986
18. Bennett RM, Kotzin BL, Merritt MJ: DNA receptor dysfunction in systemic lupus erythematosus and kindred disorders: induction by anti-DNA antibodies, anti-histone antibodies and anti-receptor antibodies. J Exp Med 166:850–863, 1987
19. Reid BL, Charlson AJ: Cytoplasmic and surface deoxyribonucleic acids with consideration to their origin. Int Rev Cytol 60:27–52, 1979
20. Lerner RA, Meinke W, Goldstein DA: Membrane associated DNA in the cytoplasm of diploid human lymphocytes. Proc Natl Acad Sci USA 68:1212–1216, 1971
21. Meinke W, Hall MR, Goldstein DA: Physical properties of cytoplasmic membrane-associated DNA. J Mol Biol 78:43–56, 1973
22. Meinke W, Goldstein DA: Reassociation and dissociation of cytoplasmic membrane associated DNA. J Mol Biol 86:757–773, 1974
23. Rogers JC: Characterization of DNA excreted from phytohemagglutinin-stimulated lymphocytes. J Exp Med 143:1249–1264, 1976
24. Bankhurst AD, Williams RC: Identification of DNA binding lymphocytes in patients with systemic lupus erythematosus. J Clin Invest 56:1378–1385, 1975
25. Okudaira K, Yoshizawa H, Williams RC: Monoclonal murine anti-DNA antibody reacts with living mononuclear cells. Arthritis Rheum 30:669–678, 1987
26. Abdou NI, Wall H, Clancy J: The network theory of autoimmunity: in vitro modulation of DNA-binding cells by anti-idiotypic antibody present in inactive lupus sera. J Clin Immunol 1:234–240, 1981
27. Sudar F, Csaba G, Robenek H, Themann H: Localization and internalization of cell surface DNA in macrophages. Cell Mol Biol 32:87–91, 1986
28. Emlen W, Rifai A, Magilvavy D, Mannik M: Hepatic binding of DNA is mediated by a receptor on nonparenchymal cells. Am J Pathol 133:54–60, 1988
29. Jerne NK: Towards a network theory of the immune system. Ann Immunol (Paris) 125:373–389, 1974