Decreased 19S Antibody Response to Bacterial Antigens in Systemic Lupus Erythematosus

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ABSTRACT The antibody response to immunization with *Brucella* and the levels of natural antibody to *Escherichia coli* and *Shigella* were compared in patients with systemic lupus erythematosus and control groups. After *Brucella* immunization, SLE patients showed a significantly lower antibody response in whole serum and in the macroglobulin antibody fraction separated by sucrose density gradient centrifugation.

Sucrose gradient fractionation of natural antibodies to *E. coli* and a polyvalent *Shigella* antigen showed a significant decrease in macroglobulin antibody against four of the five *E. coli* antigens tested and the *Shigella* polyvalent antigen in SLE patients when compared with a group of normal individuals and a matched control group with pulmonary tuberculosis. Whole serum natural antibody titers against 5 of 13 Shigella antigens were significantly lower in the SLE patients when compared with the normal group, and against 7 of 13 when compared with the matched tuberculosis controls. Whole serum titers against 8 of 13 E. coli antigens were significantly lower in the SLE patients when compared with normal subjects.

The observed decreased antibody response to bacterial antigens in SLE patients, occurring mainly in the macroglobulin fraction, is discussed in relation to the increased incidence of infection commonly observed in these patients.

INTRODUCTION

Previous studies have reported an increased antibody response in patients with systemic lupus erythematosis (SLE) after immunization with *Brucella* (1) and incompatible erythrocytes (2), and a normal or decreased response after immunization with tetanus (3). Spontaneous isoagglutinin levels and antibody to Proteus OX-2 were found by Muschel (4) to be normal whereas ASO levels were lower than in controls. The class of immunoglobulin response has not been measured in any of these studies, though it has been shown to be predominantly of the 7S variety in the case of the antinuclear antibody (5, 6). In the present study, the response of SLE patients and a group of normal individuals to stimulation with *Brucella* antigen has been determined, and the titers of 19S (IgM) and 7S (IgG) *Brucella* antibody measured. Since natural antibodies to *E. coli* (7) and *Shigella* (8) are frequently found, the whole serum, 19S and 7S antibody titers of these antibodies were compared with the patient and control groups.

METHODS

Brucella immunization. 23 female patients with SLE were immunized (Table I). 18 were black, 4 white, and one, Latin-American. Every patient had had a positive antinuclear test and all had positive LE cell tests at some time in their disease course. The mean age was 34 yr (range, 14–66 yr). 16 patients were receiving Prednisone, the average dosage being 13 mg daily (2.5–25 mg). Three patients were receiving 6-mercaptopurine, two 25 mg and one, 75 mg daily. Six patients were immunized within the 1st 6 months of their illness, whereas one patient had known disease for 12 yr. The average duration of disease was 32 months. The control group was composed of 18 student nurses with an average age of 21 yr (range, 20–23 yr). 17 were white and one was black.

Immunization was performed with undulant fever vaccine (Parke, Davis & Co.), injecting 0.1 ml in a single subcutaneous immunizing dose containing 2×10^8 organisms (10^8 *Brucella abortus* and 10^8 *Brucella melitensis*). Controls and patients were bled before immunization, and at 1, 4, and 12 wk postimmunization. Three patients were not bled until 16 wk postimmunization. 15 of the patients with SLE were reboosted with the same dosage of *Brucella* antigen. They were bled as before at 1, 4, and 12 wk, and the *Brucella* antibody titers were again measured. Antibody titers were determined by a modification of the standard agglutination method (9). One batch of antigen (Lederle, febrile diagnostic *Brucella abortus* antigen No. 110–128) gave repro-

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TABLE I

Clinical and Laboratory Data on Patients with Systemic Lupus Erythematosus

Patient	Race	Age	Prednisone	Gamma globulin	Alpha-2 globulin	Complement β_{1c}/β_{1a}	Duration of disease	Present or absent renal disease	BUN
			mg/day	*mg/100 ml*	*mg/100 ml*	*mg/100 ml*	*months*		*mg/100 ml*
E. H.	B	36	15	—	—	—	19	+	12
*C. C.	B	42	2.5	3.0	0.7	264	21	+	9
*F. S.	LA	24	5	1.6	0.6	105	39	+	80
P. B.	B	21	12.5	3.8	0.9	47	18	+	15
D. G.	B	32	0	2.4	1.3	165	144	+	9
H. C.	W	19	5	1.4	0.9	83	48	0	—
I. C.	B	44	0	2.6	0.6	155	96	+	33
H. H.	B	39	20	2.8	1.0	55	27	0	14
J. I.	B	35	20	3.0	0.6	130	26	0	14
G. A.	B	16	0	3.8	0.6	23	2/4	+	13
W. W.	B	37	5	4.1	0.3	90	36	0	23
M. P.	B	15	25	4.9	1.1	55	2	+	11
L. C.	B	29	10	3.4	0.8	110	72	0	10
C. M.	B	53	20	2.7	1.1	65	16	0	10
E. M.	B	38	15	1.8	0.9	165	29	+	13
L. T.	B	49	20	3.1	1.2	83	5	+	39
‡M. B.	W	35	15	1.1	1.0	40	7	+	20
L. B.	B	52	10	2.2	0.9	165	96	+	10
L. H.	B	40	0	4.0	0.8	112	20	0	12
M. H.	W	66	7.5	1.7	1.0	175	24	0	10
D. J.	B	29	0	3.0	0.8	89	1/4	+	10
W. M.	B	25	0	5.7	0.5	40	2/4	0	9
L. F.	W	14	0	1.9	0.8	35	1	+	39

* 6-MP, 25 mg daily.

‡ 6-MP, 75 mg daily.

ducible agglutination patterns, and was therefore used for all the tests in this study. Agglutination was read after 48 hr of incubation at 37°C.

E. coli and Shigella antibodies. 18 of the group of 23 SLE patients were matched for race, age, and sex with a group of hospitalized patients with pulmonary tuberculosis in the Tyler State Hospital, Tyler, Tex. All patients had confirmed disease of at least 6 months duration and were receiving antibiotic therapy for this condition. Sera were supplied by Dr. George Hurst, director of the Tyler State Hospital. The 18 pairs of SLE sera and matched tuberculosis sera were tested at the same time. Different lots of *E. coli* antigen were used, although the same lot was always used when SLE sera were compared with individual control groups. Antibody titers were determined by a passive hemagglutination method using a microtitrator system (10), as modified by Wegmann and Smithies (11). Endotoxins for testing of antibody to *E. coli* types 011 and 014 were prepared by phenol extraction of bacterial cultures followed by ethanol fractionation (12). The endotoxin prepared in a single batch process gave consistent results at coating concentrations of 0.5% in the case of *E. coli* 011 and 0.1% in the case of *E. coli* 014. A final concentration of 1.25% human type O erythrocytes was used for agglutination tests. Other *E. coli* endotoxins were obtained from Difco, Inc. They were used in a 1:1 dilution of the stock solution. Several different batches were used, accounting for some variation in titer when the SLE were compared with different control groups. *Shigella* polyvalent antigen and specific *Shigella* serotypes, supplied through the courtesy of Doctors John Nelson and Kenneth C. Haltalin, were utilized as described by them (13). The *Shigella* polyvalent antigen was a composite of equal amounts of all of the known individual types.

Statistical methods. The entire group of 23 SLE patients and 18 normal females were compared for significant differences in antibody titer. The results of the comparison of 18 SLE and 18 tuberculosis patients were carried out, using a method for matched pairs. All elements of the data in Table I were studied for significant correlations, using Pearson product moment correlation coefficients.

Other methods. 19S and 7S antibody titers were determined on fractions separated by sucrose density gradient centrifugation (15). All sera showing the presence of antibody were fractionated. Continuous gradients between 10 and 40% sucrose were prepared in 0.01 M phosphate buffer, pH 7.2. 0.5 ml of serum was diluted to 1 ml with phosphate-buffered saline and layered on top of the gradient. Centrifugation was carried out in an SW-39 swinging bucket rotor for 15 hr at 35,000 rpm at 4°C. Gradients were separated by puncture of the bottom of the tube and collected in 10 fractions of 0.5 ml each. The fractions were dialyzed in the cold against a large volume of phosphate-buffered saline, pH 7.2, for 24 hr. Fractions one through nine were then tested for the presence of antibody. The positions of fractions containing 19S and 7S antibody were verified by sev-

TABLE II

Reciprocal Titer of Brucella Antibody in SLE Patients

Patient	Week 0 time	1	4	12
G. A.	20	40	40	—
L. F.	20	20	80	0
W. M.	0	0	20	—
D. J.	0	0	0	20
M. H.	0	0	—	40
L. H.	80	—	0	0
L. B.	40	80	80	20
F. S.	160	160	320	80
C. C.	0	0	0	0
E. H.	—	320	640	80
M. B.	0	80	80	0
L. T.	0	0	20	—
E. M.	0	—	0	20
C. M.	0	40	0	80
L. C.	0	80	40	0
M. P.	0	80	80	20
W. W.	0	—	320	—
J. I.	0	0	80	40
H. H.	0	80	160	40
H. C.	160	—	160	160
I. C.	0	0	160	20
D. G.	0	0	0	0
P. B.	0	40	80	80

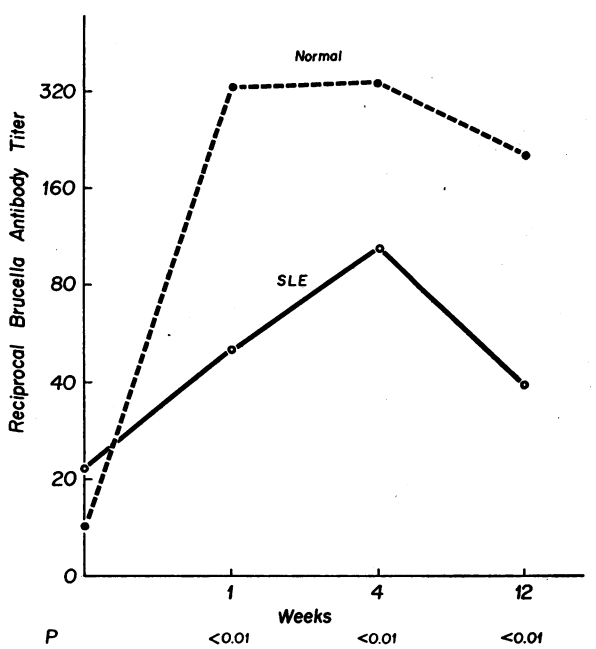

FIGURE 1 Whole serum *Brucella* antibody titer at 0, 1, 4, and 12 wk in 23 patients with SLE and 18 normal controls. The patients with SLE show a decreased titer at all time periods. The *P* values at each period are at the bottom of the figure.

TABLE III

Reciprocal Titer of Brucella Antibody in Normal Controls

Patient	Week 0 time	1	4	12
L. B.	0	320	320	80
J. G.	160	640	640	160
P. J.	0	160	320	320
C. G.	0	320	640	320
S. P.	0	160	320	160
L. D.	0	80	160	20
L. L.	0	160	160	320
G. T.	0	640	160	640
R. D.	0	320	160	—
J. S.	0	640	640	640
W. S.	20	320	320	—
K. A.	0	160	160	160
B. C.	0	640	320	640
C. S.	0	160	80	80
R. C.	0	160	80	80
L. M.	0	160	320	40
L. S.	0	640	320	320
C. J.	0	320	640	—

eral methods: (*1*) recovery of preparations of known 7S and 19S anti-DNP antibodies (prepared by Dr. J. LoSpalluto); (*2*) recovery of activity from sera containing rheumatoid and antinuclear factors (the 7S nature of the latter was established by DEAE cellulose chromatography); and (*3*) elimination of activity in fractions containing macroglobulin antibody by 2-mercaptoethanol treatment.

Levels of the C'3 (β_{1c}/β_{1a} globulin) component of complement were performed on whole sera, using Hyland immunoplates (Hyland Laboratories, Los Angeles, Calif.). Gamma globulin and α_2-globulin levels were determined by serum paper electrophoresis.

RESULTS

Brucella antibody response. Table II shows the reciprocal antibody titers to *Brucella* antigen in the patients with SLE and Table III the results in the normal group. In Table IV are seen the geometric mean titers of antibody in both groups. It can be seen (Table IV) that the antibody levels at 1, 4, and 12 wk were significantly reduced in the SLE patients. Whereas the

TABLE IV

Geometric Mean Titer of Brucella Antibody

Weeks	Normals	SLE patients	*P*
1	333	54	<0.01
4	347	107	<0.01
12	254	39	<0.01

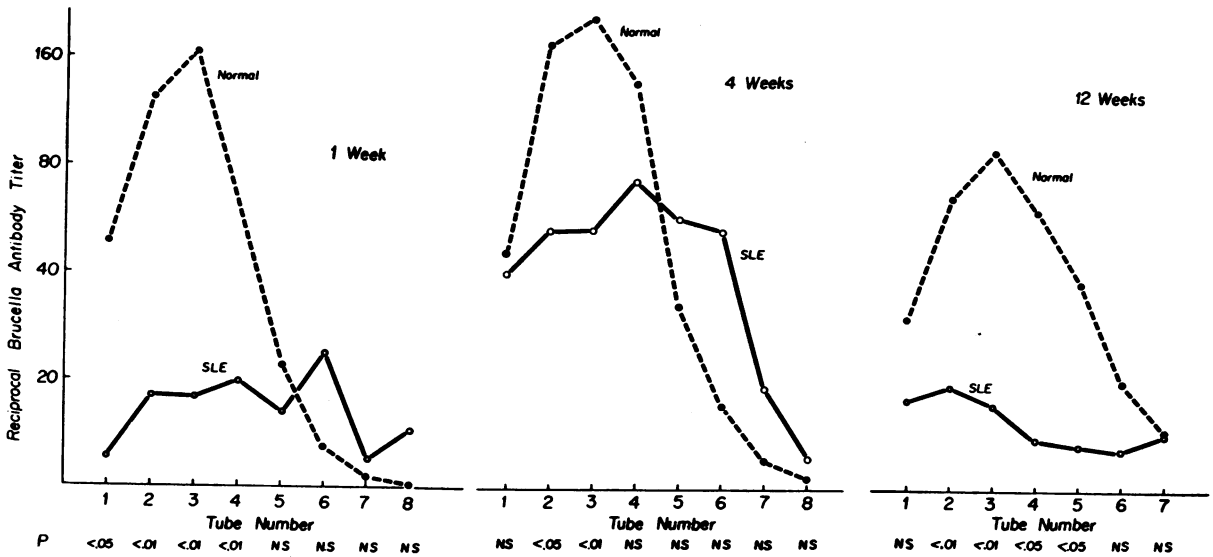

FIGURE 2 Mean *Brucella* antibody titers at 1, 4, and 12 wk postimmunization of serum fractions of 23 patients with SLE and 18 normal control subjects separated by sucrose density gradient centrifugation. Tube numbers start at the bottom of the gradient so that 19S antibody is in the initial fracions and 7S antibody in the later fractions. Note the significantly lower levels of 19S antibody in the SLE patients at all three time periods. *P* values are at the bottom of the figure.

normal subjects showed their maximal response early (Fig. 1), with a fall-off in titer after 4 wk, the patients showed a slow rise with attainment of maximum levels only at 4 wk.

When the response of the 19S and 7S immunoglobulins at each time period was studied, at 1 wk (Fig. 2), maximal antibody titers of the normal controls were noted in the lower part of the gradient where macroglobulin antibody is found. Little or no antibody titer was measured in tube six of the gradient where the 7S antibody is found. The SLE group showed low titers in the 19S end of the gradient and a slight, although not statistically significant elevation, in the 7S immunoglobulin area. The difference between the two groups was highly significant in tubes two, three, and four. At 4 wk (Fig. 2), the antibody levels of the normals were virtually identical with those found at 1 wk, but the values of the SLE group had shown a twofold increase in tubes one to six, indicating an increase in both the 19S and 7S antibody. Significantly less antibody was still present in the macroglobulin region. At 12 wk (Fig. 2), the normal group showed a fall in the level of macroglobulin antibody with somewhat increased levels in the light antibody region. The SLE patients showed a pronounced fall in all tubes with significantly lower levels again in the macroglobulin region (tubes two, three, and four).

Comparison of the macroglobulin antibody (tube three) and light antibody (tube six) titers showed that macroglobulin antibody was responsible for most of the agglutinating activity in the normal subjects. Essentially maximal titers of 19S antibody were reached at 1 wk in this group. In the SLE group, 19S and 7S titers were similar throughout the test periods. Peak titer of 19S antibody was not reached in this group until 4 wk, however, indicating a delay in attainment of maximum antibody activity in the SLE patients.

Correlation coefficients were calculated between each of the factors tabulated in Table I and the *Brucella* antibody levels at wk 4. Only the correlation between titer and serum alpha$_2$-globulin concentration was significant ($P < 0.05$). With higher levels of alpha$_2$-globulin, less *Brucella* antibody was made at the 4 wk period. No correlation ($P > 0.10$) was seen between both 19S and 7S antibody levels and Prednisone dosage (Table V), indicating that the steroid therapy did not influence the antibody response in the doses used.

No significant relationship was found between *Brucella* whole serum antibody titers and the presence of renal involvement or with the level of the BUN ($P = 0.10$) (Table II). Only four of the SLE patients had BUN levels above 30 mg/100 ml, and all of these produced significant amounts of antibody. In fact, the patient (F. S.) with the highest level, 80 mg/100 ml, demonstrated large amounts of antibody.

14 of the SLE patients were reimmunized with the same dosage of *Brucella* antigen. The increases in titer between 4 and 12 wk (Table VI) were very similar to those of the initial immunization. Peak titer was again

TABLE V

Relationship of Reciprocal Titers of Brucella Antibody to Steroid Dosage

Patient	Prednisone	Reciprocal antibody titer	
		19S	7S
	mg/day		
M. P.	25	40	0
C. M.	20	0	0
L. T.	20	0	0
H. H.	20	40	40
J. I.	20	160	20
E. H.	15	320	320
E. M.	15	0	0
M. B.	15	40	40
P. B.	12.5	80	0
L. C.	10	20	0
L. B.	10	20	20
F. S.	5	320	640
H. C.	5	80	20
W. W.	5	320	80
C. C.	2.5	20	0
D. G.	0	0	0
I. C.	0	40	20
G. A.	0	20	0
L. H.	0	0	0
D. J.	0	0	0
W. M.	0	20	20
L. F.	0	80	0

TABLE VI

Reciprocal Titer of Brucella Antibody in SLE (Second Immunization)

Patient	Week			
	0 time	1	4	12
E. H.	80	80	80	0
C. C.	0	0	0	0
F. S.	160	160	80	0
P. B.	40	20	80	0
D. G.	20	40	—	80
I. C.	20	20	20	0
H. H.	0	20	—	0
J. I.	40	0	—	0
W. W.	0	20	640	40
M. P.	20	40	40	0
L. C.	40	0	0	0
C. M.	40	—	—	40
E. M.	0	0	0	0
M. B.	40	40	160	80
Geometric mean titers	28	26	48	14

TABLE VII

Reciprocal Titers of Natural Antibodies to E. coli Antigens SLE Compared with Normal Controls*

Antigen	SLE (23 patients)	Normals (18 subjects)	*P* value
011	8	13	NS
014	3	6	NS
026	29	64	0.01
055	18	44	0.005
086	42	141	<0.01
0119	7	12	NS
0112	22	96	<0.01
0124	50	248	<0.005
0125	20	60	<0.01
0127	20	92	<0.001
055:B5	48	70	NS
0126:B16	21	60	<0.01

* Geometric mean titers.

not reached for 4 wk. Study of the individual immunoglobulin titers by testing of sucrose density gradient–separated fractions showed low responses in both IgM and IgG with no gross differences noted.

Studies on natural antibodies. These studies were carried out on the 23 patients with SLE and the 18 normal subjects previously immunized with *Brucella* antigen. In addition, to provide a matched control group with a chronic disease, 18 patients with active pulmonary tuberculosis, who were matched for age, sex, and race with the SLE group, were also studied. The patients with SLE showed significantly lower antibody levels

TABLE VIII

Reciprocal Titers of Natural Antibodies to E. coli Antigens SLE Compared with Pulmonary Tuberculosis Group*

Antigen	SLE (18 patients)	Pulmonary Tbc‡ (18 patients)	*P* value
011	7	9	NS
014	3	6	NS
026	18	30	NS
055	19	34	NS
086	90	96	NS
0112	21	31	NS
0119	54	45	NS
0124	31	53	NS
0125	20	29	NS
0127	19	54	<0.01
055:B5	27	22	NS
0126:B16	20	39	NS

* Geometric mean titers.

‡ Patients matched for race, sex, and age.

TABLE IX

Reciprocal Titers of Natural Antibodies to E. coli and Shigella Antigens on Whole Serum and Gradient Elution Fractions* (±SEM)
SLE Compared with Normal Controls

Antigen	Serum and fractions	SLE (23 patients)	Normals (18 subjects)	*P* value‡
E. coli 011	WS	8 +1.4	13 ±3.0	NS
	19S	0.2 ±0.1	5 ±1.4	<0.001
	7S	2.2 ±0.4	2.4 ±0.6	NS
E. coli 086	WS	42 ±10	141 ±58	<0.01
	19S	2.2 ±0.5	21 ±8	<0.001
	7S	3 ±0.5	3 ±0.5	NS
E. coli 0119	WS	7 ±1.7	12 ±2.9	NS
	19S	1.3 ±0.3	3.6 ±0.6	<0.01
	7S	1.6 ±0.4	1.2 ±0.1	NS
E. coli 055:B5	WS	48 ±10	70 ±20	NS
	19S	0.7 ±0.4	2.6 ±0.6	0.01
	7S	1.8 ±0.5	1.0 ±0.3	NS
E. coli 014	WS	3.0 ±0.8	5.6 ±0.6	NS
	19S	0.8 ±0.2	1.7 ±0.9	NS
	7S	3.2 ±0.5	3.4 ±0.6	NS
Shigella polyvalent	WS	205 ±28	230 ±24	NS
	19S	8 ±1.1	19 ±4	<0.01
	7S	10 ±2.6	4 ±0.5	<0.01

* Geometric mean titers.
‡ Student's *t* test.

TABLE X

Reciprocal Titers of Natural Antibodies to E. coli and Shigella Antigens on Whole Serum and Gradient Elution Fractions* (±SEM)
SLE Compared with Pulmonary Tuberculosis Group

Antigen	Serum and fractions	SLE (18 patients)	Pulmonary Tbc (18 patients)	*P* value‡
E. coli 011	WS	7 ±2	9 ±4	NS
	19S	0.2 ±0.3	4 ±0.6	<0.01
	7S	1.7 ±0.6	0.2 ±0.3	0.01
E. coli 086	WS	90 ±41	96 ±41	NS
	19S	2 ±1.3	23 ±10	<0.001
	7S	3 ±1	2 ±1	NS
E. coli 0119	WS	54 ±25	45 ±25	NS
	19S	1.1 ±0.7	4 ±0.7	<0.01
	7S	1.6 ±0.5	0.2 ±0.5	0.02
E. coli 055:B5	WS	27 ±8	22 ±8	NS
	19S	0.8 ±0.8	5 ±1.6	<0.001
	7S	1.6 ±0.6	0.7 ±0.6	NS
E. coli 014	WS	3 ±1.2	6 ±2.2	NS
	19S	1 ±0.6	1.9 ±0.6	NS
	7S	2.6 ±0.7	1.1 ±0.7	0.03
Shigella polyvalent	WS	167 ±55	192 ±55	NS
	19S	8 ±1.7	21 ±6.9	0.01
	7S	13 ±3.8	8 ±1.7	NS

* Geometric mean titers.
‡ Student's *t* test.

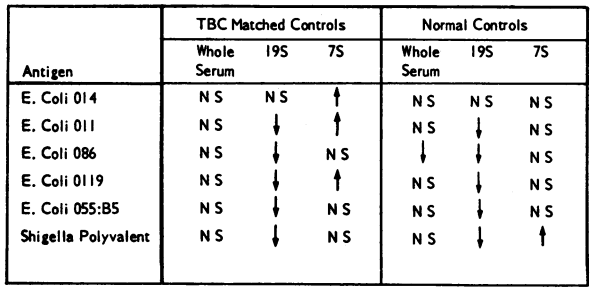

FIGURE 3 Comparison of hemagglutination titer in SLE patients and control groups. NS = not significant; ↑ = significantly higher; ↓ = significantly lower.

than the normal subjects against 8 of the 12 *E. coli* antigens examined (Table VII). However, when compared with the tuberculosis group (Table VIII), the SLE patients showed a significantly lower titer in the case of only one antigen, *E. coli* 0127. Antibody titers to all the other *E. coli* antigens showed no significant differences.

All test sera were fractionated by sucrose density gradient centrifugation and the antibody titers to five *E. coli* antigens and a *Shigella* polyvalent antigen were determined on the separated fractions. Although comparison of whole serum titers of the SLE patients and normal controls (Table IX) showed a significant reduction of the mean titer in the SLE patients against only one of the five *E. coli* antigens tested, the 19S antibody titer in the SLE group was significantly reduced against four of the five antigens. The 7S antibody titers did not differ significantly in the two groups. Comparison of the SLE patients with the matched pulmonary tuberculosis controls showed a similar pattern (Table X). Though none of the whole serum titers differed significantly, 19S antibody titers against four of the five *E. coli* antigens were significantly reduced in the SLE groups. Against three antigens, there was a significant increase of 7S antibody in the SLE patients.

Studies of natural antibodies to Shigella antigens. Whole serum antibody titers were measured against 13 individual *Shigella* antigens and against a polyvalent antigen. Against 5 of the 13 individual antigens, the SLE sera showed significantly lower antibody levels than did the normal controls (Table XI). In the case of the polyvalent antigen, the difference was not statistically significant. Only with respect to one antigen, *S. sonnei,* did the patients with SLE appear to have a significantly higher antibody level than the normal controls.

When the whole serum antibody levels to the same antigens were compared in patients with SLE and matched controls with pulmonary tuberculosis, significantly lower levels were observed against 7 of 13 antigens (Table XII). On testing the polyvalent antigen, the difference between the SLE patients and the pulmonary tuberculosis group again did not show a significant difference.

When density gradient ultracentrifugal analysis of the sera was carried out and the separated fractions tested with the *Shigella* polyvalent antigen, as found previously with the *E. coli* antigens, there was a statistically significant decrease in titer of macroglobulin antibody in the patients with SLE when compared with the normals (Table IX). A significant increase of 7S antibody was also observed in the SLE patients. The matched

TABLE XI

Titers of Natural Antibodies to Shigella Antigens SLE Compared with Normal Controls

Antigen	SLE (23 patients)	Normals (18 patients)	*P* value
1A	17	34	<0.01
1B	21	38	NS
2A	22	38	<0.01
2B	27	50	<0.01
3A	23	55	<0.001
3B	25	38	NS
3C	31	34	NS
4	12	25	NS
4A	24	44	0.01
4B	39	41	NS
5	21	34	NS
6	55	75	NS
Sonnei	63	34	0.02
Polyvalent	205	230	NS

TABLE XII

Titers of Natural Antibodies to Shigella Antigens SLE Compared with Pulmonary Tuberculosis Group

Antigen	SLE (18 patients)	Pulmonary Tbc* (18 matched patients)	*P* value
1A	19	42	0.01
1B	24	54	<0.01
2A	21	38	NS
2B	29	90	0.01
3A	22	77	<0.001
3B	26	90	<0.01
3C	30	102	<0.001
4	14	10	NS
4A	27	18	NS
4B	38	51	NS
5	21	51	0.01
6	61	102	NS
Sonnei	70	58	NS
Polyvalent	167	192	NS

* Patients matched for race, sex, and age.

tuberculosis controls (Table X) also showed a significantly lower level of macroglobulin antibody than the SLE patients but no significant difference in the light antibody. A summary of the antibody titers of whole serum and separated gradient fractions is compiled in Fig. 3 for the *E. coli* antigens tested and the *Shigella* polyvalent antigen.

DISCUSSION

There have been a number of studies of the antibody response in SLE. Sarkany (3) found no significant difference from normal controls in the response to tetanus toxoid in 18 patients with discoid lupus erythematosus and 5 with SLE. Muschel (4), studying patients with SLE, hospitalized individuals and healthy young men, found no difference in titer of blood group isoagglutinins and antibody to *Proteus* O X-2 in the three groups, although there was a somewhat reduced titer of antistreptolysin O in the SLE patients. Lee and coworkers (14) found no difference in the response to a Rickettsial antigen between SLE patients and a control group. Meiselas, Lee, Zingale, and Richman (1), on the other hand, observed a greater response to stimulation with *Brucella* in patients with SLE than in a control group with other diseases, although not as high as in patients with rheumatoid arthritis. In this study, the inoculum used was five times as large as the dose employed in the present investigation. Two studies (16, 17) have subsequently failed to confirm the increased *Brucella* antibody response of patients with rheumatoid arthritis.

Whereas chronic *Brucella* infection is associated mainly with 7S antibody, the serum contains IgM antibody early in the course of infection (18–20). In the present experiments, and as previously noted by Waller, Ellman, and Toone (17), most of the antibody produced in the normal subjects in response to immunization with *Brucella* was macroglobulin antibody. In the SLE patients, the levels of macroglobulin antibody were significantly lower, and the levels of 7S antibody at 1 and 4 wk were slightly but not significantly higher in the patients with SLE than in the controls.

The effect of steroid administration on antibody formation is a factor for consideration in this study since 16 of the 23 SLE patients were receiving steroids at the time of immunization. The dosage administered to these patients was relatively low (average, 13 mg; range, 2.5–25 mg). However, no significant correlation between the steroid dosage and whole serum, 19S or 7S antibody titers to *Brucella* antigen was found in the SLE group. Inhibition of antibody production by steroids has been investigated in a number of species, using a variety of antigens (21, 22). Mild suppression of the primary response in the mouse and rat has usually required doses of from 100 to 200 mg/kg of prednisolone. It is of interest that a study of the effect of Prednisone on *Brucella* antibody formation in the rat has actually shown an enhancement of antibody formation (23). In man, dosages equivalent to or greater than those used in the present study did not affect antibody production (24, 25).

Patients with renal disease in a state of advanced uremia have been shown to have a diminished humoral antibody response (26). In the present study, however, the response to *Brucella* antigen appeared to be uninfluenced by the presence of renal involvement. This was presumably due to the fact that only one patient in the SLE group (F. S.) had a BUN level over 40 mg/100 ml. In fact, this patient, whose BUN was 80 mg/100 ml, produced large amounts of antibody. Further evidence for lack of influence of the renal disease on antibody titer was the failure to observe any statistical correlation between the BUN and *Brucella* antibody titer.

Since it has been observed that immunoglobulin levels in blacks tend to be higher than in whites (27), it is of interest that the predominantly white control subjects had a higher antibody response to *Brucella* antigen than the predominantly black SLE group, providing evidence that race was not a factor in the reduced response observed in the SLE patients. Nevertheless, since the control subjects in the *Brucella* immunization studies were mainly white, for the natural antibody studies, an additional control group was carefully matched for race as well as age and sex. In order to take into account the factor of chronic disease, moreover, this group was selected from patients with active pulmonary tuberculosis under treatment.

The titers of the *E. coli* natural antibodies of the control subjects were similar to those reported by Kunin (28) and Neter and associates (7) in normal populations. The latter investigations tested large groups of normal subjects with several enteropathogenic *E. coli* and found that almost all individuals above the age of 12 had antibody to the organisms tested. Similarly, the titers to the *Shigella* organisms measured in the control subjects of the present study are consistent with those reported by Young, Lee, and Branche (8) in their population studies. It is of interest that when antibody titers to rat feces antigen, which cross-reacts with *E. coli* 014 (29), were measured in patients with thyroiditis, rheumatoid arthritis, scleroderma, and SLE, the antibody levels of the patients with SLE were lower than in the other groups (30).

In the present experiments, the natural antibody titers to *E. coli* in the normal controls were significantly higher than those of the SLE patients against 8 of 12 antigens studied. There was little difference in whole serum titer, however, in the case of the matched tuberculosis controls. With regard to the individual *Shigella* antigens, the SLE patients showed significantly lower titers than

the normal group against 5 of 13 of the antigens studied, and when compared with the tuberculosis controls against 7 of the 13 antigens.

To compare the titers of the 19S and 7S natural antibodies to *E. coli* and *Shigella* in SLE with those of the control groups, five *E. coli* antigens and a *Shigella* polyvalent antigen, all selected on the basis of availability, were used. The SLE patients showed significantly lower 19S antibody titers compared with both control groups against four of the five *E. coli* antigens and the *Shigella* polyvalent antigen. It is of interest that against four of the antigens, the titer of 7S antibody was significantly greater in the SLE patients than in the controls (Fig. 3).

Since patients with SLE are prone to elaborate a number of autoantibodies (31), the possibility must be considered that the decreased antibody formation noted in SLE may be a result of antigen competition (32) provided by the synthesis of these autoantibodies. This would presume that the SLE patient does not respond adequately to an exogenous antigen because a significant fraction of available immunologically competent cells is already committed to autoantigens. The fact that macroglobulin antibody is most affected could be a consequence of the fact that the 19S antibody compartment may be capable of only limited expansion in the face of antigenic stimulation by multiple cellular antigens. It is of interest that animals with Aleutian mink disease, a viral disease associated with marked hypergammaglobulinemia, which has features similar to those seen in human connective tissue diseases, have been noted by Kenyon (33) to respond with decreased antibody formation to a bacterial antigen.

The decreased whole serum titers and 19S antibody response to bacterial antigens noted in the patients with SLE may be of significance in relation to the common occurrence of infection in this disease. Specific IgM deficiencies have been reported in association with infection, especially respiratory infection (34, 35). Stiehm and Fudenberg (34) have found low IgM levels and increased IgG and IgA levels in mongoloids who have a high death rate due to pneumonia. Multiple infections have also been seen (36–39) in patients with deficiencies of IgA and IgM but with normal IgG levels. Clinical evidence for decreased resistance to infection in SLE has been given by a number of authors. Dubois (40) has reported the occurrence of bacterial pneumonia in 31% of 520 cases and Talbott and Ferrandis (41) have concluded that the most common cause of death in SLE in the preantibiotic and presteroid era was infection. Similarly, Jessar, Lamont-Havers, and Ragan (42) in a review of cases of SLE seen between 1937 and 1952 noted that lobar or bronchial pneumonia was a frequent finding at postmortem. Tumulty (43) and Harvey and coworkers (44), have also emphasized the deficient capacity of patients with SLE to resist infection. Thus, infection has been shown to play a prominent role in the morbidity and mortality of SLE and this has been found to be true not only in those series collected after the introduction of steroid therapy (31, 41), but also in series accumulated mainly in the presteroid era (42, 44, 45).

The role of macroglobulin antibody in protection against bacterial infection has been supported by several recent studies (46, 47). IgM antibodies are more active than IgG antibodies in agglutination and in lytic reactions (48). Therefore, macroglobulin antibody would appear to be of greater importance in the initial defense of the host against bacterial invasion (49). For these reasons, the observed lowered macroglobulin antibody levels in the patients with SLE may bear significantly upon the increased frequency of infection described.

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