Monoclonal Lupus Autoantibody Secretion by Human-Human Hybridomas

SELECTION OF HYBRIDS BY CONVENTIONAL AND NOVEL TECHNIQUES

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A B S T R A C T   Autoantibody-secreting hybridomas were produced by somatic cell fusion of B lymphocytes from a patient with systemic lupus erythematosus with two different human myeloma lines. Selection of hybrids formed from one of these cell lines was performed by using aminopterine-containing culture medium as this cell line was deficient in hypoxanthine-guanine-phosphoribosyl transferase (HGPRT). The second myeloma line was not HGPRT-deficient but instead was treated with diethylpyrocarbonate, which assured death of unfused myeloma cells. This novel technique has wide applicability. Hybridomas were found to secrete antibodies to native DNA and to extractable nuclear antigen. The binding specificities of one IgM anti-DNA antibody was characterized and found to be specific for double-stranded DNA and had particular binding affinity for poly(dG) · poly(dC).

INTRODUCTION

Patients with systemic lupus erythematosus (SLE)[1] produce a wide variety of autoantibodies. The hallmark of this disease is the presence of antibodies to DNA, although many patients have antibodies specific for other nuclear and cytoplasmic antigens (1). Until recently, the study of autoantibodies has been limited to those naturally occurring in patients' sera and therefore has been restricted to an analysis of polyclonal immunoglobulin molecules.

As a first step toward the study of potentially cross-reactive idiotypes common to autoantibodies produced by patients with SLE, we have produced monoclonal autoantibodies by hybridizing B lymphocytes from a patient with this disease with cells from two human myeloma cell lines. Using one of these lines and B lymphocytes from a patient with subacute sclerosing panencephalitis, Croce et al. (2) succeeded in producing anti-measles antibody-secreting hybridomas (4). In simultaneous preliminary reports, our group (3) and Shoenfeld et al. (4) successfully used this line to produce autoantibody-secreting hybridomas by fusing the line with lymphocytes from patients with SLE. More recently, Shoenfeld et al. (5, 6) detailed the binding characteristics of their resultant monoclonal autoantibodies. Others have produced human B lymphocyte hy-

bridomas by using a different myeloma line (7). Both of these myeloma lines are mutant lines; this allows for the selection of hybrids in selective medium. In addition to this method, we also produced hybridomas using a nonmutant myeloma line and a technique of hybrid selection with wide applicability.

Peripheral blood mononuclear cells from patients with SLE include a large number of spontaneous immunoglobulin-secreting cells (8). The number of such cells is directly correlated with disease activity (9). Others have also demonstrated that some of these cells secrete autoantibody (10). Therefore, hybridomas produced by fusion of peripheral blood B lymphocytes from patients with active SLE may be expected to produce autoantibodies representative of the immunoglobulin products of these activated cells. In the present work we screened hybridomas for those secreting antibodies to DNA and to extractable nuclear antigen (ENA). These two autoantibodies have been shown to be among the most prevalent in the sera of patients with severe SLE and were present in the serum of the B lymphocyte donor (1).

METHODS

SLE patient B lymphocytes. A patient with active SLE and high titers of anti-ENA and anti-DNA antibodies donated B lymphocytes for fusion. 500 ml of heparinized blood was obtained, the buffy coat was isolated, and the erythrocytes were reinfused. Mononuclear cells were then separated from the buffy coat by Ficoll-Hypaque (LSM, Litton Bionetics, Kensington, MD) density-gradient centrifugation (11). These cells were then depleted of T lymphocytes by two cycles of rosette formation with sheep erythrocytes. Nonrosetted cells were depleted of monocytes by plastic adherence (12). 40 million B lymphocyte-enriched cells were obtained from 150 million mononuclear cells.

Fusion method: conventional mutant myeloma line. A hypoxanthine-guanine-phosphoribosyl transferase (HGPRT)-deficient myeloma cell line, GM 1500-6TG-AL2, was kindly provided by Dr. Carlo Croce of the Wistar Institute, Philadelphia, PA, and was routinely cultured in RPMI 1640 containing 10% heat-inactivated fetal calf serum (FCS) supplemented with 10^{-4} M 6-thioguanine (2). 20 million B lymphocyte-enriched cells in 2.5 ml of RPMI 1640 were combined with 2.0 ml RPMI 1640 containing 20 million GM 1500-6TG-AL2 cells in log-phase growth. This combined cell population was then centrifuged at 200 g for 10 min. 1 ml of solution containing 500 mg/ml of polyethylene glycol (PEG 4000; Merck AG, Darmstadt, Federal Republic of Germany) and 0.05% vol/vol dimethyl sulfoxide in RPMI 1640 was added dropwise to the cell pellet while the pellet was being gently stirred. The cells were incubated with gentle swirling for 2 min at 37°C in a 15-ml centrifuge tube. Warm RPMI 1640 was then added to the cells; this was followed by centrifugation at 130 g for 5 min. The supernate was decanted and the cells were gently resuspended in 10% FCS containing RPMI 1640. After a second wash, the cell pellet was resuspended at 1 million viable cells/ml in RPMI 1640 containing 20% FCS and supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, nonessential amino acids, 10% NCTC 109 (M. A. Bioproducts, Bethesda, MD), 2.0 mM L-glutamine, 1.0 mM oxaloacetic acid, 0.5 mM sodium pyruvate, and 0.2

U/ml bovine crystaline insulin (Sigma Chemical Co., St. Louis, MO) (13; this medium is called supplemented RPMI). In addition, 10^{-4} M hypoxanthine, 4×10^{-7} M aminopterine, and 1.6×10^{-5} M thymidine (HAT) were added to prevent growth of unfused myeloma cells. Immediately after fusion, >85% of the cells excluded trypan blue (viable), and many multinucleated cells were present. 30 cultures of 1 ml each were established in 12-mm diam wells in 24-well plates (Costar, Cambridge, MA). After 2 d incubation at 37°C in humidified air containing 7% carbon dioxide, 1 ml of the same medium was added to each well. Thereafter, 1 ml of medium was removed and replaced with 1 ml of fresh medium three times per week. After cell growth was established (26 d), cultures were fed with supplemented medium without aminopterin. By this time, control cultures of unfused GM 1500-6TG-AL2 cells were dead (100% trypan blue positive). After 7 d more of culture, hypoxanthine and thymidine were also omitted from the culture medium. Cultures were expanded in 50-ml tissue culture flasks before cloning.

Fusion method: nonmutant myeloma cell line. 8226 myeloma line obtained from the American Type Culture Collection, Silver Spring, MD, was maintained in RPMI 1640 supplemented as above but without 6-thioguanine. As originally described by Wright (14), diethylpyrocarbonate (DEPC, Sigma Chemical Co.) was used to render these cells incapable of growth unless they were successfully rescued by fusion. Tubes containing 8226 myeloma cells with varying dilutions of DEPC were incubated for 30 min in ice water to define the lowest concentration that could prevent cell division but maintain short-term viability. Cells were first suspended in sterile phosphate-buffered saline (PBS), pH 7.4, at 10 million per milliliter. DEPC was diluted in absolute ethanol to 10-fold the desired final treatment concentration. $\frac{1}{10}$ vol of DEPC was then added to $\frac{9}{10}$ vol of cells and mixed. This was performed in a fume hood using a 50-ml centrifuge tube. After treatment the tube was filled with RPMI 1640 containing 10% FCS. Cells were washed three times to remove unhydrolyzed DEPC. In preliminary experiments, cell counts and viability determinations were performed. Cells were cultured for 2 wk after DEPC exposure to ensure adequate treatment. The ability of treated cells to incorporate tritiated thymidine was determined by pulsing a 0.1-ml culture containing 50 thousand cells with 1 μCi of tritated thymidine for 4 h in quadruplicate microtiter plate wells. The results of these preliminary studies indicated that the optimal concentration of DEPC was 1×10^{-4} vol/vol. In practice, myeloma line 8226 was treated with this concentration of DEPC and with concentrations one-half and twice this amount. Unfused treated cells were always incubated as a control for adequate treatment with DEPC. In the fusion described, DEPC treatment was at 2×10^{-4} vol/vol and unfused 8226 cells exposed to this DEPC concentration failed to grow.

Fusion of line 8226 with B lymphocytes was performed identically to the method described above for fusion with GM 1500-6TG-AL2, except HAT was not required. Culture conditions for these hybrid cells were also as described above.

Cloning procedures. Cloning was performed by limiting cell dilution in 96-well microtiter plates (Costar). Cells were suspended immediately before use such that 50 μl of supplemented RPMI 1640 contained 1,000 irradiated (2,500 rad) rat fibroblasts and 0.5, 1, 5, 10, or 20 hybrid cells. This volume was then distributed to each well and incubated at 37°C in 7% carbon dioxide in humidified air. After 2 d and 7 d the wells were fed with additional fresh medium. Macroscopic growth was observed after approximately 3 wk of culture.

Screening procedures: enzyme-linked immunoassay (ELISA). Initial screening for hybridomas producing immunoglobulin

and specific antibodies was performed using a solid-phase micro-ELISA and alkaline phosphatase-conjugated, affinity-purified, heavy chain-specific, goat anti-human immunoglobulin (Sigma Chemical Co.) (15). IgA, IgG, and IgM concentrations were determined by incubating 150 μl of 1:1,000 goat anti-human immunoglobulin (heavy chain class-specific, Sigma Chemical Co.) in bicarbonate buffer, pH 9.6, in each polystyrene plastic microtiter plate well (Immulon Plate, Dynatech Corp., Alexandria, VA) overnight. Wells were washed repetitively with PBS, pH 7.2, containing 0.5% Tween 20. 100 μl of supernate to be tested or of a known amount of human immunoglobulin was added to each well. Human immunoglobulin standards were diluted in tissue culture medium with the same concentration of FCS as the supernates. Plates were incubated for 2 h at room temperature and washed. 100 μl of the appropriate alkaline phosphatase-conjugated reagent (diluted in 1% FCS containing PBS-Tween 20 buffer) was added to each well and the plates were incubated another 2 h at room temperature. After washing, 100 μl of substrate, p-nitrophenol phosphate (Sigma Chemical Co.) diluted in diethanolamine buffer, pH 9.8, was added to each well and incubated for 45 min. The optical density at 405 nm was then determined for each well. After construction of a standard curve, the class-specific immunoglobulin concentration of each supernate was determined. This assay was sensitive to <1 ng/ml.

Antibodies to ENA were detected using a similar ELISA. ENA, prepared according to the method of Sharpe et al. (16) was the kind gift of Dr. Marion Waller (Medical College of Virginia, Richmond, VA). The ENA was diluted to 40 μg/ml in bicarbonate buffer and allowed to adhere to the polystyrene plates as described. Supernates to be tested were assayed by ELISA as in the immunoglobulin assay except that the positive control was a 1:200 dilution of the patient's serum. Results were expressed as the optical density of the supernate tested in an ENA-containing well minus the result using a control well without ENA. This difference was also expressed as a percentage of the positive control. The test was found to be very reproducible provided that the ENA was dissolved in bicarbonate buffer immediately before each use.

The ELISA assay for anti-DNA antibody was performed by using modifications of previously reported methods (17–19). Calf thymus DNA (Sigma Chemical Co.) was first dissolved in 0.1 M Tris–buffered saline (TBS), pH 7.4. 100 μl of poly-L-lysine (PLL) at 50 μg/ml in distilled water (Sigma Chemical Co.) was added to each well of a flexible polyvinyl chloride microtiter plate (Dynatech Corp.). Plates were incubated for 1 h at room temperature and washed with TBS. 50 μl of DNA at 2 μg/ml was added to each well. Control wells were coated with PLL, but 50 μl of TBS without DNA was added. Plates were dried overnight at 37°C, covered, and stored at 4°C. Before use, plates were washed extensively with TBS and 250 μl of 1% bovine gamma globulin (BGG) in TBS was added to each well and incubated at room temperature for 1 h. Plates were washed five times with TBS. 100 μl of supernate or 1:500 diluted positive control serum was placed in each well. Each sample was tested in a control well and a DNA-containing well. No effort was made to remove single-stranded regions from the calf thymus DNA. After a 2-h incubation at room temperature, plates were washed four times with TBS and 100 μl of alkaline phosphatase-conjugated goat anti-human reagent (same as above) diluted in 1% BGG-TBS was added to each well. Plates were incubated overnight at room temperature and washed four times with TBS. 100 μl of substrate was added to each well and the plates were incubated 30 min at room temperature

before determining the optical density at 405 nm for each well. The result for each sample's control well was subtracted from that of the DNA-containing well. Supernates without anti-DNA antibody activity uniformly produced a negative result in this assay although some contained large amounts of immunoglobulin. Anti-DNA-containing patient sera consistently produced a positive result.

Filter assay for DNA-binding activity. Antibodies to double-stranded DNA (ds-DNA) and heat-denatured single-stranded DNA (ss-DNA) were measured by a cellulose ester filter-binding assay. Aliquots of serum, supernate, or purified antibody were incubated with tritium-labeled *Escherichia coli* DNA (Electro-Nucleonics, Bethesda, MD). The source of antibody was used at different concentrations or volumes and mixed with 15 ng of DNA (30,000 dpm/μg) in a volume of 100 μl. The mixture was incubated for 30 min at 37°C and then overnight at 4°C. The antigen-antibody complexes were collected onto cellulose ester filters (Millipore Corp., Bedford, MA). The filters were placed in counting fluid and the radioactivity determined by liquid scintillation. In each assay, known positives and negatives gave the expected results. All data were initially expressed as the percentage of the total radioactive DNA that was bound by antibody and retained on the filter. To allow comparison of results from different experiments, this value was then expressed as a percentage of a known positive used in each experiment.

Specificity of anti-DNA-containing supernate: DNA absorption. ss-DNA was prepared from the calf thymus ds-DNA preparation by heating at 100°C for 10 min followed by rapid cooling in ice water (20). 200 μl of ds-DNA (1.0 mg/ml), ss-DNA (1.0 mg/ml), or TBS was added to 200 μl of TBS-containing monoclonal IgM anti-DNA (clone No. 38) at different concentrations. After overnight incubation (37°C for 1 h followed by 4°C), these mixtures were ultracentrifuged at 150,000 g for 45 min to remove the immunoprecipitate from the fluid phase. The supernates were then tested in the standard anti-DNA ELISA for remaining anti-DNA activity.

PEG precipitation of radiolabeled DNA by monoclonal anti-DNA. Radiolabeled calf thymus tritiated DNA (Electro-Nucleonics), ^{14}C-DNA (New England Nuclear, Boston, MA), or synthetic ^{14}C-DNA of defined nucleotide sequence (P-L Biochemical Co., Milwaukee, WI) were used. Synthetic radiolabeled DNA evaluated included poly(dC)·poly(dG); poly(dC-dG)·poly(dC-dG), poly(dT)·poly(dA), and poly(dT-dA)·poly(dT-dA). In experiments using these synthetic ds-DNA preparations, concentrations were adjusted such that a constant amount of DNA was used for each on a nanogram for nanogram basis. Since nucleotide length was unknown, equimolar amounts could not be calculated. 50 μl of radiolabeled DNA was incubated with 50 μl of monoclonal anti-DNA diluted in 0.01 M phosphate-buffered 0.14 M NaCl (PBS), pH 7.4 together with 50 μl of 1.6% BGG in PBS and 50 μl of 0.2 mg/ml dextran sulfate in PBS. The total volume was 200 μl. Control tubes contained PBS without anti-DNA. After a 2-h incubation at 37°C, 200 μl of 7% (wt/vol) PEG 6000 was added. Tubes were vortexed and incubated overnight at 4°C. After centrifugation, 100-μl samples of the supernate were removed and the radioactivity in each was determined by liquid scintillation. The results were expressed as percentage bound DNA (21).

RESULTS

Hybridomas. Both selection methods described resulted in growth of functional B lymphocyte hybridomas secreting specific antibody and death of unfused myeloma cells. With both methods hybrid growth occurred

by 3 wk after fusion. The results of cell counts performed 42 after fusion are given in Table I. Only one of 30 culture wells resulting from fusion of DEPC-treated 8226 cells exhibited growth, while all 30 wells from the GM 1500-6TG-AL2 fusion contained viable cells. Supernates of these cultures were screened for immunoglobulin 18 d after fusion. All 30 culture wells from the GM 1500-6TG-AL2 fusion were positive for IgG (also produced by the GM 1500-6TG-AL2 line), 10 of 30 were positive for IgA, and 23 of 30 were positive for IgM. At this time several wells from the line 8226 fusion contained viable cells, 15 of 30 were positive for IgG, and 6 of 30 were positive for IgA. Later, only one well from this fusion continued to exhibit stable growth and the supernate of this culture contained IgG. The 8226 myeloma line did not produce immunoglobulin heavy chains but did produce kappa light chains. In addition to screening for immunoglobulin production, supernates from all fusion culture wells were tested for anti-DNA activity. Both the ELISA assay and the DNA-binding nitrocellulose filter assay were used. Results are shown in Table II. 20 supernates were positive for ds-DNA binding in the nitrocellulose filter assay while 13 were positive in the ELISA assay. Some supernates positive in one assay were negative in the other. Patient sera were positive in both assays, indicating potential differences in the behavior of monoclonal anti-DNA antibodies as compared with that of polyclonal anti-DNA antibodies in these two assays. Differences were not explained by greater sensitivity of one assay since for each assay some supernates were positive in one assay and negative in the other (Table III). As discussed later, these results could possibly be explained by low affinity anti-DNA activity.

Anti-ENA activity was present in four supernates. The results of screening for anti-ENA antibodies are given in Table IV. The optical density at 405 nm using the patient's serum, a positive supernate, and one IgG-containing negative supernate are also given in Table IV to demonstrate the specificity of this assay.

Cloned anti-DNA-producing hybridomas. Cells from wells with supernates positive for anti-DNA ac-

tivity were cloned by limiting dilution. Cloning efficiency was ~10%. One cloned anti-DNA-producing hybridoma, clone No. 38, was selected for further study.

This cloned hybridoma was found to have a doubling time of 48 h and supernates routinely had 200–250 μg/ ml of IgM. IgM was the only human serum protein detectable by immunoelectrophoresis of the hybridoma supernate but some IgG, 2–3 ng/ml, was also detected when more sensitive methods were used (see below). IgG is also produced by the parental myeloma line. Cultures of clone No. 38 produced 30–40 μg of IgM per million cells per day. Calculations based on this data indicated that clone No. 38 secreted ~250 thousand molecules of IgM per cell per second.

Evidence of hybridoma formation. Since autoantibody-secreting cell lines have been established by infection of B lymphocytes from SLE patients with Epstein-Barr (E-B) virus, clone No. 38 and the hybrid cells produced in the 8226 fusion were tested for the E-B viral nuclear antigen by Dr. John Sixbey (University of North Carolina, Chapel Hill, NC) and found to be negative.

HLA typing was performed on clone No. 38 cells, GM 1500-6TG-AL2 cells, and the mononuclear cells from the SLE patient. The A11 and B17 antigens were present on the patient's lymphoid cells and the hybrid cells but not on GM 1500-6TG-AL2 cells.

Karyotypes were performed by Malloy Laboratories, Bethesda, MD on clone No. 38 cells and GM 1500-6TG-AL2 cells. The chromosome frequency distribution of 50 cells was determined for each. There was no significant difference between these two cell types with respect to chromosome number.

Lastly, since GM 1500-6TG-AL2 secretes IgG and clone No. 38 secretes predominantly IgM, the fact that clone No. 38 also secretes small amounts of IgG (as noted above) favors the assumption that clone No. 38 is a hybridoma. This kind of evidence was also used by Shoenfeld et al. (6) to prove that their IgM autoantibody-secreting cells were hybrids. To be certain that the 2–3 ng/ml of IgG measured in supernates containing 500-fold more IgM was not due to cross-reactivity of

TABLE I
Growth of Uncloned Hybridomas

| Fusion partner | Growing cultures/total* | Number of cultures with each cell number × 10⁻⁴/culture‡ | | | | | | |
		1–5	5–10	10–15	15–20	20–25	25–30	30+
GM 1500-6TG-AL2	30/30	2	0	2	7	12	6	1
8226	1/30	0	0	0	0	0	0	1

* Observation 25 d after fusion.
‡ Observation 42 d after fusion.

TABLE II
Anti-DNA Antibodies in Uncloned Hybridoma Supernates

ds-DNA binding (nitrocellulose filter)			Anti-ds-DNA ELISA		
Score	% Bound	Number positive/total	Score	% Reference serum	Number positive/total
+++	81–100	8/31	+++	>10	2/30
++	61–80	9/31	++	2–10	6/30
+	41–60	6/31	+	0.5–2	5/30
−	<40	8/31	−	<0.5	17/30

the reagents used in the ELISA assay, we passed the clone No. 38 supernate through an anti-μ immunoabsorbant column and then desorbed the IgM with glycine buffer at pH 2.5. This material gave background readings in the IgG ELISA even with values of 20 μg/ml in the IgM ELISA. The very small amounts of IgG detected in the clone No. 38 supernate was therefore not due to reagent cross-reactivity. It is also below the level of sensitivity of immunoelectrophoresis and therefore was not detected by this method.

Specificity of clone No. 38 anti-DNA. 100 μg of ds-DNA or ss-DNA was added to clone No. 38 IgM anti-DNA diluted with TBS to contain varying amounts of IgM. Immune complexes formed in fluid phase were removed by ultracentrifugation and the remaining unbound anti-DNA activity was measured in the anti-DNA ELISA assay using calf thymus DNA in microtiter plate wells. The results of this experiment are given in Fig. 1. With 5 μg or less of IgM, 60–70% of anti-DNA activity was lost after preincubation with the ds-DNA and not after preincubation with the ss-DNA. With 10 μg of clone No. 38 IgM, anti-DNA activity was no longer absorbed by 100 μg of ds-DNA.

The anti-ds-DNA activity of this antibody was confirmed by immunoprecipitation of tritiated ds-DNA and ^{14}C-ds-DNA in PEG after incubation with clone No. 38 IgM. ^{14}C ss-DNA was not precipitated by this antibody (see Fig. 2, top). Lastly, as illustrated on the bottom of Fig. 2, clone No. 38 IgM was found to react preferentially with the synthetic double-stranded polydeoxynucleotide poly(dC)·poly(dG). There was little reactivity with poly(dC-dG)·poly(dC-dG), poly-(dA)·poly(dT), or poly(dA-dT)·poly(dA-dT). For

TABLE III
Correlation of DNA Bindings vs. ELISA

DNA binding score	ELISA score	Number of supernates
++ or +++	++ or +++	4
++ or +++	−	9
−	++ or +++	3
−	−	3

purposes of comparison, the same clone No. 38 IgM preparation was used in all experiments using the PEG precipitation method.

DISCUSSION

We succeeded in producing human-human B cell hybridomas that secrete immunoglobulin and autoantibodies by fusing the B lymphocytes from a patient with SLE with two different human myeloma lines. While one of these lines was HGPRT-deficient, allowing for selection of hybrids with aminopterin, the other myeloma line was not a mutant. Instead, hybrids were selected by treatment of the myeloma fusion partner with DEPC. Since unstimulated B lymphocytes will not grow in culture, only myeloma cells that were rescued by fusion with a B lymphocyte were able to grow. This method of selection has wide applicability. In the fusions reported here, however, many fewer hybridomas resulted from fusion with DEPC-treated myeloma line 8226 than from hybridization with the HGPRT-deficient line GM 1500-6TG-AL2. It is possible that use of less toxic concentrations of DEPC would result in better rescue efficiency.

The ability to produce human hybridomas that secrete autoantibodies is an important step toward understanding the binding characteristics of these antibodies. It also allows for the sampling of the immunoglobulin product of individual B lymphocytes that are present in the blood of a patient with SLE (or another autoimmune disease) and that are activated for immunoglobulin secretion at that point in time. Lastly, it will facilitate the study of idiotypes on human autoantibodies, a study which may enhance our understanding of B lymphocyte abnormalities in autoimmune diseases such as SLE.

Characterization of the binding specificities of one monoclonal anti-DNA, clone No. 38 IgM, serves as an example. We found this antibody to be reactive with ds-DNA. Anti-DNA activity was absorbed by ds-DNA and not by ss-DNA. This antibody precipitated radiolabeled ds-DNA and not ss-DNA in the presence of PEG. This could be the result of either antibody spec-

TABLE IV
Anti-ENA Activity of Supernates

	IgG			IgA			IgM		
	−ENA	+ENA	Average difference	−ENA	+ENA	Average difference	−ENA	+ENA	Average difference
Patient serum 1:200	−0.020 −0.019	0.907 0.991	0.968	0.002 −0.007	0.043 0.033	0.042	−0.015 −0.017	0.097 0.104	0.116
Positive hybridoma supernate	−0.006 −0.008	0.307 0.305	0.313	0.020 0.002	0.036 0.015	0.014	0.004 −0.013	0.013 0.004	0.013
Negative hybridoma supernate*	0.002 −0.019	0.003 0.004	0.012	0.001 −0.004	0.005 0.001	0.004	−0.016 −0.014	0.019 0.015	0.032

Anti ENA in uncloned supernates

Positive in anti-ENA ELISA‡

	(IgG, IgA, and IgM)
GM 1500-6TG-AL2 fusion	3/30
8226 fusion	1/1

* IgG > 100 μg/ml and IgM = 69.3 μg/ml.
‡ Culture supernates containing immunoglobulin binding to ENA/number of cultures.

ificity for ds-DNA or preferential affinity for ds-DNA over ss-DNA determinants. However the affinity of binding was probably low since the immune complex could not be precipitated in a Farr assay using 50% saturated ammonium sulfate (data not shown). This observation has previously been shown to be due to the high salt concentration that dissociates low affinity anti–DNA-DNA complexes (21).

Lastly, this antibody was found to be reactive with poly(dG)·poly(dC), a synthetic ds-DNA with polydeoxyguanylic acid on one chain and polydeoxycytidylic acid on the other. It was not reactive with three other synthetic ds-DNA. Others have reported that anti-DNA from a patient with SLE reacted specifically with a double-stranded polyribonucleotide composed of the same bases (22). Patients with SLE may preferentially produce antibody reactive to a helical determinant present only in areas of DNA that are rich in guanosine and cytidine. This possibility is supported by the finding
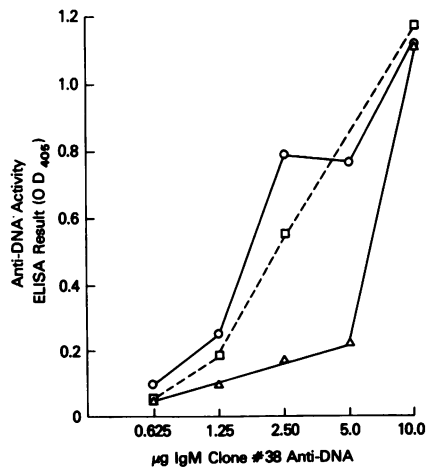

FIGURE 1 Competitive inhibition of the anti-DNA activity of clone No. 38 IgM by preincubation with 100 μg of ds-DNA (△) and not by preincubation with 100 μg of ss-DNA (○) or buffer (□).
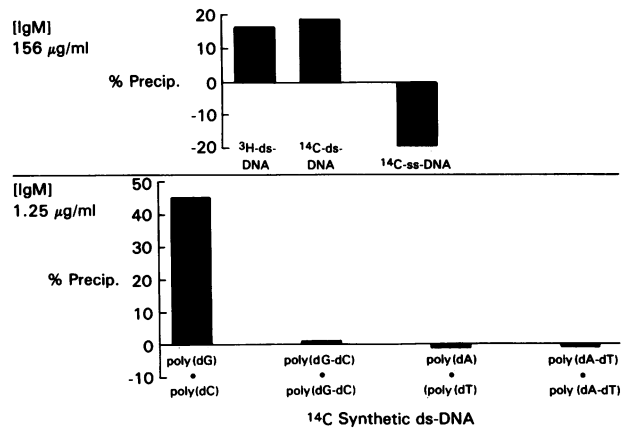

FIGURE 2 (Top) Immunoprecipitation of radiolabeled ds-DNA and ss-DNA preparations by clone No. 38 IgM in the presence of 3.5% (wt/vol) PEG 6000. (Bottom) Immunoprecipitation of radiolabeled synthetic ds-DNA polymers by clone No. 38 IgM in the presence of 3.5% PEG 6000.

of Sano and Morimoto (23) that DNA isolated from immune complexes present in the serum of patients with SLE is rich in guanine-cytosine. Fish and Ziff (18) and others (20) found that anti-DNA containing whole sera from patients with SLE reacted with poly(dA-dT) · poly(dA-dT), although this synthetic ds-DNA does not display all antigenic determinants present on native DNA. However, the helical structure of this synthetic polynucleotide most closely resembles native DNA (24).

More recently, Shoenfeld et al. (6) selected 30 human monoclonal anti-DNA antibodies reactive with ss-DNA. Some of these monoclonal antibodies also reacted with ds-DNA as well as synthetic polynucleotides. 10 of 30 reacted with the phospholipid cardiolipin. Others reacted with the "Z" form of DNA. The binding characteristics of monoclonal antibodies reactive with only ds-DNA were not studied.

A number of studies over the past 20 yr have indicated the exquisite specificity possible for antibodies to DNA. Experimental immunization of animals has led to antibodies capable of distinguishing thymidylic acid from thymidine and other nucleotides (25, 26). Also, sera of patients with SLE have been found to contain, in addition to antibodies to native and denatured DNA, antibodies specific for nucleotides, nucleosides, and bases (27–29). Finally, both induced and naturally occurring antibodies are capable of distinguishing polymers (30). Thus, antibodies reactive with poly(dA-dT) did not react with poly(dG) · poly(dC). Antibodies induced in rabbits to poly(rA) · poly(rU) did not react with poly(rG) · poly(rC) (31). More recent studies have emphasized that determinants specific for the DNA double helix structure may be recognized by specific antibodies (6, 32). In view of these studies, it is not surprising that we found an antibody with specificity for poly(dG) · poly(dC).

Taken together, these observations may also explain why many monoclonal anti-DNA in this study behave differently in different assays. For example, the highly specific antigenic determinant on poly(dG) · poly(dC) ds-DNA recognized by clone No. 38 IgM must be present in only a few areas of native DNA polymers. Assays based on the ability of anti-DNA to cross-link DNA may not detect a monoclonal antibody with very restricted DNA reactivities. Such an antibody would bind to native DNA with low affinity.

As noted above, the monoclonal antibodies obtained by hybridizing B lymphocytes from a patient with SLE represent a sample of the potential immunoglobulin products from those B lymphocytes that were activated in vivo and destined to secrete immunoglobulin. In the present work, the large number of immunoglobulin and autoantibody-secreting hybridomas obtained favors the presence of a high percentage of activated B lymphocytes with these same specificities in vivo. The

presence of large numbers of spontaneous immunoglobulin-secreting mononuclear cells in the blood of SLE patients with active disease supports this conclusion (8, 9).

Recently, Solomon et al. (33) produced murine monoclonal anti-idiotypic antibody directed against anti-DNA from one patient with SLE and found that one such monoclonal anti-idiotypic antibody also reacted with anti-DNA containing sera from eight of nine patients with SLE. In murine lupus models as well, anti-DNA immunoglobulins have been shown to share common idiotypes (34, 35). Additionally, there is accumulating evidence for underlying B lymphocyte defects in lupus mice (36). SLE patients may also share common idiotypes on their other autoantibodies. We theorize that semiautonomous B lymphocytes, which are relatively unresponsive to normal immunoregulatory signals, are responsible for autoantibody synthesis. These cells may be members of a clone descended from a cell affected by some event during B lymphocyte ontogeny that rendered that cell and its subsequent progeny semiautonomous. We plan to utilize these monoclonal autoantibodies to produce heterologous anti-idiotypic antibody. This could then be used to search for common idiotypes in the sera of SLE patients and isolate B lymphocytes bearing this idiotype. Such a finding would support this theory, have therapeutic implications, allow further study of autoantibody-producing cells, and possibly aid in early identification of potential SLE patients.

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