Neutrophil function in systemic lupus erythematosus and other collagen diseases

H. AL-HADITHY, D. A. ISENBERG,* I. E. ADDISON, A. H. GOLDSTONE, AND M. L. SNAITH†

From the Departments of Haematology and †Rheumatology, University College Hospital, London

SUMMARY Using a whole blood technique we assessed neutrophil migration, phagocytosis, and killing in a group of 20 patients with systemic lupus erythematosus (SLE) and in 8 patients with other connective tissue disorders. In the untreated cases of SLE neutrophil migration was significantly depressed, but it was usually normal in the treated group. This may be attributable either to an intrinsic neutrophil abnormality or to a humoral factor. Although isolated abnormalities of phagocytosis and killing were observed in SLE, these functions were normal when the patients were considered as a group. The treated patients with other collagen diseases showed enhanced migration in both autologous and control plasma, normal phagocytosis, and enhanced killing in autologous plasma only. The small group of untreated, non-SLE patients showed some depression of all 3 functions. There was no correlation between neutrophil function and clinical activity of disease. In the SLE patients there was no correlation between neutrophil function and circulating immune complexes.

Most reports of neutrophil functions in systemic lupus erythematosus (SLE) agree that abnormalities occur but disagree about their nature and aetiology. For example, Brandt and Hedberg¹ and Notani and colleagues² found impaired phagocytosis, whereas Hällgren *et al.*³ found it to be increased. Similarly, while Zivkovic and Baum⁴ reported normal chemotaxis in SLE, Clark *et al.*⁵ showed that chemotaxis was defective, especially in a subset of their SLE patients who tended to be younger, with earlier onset of the disease and higher DNA binding.

Several previous authors^{2 6-8} have postulated the existence of a serum inhibitor in SLE. Others, including Brandt and Hedberg¹ and Landry⁹ have suggested that a cellular defect is responsible for neutrophil dysfunction.

Conflicting results and postulates also abound in the reports of neutrophil function in rheumatoid arthritis. Brandt and Hedberg¹ and Hällgren *et al.*³ both found normal phagocytosis, but Corberand *et al.*¹⁰ and Wilton *et al.*¹¹ noted impaired phagocytosis.

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*D.A.I. is a Jules Thorn research fellow.

Correspondence to Dr A. H. Goldstone, Department of Haematology, University College Hospital, Gower Street, London WC1E 6JJ.

In the present paper, with the development of a highly reproducible whole blood technique to assess neutrophil migration, phagocytosis, and killing,^{12 13} we re-evaluate neutrophil function in SLE and other connective tissue disorders. Furthermore, an attempt was made to correlate abnormalities of neutrophil function with disease activity, with treatment, and with levels of circulating immune complexes, since the latter have been implicated in the pathogenesis of both SLE and rheumatoid arthritis.^{14 15}

Patients and methods

SLE PATIENTS

Twenty patients with SLE were studied repeatedly, with the result that 71 samples were tested. Each fulfilled 4 or more of the American Rheumatism Association's criteria for the classification of the disease.¹⁶ Disease activity was classified as inactive, moderately active, or severely active at each of the consultations before the results of any investigations were available. Severe activity was judged to be present if 3 or more of the following features were present: pleuritis, pericarditis, arthralgia, myalgia, vasculitis, cerebral, or renal disease. Patients with 1 or 2 of these features were considered to have moderately active disease and to be 'inactive' when

none of these problems was present. Treated patients received various combinations of prednisone, azathioprine, and/or levamisole. A few patients were on other medication such as nonsteroidal anti-inflammatory drugs, but these were not separated in the analysis.

Patients with other collagen diseases. Eight patients, 5 with rheumatoid arthritis, 2 with Sjögren's syndrome, and 1 with scleroderma, were studied. These patients were studied repeatedly, with the result that 24 samples were tested. Disease activity was assessed clinically as inactive, moderately active, or severely active. Four patients were receiving treatment with levamisole and one with prednisolone.

METHODS

In brief, blood was mixed 1 : 1 with Hanks's balanced salt solution. Migration was measured by placing the diluted blood in plastic caps which were inverted on to 3 µm micropore membranes resting on filter paper soaked in medium. After 2 hours' incubation at 37°C in a moisture chamber the membranes were rinsed with saline, fixed with propan-2-01, stained with haematoxylin, and cleared with cedar wood oil, so that migration could be measured microscopically by the leading front method.¹²

Phagocytosis and killing were assessed by mixing blood and *Candida guilliermondii* at a ratio of 2 : 1 candida : neutrophil and 60-minute incubation at 37°C with end-over-end rotation. Cyto-centrifuge preparations were stained with May-Grünwald-Giemsa to estimate the number of candida per 100 polymorphonuclear leucocytes and the percentage showing the colour change indicating cell death.¹³

Haematological parameters. The haemoglobin and leucocyte counts were measured on a Coulter S counter and the neutrophil count derived by scoring the percentage neutrophils from a 100-cell differential count. The haemoglobin levels ranged from 5.0 g/dl to 16.3 g/dl and the neutrophil count from 1.0 to 14.1 × 10⁹/l.

Circulating immune complexes (CIC). The CIC levels were measured by a modification¹⁷ of the

polyethylene glycol precipitation technique.¹⁸ The IgG content of the redissolved precipitate was assayed using a laser nephelometer (Hyland Laboratory, Thetford, Norfolk). The upper limit of normal established in 30 healthy volunteers is 100 µg l⁻¹ IgG.

Statistical methods. Statistical analysis of our data was carried out with the help of the MRC Biostatistics Unit, Cambridge. Use was made of paired *t* test and 2-sample *t* test and, where appropriate, of analysis of variance and rank correlation techniques. The 1% level of significance was used throughout as a convenient guide to assessing the significance of departures from a null hypothesis.

Results

Normal controls. A total of 39 samples from healthy volunteers were employed in the neutrophil migration studies and 47 in the candida phagocytosis and killing experiments. In all instances the results fell within the previously established normal ranges.^{12 13}

Neutrophil function in SLE. When all the SLE patients were considered together (i.e., treated and untreated), no significant abnormality in neutrophil migration, phagocytosis, or killing was detected in either autologous or control plasma (Table 1). For all 3 neutrophil functions no significant differences were detected between tests performed in autologous and control plasma (*p* always > 0.1).

However, in the untreated cases considered as a group (Fig. 1) neutrophil migration was significantly depressed; in 40% and 32% of samples (in autologous and control plasma respectively), migration < 80% of the normal control.

Although candida phagocytosis and killing were normal overall in the untreated group, around 20% of samples showed impaired uptake and killing (< 80% the normal control). Among the samples showing reduced phagocytosis and killing, in approximately 50% phagocytosis and killing returned to the normal range when the tests were performed in control plasma.

Table 1 Neutrophil function (mean ± 1 SD) in SLE

Neutrophil function	Plasma in which test performed	Patients		
		All (n = 71)*	Treated (n = 45)	Non-treated (n = 26)
Migration	Autologous	0.98 ± 0.30 (NS)†	1.08 ± 0.31 (NS)	0.8 ± 0.21 (P < 0.001)
	Control	0.98 ± 0.25 (NS)	1.04 ± 0.25 (NS)	0.87 ± 0.21 (P < 0.01)
Phagocytosis	Autologous	0.97 ± 0.18 (NS)	1.01 ± 0.18 (NS)	0.91 ± 0.14 (NS)
	Control	0.93 ± 0.16 (NS)	0.93 ± 0.175 (NS)	0.93 ± 0.13 (NS)
Killing	Autologous	0.94 ± 0.19 (NS)	0.95 ± 0.21 (NS)	0.93 ± 0.14 (NS)
	Control	0.93 ± 0.18 (NS)	0.92 ± 0.19 (NS)	0.945 ± 0.16 (NS)

* n = Number of samples tested (69, migration; 71, phagocytosis and killing).

† NS = No significant difference from normal control.

Fig. 1 Neutrophil migration, phagocytosis, and killing in the untreated group of SLE patients.

Fig. 2 Neutrophil migration, phagocytosis, and killing in the treated group of SLE patients.

Relationship between neutrophil function and activity of SLE. There was no correlation between disease activity and neutrophil function.

Effect of treatment in SLE. When the treated patients were considered as a group, neutrophil migration was found to be normal. However, in 9

samples from 2 patients migration was $<80\%$ of the normal control in autologous plasma (Fig. 2); in 1 of these patients (3 samples) migration was corrected in control plasma. In the other patient, who was being treated with levamisole, no correction was seen in 5 out of 6 (Fig. 3).

In the treated group phagocytosis and killing

Fig. 3 Neutrophil migration in a treated (with levamisole) case of SLE, showing a lack of response to treatment when the test was performed in both autologous and control plasma.

were normal overall whether the tests were performed in autologous or control plasma. However, in autologous plasma 13.3% and 28.9% of samples showed abnormal (<80%) phagocytosis and killing respectively. The figures for control plasma were 22.2% and 26.7% respectively.

Circulating immune complexes in SLE. Twelve of the 20 SLE patients had raised CIC levels. There were no correlations between disease activity or neutrophil function tests and levels of CIC.

Neutrophil function in other collagen diseases. Neutrophil migration was significantly enhanced (Fig. 4) in both autologous and control plasma (mean 1.18 ± 0.19 and 1.17 ± 0.15 respectively; $p < 0.001$), but phagocytosis was normal (1.02 ± 0.19 and 0.96 ± 0.18 in autologous and control plasma respectively). Killing was enhanced (1.18 ± 0.22 , $p < 0.005$) in autologous plasma, but normal (1.03 ± 0.16) in control plasma. There was no correlation between neutrophil function and disease activity.

Only 3 patients were not being treated at the time of the study. It was thus not possible to perform an adequate statistical analysis of the effect of treatment on neutrophil function in these disorders. However, the results in the 6 samples obtained from the untreated patients showed the following results in autologous and control plasma respectively: mean

Fig. 4 Neutrophil migration, phagocytosis, and killing in the treated group of patients with other collagen diseases.

neutrophil migration 0.60 ± 0.29 and 0.72 ± 0.25 ; mean candida phagocytosis 0.92 ± 0.32 and 0.91 ± 0.28 ; mean candida killing 0.90 ± 0.20 and 0.82 ± 0.22 .

Haematological correlations. There were no correlations between the haemoglobin level and any of the neutrophil functions. However, there were correlations between the neutrophil count and migration in both autologous and control plasma ($r = 0.53$, $p < 0.001$, and $r = 0.49$, $p < 0.001$ respectively). Neither phagocytosis nor killing in either autologous or control plasma could be correlated with the neutrophil count.

Discussion

In the present study it is clearly shown that neutrophil migration is consistently reduced in untreated SLE. The effect of treatment by various combinations of prednisolone, azathioprine, and levamisole was to normalise migration.

In approximately half the cases reduced migration was not corrected by autologous plasma. The present study therefore provides evidence of both an intrinsic neutrophil defect and of a serum inhibitor of neutrophil migration. Our study is in accord with most previous work, which has shown reduced neutrophil migration in SLE^{5 7-9} but reconciles studies which have demonstrated either the existence of a serum inhibitor^{5 7 8} or of an intrinsic neutrophil defect.⁹

Although, overall, there were no statistically significant differences in candida phagocytosis or killing compared with normal controls, in either autologous or control plasma, some patients did show marked defects in these tests.

Previous authors have reported both overall decreased^{1 2 6 9} and increased³ phagocytosis in SLE, and killing has received little previous attention. The reason for the discrepancy between the present study and that of others is not clear, but it may relate to the difference types of SLE patients studied and to the different techniques used.

No correlation of neutrophil function was found in the SLE patients with clinical states. This lack of correlation agrees with the observations by Zurier,⁶ who studied particle uptake by neutrophils and lysosomal enzyme release.

The present study did not confirm the association of decreased neutrophil phagocytosis in SLE with neutropenia.¹ In contrast, neutropenia was significantly associated with impaired neutrophil migration.

Like some authors,^{1 9} though unlike others,^{2 0} we

found no relationship between CIC levels and either neutrophil function or disease activity.

In the treated nonlupus group neutrophil migration was enhanced in both autologous and control plasma. This supports an earlier study on rheumatoid arthritis by Jacobs and Goetzal²¹ and is in accord with the stimulation of chemotaxis by levamisole as previously noted in normal volunteers²² and in patients with rheumatoid arthritis.²³ In our treated nonlupus patients neutrophil phagocytosis was normal. In contrast previous authors^{10 11 24} have shown reduced phagocytosis in rheumatoid arthritis; this discrepancy may be the result of the different drug therapy our patients received.

In conclusion our results indicate that neutrophil function is frequently abnormal in SLE and other collagen diseases and that it is often improved by therapy. However, the precise significance of such neutrophil defects in the pathogenesis of collagen disease remains obscure.

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