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Research Article

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Cellular Immunity to Nuclear Antigens in Systemic Lupus Erythematosus

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ABSTRACT Cellular immune repsonses were determined by skin testing and mitogen- and antigen-induced blastic transformation of peripheral blood lymphocyte cultures in 24 patients with systemic lupus erythematosus (SLE) and 24 normal subjects. The incidence of positive skin tests with *Candida albicans,* PPD (tuberculin-purified protein derivative) intermediate strength, *Trichophyton* and histoplasmin was not significantly different in the two groups nor was lymphocyte stimulation by the mitogen phytohemagglutinin-M (PHA-M), implying that cellular immunity is normal in SLE. However, the SLE patients had a significantly increased incidence of positive skin tests and stimulated lymphocyte cultures to a number of nuclear antigens compared with normal subjects. No correlation could be made between the test results and the activity of the SLE at the time of study except for a significant association between lymphocyte culture stimulation by rabbit thymus native DNA and active SLE nephritis. Patients with a membranous antinuclear factor (ANF) pattern had positive skin tests with rabbit thymus native DNA and usually had active disease.

INTRODUCTION

Characteristically, patients with systemic lupus erythematosus (SLE)[1] make immune responses to numerous self-antigens. Most research in this disease has concentrated on the humoral aspects of these responses, particularly those made against nuclear constituents. Cellular immune reactions in SLE have received far less attention.

A number of investigators have noted in SLE patients, delayed type skin test reactions to the intradermal injection of autologous and homologous leukocytes (1–5). Similar skin test findings have also been obtained using more chemically defined heterologous nuclear antigens, particularly calf thymus deoxyribonucleic acid (DNA) (5–12). Lymphocyte culture stimulation with DNA has been reported in a group of SLE patients (13). However, discordant findings were reported by another group of investigators who found the incidence of positive skin tests to calf thymus DNA in SLE patients was similar to that found in their control population (11). This same group also noted anergy to tuberculin in SLE patients.

This present study was undertaken to investigate a number of aspects of cellular immunity in SLE patients including pre-existing delayed hypersensitivity to a number of "universal" antigens, skin testing with various types and sources of nuclear antigens and the stimulation induced in peripheral blood lymphocyte cultures by these nuclear antigens and phytohemagglutinin-M (PHA-M).

METHODS

Study subjects. 24 patients with SLE from the Arthritis Clinic and In-patient Service of the University of Cincinnati Medical Center were studied. Their ages ranged from 13 to 67 yr with a mean of 35.7 yr. Only three patients were men. All had positive antinuclear factor tests (ANF) at the time of study. 24 healthy volunteers with negative ANF tests served as controls. Their ages ranged from 20 to 56 yr with a mean of 32.6 yr. Three volunteers were men. 13 patients and 7 controls were Negroes. 12 patients were on treatment other than aspirin including 8 with prednisone, 3 with prednisone and azathioprine, and 1 with hydroxychloroquine. The other patients were either receiving no treatment or taking aspirin. Those on aspirin had the drug discontinued 24–48 hr before testing.

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[1] *Abbreviations used in this paper:* ANF, antinuclear factor; DNH, deoxyribonucleohistone; DNP, deoxyribonucleoprotein; PHA-M, phytohemagglutinin-M; PPD, tuberculin-purified protein derivative; SLE, systemic lupus erythematosus.

Antigens. Antigens used for testing pre-existing delayed hypersensitivity included the following: tuberculin-purified protein derivative (PPD) 5 TU,[2] *Candida albicans* 1:100, *Trichophyton*[3] 1:100, and histoplasmin.[4] Nuclear antigens used for testing included rabbit thymus native and heat-denatured DNA 45–75 μg/0.10 ml, rabbit thymus deoxyribonucleoprotein (DNP) 56–80 μg/0.10 ml (concentration expressed as DNA content), rabbit thymus DNP at a 1:5 dilution of the preceding antigen, rabbit thymus deoxyribonucleohistone (DNH) 11–25 μg/0.10 ml (concentration expressed as DNA content), calf thymus native and heat-denatured DNA 100 μg/0.10 ml, and the diluent used in preparing the nuclear antigens (0.02 M Tris, 0.04 M K_2SO_4 at pH 7.4).

Skin testing method. 0.10 ml of the antigens was injected intradermally except for *Candida* and *Trichophyton* which only had 0.02–0.04 ml injected initially to protect against severe reactions. If the *Candida* or *Trichophyton* skin tests were negative at 24 hr, a further 0.10 ml was then injected. All skin tests were read at 20 min, 4, 24, and 48 hr. Both induration and erythema were recorded by measuring perpendicular diameters and averaging the readings. Erythema was partially masked on black skin and although recorded, was not used for the study. Criteria for a positive skin test were 5 mm of induration appearing at 24 or 48 hr postapplication for *Candida, Trichophyton,* PPD, histoplasmin, and rabbit thymus native and denatured DNA. 10 mm or more induration at 24 or 48 hr postapplication was the criteria used for all other nuclear antigens.

Preparation of nuclear antigens

Rabbit thymus DNA. High molecular weight DNA was isolated from thymus tissue of 4–6 lb. New Zealand white rabbits by a modification of the method of Kay, Simmons, and Dounce (14). Intrinsic viscosities measured in a low shear instrument were in the range 100–180 dl/g. Protein content was less than 1%. DNA concentrations, based on DNA in the acid form, were determined by measurement of absorbance at 260 mμ taking $A^{1\ mg/ml} = 21.3$. Denatured rabbit thymus DNA was prepared by heating the native DNA at 100°C for 10 min and then rapidly cooling.

Rabbit thymus DNP. The compact type of DNP used in this work was prepared from rabbit thymus nuclei as described by Rees and Krueger (15). Purification was accomplished by chromatography on Sephadex G-200 using as eluting solvent a buffer consisting of 0.04 M K_2SO_4–0.02 M Tris sulfate at pH 7.4. The sedimentation constants were determined in a Spinco model E ultracentrifuge (Spinco Div., Beckman Instruments, Inc., Palo Alto, Calif.) using schlieren optics and were corrected to 20°C and water. Values ranged from 123S to 173S. The DNA content of the DNP preparations was determined by measuring absorbance at 260 mμ taking $A^{1\ mg/ml} = 25.6$. As judged by their eluting characteristics, sedimentation properties, ultraviolet spectra, and intrinsic viscosities in 2 M NaCl, the DNP preparations were similar to those described earlier (15). Chemical analysis has shown this DNP to consist of 1 part DNA, 1 part histone, and 0.3 parts nonhistone-protein.

Rabbit thymus DNH. The water soluble type DNH was prepared according to the following procedure. 4- to 6-lb. New Zealand white rabbits were the source of the thymus tissue. All operations were carried out in the cold. A single thymus was the source of each DNH preparation. Each thymus was homogenized in 80 ml of a buffer consisting of 0.10 M NaCl and 0.01 M EDTA at pH 7.4. This was done in a glass Teflon, Potter-Elvehjem homogenizer. The homogenate, consisting of whole nuclei at this point, was filtered through silk and centrifuged at 2000 rpm for 10 min to collect nuclei. The sediment was washed several times with salt-EDTA by suspension and centrifugation. During this process, the nuclei lyzed and the final sediment consisted of washed chromatin fibers. This sediment was then suspended in 40 ml distilled H_2O and agitated frequently for an hour. A viscous gel was formed which was sedimented and suspended in 300–400 ml H_2O and stirred for 3 hr. At this point, the gel was well dispersed into the total water volume yielding a highly viscous solution. This material was next sheared by homogenization (40 ml at a time) in a Potter-Elvehjem homogenizer using 36 strokes at 1600 rpm and applying intermittent cooling. The solution was then centrifuged for 1 hr at 15,000 rpm. DNH was in the top clear layer which was carefully pipetted off from any gel residue. The concentration of DNA in the DNH preparations was determined by measurement of the absorbance at 260 mμ assuming $A^{1\ mg/ml} = 27.0$. Intrinsic viscosity of this type of preparation was of the order of 20 dl/g DNA.

Calf thymus DNA. The calf thymus DNA (commercially obtained)[5] was solubilized in 0.15 M NaCl. The native DNA was denatured to single stranded molecules by heating at 100°C for 10 min and then rapidly cooling.

Lymphocyte culture technique. 100 ml of venous blood in heparin was drawn from each subject immediately before skin testing. The heparinized blood was allowed to sediment at 37°C at a 45° angle for 30 min to 1 hr. The plasma was withdrawn and mixed with an equal amount of Eagle's minimal essential medium containing 100 μg/ml of streptomycin (MEM-S). This mixture was centrifuged at 500 *g* and the resulting cell pellet washed twice more with MEM-S. The cells were then diluted with an appropriate amount of MEM-S which had been mixed with 20% fetal calf serum and L-glutamine 0.2 mm/100 ml[6] to yield a final concentration of 750,000 mononuclear cells/ml. 4-ml portions were placed in sterile disposable glass culture tubes and the antigens or PHA-M[7] added. The tubes were covered with Morton caps and placed upright in an incubator at 37°C in an atmosphere of 95% air and 5% CO_2. After the proper incubation period (5 days for antigens and controls and 3 days for PHA-M) the cells were pulsed for 2 hr with 4 μCi of thymidine-^{3}H (11 Ci/mM). After 2 hr, the cultures were chilled, centrifuged, and the cell buttons mixed with 2 ml of ice-cold 5% trichloroacetic acid, the resulting precipitate washed twice more with 1 ml of ice-cold 5% trichloroacetic acid and then washed once with 2 ml of ice-cold methanol after which it was digested with hydroxide of hyamine (10 ×). The digested material was incubated at 70°C in an oven for 15 min and transferred to glass counting vials with the addition of 19.5 ml scintillation fluid (0.3% 2,5-diphenyoxazole (PPO) and 0.01% 1,4-bis[2-(5-phenyloxazolyl)] benzene (POPOP) in toluene). The vials were counted in a liquid scintillation counter (Nuclear-Chicago Corp., Des Plaines, Ill.) with quenching determined by use of an external standard. All antigen and control cultures

[2] Center for Disease Control, Atlanta, Ga. (bottled by Parke, Davis & Company, Detroit, Mich.

[3] Hollister-Stier Laboratories, Downers Grove, Ill.

[4] Parke, Davis & Company.

[5] Schwarz/Mann, Orangeburg, N. Y.

[6] Microbiological Associates, Inc., Bethesda, Md.

[7] Difco Laboratories, Detroit, Mich.

were done in duplicate or in most cases triplicate, depending on the lymphocyte yield.

The concentrations of nuclear antigens used in lymphocyte cultures were those described for the skin tests. PHA-M was diluted with sterile, buffered saline and used in a concentration of 0.10 ml/culture. Significant blastic transformation was arbitrarily judged to be present when an antigen or mitogen culture had greater than twice the counts per minute (cpm) of the control cultures:

$$\frac{\text{cpm antigen or mitogen cultures—background}}{\text{cpm control cultures—background}} = 2 \text{ or more}$$

Antibody studies. Antinuclear factors were determined according to the immunofluorescent technique of Coon's using human thyroid as the antigen source and fluorescein isothiocyanate-labeled goat anti-human immunoglobulin (G, A, M) as the antiserum. A titer of 1:10 was considered a positive reaction.

Statistical analysis. Statements of statistical significance are based on chi square analysis using Yates' correction and Fisher's exact test where applicable.

RESULTS

Skin tests. The majority of skin test reactions to nuclear antigens reached maximum induration by 24 hr after application. Most tests began to resolve at 36 or 48 hr. A few tests did continue to enlarge until 48 hr. The initial injection of nuclear antigens as well as the diluent was quite irritating and by 20 min most of the subjects had evidence of a wheal and flare at the injection site. This flare had resolved by the 4 hr reading but the area of induration at 4 hr was often the same or slightly larger than the 20 min reading. Since this reaction occurred in both patients and controls and with both antigens and diluent, the 4 hr reaction was thought to be due to irritation. After 24 hr, the diluent reaction had resolved.

TABLE I
Skin Test Results

Antigen	SLE patients No. positive/ No. tested	Controls No. positive/ No. tested	P value
Candida	19/23	22/23	NS*
Trichophyton	3/7	7/13	NS
PPD	8/23	5/24	NS
Histoplasmin	3/7	8/14	NS
DNP	15/24	5/24	<0.01
DNP 1:5	5/7	0/13	<0.001
DNH	5/23	1/24	NS
Rabbit DNA-native	7/24	0/24	<0.025
Rabbit DNA-SS	6/23	1/24	NS
Calf DNA-native	6/14	0/20	<0.01
Calf DNA-SS	5/13	0/14	<0.05

* NS, not significant.

TABLE II
Frequency of Positive Skin Tests with Nuclear Antigens

	No. tested	1 or more +	2 or more +	3 or more +	4 or more +	5 or more +
SLE patients	24	20	13	9	6	1
Controls	24	6	1	0	0	0

The incidence of positive skin tests to *Candida,* PPD intermediate strength, *Trichophyton,* and histoplasmin was not significantly different in the two groups (Table I). However, the incidence of positive skin tests to five of the seven different nuclear antigen preparations (DNP at both concentrations, rabbit native DNA, calf native DNA, and calf heat-denatured DNA) was significantly increased in the SLE patients (Table I).

Five SLE patients who exhibited a membranous pattern in the ANF test had a positive skin test to rabbit thymus native DNA ($P < 0.005$). Another important finding was that the SLE patients frequently had positive skin tests to more than one nuclear antigen while this was observed only once in the control group (Table II). The skin reactions observed in the SLE patients were also of greater intensity, as the mean induration of the skin tests with each nuclear antigen exceeded twice that found in the control subjects except for DNH (Table III).

A biopsy of one 24 hr DNP skin test obtained from an SLE patient (Figs. 1 and 2) showed evidence of a perivascular and intracollagenous round cell infiltrate. There was no evidence of vascular thrombosis. Small mononuclear cells and monocytes predominated with only rare granulocytes seen. These findings are typical of delayed hypersensitivity. A skin test biopsy of a rabbit native DNA skin test from another SLE patient 24 hr after application showed similar findings.

The skin test response to nuclear antigens did not seem to be altered by treatment in the SLE patients

TABLE III
*Mean Induration of Skin Tests with Nuclear Antigens in All Subjects Tested**

Antigen	SLE patients	Controls
	mm	*mm*
DNP	17.3	7.1
DNP (1:5 dilution)	26.0	1.0
DNH	7.4	5.0
Rabbit DNA-native	3.3	1.3
Rabbit DNA-SS	7.8	1.2
Calf DNA-native	6.4	1.4
Calf DNA-SS	6.4	1.6

* Maximum induration at 24 or 48 hr postapplication.

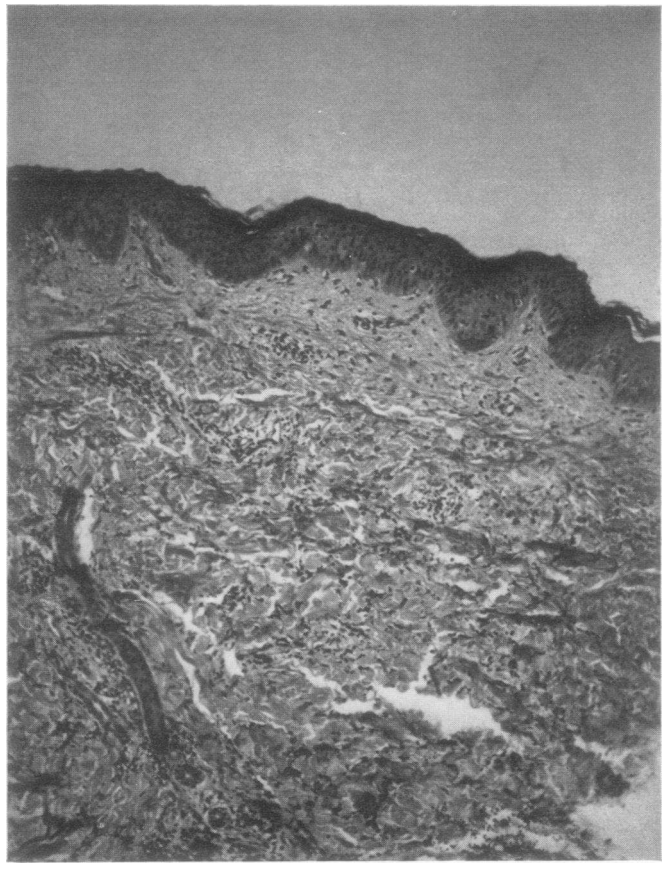

FIGURE 1 Biopsy of DNP skin test site 24 hr after injection in SLE patient. (× 80)

as 10 of 12 treated and 10 of 12 untreated patients reacted to at least one antigen. Also, 5 of 12 treated and 7 of 12 untreated patients had positive skin tests to at least two different nuclear antigens.

Peripheral blood lymphocyte cultures. Another acceptable method of assessing cellular immunity is mitogen- and antigen-induced blastic transformation of peripheral blood lymphocyte cultures (16–18). In Table IV, results of lymphocyte transformation tests obtained with PHA-M and nuclear antigens in SLE patients are compared with those found in the control individuals. The mean cpm for cultures which were incubated for 5 days without any antigen or mitogen was 654 cpm for SLE patients and 599 cpm for the control group. PHA-M stimulated the cultures in 22 SLE patients and in all the controls with the mean ratios being 134 and 135, respectively. The two SLE patients not stimulated by PHA-M were both on corticosteroids, with one receiving azathioprine.

TABLE IV
Lymphocyte Culture Results

Antigen or mitogen	SLE patients No. positive/ No. tested	Controls No. positive/ No. tested	
PHA	22/24	24/24	
DNP	5/24	1/24	
DNP (1:5)	0/3	0/10	
DNH	4/23	0/23	
Rabbit DNA-native	3/23	0/22	
Rabbit DNA-SS	2/23	0/23	
Calf DNA-native	2/13	0/19	
Calf DNA-SS	0/13	0/19	
Total to all nuclear antigens	16/122	1/140	($P < 0.005$)

Out of a total of 118 lymphocyte tests with nuclear antigens in SLE patients, 16 were positive whereas only 1 out of a total of 140 tests were positive in the controls, a significant difference. The mean ratios in patients who had cultures stimulated by the various nuclear antigens were rabbit DNA 3.9, rabbit-denatured DNA 2.5, DNP 3.0, DNH 2.3, calf thymus native DNA 2.6. 11 of the 24 patients had a positive response to

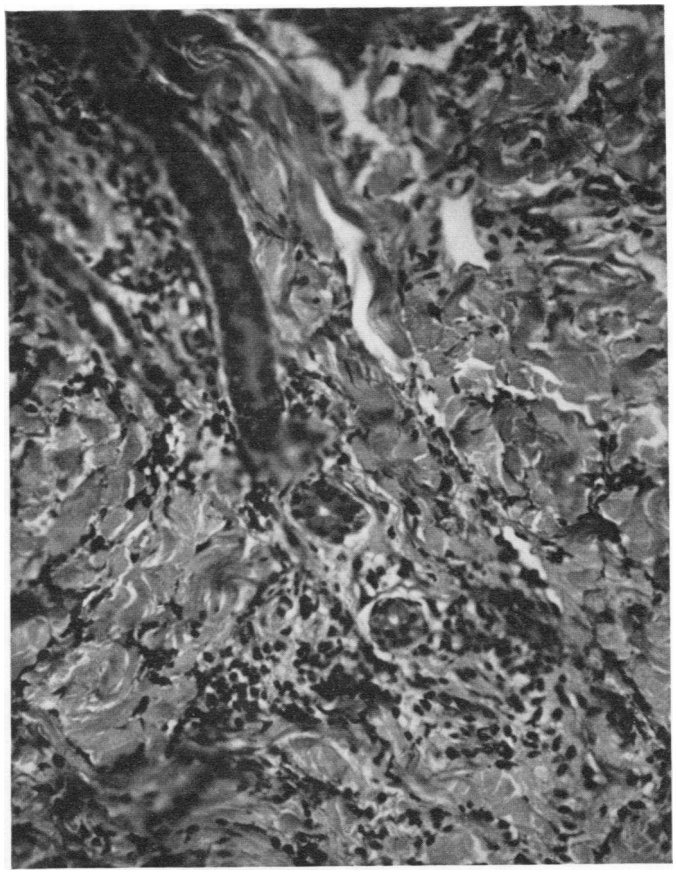

FIGURE 2 Higher magnification of biopsy shown in Fig. 1 (× 200), demonstrating perivascular lymphocytic infiltration.

one or more of the nuclear antigens and 3 responded to at least 2 antigens (Table V). Of these 11 SLE patients who had lymphocyte cultures stimulated by one or another of the nuclear antigens, 7 had positive skin tests to 1 or more of the corresponding antigens. Of the total of 16 positive lymphocyte cultures in all the SLE patients, 8 had corresponding skin tests. However, of the 8 which did not correspond, 3 did have borderline skin reactions between 5 and 10 mm induration to the antigen.

The lower incidence of stimulation of lymphocyte cultures with the nuclear antigens in the SLE patients as compared with the incidence of positive skin tests with the same antigens is at least partially an effect of treatment (prednisone and/or azathioprine and hydroxychloroquine). 4 of 12 treated patients had lymphocyte stimulation with at least one nuclear antigen whereas 7 of 12 untreated patients had at least one positive test.

Correlation of skin tests, lymphocyte stimulation, and ANF tests with clinical abnormalities in the SLE patients. Many of the SLE patients had been followed for a number of years with fluctuations in severity and organ system involvement during their clinical course, for example, several had had clinical and histologic evidence of renal disease earlier in their course. Other patients were new to the medical center with less well-documented past histories. Therefore it was decided to attempt to correlate only those clinical abnormalities present at the time of study with the skin test and lymphocyte culture results. In Table VI, each individual patient is listed with his or her major clinical disease manifestations, skin test, and lymphocyte culture results and the pattern of the ANF.

Table VII lists the four major disease manifestations

TABLE V

Frequency of Stimulated Lymphocyte Cultures with Nuclear Antigen

	No. tested	1 or more +	2 or more +	3 or more +
SLE patients	24	11	3	2
Controls	24	1	0	0

TABLE VI

Summary of Skin Tests, Lymphocyte Cultures,

SLE patient	Skin tests											Lymphocyte cultures			
	Candida	*Trichophyton*	PPD	Histoplasmin	DNP	DNP 1:5	DNH	Rabbit DNA native	Rabbit DNA S-S	Calf DNA native	Calf DNA S-S	PHA-M	DNP	DNP 1-5	DNH
1. B. G.	N.D.	N.D.	N.D.	N.D.	+	N.D.	N.D.	0	N.D.	N.D.	N.D.	+	+	N.D.	0
2. G. S.	+	N.D.	0	N.D.	+	N.D.	0	0	0	N.D.	N.D.	+	+	N.D.	0
3. R. B.	+	N.D.	+	N.D.	+	N.D.	0	0	+	N.D.	N.D.	+	0	N.D.	+
4. B. E.	+	N.D.	0	N.D.	+	N.D.	+	+	0	N.D.	N.D.	+	0	N.D.	0
5. C. W.	+	N.D.	0	N.D.	+	N.D.	0	0	0	N.D.	N.D.	+	0	N.D.	0
6. C. J.	0	N.D.	0	N.D.	+	N.D.	0	+	0	N.D.	N.D.	+	0	N.D.	0
7. A. J.	+	N.D.	0	N.D.	+	N.D.	+	+	+	N.D.	N.D.	+	0	N.D.	+
8. O. G.	+	N.D.	+	N.D.	0	N.D.	0	0	0	N.D.	N.D.	0	0	N.D.	0
9. R. D.	+	N.D.	+	N.D.	0	N.D.	0	0	0	N.D.	N.D.	+	0	N.D.	+
10. M. D.	+	N.D.	0	N.D.	0	N.D.	0	0	0	N.D.	N.D.	+	0	N.D.	0
11. J. H.	+	N.D.	0	N.D.	+	N.D.	+	0	0	0	N.D.	+	+	N.D.	0
12. L. V.	+	N.D.	0	N.D.	+	N.D.	0	0	0	+	+	+	0	N.D.	0
13. F. M.	+	N.D.	0	N.D.	+	N.D.	0	0	+	+	+	+	+	N.D.	+
14. B. S.	0	N.D.	+	N.D.	+	N.D.	0	0	+	+	+	+	0	N.D.	0
15. L. P.	0	N.D.	0	N.D.	+	N.D.	0	0	0	0	0	+	0	N.D.	0
16. O. W.	+	N.D.	0	N.D.	+	N.D.	+	0	0	+	+	0	0	N.D.	0
17. P. W.	+	N.D.	+	N.D.	+	N.D.	0	+	+	+	+	+	0	N.D.	0
18. W. S.	+	0	+	+	0	+	0	+	0	+	0	+	0	0	0
19. M. H.	+	0	0	0	+	+	0	+	+	0	0	+	0	0	0
20. M. M.	+	+	+	+	0	+	0	0	0	0	0	+	0	0	0
21. W. S.	+	+	0	+	0	+	+	0	0	0	0	+	0	N.D.	0
22. O. S.	+	+	+	0	0	+	0	0	0	0	0	+	+	N.D.	0
23. P. B.	0	0	0	0	0	0	0	+	0	0	0	+	0	N.D.	N.D.
24. C. G.	+	0	0	0	0	0	0	0	0	0	0	+	0	N.D.	0
Total	19/23	3/7	8/23	3/7	15/24	5/7	5/23	7/24	6/23	6/14	5/13	22/24	5/24	0/3	4/23

* P, prednisone; A, azathioprine; C, chloroquine (hydroxy).

present in this group and their correlation with the tests. The category arthritis represents nonerosive arthritis; oral-cutaneous lesions refer to oral ulcers, skin rashes of various types seen in SLE; hematologic represents anemia, leukopenia and/or thrombocytopenia; renal disease refers to *active* clinical disease at the time of the study. Some manifestations of SLE were present too infrequently in this group of patients at the time of study to attempt correlation with the tests, i.e. Raynaud's phenomenon in three patients, fever in three patients, etc. were not correlated. Erythrocyte sedimentation rates (Westergren method) are also listed in Table VI but they were elevated in most patients. As noted in Table VII, only one test exhibited a high degree of correlation with a disease manifestation. Active renal disease was significantly associated with a positive lymphocyte culture response to rabbit native DNA (3/6). Of the six patients with active renal disease, five had the diagnosis verified by renal biopsy. Of the three major ANF patterns, only the membranous pattern correlated with skin tests or lymphocyte culture responses to the nuclear antigens. Skin test response to rabbit native DNA had a highly significant association ($P < 0.005$) with the membranous staining pattern. Lymphocyte response to rabbit native DNA also correlated with the membranous pattern when left uncorrected but failed to correlate when the Yates correction was used.

It should be noted that 5/11 patients on steroid/azathioprine therapy had positive PPD skin tests and 8/11 a positive *Candida* reaction. The four patients who did not react to *Candida* were all young with acute active SLE; three did not react to PPD. However, all four patients reacted to one or more of the nuclear antigens on skin testing. All reacted to PHA-M and two had stimulated lymphocyte culture with rabbit-native DNA (only three patients in the entire study reacted).

DISCUSSION

With the parameters employed in this study, i.e. PHA-M stimulation of lymphocyte cultures and skin tests with PPD, histoplasmin, *Candida,* and *Trichophyton,* the cel-

Clinical Data, and ANF Type in SLE Patients

Lymphocyte cultures				Clinical manifestations						Pattern of ANF				
Rabbit DNA native	Rabbit DNA S-S	Calf DNA native	Calf DNA S-S	Renal	Arthritis	Hemato-logic	Cutane-ous/oral	Therapy*	ESR	Diffuse	Speckled	Mem-branous	Age time of study	Age of onset
0	0	N.D.	N.D.	0	0	+	0	0	38	0	+	0	49	48
0	N.D.	N.D.	N.D.	0	+	+	+	0	25	+	+	0	33	32
0	0	N.D.	N.D.	0	0	0	0	P	8	+	0	0	41	35
0	0	N.D.	N.D.	0	+	0	0	0	100	+	0	+	64	55
0	0	N.D.	N.D.	0	+	+	0	P	83	+	0	0	38	32
+	+	N.D.	N.D.	+	+	+	+	0	N.D.	+	0	+	14	14
N.D.	0	N.D.	N.D.	+	0	+	0	0	52	+	0	+	23	17
0	0	N.D.	N.D.	+	0	0	0	P,A	54	+	0	+	39	35
0	0	N.D.	N.D.	0	0	0	+	P,A	62	+	+	0	46	44
0	0	N.D.	N.D.	0	0	0	0	0	20	+	+	0	30	19
0	+	+	0	0	+	0	0	P	45	+	0	0	19	16
+	0	0	0	+	+	+	+	0	43	+	+	0	18	17
0	0	+	0	0	+	0	0	0	30	0	+	0	34	30
0	0	0	0	0	0	0	0	P	28	+	+	0	18	17
0	0	0	0	+	0	0	+	P,A	28	+	+	0	17	15
0	0	0	0	0	+	0	0	P	46	+	0	0	67	59
0	0	0	0	0	0	+	+	0	18	0	+	0	48	38
0	0	0	0	0	+	0	0	0	42	+	+	+	28	27
0	0	0	0	0	+	0	0	P	42	+	+	+	48	38
0	0	0	0	0	+	0	+	P	12	+	0	0	42	35
0	0	0	0	0	+	0	+	C	8	+	+	0	45	40
0	0	0	0	0	+	+	0	0	78	+	0	0	61	51
+	0	N.D.	N.D.	+	0	+	+	P	35	+	0	+	13	13
0	0	0	0	0	+	0	0	0	3	+	0	0	23	21
3/23	2/23	2/13	0/13	6/24	14/24	10/24	10/24	12/24		21/24	11/24	6/24		

lular immune system in the SLE patients appeared intact. Also, as has been found with the humoral limb of immune responses, cellular immune reactions were found with a variety of nuclear antigens, both on skin testing and with antigen-induced blastic transformation of peripheral blood lymphocyte cultures. As has been found with antibody responses to nuclear antigens, nucleoproteins produced the greatest number of positive skin tests and stimulated lymphocyte cultures. However, there were a number of exceptions in that some patients who failed to respond to nucleoproteins, did so to one of the types of DNA. This indicates that a battery of nuclear antigens is needed to detect all of the potential reactors. In the present study which used such a variety of antigens, 83% of the patients with SLE had at least one positive response on skin testing and 46% one positive response with lymphocyte cultures. Only one of the controls with a positive skin test reacted in lymphocyte culture. The results of other investigators using primarily DNA as the antigen, are in agreement with the present skin test findings (5–11). Only Block, Gibbs, Stevens, and Shulman found no differences in skin test reactivity between calf thymus DNA and their control group (11). However, the incidence of positive reactions in SLE with either calf thymus DNA or rabbit thymus DNA in this study is in the same range as Block et al. found and contrasts sharply with a much higher incidence of positive reactions reported by other groups with DNA. Azoury,

TABLE VII

Main clinical disease manifestations at time of study		Correlative tests
Arthritis	14/24	None
Hematologic abnormalities	10/24	None
Oral or cutaneous lesions	10/24	None
Active renal disease	6/24	Positive lymphocyte response to rabbit native DNA ($P < 0.01$)

Jones, Derbes, and Gum also noted an association between skin reactivity to calf thymus DNA and a membranous pattern of the ANF test (5). In the present study, the association occurred with rabbit thymus DNA; only one of the patients with membranous ANF in this study was also tested with calf thymus DNA and had a positive skin test. The response to nuclear antigens in SLE using peripheral blood lymphocyte cultures has been examined only by Patrucco, Rothfield, and Hirschhorn (13). The lymphocytes of 11 of their 12 SLE patients responded to either native or denatured DNA, a much higher incidence than was found in the present study. However, their study used morphological techniques to assess lymphocyte transformation and we have found it difficult to distinguish blast cells from macrophages using this method of cell fixation and acetic orcein staining.

The lower incidence of response of lymphocyte cultures as compared with skin tests with the same antigen and the lack of a high degree of correlation between skin tests and lymphocyte transformation has been noted by others (19, 20). It is now recognized that a number of lymphokines exist and that the absence of some of these may result in a negative skin test (21). Part of this effect in this study was possibly due to treatment. Prednisone, azathioprine, and chloroquine may depress blastogenesis of lymphocytes in vitro (22–25). Another factor in this study for the low correlation was that only one dose of antigen was employed for either skin testing or lymphocyte transformation. Obviously a range of doses for each antigen would have provided a greater chance of inducing a positive reaction. However, this was impractical because of the large number of antigens.

The present study contrasts responses to two different nucleoprotein preparations, DNP and DNH. These two nucleoproteins are thought to differ in tertiary structure, with DNP being a much more compact molecule as evidenced by its lower viscosity and higher sedimentation coefficient compared with DNH. Although this would suggest that antigenic sites might be more available on DNH, this molecule is less soluble in ionic solutions. This may account for the low incidence of positive skin tests with this antigen. Although these two nucleoproteins were employed at different DNA concentrations, the difference found in the skin tests does not seem to be related to this factor as a dilution of DNP did not decrease its heightened skin test reactivity. In vitro, both nucleoproteins induced a similar frequency of stimulated lymphocyte cultures.

Renal disease was the only major manifestation of SLE which correlated with any of the tests. As previously pointed out, treatment may have masked some of the correlations by suppressing disease manifestations, as well as suppressing tests. Other groups have presented conflicting data on this point. Tromovitch and March noted that the incidence of positive skin tests to autologous leukocytes paralleled the severity and acuteness of the disease (3), whereas Block and associates did not observe such an association with calf thymus DNA (11). Again, most investigators have noted some effect on immune responsiveness to skin testing by treatment with immunosuppressive agents (4, 7, 12). This study did not find any obvious depression of the responses in patients on treatment. Only 2 of 12 on treatment failed to respond to PHA-M.

Recently, Bitter, Bitter, Silberschmidt, and Dubois examined 10 patients with early active SLE who were not on treatment and found that these patients were essentially anergic to a battery of antigens including intermediate PPD, streptodornase-streptokinase, mumps, coccidiodin, histoplasmin, *Candida albicans,* and *Trichophyton* and found no significant stimulation with phytohemagglutinin in lymphocyte cultures (26). Although most patients in the present study had SLE for a longer period of time, such marked anergy was not noted even in two patients with early acute active disease of less than 6 wk duration. One patient, a 13 yr old white female who was seen within 4 wk of onset of her disease, although negative to *Candida, Tricophyton,* PPD, and histoplasmin had positive skin tests and lymphocyte cultures to rabbit native DNA. Her PHA-M response was 102 times the control. The second patient, a 14 yr old white female seen within 1 month of the onset of the disease, had a positive skin test to rabbit native DNA and DNP and positive lymphocyte cultures to rabbit native DNA and single-stranded DNA. Her PHA-M response was 65 times the control. Both of these patients had membranous pattern ANF reactions.

The finding of positive skin tests to nuclear antigens in normal individuals has been observed in this study and by others (3, 7). Obviously, the significance of these findings is unknown. If in the normals, these represent true immunologic reactions, then one interpretation of these findings could be that an inheritable "lupus diathesis" is present. The cellular response to nuclear antigens found in SLE patients is in accord with a recent hypothesis of autoimmunity (27). The presence of the thymus is necessary for the production of immune tolerance as well as cellular immunity (28). The experience with New Zealand black mice has shown that transfer of thymus cells from young to older animals with hemolytic anemia will suppress the anemia (29). Teague and Friou noted that aging A/Jax mice frequently have positive ANF titers but these titers disappear or are reduced by the transfer of thymus cells from young to old syngeneic animals (30). Such data support the concept that the thymus controls

autoimmunity and in the normal state prevents immune reactions against self-antigens. Therefore, in SLE, abnormal T-cell functions would be expected and this is confirmed in this study by the development of cellular immune reactions to nuclear antigens. The exact role of these cellular immune reactions in the production of tissue injury in SLE could not be determined from these experiments but obviously could be of some consequence as is suggested by finding a correlation between renal disease and blastic transformation to rabbit native DNA.

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