Neutrophil chemotaxis in systemic lupus erythematosus

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A high incidence of infections in patients with systemic lupus erythematosus (SLE) has frequently been commented upon (Harvey, Shulman, Tumulty, Conley, and Schoenrich, 1954; Jessar, Lamont-Havers, and Ragan, 1953; Carpenter and Sturgill, 1964; Ropes, 1964) and a recent study has offered some further support for this contention (Staples, Gerding, Decker, and Gordon, 1970). At least part of the explanation for the apparent increase in susceptibility to infections in SLE may be related to the frequent therapeutic use of immunosuppressive agents in these patients. In addition, renal failure, a clinical development associated with a high incidence of infections (Montgomerie, Kalmanson, and Guze, 1968), is common in SLE. Hypocomplementaemia is a frequent finding, particularly when active renal disease is present (Schur and Sandson, 1968) and decreased antibody responses to bacterial antigens have been described (Baum and Ziff, 1969). Granulocytopenia may occur and bone marrow granulocyte reserves have been shown to be diminished (Kimball, Wolff, Talal, Plotz, and Decker, 1973). In some patients phagocytosis has been found to be defective (Brandt and Hedberg, 1969; Orozco, Jasin, and Ziff, 1970). A cellular defect in chemotaxis has been described in a single patient with SLE (Mowat and Baum, 1971a). Another study reported normal neutrophil chemotactic responses, but serum chemotactic activity was not evaluated (Zivkovic and Baum, 1972). *In vivo* leukocyte migration has been reported to be diminished in patients with SLE (Gewurz, Page, Pickering, and Good, 1967).

The current study was undertaken to examine both serum and cellular factors in neutrophil chemotaxis in a group of 23 patients with SLE. Utilizing the *in vitro* Boyden chamber technique, experiments were designed to evaluate the ability of the patients' serum to generate chemotactic factors and the capacity of the patients' neutrophils to migrate in response to a

chemotactic stimulus. Results were correlated with various clinical parameters of disease activity.

Methods

PATIENTS

All patients studied were hospitalized at the Clinical Center of the National Institutes of Health during the period from 1970 to 1972. The criteria for diagnosis of SLE were those of the American Rheumatism Association (Cohen, Reynolds, Franklin, Kulka, Ropes, Shulman, and Wallace, 1971). Clinical data were gathered prospectively as part of an ongoing study of patients with SLE. The data recorded were those obtained on the same day as or within 1 or 2 days at most of the date of study of chemotactic function. Patients were not studied during or for a period of 2 months following any bout of acute infection.

CHEMOTAXIS

Polymorphonuclear leucocyte (PMN) chemotaxis was studied by a previously described modification (Clark and Kimball, 1971) of the method of Boyden (1962). Generation of chemotactic factors was evaluated as follows: blood from patients and control subjects was allowed to clot at room temperature for 1 hour and the serum was used immediately or stored at -70°C . for future use. Fresh or fresh-frozen serum 0.05 ml. was added to a mixture of 0.85 ml. gelatin-veronal-buffered saline (calcium 7×10^{-5} M, magnesium 5×10^{-4} M) and 0.1 ml. 0.85 per cent. NaCl containing 30 μg . endotoxin (*E. coli* 0127:B8 lipopolysaccharide B, Difco Labs, Detroit, Mich.). In some cases 10 μg . of insoluble immune complexes (rabbit anti-human serum albumin combined with human serum albumin at equivalence) was used in place of the endotoxin. Chemotactic factors were generated by incubating this mixture at 37°C . for 60 min. and residual complement was then inactivated by heating at 56°C . for 30 min. After dilution in 1.0 ml. Gey's medium (Clark and Kimball, 1971), these samples were tested for chemotactic activity.

Blood granulocytes from patients and control subjects were harvested by a previously described method (Clark

and Kimball, 1971) employing dextran sedimentation and hypotonic lysis of erythrocytes. Final cell suspensions (2.3×10^6 granulocytes/ml. Gey's medium) averaged 75–80 per cent. granulocytes in patients and controls. These cells were tested for their ability to respond to chemotactic factor generated from autologous patient serum and normal control serum.

Chemotaxis chambers consisted of an upper compartment containing the cell suspension (0.8 ml.) and separated from the lower compartment containing the chemotactic factor by a 3μ micropore filter (Sartorius Membran-filter, Gottingen, West Germany). After a 3-hour incubation at 37°C . in 5 per cent. CO_2 with 100 per cent. humidity, the filters were removed and stained. The number of cells having migrated through to the lower surface of the filter were counted in five to ten random fields and the chemotactic activity determined as the average number of cells/high power field (HPF) in four duplicate chambers. Patient data was expressed as a percentage of the concomitantly studied controls. Control subjects were selected from healthy laboratory personnel of both sexes ranging in age from 22 to 45 years. Cells and serum from two or more SLE patients were tested concomitantly with cells and serum from two different normal subjects. Control chambers in which the chemotactic factor was replaced by buffer were run in every experiment and consistently showed migration of less than 20 cells/HPF.

CLINICAL PARAMETERS

DNA binding was determined by the Farr assay (Steinberg, Pincus, and Talal, 1971) and greater than 36 per cent. binding was considered abnormal. Immunodiffusion plates were used to measure C3 (Hyland Laboratories, Los Angeles, Cal.; normal 119–175 mg. per cent., C4 (Meloy Laboratories, Springfield, Va.; normal 20–60 mg. per cent., and immunoglobulins (Meloy Laboratories; normal 7.7–11.3 mg./ml. for IgG, 0.8–2.0 for IgA, and 0.9–1.7 for IgM). The presence of tubuloreticular structures was evaluated as described elsewhere (Grimley, Decker, Michelitch, and Frantz, 1973). Haematological and renal parameters were determined by standard techniques.

STATISTICAL METHODS

Standard error was used throughout as an estimate of variance and means were compared using the *t* test. Correlation coefficients were calculated by the Spearman rank correlation test and non-parametric data were analysed by the χ^2 test with the Yates correction or by the Fisher exact test.

Results

Generation of chemotactic activity in SLE sera

The amount of PMN chemotactic factor generated by endotoxin activation of serum from 23 patients with SLE is tabulated in Table I (col. 1). Chemotactic activity in ten of these sera was significantly reduced from normal (25 to 58 per cent. of control). Three additional sera were defective to a lesser degree (71 to 81 per cent. of control), although in these instances the reduction was not significant. Ten sera were clearly normal (97 to 124 per cent. of control).

Two of the defective sera were activated with immune complexes as well as endotoxin; similar reductions in the amount of chemotactic activity generated were observed with both activating agents (Table I).

Table I *PMN chemotaxis in systemic lupus erythematosus†*

Subject	Serum chemotactic activity‡	PMN chemotactic response
1	25* (IC 25*)§	81
2	29*	—
3	35*	85
4	43*	118
5	47*	99
6	48**	—
7	49*	—
8	50**	101
9	56** (IC 35*)§	175
10	58**	—
11	71	60
12	78	77
13	81	81
14	97	27*
15	99	121
16	100	—
17	100	108
18	100	—
19	104	—
20	108	86
21	108	—
22	110	91
23	124	91

* $P < 0.01$.

** $P < 0.05$.

† Data expressed as percentage of control. — = not done.

‡ Endotoxin activation.

§ Immune complex activation in parentheses.

Serial samples of serum were studied in seven of the patients. In six of these no major changes were observed, but in one subject (case 5) five sera obtained over a 3-month period showed a gradual decrease from 62 to 33 per cent. of normal. The patient's clinical course during this interval remained stable.

Several clinical parameters were tabulated for the entire study group. In Table II the group of thirteen patients with depressed serum chemotactic activity are compared with the ten patients with normal activity. The three subjects with intermediate activity were considered with the abnormal group for this analysis, but no major effect on the data was observed when these three were either omitted or included with the normal activity group. As can be seen, several parameters correlated with serum chemotactic activity. The group with depressed activity was significantly younger at the time of study and were younger at the time of onset of disease. Age at onset of renal involvement correlated similarly. Duration of disease tended to be longer, but this was not significant. The level of serum DNA-binding activity showed a significant negative correlation with serum chemotactic activity. Both IgG and IgA levels in serum showed strong positive correlations

Table II Correlation of clinical parameters with serum chemotactic activity

Parameter	Chemotaxis		P†	r*	p*
	Abnormal (13)	Normal (10)			
Sex (Female/Male)	10/3	8/2	NS‡	—	—
Age (yrs)	26.0 ± 2.7	39.7 ± 4.8	< 0.02	+ 0.514	< 0.01
Age at onset of SLE (yrs)	19.8 ± 2.6	36.0 ± 5.3	< 0.01	+ 0.581	< 0.005
Duration of SLE (yrs)	6.1 ± 1.5	3.8 ± 0.9	NS	—	—
Extent of disease					
Joints	13/13	8/10	NS	—	—
Skin	12/13	8/10	NS	—	—
Kidney	12/13	9/10	NS	—	—
CNS	6/13	3/10	NS	—	—
Haematological parameters					
Hematocrit (vol. per cent.)	32.8 ± 1.9	32.0 ± 1.8	NS	—	—
Leucocytes/cmm.	6292 ± 908	6250 ± 848	NS	—	—
Granulocytes/cmm.	4416 ± 725	4230 ± 568	NS	—	—
Platelets × 10 ⁻³ /cmm.	288 ± 25	253 ± 25	NS	—	—
ESR (mm./hr)	64 ± 9	84 ± 9	NS	—	—
Renal parameters					
Urea nitrogen (mg. per cent.)	22.4 ± 3.0	28.8 ± 3.7	NS	—	—
Creatinine (mg. per cent.)	1.08 ± 0.18	0.97 ± 0.09	NS	—	—
Creatinine clearance (ml./min.)	80 ± 8	74 ± 8	NS	—	—
Urine protein (g./24 hrs)	2.7 ± 0.7	2.2 ± 1.0	NS	—	—
Immunological parameters					
LE preparation§	11/13	6/10	NS	—	—
Increased DNA-binding	11/13	2/10	< 0.01	- 0.472	< 0.02
C'3 (mg. per cent.)	101 ± 11	98 ± 15	NS	—	—
C'4 (mg. per cent.)	18.4 ± 2.7	18.9 ± 3.6	NS	—	—
IgG (mg./ml.)	5.55 ± 0.89	13.40 ± 1.75	< 0.01	+ 0.533	< 0.005
IgA (mg./ml.)	1.09 ± 0.17	2.68 ± 0.31	< 0.01	+ 0.709	< 0.0005
IgM (mg./ml.)	1.25 ± 0.29	1.72 ± 0.28	NS	+ 0.309	< 0.08
Tubulo-reticular structures	10/10	8/9	NS	—	—
Drugs					
Prednisone	12/13	8/10	NS	—	—
Dose (mg./day)	24 ± 3	25 ± 6	NS	—	—
Aspirin	5/13	5/10	NS	—	—
Azathioprine	3/13	0/10	NS	—	—

* r = Spearman rank correlation coefficient; P = significance level of correlation.

† Significance level of difference using t test or χ^2 (see methods).

§ Number positive at time of study; all patients had positives at some time.

‡ Not significant.

with chemotactic activity, and mean levels in the abnormal group were significantly lower than in the normal group. A similar tendency was seen with IgM levels, but this did not prove to be significant. Thus, the patients with depressed serum chemotactic activity as a group were younger, had an earlier age at onset of disease, high DNA-binding, and low immunoglobulin levels.

Other factors, such as sex, presence of joint, skin, kidney, or central nervous system involvement, various haematological parameters, indices of renal function, LE preparation positivity, complement levels (C3 and C4), presence of tubuloreticular structures, and drug therapy, did not correlate with serum chemotactic activity. Additional factors (not

shown) which showed no differences between the two groups were body temperature (all afebrile at time of study), urine sediment findings, renal biopsy results, presence of rheumatoid factor, cryoglobulinaemia, and the use of various antihypertensive agents, diuretics, and tranquilizers. Co-existing diseases were present in only three subjects: case 3 had chronic pyelonephritis, case 14 had sarcoidosis and diabetes mellitus (see below), and case 21 had thyrotoxicosis treated with radioactive iodine (¹³¹I).

Data were also gathered on the relative incidence of infections in the two groups of subjects during the 2 years preceding the study. In the group of thirteen patients with depressed chemotactic activity, there was a history of five serious infections (pneumococcal

pneumonia with bacteraemia [2 patients], *Salmonella* sepsis with meningitis, *Moraxella* endocarditis, and tuberculosis with involvement of meninges, joints, and genitourinary tract) and seventeen minor infections (urinary tract, pyoderma, otitis media, hepatitis, conjunctivitis, sinusitis, prostatitis). In the group of ten patients with normal serum chemotactic activity, there were no serious infections and only four minor infections. Since some patients had more than one infection, statistical analysis was performed using the number of individuals who had at least one infection. For serious infections, there were four of thirteen subjects in the abnormal chemotaxis group and none of ten in the normal chemotaxis group with infection ($P = 0.10$; Fisher exact test). For minor infections, there were ten of thirteen subjects in the abnormal chemotaxis group and three of ten in the normal chemotaxis group with infection ($P = 0.04$). Combining the P values for serious and minor infections, the overall difference in the incidence of infection in the two groups was significant at the 0.03 level.

Chemotactic responses of SLE neutrophils

The *in vitro* chemotactic responsiveness of PMN from fifteen patients with SLE was evaluated (Table I, col. 2) using endotoxin activated normal and autologous patient serum as the chemotactic stimulus. Normal migration was observed with all subjects but one. This patient, whose PMN response averaged only 27 per cent. of normal, was a 43-year-old female who had severe disease including central nervous system involvement, but with respect to the clinical parameters in Table II, she did not differ significantly from the group as a whole. She did, however, have sarcoidosis and poorly controlled adult onset diabetes mellitus with blood sugar levels frequently exceeding 400 mg. per cent. This patient's serum chemotactic activity was normal (97 per cent. of control). In addition, for the entire group, there was no correlation between PMN chemotactic responsiveness and serum chemotactic activity.

Discussion

The data presented demonstrate defective serum generation of PMN chemotactic factors in approximately half of a group of 23 patients with SLE. This defect seemed to occur in a sub-group of patients who were younger, had an earlier age at onset of disease, high DNA-binding, and low immunoglobulin levels. It is difficult to establish cause and effect relationships among these various correlated factors. However, the finding that the group of patients with abnormal generation of serum chemotactic factors had a significantly higher incidence of infection suggests that this defect may contribute to the increased susceptibility to infection in SLE.

Some comments regarding the immunoglobulin

levels and their correlation with serum chemotactic activity are in order. Previous studies have reported elevated globulins, especially IgG, in a certain percentage of SLE patients (Cass, Mongan, Jacox, and Vaughan, 1968). Similar elevations were found in the present group of subjects. Of the 23 patients, increased levels of IgG, IgA, and IgM were noted in six, eight, and seven respectively. The IgG elevations were the most striking, with levels as high as 21.8 mg./ml. Contrasting with previous reports, however, was the unexpected finding of diminished globulin levels in some patients (IgG twelve subjects; IgA five subjects; and IgM nine subjects). This was occasionally quite striking with levels for IgG as low as 1.13 mg./ml. These determinations were all performed on at least two separate occasions. In only two of the patients with low IgG levels could this be ascribed to treatment with immunosuppressive agents. As noted previously and documented in Table II, there was a significant correlation between the hypogammaglobulinaemia and decreased serum chemotactic activity. Although serum defects in chemotaxis have been reported in association with hypogammaglobulinaemia (Clark and Kimball, 1971; Steerman, Snyderman, Leiken, and Colten, 1971), there is no evidence that low globulin levels *per se* could account for depressed serum chemotactic activity.

The lack of correlation between serum levels of C3 and C4 and chemotactic activity is of interest in view of the fact that generation of chemotactic factors by endotoxin or immune complexes is a complement mediated process (Snyderman and Mergenhagen, 1972). Endotoxin which was used for most of the studies utilizes the alternate pathway of complement activation which involves C3, but not C4 (Frank, May, Gaither, and Ellman, 1971). In general the C3 and C4 levels in the patients were not so low as to be expected to limit the rate of these reactions, but even with a C3 level as low as 38 mg. per cent. in one subject, normal serum chemotactic activity was generated.

The correlations between diminished serum chemotactic activity and younger age, earlier onset of disease, and high DNA-binding are difficult to explain satisfactorily, although these parameters may simply relate to more severe disease. Corticosteroids in the doses employed had no appreciable effect on serum chemotactic activity. In animals, advanced renal failure has been shown to cause depressed serum chemotactic activity (Clark, Hamory, Ford, and Kimball, 1972). A lack of correlation between various parameters of renal function and serum chemotactic activity in the current study is most likely due to the relatively mild degree of azotaemia present (highest urea nitrogen 45 mg. per cent.) in spite of documented kidney involvement in all but two patients. In fact, it should be noted that patients with severe fulminant SLE were not heavily represented in this study.

It is interesting to speculate on a possible relationship between the *in vivo* serum chemotactic defect described here and the *in vivo* defect in leucocyte migration in SLE noted by Gewurz and others (1967) using the skin-window technique. It is tempting to ascribe both of these observations to a common serum defect, such as partial or complete absence of a necessary serum component or the presence in the serum of an inhibitory factor. However, the exact nature of the chemotactic factors involved in the skin-window response and whether serum components such as complement are involved in this system have not been firmly established. Therefore, no necessary connection between the *in vitro* and *in vivo* defects can be asserted at the present time.

In contrast to the serum chemotactic defect described, neutrophils from SLE patients exhibited normal chemotactic responses in all but one subject. As noted, this patient also had sarcoidosis and diabetes mellitus. Normal neutrophil chemotaxis has been reported in sarcoidosis (Clark and Kimball, 1971), but a cellular defect in PMN chemotaxis has been described in diabetes mellitus (Mowat and Baum, 1971b). Thus, the abnormal findings in this patient may be related to her diabetes rather than her SLE. The finding of normal neutrophil responses in

all of the other patients should be interpreted cautiously, since we are dealing primarily with patients having relatively mild and partially treated disease. This does, however, agree with a previous report describing normal cellular chemotactic function in SLE (Zivkovic and Baum, 1972).

Summary

Polymorphonuclear leucocyte chemotaxis was evaluated *in vitro* in 23 patients with systemic lupus erythematosus. Serum generation of chemotactic factors was clearly depressed in ten subjects and reduced to a lesser degree in three others. This defect correlated with several clinical parameters, including young age at onset of disease, high DNA-binding activity, low immunoglobulin levels, and a high incidence of infection. In contrast to the serum defect, cellular responses to chemotactic factors appeared to be normal. It is suggested that depressed generation of serum chemotactic factors may contribute to the increased susceptibility to infection observed in systemic lupus erythematosus.

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