Systemic Lupus Erythematosus and Normal Antibodies. (26422)

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Systemic lupus erythematosus (SLE) is a disease marked by an abnormality in immunological reactivity and is generally considered to represent an autoimmune disease *par excellence*. The sera of patients with this condition are capable of giving typical serological reactions with cell nuclei, nucleoprotein and deoxyribonucleic acid, cytoplasmic constituents, saline extracts of kidney, liver, and thymus tissues, thrombocytes and clotting factors(1,2). Syphilitic reagin or Wassermann antibody, unrelated to leutic infection, directed against lipid substances of tissue origin is also present occasionally. The serum substances responsible for these reactions are globulins, relatively heat stable and capable of complement fixation; presumably, they are antibodies.

The impression has arisen, therefore, that the SLE patient is a prolific antibody producer. Antibodies described in SLE patients are all directed against autologous antigenic substances, but little evidence is available if enhanced amounts of circulating antibodies to substances antigenically unrelated to mammalian tissue are also present. Recently, 4 of 12 SLE patients have been reported to possess hemagglutinating antibody to penicillin in contrast to the absence of detectable levels of this antibody in 31 other patients after completing a course of penicillin therapy(3). On the other hand, after inoculation with vaccines of brucellae and rickettsiae, antibacterial and antirickettsial antibodies appeared in patients with SLE at comparable times and in comparable titers to those observed in a control group of patients (4).

The present study was undertaken to determine whether the enhanced antibody levels in patients with systemic lupus erythematosus were restricted to autoantigens or whether their altered immunological reactivity extends to other antigenic materials as reflected by so-called normal antibody concentrations. The origin of the blood group isoantibodies and of other normal antibodies against microbes or microbial products is not known. In some instances, they may arise by immunization against the homologous or related microbes which find their way into the tissues during life(5). Other normal antibodies are possibly merely normal physiological constituents of serum which fortuitously possess a complementary configuration to microbial antigens(6). In any event, determinations were made of the relative levels of blood group isoantibodies and of other so-called normal antibodies to a gram-negative bacterium selected at random, *Proteus* OX-2, and to streptolysin O, an enzymic product of the gram-positive *Streptococcus* in a group of SLE patients, other hospital patients and normal individuals.

Materials and methods. Sera. The test specimens consisted of 11 sera from SLE patients all of which showed marked complement fixation reactions with calf thymus nucleohistone antigen(7). Control specimens consisted of 12 sera from hospital patients selected at random in whom clinical and serological evidence of systemic lupus erythematosus was lacking and 12 sera from healthy young men who had passed a rigorous physical examination for entrance into the U. S. Military Academy at West Point, N. Y.

Saline isoagglutinins: Serial dilutions of the serum in 2-fold steps were made in 0.85% NaCl starting at 1/4. Each dilution was contained in 0.1 ml. To each dilution, 0.1 ml of a 2% saline suspension of group A_1 red cells was added and the test tubes containing the mixtures were shaken and allowed to stand at room temperature for 30 min, centrifuged lightly (500 g) for 2 min and read macroscopically with the aid of a microscope mirror. The reciprocal of the highest dilution

TABLE I. Blood Group Isoagglutinin Titers in SLE Patients, Other Patients and Normal Individuals.

Anti-A

SLE			Other patients			Normal		
Serum No.	Blood group	Saline titer	Serum No.	Blood group	Saline titer	Serum No.	Blood group	Saline titer
1	O	32	3	B	64	3	B	64
5	O	256	4	O	128	4	B	32
10	O	32	6	O	32	5	O	64
11	B	8	8	O	16	6	O	32

Anti-B

SLE				Other patients			Normal			
Serum No.	Blood group	Saline titer	Indirect Coombs	Serum No.	Blood group	Saline titer	Serum No.	Blood group	Saline titer	Indirect Coombs
1	O	16		1	A	8	1	A	64	
2	A	32		2	A	64	2	A	16	
3	A	8	32	4	O	32	5	O	8	32
4	A	8	16	5	A	8	6	O	2	8
5	O	8	8	6	O	32	7	O	32	64
6	A	16	16	7	A	8	8	O	16	32
8	A	128		8	O	16	9	O	32	
10	O	64		9	O	16	11	A	8	
11	B	32		10	A	8	12	O	8	
				12	O	16				
Geometric mean		22				16			14	

Note: SLE sera No. 7 and 9 and normal serum No. 10 were from Group AB patients. Other patients serum No. 11 was from a Group B individual.

giving visible agglutination was considered the titer. A limited number of sera were also subjected to the indirect Coombs technic. The unagglutinated saline suspended cells were washed 3 times, and one drop of anti-human serum was added to each tube. The tubes were then allowed to incubate at 37°C in a water bath for 30 min, and final readings were made after centrifugation in the same manner as for the saline isoagglutinins. *Antistreptolysin O* determinations were performed with streptolysin O reagent, product of Difco Laboratories, as described by Rantz and Randall(8). *Proteus* OX-2 agglutinin levels were determined by mixing equal volumes of 2-fold serial dilutions of test sera with the bacterial suspension and incubating the mixtures at 52°C for 16 hours. The tests were read macroscopically and the titers determined in a standardized manner (9).

Results. Blood group isoantibodies. Anti-A isoagglutinin determinations were made on sera from individuals of blood groups O and B and anti-B determinations on groups O and A. This was necessary because of the absence of detectable anti-A in the blood groups A and AB individuals and absence of anti-B in those of blood groups B and AB. The results of the isoagglutinin determinations are given in Table I. The differences between SLE and other sera were obviously not marked. Mean anti-A level of the SLE sera was not significantly lower than those of the other groups and although anti-B levels were higher with the SLE sera, analysis of variance of anti-B results indicated greater variation within the 3 groups of sera than between them. Moreover, the indirect Coombs test results performed on 4 random SLE and 4 normal sera (Table I) did not indicate that SLE patients produced inordinately large amounts of incomplete antibody.

Proteus and Streptolysin O antibody determinations. The results of the tests for *Proteus* OX-2 agglutinins and antistreptolysin O (Table II and III) demonstrated that SLE patients do not possess elevated levels of these antibodies; in fact, their antistreptolysin O titers were lower than those of the normal subjects.

Finally, no association was observed be-

TABLE II. *Proteus* OX-2 Agglutinin Titers in SLE Patients, Other Patients, and Normal Individuals.

SLE		Other patients		Normal	
Serum No.	Titer	Serum No.	Titer	Serum No.	Titer
1	<10	1	20	1	20
2	80	2	40	2	20
3	20	3	20	3	10
4	160	4	320	4	80
5	10	5	80	5	10
6	<10	6	20	6	10
7	20	7	20	7	80
8	20	8	20	8	40
9	10	9	<10	9	10
10	40	10	<10	10	20
11	10	11	20	11	<10
		12	20	12	20
Geom. mean*	19		24		19

* Geometric mean.

tween antibody levels determined in this study and nucleohistone antibody levels of the SLE patients. SLE patients No. 2, 3, 11 had nucleohistone complement fixation titers of 243 or greater, whereas serum titers of the other SLE patients were not greater than 27. Yet sera of patients No. 2, 3, 11 were among the lowest in *Proteus* OX-2 agglutinins and antistreptolysin O.

Discussion. SLE has been considered to be a disease associated with an unusually productive antibody synthesizing system. That the SLE patient possesses serum factors, often at extremely high levels, capable of reacting with autogeneous materials has been amply documented. Limited evidence for hyperreactivity, particularly to rare blood group substances(10) and antibiotics (3,11) has also been reported. Although the sample tested was small and the technics have a large inherent error, the results of this study do not, however, lend support to the concept that SLE patients produce abnormally large amounts of circulating antibodies directed against foreign antigens. If the reasonable assumption is made that the exposure of SLE patients to *Proteus, Streptococcus* and blood group antigens was comparable to that of the control groups, then SLE patients do not possess unusual reactivity against these foreign antigens, at least as far as circulating antibodies are concerned. The studies involving deliberate immunization with bacterial and rickettsial vaccines(4) provide even stronger evidence for this premise. However, if normal antibodies determined in this study are unrelated to immune processes, the conclusion must be reached that the cells or processes involved in their production are probably normal in the SLE patient in contrast to other immunological abnormalities in these patients. It would seem reasonable, therefore, to investigate the possibility that the most significant immunological aberration in SLE patients will be found in the delayed or cellular hypersensitivity reaction or in reactions unknown at present. Preliminary findings for an autoimmune reaction of a delayed type to leucocyte homogenates in SLE patients has been reported(12), but it would be desirable to extend these observations to foreign antigens.

These results that indicate that SLE patients do not produce abnormally large amounts of circulating antibody to foreign antigens may be related to certain phenomena in immune tolerance. Immune tolerance is relatively easily induced to implantable cells and to soluble antigens of vertebrate origin but impossible with most microbial antigens(13). Thus, normal individuals do not ordinarily form autoantibodies because they are "exposed" to their own cells *in utero* and thus become incapable of reaction against these cellular antigens. In SLE

TABLE III. Anti-Streptolysin O Titers in SLE Patients, Other Patients, and Normal Individuals.

SLE		Other patients		Normal	
Serum No.	Titer	Serum No.	Titer	Serum No.	Titer
1	12	1	166	1	12
2	12	2	100	2	12
3	12	3	250	3	125
4	100	4	125	5	12
5	100	7	125	7	12
6	166	8	100	9	50
7	12	9	12	10	12
8	<12	10	333	11	100
9	12	11	166	12	100
11	<12	12	125		
Geom. mean*	21		118		29

* Geometric mean.

The sera not tested were in insufficient quantity for the anti-streptolysin O determination.

patients, certain immunologically competent cells may "forget" their early exposure by mutation to a pattern corresponding to a tissue antigen with a concomitant loss of homeostatic controls on number of cells(13). Precisely how such cells arise and proof of their existence remain to be determined. On the other hand, the abnormal immunological reactions in SLE may result simply from autologous antigens altered by contact with microbial agents or their toxins. Such alterations may change certain cellular structures so that they are no longer recognizable by antibody producing cells, and these altered autologous antigens may provide the direct incitant for autoimmune disease. The antibodies produced may be cross reactive with normal structures and thus the auto-immunization process initiated. Certain disease processes elicit elevated levels of tissue autoantibodies(14), but whether these autoantibodies are similar to those seen in SLE patients remains to be determined. The normal agglutinin and antistreptolysin O levels found in SLE patients in this study represent antibodies to foreign antigens and cannot contribute to the question whether the etiology of autoimmune disease is to be attributed to altered normal cellular constituents. The results indicate, however, that SLE patients are not greatly abnormal in their production of so-called normal antibodies to foreign antigens.

Summary. SLE patients do not possess higher levels of blood group isoagglutinins, *Proteus* OX-2 agglutinins or antistreptolysin O than other patients or normal individuals. The immunological hyperreactivity of these patients may be restricted, therefore, to circulating autoantibodies and possibly to the delayed or cellular hypersensitivity reaction.

1. Miescher, P., Strassle, R., in *Immunopathology,* 1st Internat. Symposium, 1958, ed. by P. Grabar, P. Miescher, Basel/Stuttgart, 1959, Benno Schwabe Co., p. 454.

2. Asherson, G. L., *Brit. J. Exp. Path.,* 1959, vXL, 209.

3. Harris, J., Vaughn, J., *J. Clin. Invest.,* 1960, v39, 995.

4. Lee, S. L., Mieselas, L. E., Zingale, S. B., Richman, R., *ibid.,* 1960, v39, 1005.

5. Springer, G., Horton, R. E., Forbes, M., *J. Exp. Med.,* 1959, v110, 221.

6. Wilson, G. S., Miles, A. A., Topley and Wilson's *Principles of Bacteriology and Immunology,* 4th ed., Baltimore, 1955, Williams & Wilkins, vII, 1234.

7. Scalettar, R., Marcus, D. M., Simonton, L. A., Muschel, L. H., *New Eng. J. Med.,* 1960, v263, 226.

8. Rantz, L. A., Randall, E., PROC. SOC. EXP. BIOL. AND MED., 1945, v59, 22.

9. *Methods for Medical Laboratory Technicians,* Dept. of the Army Technical Manual 8-227, Washington, 1951, U. S. Govt. Printing Office, p387.

10. Callender, S., Race, R., *Ann. Eugenics,* 1946, v13, 102.

11. Muelling, R., Beven, T., Samson, R., Jenevein, E., Guillory, J., *Am. J. Clin. Path.,* 1958, v29, 503.

12. Holman, H. R., *Am. J. Med.,* 1959, v27, 525.

13. Burnet, F. M., *Perspectives in Biol. & Med.,* 1960, v3, 447.

14. Muschel, L. H., Simonton, L. A., Wells, P. A., Fife, E., *J. Clin. Invest.,* 1961, in press.

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Localization of Acetylcholinesterase in the Neurohypophysis and its Functional Implications.* (26423)

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The neurosecretory theory of functioning of the hypothalamico-neurohypophyseal system is now generally accepted. According to this concept, vasopressin and oxytocin, or their precursors, are elaborated within cell bodies of neurons of the supraoptic and paraventricular nuclei, and transported *via* the

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