Serum Heat-Labile Opsonins In Systemic Lupus Erythematosus

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ABSTRACT To study possible mechanisms responsible for the increased susceptibility to infection of patients with active systemic lupus erythematosus (SLE), a study of the serum heat-labile opsonic capacity (HLOC) in such patients was undertaken. With leukocytes from normal donors, the sera of 12 of 30 patients with active SLE demonstrated decreased HLOC for *E. coli 075*. The phagocytic activity was partially restored by normal serum, suggesting that decreased HLOC was responsible for the defective phagocytosis. While 8 of 10 patients with active SLE and concomitant infections showed deficient opsonic capacity to *E. coli 075*, only 4 of 20 such patients without infections showed the defect ($P = 0.01$). None of 12 patients with inactive disease had deficient opsonic capacity. Similar results were obtained with *S. aureus 502A* as the test bacterium. In the patients surviving infection, recovery of normal serum opsonic capacity was rapid and usually coincided with an increase of serum complement to normal levels.

In three patients with active SLE and infection, the causative microorganisms were isolated and opsonic capacity for these organisms tested with the individual patients' sera. In each case, sera obtained at the onset of the infectious episode had low opsonic capacity when compared with normal sera.

Serum C3 proactivator levels were low in 9 of 11 sera with deficient opsonic capacity. However, similar low values were found in other SLE sera with normal HLOC, suggesting that other factors of the opsonic system were also depleted. Addition of the classical complement components C1, C4, C2, C3, and C5 to sera with deficient HLOC failed to restore activity. Addition of pure C3 proactivator also failed to restore activity. However, addition of C3 proactivator together with 50°C-heated normal serum restored activity, indicating that factors active at the early steps of opsonic activation via the alternate pathway of complement are necessary to restore opsonic activity.

These findings indicate that in active SLE, a decrease of components of the alternate pathway of complement activation results in an acquired defect of serum HLOC and perhaps other related complement-mediated functions. This defect may be an important factor in the increased susceptibility to infections of patients with active systemic lupus erythematosus.

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INTRODUCTION

The increased susceptibility of patients with active systemic lupus erythematosus (SLE)[1] to bacterial and mycotic infections has been well documented (1–8). Although the introduction of corticosteroid therapy may currently be a contributing factor, infections constituted a frequent cause of death in the precorticosteroid era (1–5). Furthermore, infections frequently occur at times of overt clinical activity (8).

The possible causes of the increased susceptibility to infections in SLE are not known. Nevertheless, some of the known cellular and humoral derangements found in this disease may provide a pathophysiologic mechanism. A decrease in IgM antibody titers to bacterial antigens has been reported by Baum and Ziff (9). Deficient cellular phagocytic function may play a role

[1] *Abbreviations used in this paper:* C3PA, C3 proactivator; C'H50, hemolytic complement; HLOC, heat-labile opsonic capacity; SLE, systemic lupus erythematosus.

(10), since leukopenia and cytotoxic antibodies to circulating white cells are commonly present in active SLE (11, 12). Furthermore, low serum complement levels are characteristic of active SLE; and this acquired deficiency may also be responsible for increased susceptibility to infection (13).

Since the development of an infectious episode may depend on the early events after invasion by the infectious agent, nonspecific defense mechanisms, independent of specific antibody may be of particular importance in the susceptibility to infection in this disease. Therefore, a study of the phagocytic function mediated by the serum heat-labile opsonin system was undertaken in patients with SLE. It was considered desirable to study the heat-labile opsonin system, because complement components are known to participate in its action (14–17) and because heat-labile opsonization of bacteria and fungi, presumably in the absence of specific antibodies

TABLE I
Characteristics of 30 Patients with Active SLE

Patient	C'H50	C3*	HLOC‡	Leukocyte count	PMN‡	Prednisone	Infectious episode
	U	*mg/100 ml*		*cells/mm³*	%	*mg/24 h*	
P. C.	ND	80	↓‡	4,200	69	20	Lobar pneumonia
F. C.	ND	54	↓	4,900	62	20	Gram-negative pneumonia and septicemia
J. G.	15	64	↓	7,100	79	0	Lobar pneumonia
R. W.	20	36	↓	4,500	84	30§	Staphylococcal cellulitis
D. J.	30	42	↓	1,200	75	18	Pneumococcal pneumonia and septicemia
M. H.	5	41	↓	4,200	76	0	Staphylococcal pericarditis
J. S.	13	29	↓	3,400	84	0	Pneumococcal pneumonia
J. W.	45	66	↓	4,100	88	0	β-hemolytic streptococcal septicemia and meningitis
B. P.	40	62	Nl‡	4,600	85	15	*E. coli* meningitis and septicemia
H. H.	ND‡	150	Nl	8,400	80	30	Gram-positive bacterial meningitis
L. S.	10	36	↓	8,200	81	0	—‡
L. N.	20	47	↓	3,500	57	0	—
A. A.	ND	160	↓	5,550	69	0	—
J. U.	5	41	↓	7,200	51	20	—
P. C.	30	143	Nl	4,000	64	5	—
B. H.	ND	82	Nl	3,050	96	30	—
D. S.	ND	69	Nl	14,500	72	80‖	—
G. W.	ND	89	Nl	4,350	72	40	—
M. R.	ND	204	Nl	3,050	77	20	—
D. J.	20	68	Nl	2,500	50	20	—
N. W.	50	64	Nl	9,700	77	0	—
M. W.	30	74	Nl	8,900	73	10	—
R. A.	10	52	Nl	4,800	47	0	—
L. W.	27	75	Nl	6,800	90	0	—
S. B.	23	72	Nl	9,000	ND	60¶	—
M. E.	80	70	Nl	5,000	43	0	—
R. C.	25	84	Nl	8,000	70	40	—
G. H.	10	85	Nl	8,800	73	0	—
N. B.	55	175	Nl	15,000	91	12.5	—
D. P.	30	57	Nl	5,200	85	100	—

* Normal values: C'H50, Mean: 80 U (range 60–110). Serum C3, mean: 140 mg/100 ml (range 100–180).
‡ Tested with *E. coli 075*. PMN, polymorphonuclear leukocytes. ↓ = Decreased. Nl = Normal. ND, not done. —, no infection.
§ Received 75 mg of cyclophosphamide daily for 1 wk.
‖ Received whole blood transfusion of 2 U 3 days previously.
¶ Received 50 mg of cyclophosphamide daily for 1 wk.

(18–20), can be demonstrated in most normal sera. The results obtained in this investigation indicate that the onset of infections in active SLE coincides with a marked decrease in serum heat-labile opsonic capacity (HLOC), and that the deficiency may be the result of the functional depletion of the alternate pathway of complement activation.

METHODS

Clinical material. Serum was obtained as far as was possible from every patient with SLE admitted to Parkland Memorial Hospital, Dallas, during the period of this study. In addition, random serum specimens were obtained from patients attending the outpatient department. A total of 34 patients were studied. Of these, 8 were included at different times in both the active and inactive disease groups. Donors of normal serum and blood leukocytes were either laboratory workers or patients admitted to the orthopedic wards with no evidence of systemic disease.

Criteria for SLE activity. Clinical manifestations of SLE, such as the presence of fever not responding to antibiotic therapy, skin rash, arthritis, serositis, glomerulonephritis, organic psychosis, and focal neurological signs were used to arrive at the diagnosis of SLE activity. 30 of the patients studied were considered to be clinically active (Table I). Serum complement levels were low in 27 of these patients. 12 of the active patients were untreated at the time of testing. The rest were treated with corticosteroids, in daily dosage ranging from 10 to 100 mg of prednisolone. Two patients received cyclophosphamide for 3 and 7 days before testing HLOC (Table I). When HLOC was measured in patients with infection, serum was obtained either before or within 48 h after the onset of overt clinical symptoms of infection, with one exception. In this case serum was obtained 3 wk after the onset of pneumococcal pneumonia.

Phagocytosis. A modification of the method of Hirsch and Strauss (21, 22) was used. Peripheral blood leukocytes from heparinized blood were obtained from normal donors by differential sedimentation of erythrocytes upon addition of 10% vol/vol of a 6% solution of dextran 250 (Pharmacia Fine Chemicals, Uppsala, Sweden) in saline solution. The leukocytes were washed twice in Hanks' solution containing 0.1% gelatin (Difco Laboratories, Detroit, Mich.) and adjusted to a final concentration of 10^7/ml. Sera were obtained from clotted blood incubated at room temperature for 1 h and stored at −70°C until used. The sera from patients treated with antibiotics were dialyzed overnight against large volumes of Hanks' solution at 4°C. Serum dilutions were made with Hanks' solution containing 0.1% gelatin and 50% fetal calf serum heat-inactivated at 56°C for 30 min. For the reconstitution experiments the fetal calf serum was omitted. Measurements of HLOC were carried out with two standard microorganisms: *Escherichia coli 075* and *Staphylococcus aureus 502A*. These were cultured for 16–18 h in trypticase soy broth. *Pneumococci* were cultured in Todd-Hewitt broth. The bacterial suspensions were washed twice in cold sterile saline solution by centrifugation at 8000*g* for 30 min. Bacterial concentration was adjusted at 650 nm for *E. coli* and *Pneumococcus* and 620 nm for the *Staphylococcus* to an optical density of 0.6, corresponding to $1–8 \times 10^8$ bacteria/ml. The dilution from the stock bacterial suspension varied from 1 : 10 to 1 : 50, depending on the microorganism, and was established for each in preliminary experiments. Testing was carried out in sterile 12 × 75-mm polystyrene tubes (Falcon Plastics, Division of B-D Laboratories, Inc., Los Angeles, Calif.) containing 5×10^6 leukocytes from normal donors, test serum usually at a final concentration of 5, 2.5, or 1%, and 10^6–10^7 bacteria in a final volume of 1 ml. Duplicate samples were incubated at 37°C and rotated end-over-end at 12 rpm for 1 and 2 h in most cases. At the end of the incubation period the tubes were centrifuged at 50*g* for 5 min and the supernates used to make five 10-fold dilutions in ice-cold sterile saline solution. Bacterial quantitation was achieved by colony counting of four dilutions plated on MacConkey agar for *E. coli,* blood-agar for the *Pneumococcus,* and mannitol-salt agar or blood agar plates for the *Staphylococcus.* For most experiments, controls consisted of tubes containing serum heat-inactivated at 56°C for 30 min and tubes without leukocytes to rule out possible bactericidal effects mediated by serum alone. In addition, duplicate tubes were kept at 0°C for the determination of the initial bacterial concentration (zero time) in each experiment.

Complement components. Human C3 proactivator (C3PA) was purified from pooled normal serum by the method of Götze and Müller-Eberhard (23). The final preparation was over 98% pure as assessed by polyacrylamide electrophoresis, with a trace of IgG detected by agarose immunodiffusion. Human C3 and C5 were purified by the method of Nilsson and Müller-Eberhard (24). Functionally pure human C2 and C4 were obtained from Cordis Laboratories, Miami, Fla. A crude preparation of human C1 was obtained by the method of Nelson (25).

Hemolytic complement was measured by microtitration according to the technique of Nelson, Jensen, Gigli, and Tamura (26). Normal values ranged from 60–110 U with an average of 80 U. Radial immunodiffusion plates for the determination of C3 were obtained from Hyland Div., Travenol Laboratories, Inc., Costa Mesa, Calif. Normal values ranged from 100 to 180 mg/100 ml with an average of 140 mg/100 ml. Serum C3PA levels were measured by the radial immunodiffusion method (27) with monospecific rabbit antisera. Some of the plates used were a gift of Dr. Müller-Eberhard. Normal values ranged from 150 to 300 μg/ml with an average of 216 μg/ml.

Statistics. Student's *t* test for nonpaired samples, the chi square test, and least squares regression analysis were used.

RESULTS

Opsonic capacity for E. coli 075 *and* S. aureus 502A. Fig. 1 shows the average of results obtained with *E. coli 075* with sera and leukocytes from 23 normal subjects. In every experiment using normal serum at 5% concentration, phagocytosis of more than 90% (1 log unit) of the initial number of bacteria took place. Increasing the serum concentration did not change the results appreciably; however, a decrease to 2.5% serum concentration resulted in a marked decrease of phagocytic activity of several normal sera. It was therefore decided to use 5% as the standard concentration in experiments with *E. coli 075.* To insure that the opsonins detected were heat-labile, control tubes always included test serum previously heated at 56°C for 30 min.

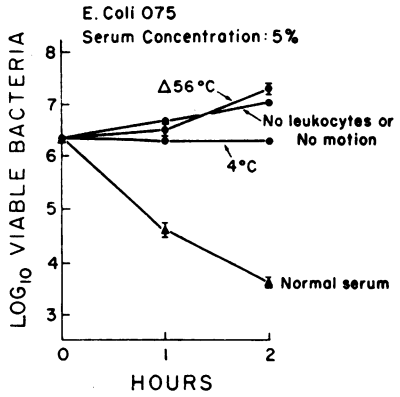

FIGURE 1 In vitro phagocytosis of *E. coli 075* with 5% normal serum and blood leukocytes from 23 normal subjects. Brackets indicate ±SEM. Notice the absence of phagocytosis when sera were previously heated at 56°C for 30 min, when leukocytes were omitted, and when tubes were kept at 4°C or not rotated.

As shown in Fig. 1, the number of bacteria in the heated controls increased over the baseline tubes kept at 4°C, indicating that growth occurred during the 2-h incubation period. Similarly, in controls omitting leukocytes or rotating motion, which were included to ensure that the decrease in the number of bacteria was not due to serum bactericidal activity, no evidence of such bactericidal activity was detected in the 17 normal sera so tested.

In order to rule out the possibility that the abnormal phagocytic function measured in patients with active SLE was due to a decrease in phagocytic activity by the patients' leukocytes, as has been reported (10), normal leukocyte donors were used in all the experiments reported here.

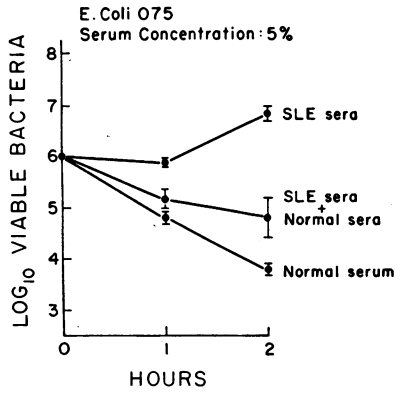

FIGURE 2 Effect of addition of equal volumes of normal serum to SLE serum with deficient HLOC. The final concentration of each serum was 5%. The mean values of 16 different experiments are shown. Brackets indicate ±SEM.

Since the leukocytes were centrifuged at the end of the incubation period, an effort was made to rule out possible cosedimentation of bacteria attached to leukocytes or the presence of live intracellular microorganisms. In five experiments using leukocytes from controls or patients with SLE, the cells were resuspended, lysed in water, and tested for the presence of viable bacteria. In all cases, bacteria were not detected, indicating that they were killed rapidly after phagocytosis.

The possibility that the sera from SLE patients contained a cytotoxic factor for leukocytes (11, 12) was also ruled out. Addition of equal volumes of normal serum to heat-labile opsonin-deficient SLE serum resulted in almost complete restoration of opsonic capacity in 12 of 16 experiments. Fig. 2 shows the average results obtained for the 16 experiments. After 2 h of incubation, there was an average decrease of more than 90% of the initial number of bacteria.

HLOC of normal sera, sera from patients with miscellaneous acute infections, and SLE sera from patients with and without infections are shown in Fig. 3, after 1 and 2 h of incubation with *E. coli 075* as the test bacterium. There were 10 patients with active SLE and concomitant infectious episodes (see Table I for types of infection). In this group, 8 of the 10 patients had decreased HLOC as indicated by the failure to reduce the initial number of bacteria by 90% or more (1 log unit) after a 2-h incubation. One of the patients (B. P.) with normal HLOC for *E. coli 075* had decreased opsonic capacity when tested with the *E. coli* responsible for her

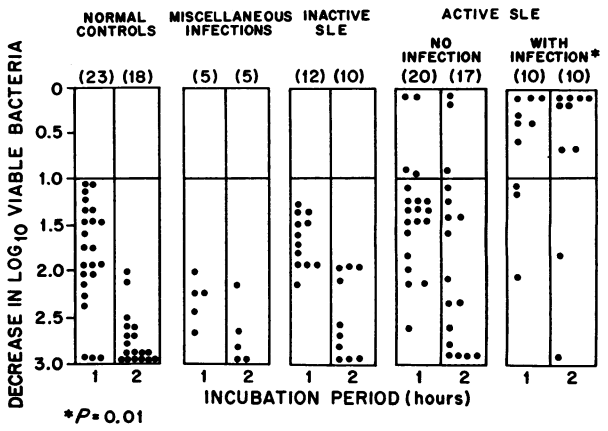

FIGURE 3 HLOC of normal and SLE sera against *E. coli 075*. Blood leukocytes obtained from normal donors were used in all experiments. A decrease of 1 log unit represents phagocytosis of 90% of the initial number of bacteria after 1 or 2 h of incubation. Individual values of normal sera, sera from patients with acute infections, and sera from SLE patients with and without concomitant infection are shown. The difference between the SLE groups with and without infection was highly significant ($P = 0.01$).

infection, as will be discussed below. Of the remaining 20 active patients without infection, only 4 had decreased HLOC ($P = 0.01$) and none of 12 inactive SLE patients had deficient opsonic capacity.

Similar results were obtained with *S. aurcus 502A* as the test bacterium (Table II). HLOC was tested at two different serum concentrations; in each case there was a statistically significant difference between the opsonic capacity of normal sera and a group of six sera from patients with active SLE and concomitant infections.

Sera from five patients with a variety of acute bacterial infections but without SLE were also tested against both *E. coli 075* (Fig. 3) and *S. aureus 502A;* these showed normal HLOC in every case.

In Table I are summarized the HLOC and the clinical and laboratory findings in the group of 30 patients with active SLE studied. Although the average serum hemolytic complement (C'H5O) and C3 values (Table III) and blood leukocyte counts were lower in the group of patients with concomitant infection than in the group without infection, the differences were not statistically significant ($P > 0.1$) in each case. However, when comparison was made between the groups with low and normal HLOC, the differences between the mean C'H5O and C3 levels of the two groups were in each case statistically significant ($P < 0.05$), while average leukocyte counts and corticosteroid dosages did not differ significantly ($P > 0.1$), indicating that the patients with low opsonic capacity tended to have lower serum complement levels. This impression was confirmed by regression analysis of the data. When HLOC and serum C3 levels of 49 sera from all groups were compared, a correlation coefficient of 0.695 ($P < 0.001$) was obtained, indicating that a positive correlation existed between serum opsonin and C3 levels (Fig. 4).

HLOC of SLE patients during and after infection. Table IV shows the opsonic capacity and C3 levels of

TABLE II

HLOC for Staphylococcus aureus 502A *of Normal Sera and Sera from Patients with Active SLE and Concomitant Infections*

Sera	Number	Decrease in number of bacteria			
		1% serum		2.5% serum	
		1 h	2 h	1 h	2 h
Normal	6	1.7*	2.5	1.8	2.6
Active SLE with infection‡	6	0.6	1.2	0.8	1.6
P		<0.005	<0.01	<0.005	<0.01

* Values represent average decrease in number of bacteria in $\log_{10}$ units.
‡ Types of infection are listed in Table I.

TABLE III

Average Serum Complement Levels, Blood Leukocyte Counts, and Corticosteroid Dosage for the 30 Patients with Active SLE

Patients	No.	C'H50	C3	Leukocyte count	Prednisone
		U	*mg/100 ml*	*cells/mm³*	*mg/24 h*
With infection	10	24.0	62.4	4,660	13.3
Without infection	20	28.3	87.3	6,855	21.9
Low HLOC	12	18.0*	58.0*	4,840	9.0
Normal HLOC	18	33.1*	93.1*	6,980	25.7

* Statistically significant difference ($P < 0.05$).

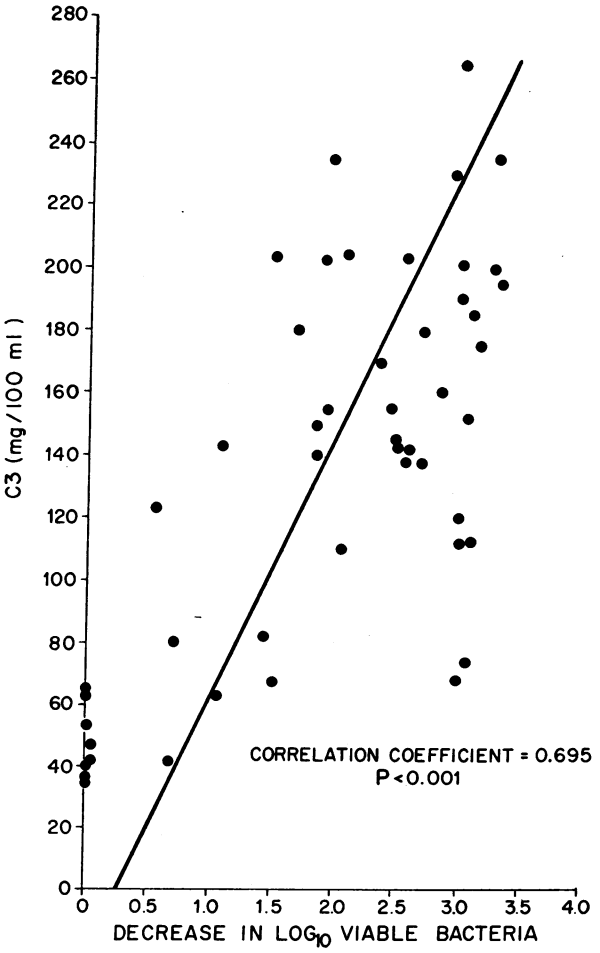

FIGURE 4 Correlation between serum HLOC and serum C3 levels. Decrease in the number of viable bacteria (*E. coli 075*) in $\log_{10}$ units after 2 h incubation plotted against serum C3 levels in milligrams per 100 milliliters in 49 sera obtained from normal donors and patients with active and inactive SLE. The correlation coefficient of 0.695 ($P < 0.001$) indicates a positive relationship.

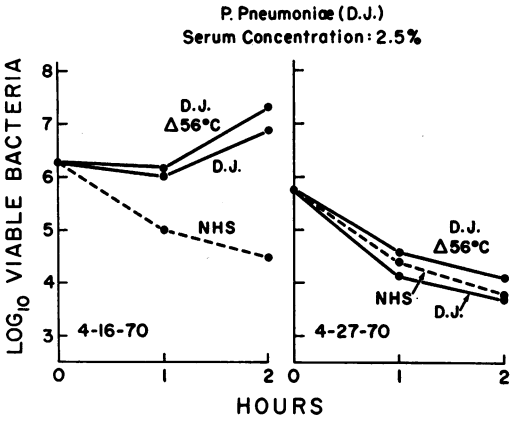

FIGURE 5 HLOC of early and late sera from patient D. J. against *Pneumococcus pneumoniae* isolated from the patient. Serum was used at a 2.5% concentration. The dotted line indicates phagocytosis with normal human serum (NHS). It is seen that the early serum (left) was deficient in HLOC while a subsequent serum obtained 11 days later (right) demonstrated the presence of heat-stable opsonins.

sera obtained early in the course of infection and sera obtained shortly after recovery from four patients with concomitant infections. In addition to antibiotic therapy, the corticosteroid dosage was increased in three of these patients. In each case, HLOC for *E. coli 075* returned to normal, and in three patients there was a concomitant elevation of serum C3. Although patient M. H. showed recovery of HLOC without increase in C3 level, the hemolytic complement level rose from 5 to 30 U. In the case of patient J. S., who was treated with antibiotics alone, low HLOC was present 20 days after the onset of pneumococcal pneumonia. HLOC became normal in this patient only after treatment with prednisone, and coincident with the subsidence of clinical evidence of SLE activity and return of the serum complement to a normal level.

TABLE IV
Opsonic Capacities for E. Coli 075 *and C3 Levels in Early Sera and Sera Obtained after Recovery from Infection*

Patients	Early serum		Late serum	
	HLOC	C3	HLOC	C3
	Δ log10*	mg/100 ml	Δ log10	mg/100 ml
M. H.	+0.8‡	41	2.5	44
J. G.	+0.7	64	1.3	140
D. J.	0.7	42	3.0	112
J. S.	+0.2	29	1.9	110

* $\Delta\log_{10}$: Decrease in the number of viable bacteria after 2 h incubation expressed in log units.
‡ Plus sign denotes an increase in the number of bacteria in $\log_{10}$ units.

Opsonic capacity for isolated causative organisms. In the case of three patients with infection, it was possible to isolate the causative organism and measure the opsonic capacity of the individual patients' sera against their own infecting organisms. In each case, serum obtained at the onset of the infectious episode had low opsonic capacity when compared with normal serum at similar dilutions. From the blood of patient D. J., the type 19 *Pneumococcus* was isolated. This type is rarely infectious in adults (28) and was avirulent when inoculated in guinea pigs and rats. Unlike virulent *P. pneumoniae* (29), the isolated organism was easily opsonized at low concentrations of normal serum. However, the early serum of D. J. showed deficient HLOC for the isolated type 19 *Pneumococcus* (Fig. 5) as well as for *E. coli 075* (Table IV). 11 days later, after recovery from the infection and control of the SLE activity, opsonic capacities for both the isolated *Pneumococcus* and *E. coli 075* returned to normal. It is of interest that a second serum drawn 11 days later developed heat-stable opsonic capacity towards the isolated organism, indicating the appearance of specific antibody.

Patient B. P. developed *E. coli* meningitis and septicemia after admission to the hospital for an exacerbation of lupus glomerulonephritis. Her serum was unable to opsonize the isolated organism, while HLOC for *E. coli 075* was found to be normal (Fig. 6). It should be pointed out that the *E. coli* isolated from this patient needed a higher concentration of normal serum for optimal opsonization. Subsequent sera developed bacteri-

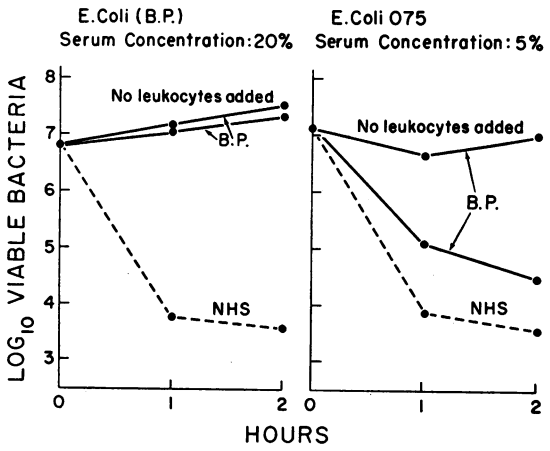

FIGURE 6 HLOC of an early serum from patient B. P. with *E. coli* isolated from the patient (left) and *E. coli 075* (right). The dotted line indicates phagocytosis with normal human serum (NHS). Sera were diluted to 20% to test for *E. coli* (B. P.). Notice the deficient serum opsonic capacity for the patient's own microorganism (left).

cidal activity to her own microorganism at low concentrations so that opsonins could not be tested further.

Patient M. H. was admitted with markedly active SLE and pericarditis that did not respond to corticosteroid treatment. A *Staphylococcus aureus* was shortly isolated from the pericardial fluid, which was poorly opsonized by the patient's early serum (3–2–71) at 1% concentration (Fig. 7). Deficient opsonization was also demonstrated with this serum at 2.5% concentration. Subsequent sera acquired HLOC demonstrable at 1% concentration. HLOC with *E. coli 075* was also shown to be deficient in early sera (3–2–71, 4–1–71). These also became normal 7 wk later, coinciding with an increase of hemolytic complement level from 5 to 30 U.

Serum C3PA levels in SLE sera. Fig. 8 shows the results of radial immunodiffusion measurements of C3PA levels in normal and SLE sera. An average value of 216 μg/ml was obtained from 18 normal sera. In contrast, 17 of the 23 sera from active SLE patients had values below one standard deviation from the normal average ($P < 0.005$). However, when the C3PA levels of sera with normal and deficient HLOC were compared, there were no significant differences between the two groups ($P > 0.3$), suggesting that other factors playing a critical role in opsonization might also be depleted.

Restoration of opsonic capacity of deficient SLE sera. Attempts to pinpoint the deficient factor or factors responsible for the defect in opsonic capacity were made by the addition of purified complement components to the sera tested. Table V summarizes the results obtained with six deficient SLE sera with (*a*) purified C3PA, (*b*) normal serum heated at 50°C for 40 min as a reagent devoid of C3PA (30), (*c*) purified C3, and (*d*) functionally pure C2. The addition of C3PA to five

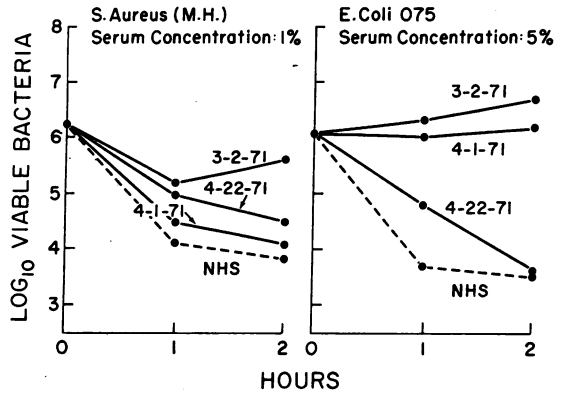

FIGURE 7 HLOC of early and late sera from patient M. H. with *Staphylococcus aureus* isolated from the patient (left) and *E. coli 075* (right). The dotted line indicates phagocytosis with normal human serum (NHS). Notice the deficient opsonic capacity of early sera for both the patient's own microorganism (left) and the test bacterium *E. coli 075* (right).

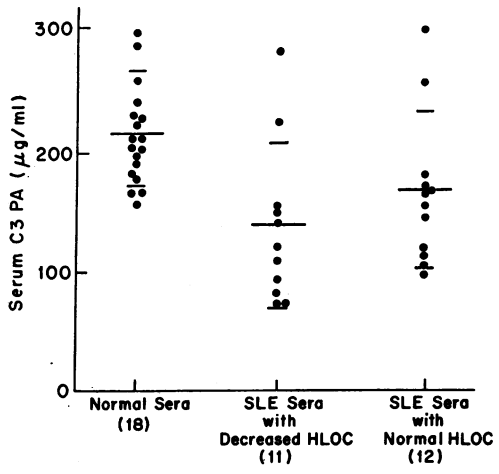

FIGURE 8 Serum C3PA levels in normal controls and patients with active SLE. The horizontal lines indicate the means and one standard deviation. The mean values for the SLE sera with low (139 μg/ml) or normal HLOC (168 μg/ml) were significantly lower than the normal values ($P < 0.005$ and < 0.05, respectively).

sera to a concentration of 300 μg/ml, a high normal level, failed to increase their opsonic capacity in all cases. When 5% serum heated at 50°C for 40 min was added as a source of factors other than C3PA, there was some restoration of opsonic activity in two of the six sera tested but only in the case of J. W. did the increase in activity exceed one log unit. In previous experiments normal serum heated at 50°C in the same concentration was completely devoid of opsonic capacity (18). When in the present experiments, however, both heated normal serum and C3PA were added, restoration of HLOC was accomplished in all six sera tested, and the degree of restoration was even greater than that achieved by the addition of 5% fresh normal serum alone.

A number of other attempts were made to restore opsonic activity to SLE sera (Table V). In all cases there was no significant increase in such activity. Addition of three different batches of purified C3 to eight deficient sera, of which five examples are shown in Table V, failed to increase HLOC in every instance. Similar negative results were obtained by the addition of 5% serum heated at 50°C for 40 min and functionally pure C2, a combination that contains normal hemolytic complement levels (18). Other negative experiments not shown in Table V included the addition of human C1, C4, C2, and C5 separately, together or in sequence. When these were added in sequence, the bacteria were washed after incubation with C1, and then C4, C2, C3, and C5 were added. No increase in HLOC was observed. Addition of C3PA and purified C3 together to three deficient sera failed to restore activity. Addition of hydrazine-treated or zymosan-incubated serum also failed to restore activity. To rule

TABLE V

Restoration of Opsonic Activity of Deficient SLE Sera: Decrease in Number of E. coli 075 in *Log_{10} Units after Addition of Various Factors**

Patient	Factors added						
	None	5% normal serum	5% heated normal serum‡	C3PA (15 μg)	5% heated normal serum‡ +C3PA (15 μg)	5% heated normal serum‡ +C2 (100 U)	C3 (50 μg)
J. U.	+1.0§	2.0	0.2	+0.5	2.3	0.2	+0.5
J. G.	+0.7	1.6	0.6	+0.8	2.0	0.4	+1.0
J. W.	+0.1	2.6	2.1	0.1	2.6	ND‖	+0.1
J. S.	+0.2	2.1	+0.7	ND	1.7	0	0.1
J. A.	+0.4	0.8	0.2	+0.7	1.7	ND	ND
M. H.	+0.8	0.3	+0.5	0.1	1.7	+0.5	+0.2

* After 2 h incubation with normal blood leukocytes.

‡ Normal serum heated at 50°C for 40 min.

§ Plus sign denotes an increase in the number of bacteria in log_{10} units.

‖ ND, not done.

out a possible deficiency of "natural antibody" (9) as a cause for the defect, 19S immunoglobulin isolated from normal serum by agarose column chromatography was also added in the concentration found in the original serum. No increase in HLOC was noted. Taken together, these findings show that C3PA and one or more as yet unidentified factors of the alternate pathway of complement activation are depleted in the deficient SLE sera tested and that this depletion is responsible for the decrease in HLOC.

The role of the alternate pathway of complement in the opsonization of one of the bacteria isolated from infected patients was studied in detail. Fig. 9 shows the results obtained for the *E. coli* isolated from patient B. P. Normal serum heated at 50°C for 40 min lost its opsonic capacity for the *E. coli* tested. Complete restoration of activity could be achieved by the addition of 30 μg of purified C3PA alone. No increase was noticed upon the addition of 200 U of C2, a concentration that restored the hemolytic activity of the heated serum to the normal level.

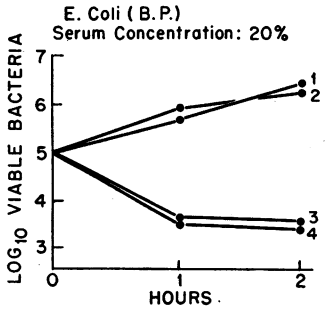

FIGURE 9 Role of the alternate pathway of complement activation in the opsonization of *E. coli* isolated from patient B. P. 1. 20% normal serum heated at 50°C for 40 min. 2. 20% heated normal serum + 200 U C2. 3. 20% heated normal serum + 30 μg C3PA. 4. 20% normal serum. Restoration of activity is achieved by the addition of C3PA to heated serum.

DISCUSSION

A number of factors may contribute to the abnormal susceptibility to infection observed in active SLE (9–12). The present work has shown that phagocytosis by normal polymorphonuclear cells was deficient when tested with diluted serum of patients with active SLE and concomitant infection. This defect was observed in the sera of 8 of 10 such patients studied. Evidence was obtained that a decrease in serum heat-labile opsonin concentration was in fact responsible for the defect. It was also shown that the defect in phagocytosis was not due to the presence of leukocytotoxic antibodies (6, 7) in the deficient SLE sera by the successful restoration of opsonic capacity upon addition of normal serum in 12 of 16 experiments. The less than complete restoration observed with occasional sera of this group may have been due to consumption of critical complement components of the added normal serum during the 1 and 2-h phagocytosis periods by the active SLE sera present, since the latter are known to contain immune complexes and to be anti-complementary (31). This possibility is also suggested by observations made on two sera (J. A. and M. H., Table V) whose opsonic capacity was not completely restored by normal serum. Addition to these sera of 50°C-heated serum and an amount of C3PA that yielded a final concentration in the high physiologic range resulted in more intense phagocytic activity.

In 9 of 10 patients with active SLE and infection, serum heat-labile opsonization was found to be defi-

cient either against *E. coli 075* or the microorganisms responsible for the infectious episode. In contrast, only 4 of 20 patients with active SLE but without infection and none of 12 with inactive SLE demonstrated low serum opsonic capacity when tested against *E. Coli 075*. Although average values for serum complement (C'H5O and C3) and blood leukocyte counts in the group with infection tended to be lower than in the group without infection, suggesting as previously observed (8) that the more active patients were most likely to become infected, the differences were not statistically significant. However, when C'H5O and C3 levels were compared in those with low and those with normal HLOC, the differences were statistically significant, indicating that the patients with low serum complement levels tended to have low HLOC. This impression was confirmed by the positive correlation obtained between serum C3 levels and HLOC in both normal and SLE sera. Since the mean corticosteroid dosage of the patients with infection was appreciably lower than in those without infection and since in all cases tested HLOC levels rose to normal as disease activity was controlled by adequate doses of corticosteroids, it is possible that the lower complement levels seen in the patients with low HLOC may have been a result of increased clinical activity due to the lower dosage taken by these patients before the onset of active disease.

If HLOC plays an important role in the defense against infection, particularly in the early "preantibody phase," it would be expected that the causative microorganisms would not be opsonized by the patient's serum at the time of infection but would be susceptible to opsonization in the presence of normal serum. This was the case with all three bacteria isolated from infected patients. Subsequently, heat-stable opsonins or bactericidal activity directed only to the infecting organism eventually appeared in two of the patients, indicating the emergence of specific serum antibodies. However, the finding in initial and early sera of decreased HLOC for the test bacteria employed and for the specific organisms isolated from infected patients indicates that low serum HLOC may contribute directly to the increased susceptibility to infection of patients with active SLE.

Since it is likely that whether an infection develops may depend on the early events after invasion by the infectious agent, the contribution of nonspecific defense mechanisms such as serum HLOC should be of particular importance at this stage. That heat-labile opsonins may mediate phagocytosis of some infectious agents in the early, pre-antibody period of infection is indicated by several studies (18–20) that have shown that activation of HLOC may not require the presence of immunoglobulins. With the test bacterium *E. coli 075* it was demonstrated in this laboratory (18) that normal opsonization occurred with agammaglobulinemic serum completely depleted of immunoglobulins and $C1_q$ by immunoabsorption. It was also shown that heat-labile opsonization for *E. coli 075* was mediated via the alternate pathway of complement activation (18). It is likely that the low HLOC for *E. coli 075* and for organisms cultured from infected patients found in sera from active SLE patients with concomitant infections reflects the functional depletion of the alternate pathway, confirming the findings previously reported by others in this disease (32–34). This impression is reinforced by the failure in the present experiments to restore heat-labile opsonin activity by the addition of the isolated components of the classical pathway, C1, C4, C2, C3, and C5 either separately, together, or in sequence. Furthermore, addition of a reagent such as 50°C-heated normal serum plus functionally pure C2, which presumably contains near normal amounts of the classical pathway components (18), failed to restore opsonic capacity in the SLE sera. In the case of one of the three microorganisms isolated, direct evidence that opsonization was mediated via the alternate pathway was observed in the restoration of HLOC to 50°C-heated normal serum by addition of purified C3PA.

Ancillary evidence for the depletion of components of the alternate pathway was provided by the finding of subnormal C3PA levels in 9 of 11 sera with deficient HLOC. That low serum C3PA was not the unique factor responsible for the defect was evident from the fact that SLE sera with normal opsonic capacity also showed similarly decreased levels. In fact, addition of purified C3PA alone to five deficient sera failed to increase HLOC in all instances, indicating that other factors of the alternate pathway, presumably acting in the earlier activation steps, were also functionally depleted. The factors provided by the 50°C-heated serum seemed to be critical in the case of patients J. G. and J. W. (Table V), since addition of this reagent alone resulted in partial restoration of opsonic activity. However, complete restoration of all sera tested was achieved only by the addition of both 50°C-heated serum and C3PA, suggesting that the functional depletion of the pathway leading to the activation of C3 involved one or more components of the alternate pathway in addition to C3PA.

Although the decrease in HLOC correlated fairly well with the serum concentration of C3 (correlation coefficient, 0.695), addition of C3 to eight deficient sera (of which five are shown in Table V) failed to increase opsonic capacity in all cases. Although C3 plays a very important role in opsonization (15, 16), as well as taking part in the activation of the alternate pathway in the form of factor A (35), the small concentrations found in the SLE sera tested seemed to be sufficient to sup-

port normal opsonization of *E. coli 075* when adequate amounts of other components of the alternate pathway were present.

Several patients with congenital absence of complement components and increased susceptibility to infection have been reported in recent years (13, 36–39). In one case (36, 37), absence of C3 inactivator allowed generation of active C3 (factor A), which in turn resulted in abnormal activation of the alternate pathway with depletion of its components including the opsonic agent C3. In the case of active SLE, consumption of complement components appears to result in a similar defect leading to a decrease in HLOC. On the same basis, other related complement-mediated functions linked to the activation of C3, such as the complement-dependent generation of chemotactic factors for polymorphonuclear leukocytes (40–43), may also be deficient in SLE.

It is unlikely that the observed decrease in HLOC of active SLE sera from patients with concomitant infections is the consequence of the infection rather than an antecedent event for a number of reasons: (*a*) There were 4 patients in the group of 20 without infection who also had low HLOC. (*b*) In the case of one patient with lobar pneumonia, HLOC remained low for 3 wk after successful treatment of the pneumonia and became normal only after treatment with prednisolone and subsidence of clinical activity and elevation of the serum complement to normal levels. (*c*) Decreased HLOC was observed in a number of sera obtained before overt clinical evidence of infection. (*d*) Others have observed a decrease in complement-dependent functions (42, 43) and in serum levels of some components of the alternate pathway (32–35) in SLE, presumably in the absence of infection.

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