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Research Article

Antigen-ginding lymphocytes capable of binding native DNA (DNA-ABC) were identified in the peripheral blood of normal controls and patients with systemic lupus erythematosus (SLE) by autoradiography with 125I-nDNA. 12 patients with active SLE had 404 +/- 273 (mean +/- SD) DNA-ABC/105 lymphocytes, while 7 inactive SLE patients and 13 normals had 120 +/- 48 and 48 +/- 36, respectively. All three groups were significantly different from one another (p less than 0.01). No significant correlation was detected between the quantity of anti-native DNA (nDNA) antibody and number of DNA-ABC; however, most patients with large amounts of anti-nDNA antibody had both active disease and large numbers of DNA-ABC. Numbers of DNA-ABC and lymphocytes with surface immunoglobulin (Ig) did not change significantly after an 18-h incubation at 37degreeC. After depletion of B-lymphocytes by passage over bead columns coated with a complex of IgG and anti-IgG, the great majority of DNA-ABC were removed in both normal subjects and SLE patients. Labeling lymphocytes sequentially with 125I-nDNA, followed by an indirect fluorescence technique for identification of surface Ig, indicated that the great mahority of radiolabeled cells had surface Ig by fluorescence microscopy in four normals (average 93%) and five patients with active SLF (average 82%). The predominance of nDNA-sensitive B-lymphocytes in the peripheral blood of both normals and SLE patients is consistent with the concept […]

Identification of DNA-Binding Lymphocytes in Patients with Systemic Lupus Erythematosus

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ABSTRACT Antigen-binding lymphocytes capable of binding native DNA (DNA-ABC) were identified in the peripheral blood of normal controls and patients with systemic lupus erythematosus (SLE) by autoradiography with ^{125}I-nDNA. 12 patients with active SLE had 404±273 (mean±SD) DNA-ABC/10^5 lymphocytes, while 7 inactive SLE patients and 13 normals had 120±48 and 48±36, respectively. All three groups were significantly different from one another ($P < 0.01$). No significant correlation was detected between the quantity of anti-native DNA (nDNA) antibody and number of DNA-ABC; however, most patients with large amounts of anti-nDNA antibody had both active disease and large numbers of DNA-ABC. Numbers of DNA-ABC and lymphocytes with surface immunoglobulin (Ig) did not change significantly after an 18-h incubation at 37°C. After depletion of B-lymphocytes by passage over bead columns coated with a complex of IgG and anti-IgG, the great majority of DNA-ABC were removed in both normal subjects and SLE patients. Labeling lymphocytes sequentially with ^{125}I-nDNA, followed by an indirect fluorescence technique for identification of surface Ig, indicated that the great majority of radiolabeled cells had surface Ig by fluorescence microscopy in four normals (average 93%) and five patients with active SLE (average 82%).

The predominance of nDNA-sensitive B-lymphocytes in the peripheral blood of both normals and SLE patients is consistent with the concept that the induction of the anti-nDNA antibody response is due to the stimulation of preexisting nDNA-specific B lymphocytes by mechanisms other than those necessarily involving participation of nDNA-specific T lymphocytes.

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INTRODUCTION

Systemic lupus erythematosus (SLE)[1] was first recognized as a disease accompanied by widespread connective tissue damage to segments of the vascular system and various serous surfaces (1). The finding that hematoxylin bodies were altered DNA (2) and later that LE cells contained ingested phagocytized nuclear material (3) indicated possible participation of nuclear substances in the genesis of the disease. Subsequently, the application of a variety of immunologic methods established that sera from patients with SLE contain a wide spectrum of antibodies to nuclear components, including nucleoprotein, extractable nuclear antigens, histones, and DNA itself (4-10). Antibodies to DNA appear to play a central role in some of the immune lesions of SLE, since several lines of evidence clearly link them to renal injury. Analyses of immunoglobulin eluted from isolated SLE glomeruli have indicated high relative concentrations of anti-DNA antibody (11, 12). A close temporal relationship between the appearance of DNA in serum and subsequent anti-DNA antibody associated with exacerbations of lupus nephritis and lowering of serum complement components (13-17) emphasizes the pathogenetic significance of such antibodies.

Other antibodies to nuclear components, as well as antibodies to various types of RNA (18), may also be of importance in the pathogenesis of SLE, yet native DNA (nDNA) and anti-nDNA antibodies have clearly been defined as an important potential source of immune complexes in this disorder.

[1] *Abbreviations used in this paper:* ABC, antigen-binding cell; DNA-ABC, antigen-binding lymphocyte for native DNA; DNP_{10}-BSA, dinitrophenylated bovine serum albumin; FCS, fetal calf serum; MEM, minimal essential medium; nDNA, native or double-stranded DNA; PBS, phosphate-buffered saline, pH 7.2; SLE, systemic lupus erythematosus; Tg, thyroglobulin.

The present study was designed to examine the types of nDNA-antigen-binding cells (DNA-ABC) present in the peripheral blood of normal subjects and lupus patients. Current data support the concept that the interaction of an antigen with a specific, predetermined lymphocyte surface receptor initiates a chain of events leading to detectable antibody formation. Studies in the mouse with heterologous isotopically labeled antigens have shown that the antigen-sensitive lymphocyte is included among the ABC identified with autoradiographic techniques (19). Previous studies identified the presence of ABC for human thyroglobulin in normal subjects (20), as well as in chronic thyroiditis (21). Since the former study suggested that the ABC for thyroglobulin (Tg) were primarily B lymphocytes, it is possible that self-tolerance to Tg may be due to the absence of helper T lymphocytes (22). It was unclear from these Tg studies whether the anti-Tg response was related to the appearance of specific T helper cells in patients with chronic thyroiditis. The current study was directed to the question whether ABC capable of binding the potential autoantigen nDNA in normal subjects and lupus patients were B and/or T lymphocytes. The evidence presented here seems to indicate that loss of tolerance to nDNA in patients with SLE may be accompanied by proliferation principally of antigen-sensitive B lymphocytes.

METHODS

Subjects. Blood samples were obtained from 12 patients with active SLE, 7 patients with inactive SLE, and 13 normal subjects. The diagnosis of SLE was based on American Rheumatism Association criteria (23). Patients with active SLE generally showed decreased serum complement (C3) and signs of activity such as synovitis, leukopenia, rash, nephritis, or serositis. Patients with inactive SLE showed normal complement and no evidence of clinical disease activity. The SLE patients generally were on a therapeutic regime that included corticosteroids (10–30 mg prednisone) and azathioprine (50–150 mg). Several untreated patients were studied, including the patients L. P. and V. C., referred to in Tables I and IV.

Lymphocyte preparation. Peripheral venous blood was collected in heparinized glass tubes. Purified lymphocyte suspensions were then prepared with Ficoll-Hypaque gradient (24) followed by three washings in PBS. The lymphocytes were incubated on 100 × 15-mm plastic Petri dishes for 45 min at 37°C in minimal essential medium (MEM) containing 10% fetal calf serum (FCS) to deplete phagocytic mononuclear cells. The nonadherent cells were aspirated from the Petri dishes and again washed three times in PBS before use in the procedures described below.

Preparation of antigens. Native calf thymus DNA (Sigma Chemical Co., St. Louis, Mo.; Type I, sodium salt, high-polymerized, D 1501) was deproteinized by three successive phenol extractions by the method of Harbeck et al. (25).The DNA was then sonicated with a Sorvall sonifer (Branson Sonic Power Co., Danbury, Conn., Model W 185 D, position 5 for 1 min). The sonicated, deproteinized DNA was finally passed through a Sepharose 4B column in 0.05 M Tris buffer, pH 8.0. Finally, the sonicated, deproteinized DNA was passed through a cellulose nitrate filter (type HAWP, Millipore Corp., Bedford, Mass.) to remove any contaminating, single-stranded DNA bound to the filter (26), dialyzed against PBS, and stored at concentrations of 0.5 mg/ml at −20°C. The resulting DNA produced a single, narrow peak on analytical ultracentrifugation with a sedimentation coefficient of approximately 22S. There was no detectable protein in this DNA preparation by the Folin method (27).

Staphylococcal protein A and human kappa light chain were used as controls in the blocking experiments. Protein A was prepared according to the method of Sjöquist et al. (28); human kappa light chains were prepared from lyophilized urine by zone electrophoresis on starch block. Both protein A and the purified light chains were dialyzed against PBS before use.

Labeling of DNA with ^{125}I. The deproteinized, sonicated nDNA was labeled with ^{125}I as previously described (29). The incubation volume was 0.15 ml and contained 20 μg nDNA, 0.1 M acetate buffer, pH 5.0, 2.5×10^{-4} M KI, 3 mCi radioactive iodine (carrier-free ^{125}I, sodium salt in 0.1 M NaOH, Amersham/Searle Corp., Arlington Heights, Ill.), and 1.5×10^{-3} M thallium trichloride (K and K Laboratories, Inc., Plainview, N. Y.). After the incubation for 1 h at 60°C, DNA was dialyzed against phosphate-buffered saline (PBS) (500:1, dialysate volume: dialysis tube volume) for 48 h with four changes of dialysis fluid. The ^{125}I-DNA was passed through a Millipore filter again, as described above, to remove any DNA denatured by the iodination procedures. The specific activity of the ^{125}I-nDNA was 4–7 μCi/μg.

Labeling of lymphocytes with ^{125}I-nDNA. Conditions for lymphocyte labeling with radioiodinated antigens have been described (30). 3×10^6 lymphocytes prepared as above were incubated in 0.5 ml of PBS, 0.1% sodium azide, with 800–1,200 ng of ^{125}I-nDNA for 30 min at 0°C. The cells were then washed alternately with PBS or FCS gradients four times. In the case of FCS gradients, the cells were introduced on the surface of a 3.0 ml gradient of FCS and PBS. The lower third of the gradient consisted of 100% FCS, the middle third 75% FCS, and the top third 50% FCS and 50% PBS. In blocking experiments with unlabeled antigens, the lymphocytes were incubated for 1 h at 0°C with either 340 μg nDNA, 100 μg protein A, or 1,700 μg human kappa light chain in a volume of 0.5 ml and washed through one FCS gradient before incubation under standard conditions with ^{125}I-nDNA. The specificity of binding of native DNA was also studied by preincubating the lymphocytes as described above with 500 μg dinitophenylated bovine serum albumin (DNP_{10}-BSA), 340 μg denatured DNA, as well as unlabeled nDNA itself. DNP_{10}-BSA was prepared by the method of Davie and Paul (31), as previously described. Denatured DNA was prepared by heating purified, sonicated nDNA to 100°C for 15 min in boiling water and then immediately cooling it in ice water. Finally, binding specificity was additionally checked with a DNase-treated preparation of ^{125}I-nDNA. The DNase (Type I deoxyribonuclease) was obtained from Sigma Chemical Co., and used as described by Harbeck et al. (25).

Autoradiographic detection of antigen-binding lymphocytes. Cell suspensions for autoradiography were spread upon gelatin-coated slides. The slides were dipped in NBT-2 liquid emulsion (Eastman Kodak Co., Rochester, N. Y.). The techniques for the fixation of cells, exposure of slides, and the development of slides have been described (30). Slides were exposed for 12–18 days. The cells were stained

with Giemsa and at least $5–10 \times 10^5$ cells were counted on each slide. A positive cell was identified by the following characteristics: (*a*) it had to be morphologically a small lymphocyte (less than 8 μm in diameter); (*b*) it had to have an intact cytoplasmic membrane with no other obvious damage; and (*c*) it could not be in contact with debris or another cell. All cells that had enough grains to obscure the underlying morphology were treated with a solution of 1% potassium ferricyanide to dissolve the grains (2.35 mg potassium ferricyanide and 30 mg sodium thiosulfate/1.0 ml). This technique was important, since debris and monocytes were occasionally labeled that would otherwise have been indistinguishable from positive lymphocytes. The background grain count was generally in the range of 0–6 grains. A positive cell was identified by a minimum of 20 grains on its surface or within one-half cell diameter from its surface.

Anti-human Ig column and production of T lymphocyte suspensions. Double-layer (anti-human Ig-Ig) columns were prepared by a minor modification of the method of Wigzell et al. (32). Degalan V-26 plastic beads (Degussa Wolfgang AG, Hanau am Main, Germany) were coated with human IgG (Cohn II fraction) and then equilibrated in Pasteur pipettes with a large excess of rabbit anti-human Ig. Generally only 0–3% of cells in the effluent from such a column were lymphocytes with detectable amounts of surface Ig.

Identification of lymphocyte surface Ig. Lymphocyte surface Ig was identified by an indirect fluorescent technique. Generally $2–3 \times 10^6$ lymphocytes were incubated at 0°C for 30 min with a 1:4 dilution in PBS of a high titer rabbit anti-polyvalent human Ig antiserum. This was followed by three washes with PBS and another 30-min incubation at 0°C with 0.1 ml of fluoresceinated goat anti-rabbit IgG (IgG fraction, 2–3 mg/ml, Meloy Laboratories Inc., Springfield, Va.). The cells were again washed three times after the second incubation, and at least 200 cells were counted under the fluorescent microscope.

Analysis of ABC by autoradiography and/or immunofluorescence. Lymphocyte suspensions were labeled successively with ^{125}I-nDNA and fluoresceinated goat anti-rabbit IgG by the indirect sandwich technique. The first step, involving the incubation with ^{125}I-nDNA, was done as described above. The cells were washed three times with FCS gradients before incubation with rabbit anti-polyvalent human Ig. The indirect sandwich technique was performed as above, except that the third wash after the incubation with fluoresceinated goat anti-rabbit IgG was done through a FCS gradient before fixation on gel slides with ethanol for 5 min. The slides were then dipped in nuclear emulsion as described above and developed 4–10 days later.

Microscopy. The preparations were examined with a Zeiss Photomicroscope equipped with a vertical Illuminator III RS and a mercury light source (Carl Zeiss, Inc., New York). The following filter combination was used for fluorochrome visualization: BG12 exciter filter, Fl 500 chromatic beam-splitter, and barrier filter 50. Experiments evaluating the percentage of labeled lymphocytes in cell suspensions were performed by counting fluorescent cells in the ultraviolet field, then switching to light-field illumination to count the total cell numbers. When fixed cells were examined under bright-field for autoradiographic labeling as well as under ultraviolet for fluorochrome labeling, the slide was covered with several drops of a mixture of glycerine and 10% Tris buffer, pH 9.5 (9:1 volume ratio), and a cover slip. When only bright-field microscopy was performed, the fixed preparations were examined without a cover slip with a high-power objective (40×), so that the grains could be dissolved when necessary. When combined bright-field-ultraviolet microscopy was done on fixed preparations, the only radiolabeled cells selected for fluorochrome examination were those with grains that did not obscure the morphology of the cell, since dissolution of grains was impossible when the cover slip was present.

Measurement of anti-nDNA antibody. This was done with [^{14}C]nDNA in a primary binding technique by the ammonium sulfate (Farr) method (33). [^{14}C]nDNA was obtained from Amersham/Searle Corp. (CFB 170). The DNA-binding capacity was expressed as percentage of total [^{14}C]nDNA bound by a 1:10 dilution of test serum (normal serum bound, 5.1±1.5%, mean±1 SD).

RESULTS

Numbers of DNA-ABC in SLE patients and normal people. A comparison was made between the numbers of DNA-ABC in normal people and patients with inactive or active SLE (Fig. 1). 12 patients with active SLE had 416±270 (mean±SD) DNA-ABC/10^5 lymphocytes, while 7 inactive SLE patients and 12 normals had 120±48 and 49±40, respectively. No clear correlation between DNA-ABC and any distinct clinical profile was detected; however, most patients with active SLE also showed lupus nephritis. The three groups were all significantly different from one another ($P < 0.01$).

The relationship between the number of DNA-ABC and the quantity of anti-nDNA antibody. No significant correlation was detected between the quantity of anti-nDNA antibody and the total number of lymphocytes that bound ^{125}I-nDNA (Fig. 2). However, the great ma-

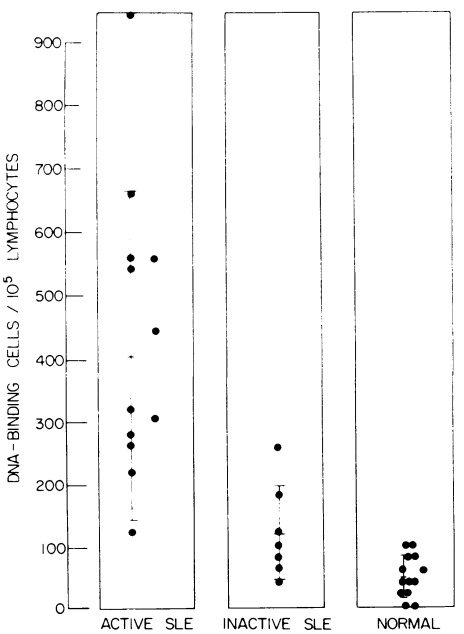

FIGURE 1 Numbers of DNA-ABC in SLE patients and normal people. The mean±SD are indicated.

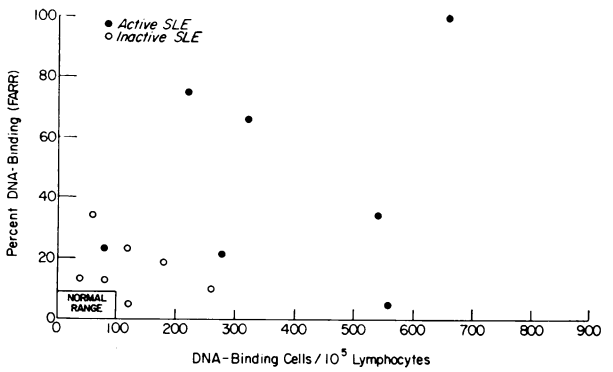

FIGURE 2 The relationship between the number of DNA-ABC and the quantity of anti-nDNA antibody. The anti-nDNA titer is expressed as the percentage of the total labeled nDNA precipitated by a 1 : 10 dilution of test serum. (Normal serum binding is 5.1±1.5%, mean±1 SD).

jority of patients with DNA-binding activity over 30% had both active disease and large numbers of DNA-ABC. Several interesting discrepancies between the amount of anti-nDNA antibody and the number of DNA-ABC were noted such as in an active SLE patient with 560 DNA-ABC and no anti-nDNA antibody and in one inactive SLE patient with a normal number of DNA-ABC but a high titer of anti-nDNA antibody (34%).

Tests for specificity of ^{125}I-nDNA binding. The specificity of the surface labeling of lymphocytes with radiolabeled nDNA was investigated by preincubating the lymphocytes with excess unlabeled nDNA, human kappa light chain, staphylococcal protein A, DNP_{10}-BSA, and denatured DNA (Table I). 100% inhibition of ^{125}I-nDNA binding was observed in active SLE patients and in normals only with nDNA, and not with controls such as protein A or human kappa light chain. Of interest was that only minor inhibition of binding (9 and 12%) was recorded with DNP_{10}-BSA. A small degree of inhibition (25-28%) was recorded with denatured DNA (Table I). Finally, prior DNase treatment of ^{125}I-nDNA completely abolished DNA binding in all instances.

The effect of prolonged incubation upon the numbers of DNA-ABC. To investigate the question of whether anti-lymphocyte antibody, anti-nDNA antibody, or nDNA-anti-nDNA complexes were present on the surface of lymphocytes, the numbers of radiolabeled lym-

TABLE I

Blocking of the Formation of DNA-ABC by Pretreatment with Unlabeled Materials

Human subject	Pretreatment	Labeled compound	Radiolabeled cells	Inhibition
			cells/10⁵ lymphocytes	%
D. F. (active SLE)	PBS	^{125}I-nDNA	400	
	DNA (340 μ)	^{125}I-nDNA	0	100
	Protein A (100 μg)	^{125}I-nDNA	320	20
	Human kappa light chain (1,700 μg)	^{125}I-nDNA	380	5
A. R. (normal)	PBS	^{125}I-nDNA	100	
	DNA (340 μg)	^{125}I-nDNA	0	100
L. P. (active SLE)	PBS	^{125}I-nDNA	800	
	Protein A (100 μg)	^{125}I-nDNA	780	2.5
	Human kappa light chain (1,700 μg)	^{125}I-nDNA	900	0
V. C. (active SLE)	PBS	^{125}I-nDNA	550	
	nDNA (340 μg)	^{125}I-nDNA	0	100
	Denatured DNA (340 μg)	^{125}I-nDNA	400	28
	DNP-BSA (500 μg)	^{125}I-nDNA	500	9
	PBS	DNase-treated ^{125}I-nDNA	0	100
J. D. (normal)	PBS	^{125}I-nDNA	80	
	nDNA (340 μg)	^{125}I-nDNA	0	100
	Denatured DNA (340 μg)	^{125}I-nDNA	60	25
	DNP-BSA (500 μg)	^{125}I-nDNA	70	12
	PBS	DNase-treated ^{125}I-nDNA	0	100

3×10^6 cells were preincubated for 30 min at 2°C with the indicated quantities of materials in a volume of 0.1 cm³. The cells were subsequently washed and incubated with ^{125}I-nDNA (1.000 μg) as described under Methods.

TABLE II

The Effect of Prolonged Incubation upon the Number of DNA-ABC and Lymphocytes with Detectable Surface Ig

Exp.	Subject	Number of radiolabeled lymphocytes‖ Before incubation*	After incubation‡
1	D. R. (normal)	40	60
	S. Z. (inactive SLE)	220	220
2	R. R. (normal)	80	100
	S. Z. (inactive SLE)	120	180
4	V. P. (active SLE)	240	260
		Percentage of cells with surface Ig§	
1	S. A. (active SLE)	21	23
2	S. Z. (inactive SLE)	16	19
3	S. O. (active SLE)	19	26
	C. O. (active SLE)	23	18
	P. C. (normal)	30	27

* Lymphocytes were prepared on Ficoll gradients and incubated in plastic Petri dishes for 45 min at 37°C. The nonadherent, washed cells were then fixed on slides after labeling with fluoresceinated antiserum or ^{125}I-nDNA.

‡ These cells were further incubated for 18–20 h at 37°C in MEM containing 10% FCS before surface labeling with either ^{125}I-nDNA or fluoresceinated antiserum. Essentially no loss of viability was observed during this incubation.

§ 200 cells on a fixed preparation were counted. No significant difference was observed between the percentage of cells before and after prolonged incubation ($P > 0.8$).

‖ At least 5,000 cells were counted. No significant difference was observed between the number of labeled cells before and after incubation ($P > 0.10$).

phocytes and lymphocytes with surface immunoglobulin detectable by fluorescent antibody were measured before and after an 18-h incubation at 37°C. There was no significant difference between the numbers of DNA-ABC (Table II, top) ($P > 0.8$) and lymphocytes with surface Ig (Table II, bottom) ($P > 0.10$) before and after incubation in active or inactive SLE patients and normals.

The identification of DNA-ABC as either B or T lymphocytes. The first approach to the question of whether DNA-ABC were B- or T-cells was to compare the number of DNA-ABC before and after depletion of B lymphocytes by passage over Degalan bead columns coated with human IgG and anti-Ig (Table III). The great majority of cells capable of binding labeled nDNA were removed by this procedure in two normals (100% and 75% depletion) and one active SLE patient (90%).

The second approach was to label lymphocytes sequentially with ^{125}I-nDNA and an indirect fluorescent technique for surface immunoglobulin (Table IV). Using such a technique, we could tell by visible, bright-field microscopy whether a cell bound ^{125}I-nDNA and whether the same cell had surface immunoglobulin. An average of 92% of DNA-ABC in four normals and 82% of DNA-ABC in five patients with active SLE showed concomitant surface Ig. One active SLE patient had only 50% of radiolabeled cells with surface immunoglobulin.

DISCUSSION

One of the most convincing associations between autoantibody and tissue lesions is exemplified by the relationship of anti-nDNA antibody and the glomerulonephritis of SLE (13-17). To cast light on the events initiating such an aberrant B-cell response, we presumably have to focus on the nDNA-sensitive lymphocyte. Identification of this functional subpopulation by morphologic criteria (nDNA-binding lymphocyte) is based on the work done by Ada and Byrt in the mouse (19). The presence of DNA-ABC in normal subjects, demonstrated in the current study, is contrary to any simplistic theory of self-tolerance that presupposes the absence of autoantigen-sensitive lymphocytes in normal individuals. In other words, the activation of the anti-nDNA response involves the triggering of preexisting specific B lymphocytes and not merely the emergence *de novo* of nDNA-sensitive B lymphocytes. The present data also showed increased numbers of DNA-ABC in patients with active or inactive SLE. Previous studies with human thyroglobulin have shown parallel changes in ABC for human

TABLE III

The Decrease in the Number of DNA-ABC after Column Depletion of Lymphocytes with a Large Amount of Surface Ig (B Lymphocytes)

Subject	Number of DNA-ABC			B Lymphocytes*	
	Before column	After column	Decrease	Before column	After column
	labeled lymphocytes/10^5 lymphocytes		%	%	%
M. K. (normal)	80	0	100	28	1
D. R. (normal)	80	20	75	25	0
S. A. (active SLE)	200	20	90	32	1

* B lymphocytes were identified by their large amount of surface Ig by ultraviolet microscopy. The depletion of B lymphocytes was effected by their passage through a double-layer bead column that possessed free anti-Ig combining sites.

TABLE IV

A Study of the Percentage of Cells that Simultaneously Have Surface Ig and Bind ^{125}I-nDNA

Subject	DNA-ABC*	Cells with surface Ig‡	^{125}I-nDNA-binding cells also having surface Ig§
		%	%
J. D. (normal)	60	18	100
F. P. (normal)	80	31	80
M. P. (normal)	80	36	90
M. F. (normal)	100	26	100
D. F. (active SLE)	550	36	80
L. P. (active SLE)	680	17	50
S. A. (active SLE)	560	20	100
V. C. (active SLE)	440	18	90
L. B. (active SLE)	300	15	90

* At least 5,000 cells were counted. Results are expressed as number of radiolabeled cells/10^5 lymphocytes.

‡ Fixed cells were examined for fluorescence by the sandwich technique described in Methods.

§ At least 10 radiolabeled cells were examined for the presence of fluorescence by the double-label technique described under Methods.

thyroglobulin in normals (20) and patients with chronic thyroiditis (21). The increase in DNA-ABC with disease activity was not unexpected since studies in mice have clearly shown that immunization causes an increase in the number of ABC for that antigen (34).

The DNA-ABC were primarily B lymphocytes in both normal individuals and SLE patients, whether the B lymphocytes were identified by column depletion or the double-labeling technique. This would suggest that the SLE patients did not differ from normals simply because of the presence of a larger number of antigen-sensitive T lymphocytes that could enable a cooperative B-T lymphocyte anti-nDNA response to occur. One might argue that the antigen-binding techniques preferentially identify B lymphocytes, and any T lymphocytes that are DNA-ABC would evade detection. However, mouse T lymphocytes that are ABC (35) and ABC in the human thymus (36) have been identified by the autoradiographic method used in this paper. Our results provide a possible explanation for some forms of tolerance to nDNA and the induction of the anti-nDNA response in SLE. The presence of DNA-ABC in normal individuals presumably excludes the simple explanation that tolerance to nDNA is due to a lack of immunocompetent cells able to react with nDNA. If we assume that the formation of antibodies against nDNA requires the participation of two types of lymphocytes, it is possible that the absence of either B or T lymphocytes could result in self-tolerance. Since the DNA-ABC in normal people were primarily B lymphocytes, the observations suggest that T lymphocytes able to react with such B-cells are relatively scarce or absent. However, the very presence of DNA-ABC in normal subjects is of considerable interest and should temper a differentiation between normals and patients with SLE only on the basis of presence or absence of such cells. ABC detection is a subject of some controversy and appears to vary according to antigen used and methodology (37). Despite these technical problems, the inhibition data as shown in Table I support apparent primary specificity of ABC for nDNA in both normal subjects and patients with SLE. The initiation of the anti-nDNA response could presumably have arisen by one of three mechanisms: (*a*) the emergence of a population of helper T lymphocytes, (*b*) the disappearance of a population of suppressor T lymphocytes, or (*c*) another mechanism that bypasses the requirement for a helper T lymphocyte. If one assumes that specific helper or suppressor T cells could be identified as ABC by autoradiography, the first two possibilities seem less likely, since there was no apparent alteration in the percentage of lymphocytes that were both B lymphocytes and DNA-ABC in normal people versus patients with active SLE. This leaves the T cell "bypass" mechanism as another possibility for the initiation of the anti-nDNA response. The need for a nDNA-sensitive T lymphocyte could be overcome if helper function could be supplied by stimulation of other T lymphocytes reactive with a cross-reacting antigen, or if the nDNA was functioning as a hapten complexed to a carrier.

Several problems are involved in the interpretation of DNA-ABC. First, the specificity of the reaction between lymphocytes and nDNA must be examined. Data presented in Table I support the primary nDNA specificity of the reactions measured here. Very little inhibition was observed with unrelated proteins such as protein A, kappa light chain, or DNP_{10}-BSA. Complete inhibition was repeatedly observed with excess unlabeled nDNA, whereas only 25-28% inhibition was recorded with heat-denatured DNA. It was recognized that the sonicated preparations of nDNA used in our experiments may have contained fragments of DNA with exposed terminal ends partially denatured by the sonication process. However, the technical requirements necessary for the antigen-binding assay required such prior treatment.

Second, one has to be certain that the DNA-ABC are lymphocytes and not monocytes that bind and/or pinocytose antigens and antigen-antibody complexes (38, 39). Morphologic separation of large monocytes versus large lymphocytes is extremely difficult without special stains, such as nonspecific esterase in the case of monocytes. However, it is well established that cells identified morphologically as small lymphocytes are never stained by the sensitive, nonspecific esterase method (40). No lymphocytes greater than approximately 8 μm were counted in the present study, so that monocyte labeling

was essentially excluded. Grains overlying cells were dissolved when necessary to facilitate positive identification of cells as small lymphocytes. Pinocytosis was prevented by the cold incubation conditions and the presence of sodium azide.

Third, it is conceivable that cytophilic anti-nDNA antibody and/or nDNA-anti-nDNA complexes may be present on the lymphocyte surface. This consideration was of special importance, since our data showed a tendency for increased numbers of DNA-ABC to be associated with a larger amount of anti-nDNA antibody. B lymphocytes are capable of binding certain types of Ig. This Ig binding is not as secure as when the Ig is complexed with antigen, and generally this cytophilic antibody can be removed by washing (41). There are several reasons why cytophilic anti-nDNA antibody is probably not important in the present study. These reasons may be listed as follows: (*a*) Patients were found with high numbers of DNA-ABC without detectable serum anti-nDNA. Obviously one would not expect this if cytophilic antibody were responsible for the elevated numbers of DNA-ABC found in SLE patients. (*b*) Incubation in cell culture for 18-20 h did not affect the number of DNA-ABC. Because of the well-documented dynamic turnover of the lymphocyte membrane (42), one would expect cytophilic antibody to be shed along with membrane components. (*c*) Lymphocyte suspensions were washed at least three times after preparation, which ordinarily removes cytophilic antibody (41). For an adherent nDNA-anti-nDNA complex to interact with radiolabeled nDNA, it is necessary that the complex be formed in antibody excess so that the complex would retain free anti-nDNA combining sites. However, it is clear from the present study that large numbers of DNA-ABC can exist in the absence of anti-nDNA antibody. Also dynamic membrane changes would presumably involve the receptors for antigen-antibody complexes and result in the shedding of the complex in a relatively short time. Our long-term incubation studies showed no significant change in the number of DNA-ABC after overnight incubation at 37°C, thus making the possibility of ^{125}I-nDNA binding by complexes unlikely.

B lymphocytes were identified in the present study by methods that detected relatively large amounts of surface Ig. Since patients with SLE have antibodies directed against both B and T cells (43, 44), the presence of surface Ig could theoretically include some T lymphocytes as well as B lymphocytes. This potential problem was overcome in the present study by an initial incubation at 37°C, which allowed any anti-lymphocyte antibody to be shed from the cell surface (45). The efficacy of such a preliminary short incubation in inducing complete antibody shedding was confirmed by the lack of a significant change in the number of cells with surface Ig after a further, prolonged, 18-h incubation at 37°C.

The identification of DNA-ABC in normal people and patients with SLE was an attempt to define the cellular mechanisms involved in the induction of the anti-nDNA antibody response. The results reported here are inconsistent with Burnet's concept (46) that the emergence of a "forbidden clone" of B lymphocytes is the primary event in the production of autoantibodies. At least in the case of the anti-nDNA antibody response in SLE, it appears that the response may be initiated by the stimulation of preexisting nDNA-specific B lymphocytes by several mechanisms other than the participation of nDNA-specific helper T lymphocytes.

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