Defective responsiveness to ascorbic acid of neutrophil random and chemotactic migration in Felty’s syndrome and systemic lupus erythematosus

EDWARD J. GOETZL

From the Departments of Medicine, Harvard Medical School and Robert B. Brigham Hospital, Boston, Massachusetts, U.S.A.

Goetzl, E. J. (1976). *Annals of the Rheumatic Diseases*, 35, 510–515. Defective responsiveness to ascorbic acid of neutrophil random and chemotactic migration in Felty’s syndrome and systemic lupus erythematosus. Polymorphonuclear (PMN) leucocytes from 4 patients with untreated systemic lupus erythematosus (SLE) showed defective random migration (P<0.05) and depressed chemotactic responses to C5a and kallikrein (P<0.01) compared to PMN leucocytes from normal subjects, or patients with rheumatoid arthritis (4) or Felty’s syndrome (4) when examined at a standardized cell concentration with a micropore filter radioassay but not with a conventional Boyden technique. Normal in vitro enhancement of PMN leucocyte random and chemotactic migration by sodium ascorbate was absent in SLE and Felty’s syndrome, but sodium ascorbate gave normal stimulation of hexose monophosphate shunt activity in the PMN leucocytes precluding a defect in ascorbate transport.

The high incidence of focal and systemic bacterial and other infections in patients with systemic lupus erythematosus (SLE) and Felty’s syndrome has been attributed in part to a deficiency in the polymorphonuclear (PMN) leucocyte component of host defence (Shearn and Pirofsky, 1952; Ruderman, Miller, and Pinals, 1968; Estes and Christian, 1971; Dubois, 1974; Staples and others, 1974). A poor correlation between the risk of infection and the extent of peripheral blood leucopenia in such patients has led to the postulation of a qualitative defect in the PMN leucocytes (Louise and Pearson, 1971; Mowat, 1972; Staples and others, 1974).

Several studies have documented distinct abnormalities of phagocytosis and nitroblue tetrazolium reduction by PMN leucocytes in SLE (Brandt and Hedberg, 1969; Orozco, Jasim, and Ziff, 1970; Wenger and Bole, 1973) and low in vitro PMN leucocytic responsiveness to a chemotactic factor in rheumatoid arthritis, Felty’s syndrome, and in some patients with SLE (Mowat and Baum, 1971; Zivkovic and Baum, 1972; Clark, Kimball, and Decker, 1974). In addition to analysing the erythrophagocytic capacity and random and chemotactic migration of PMN leucocytes in rheumatic states, this study correlates the results from two distinct in vitro assays of migration and employs ascorbate stimulation of PMN leucocyte migration as a probe for intrinsic cell defects.

Materials and methods

Thirteen adult patients were evaluated, of whom 4 have seropositive rheumatoid arthritis (RA), 1 has vasculitis, 4 have Felty’s syndrome, and 4 have SLE. An age- and sex-matched normal subject was studied concurrently with each patient to provide 13 control analyses of leucocyte migration. All patients were inpatients or outpatients at the Peter B. Brigham Hospital or Robert B. Brigham Hospital and received a comprehensive general medical evaluation at the time of the leucocyte studies. Diagnoses were based on standard criteria (Ruderman and others, 1968; Louise and Pearson, 1971; Trimble and others, 1974), and all patients with RA, SLE, and Felty’s syndrome had active disease. The patient with vasculitis had a necrotizing cutaneous angiitis documented by a characteristic skin biopsy and was free of joint or other organ system involvement. All of the patients with...
Felty's syndrome and 2 of the 4 with SLE exhibited peripheral blood leucopenia at the time of the study. None of the patients had azotaemia, liver disease, or diabetes mellitus. Each patient was receiving aspirin in doses ranging from 0·6–1·8 g/day but had not taken any other anti-inflammatory medication or any drug known to influence PMN leucocyte function in the 3-month period preceding evaluation.

Polystyrene disposable chemotactic chambers (Adaps, Dedham, Mass.) and acrylic radiochemotactic chambers (Neuprobe, Bethesda, Md.) were assembled with 3 μm and 8 μm pore size micropore filters (Millipore, Bedford, Mass.) as previously described (Goetzl and Austen, 1972a, b). Hanks's solution and medium 199 with or without ascorbic acid (Microbiological Associates, Bethesda, Md.), ovalbumin five times recrystallized (Miles-Seravac, Kankakee, Ill.) dextran, Sephadex and Ficoll (Pharmacia Laboratories, Piscataway, N.J.), sodium diatrizoate (Hypaque, Winthrop Laboratories, New York, N.Y.), two times recrystallized trypsin and soybean trypsin inhibitor (Worthington Biochemical, Freehold, N.J.), sodium 86Cr-chromate, 1-13C-glucose and 6-13C-glucose (Amersham-Searle, Arlington Heights, Ill.), sodium lauryl sulphate and ascorbic acid (Fisher Scientific, Medford, Mass.), and plastic 35–×10–mm Petri dishes (Falcon Plastics, Oxnard, Calif.) were obtained. Gamma radiation from 86Cr-containing micropore filters was measured with a dual channel gamma well counter, and β-radiation from 13C-glucose solutions was quantitated with Bray's fluid in a liquid scintillation counter (Nuclear-Chicago, Des Plaines, Ill.).

**ASSessment of Chemotaxis, Random Migration, and Phagocytosis**

Chemotaxis of human leucocytes was assayed by a modification (Goetzl and Austen, 1972a) of the Boyden micropore filter technique (Boyden, 1962) and by a radiochemotactic method using modified chambers that hold a layer of two closely apposed micropore filters between 86Cr-labelled leucocytes and the chemotactic stimulus (Goetzl and Austen, 1972b). Human peripheral leucocytes from normal subjects and patients were collected and separated from erythrocytes by dextran sedimentation (Goetzl and Austen, 1972a), and the neutrophil PMN leucocyte fraction was purified by centrifugation of the mixed leucocytes on Ficoll-Hypaque cushions (Böyum, 1968).

Neutrophils were washed twice and resuspended in ascorbate-free medium 199 with 0·4 g ovalbumin/100 ml (pH 7·4) for single micropore filter chambers of the modified Boyden technique or medium 199 without added protein for 86Cr labelling (Goetzl and Austen, 1972a, b). The 86Cr-labelled neutrophils were washed twice and resuspended in ascorbate-free medium 199-ovalbumin. The neutrophil suspensions were adjusted to a standard range of concentrations of 2·2±0·4×106/ml in the modified Boyden method with a 3 μm pore filter for the chamber and 5·0±0·5×106/0·5 ml containing 50 000–70 000 counts per 4 minutes (CP 4 min) for the radioassay with an 8 μm pore filter over a 3 μm pore filter for the chamber.

The chemotactic factors were C5a generated by trypsin digestion of highly purified human C5 (Ward and Newman, 1969) and human kallikrein produced from purified pre-kallikrein by the action of Hageman factor fragments (Kaplan, Kay, and Austen, 1972). Random migration in the absence of a chemotactic factor was assessed with the modified Boyden method by increasing the incubation interval from the 24-hour chemotactic period to 34 hours with the radioassay by increasing the interval from the 4-hour chemotactic period to 5 hours at 37°C.

The neutrophil response in the modified Boyden assay was quantitated by counting the cells in fixed and stained filters at 80 μm from their source at the top of the filter and was expressed as leucocytes per high power field (hpf) with a correction for background migration in the chemotactic assay. The neutrophil response in the radioassay was calculated as CP 4 min in the lower filter per 50 000 CP 4 min in the initial neutrophil suspension with a correction for background migration in the chemotactic assay. The enhancement of random or chemotactic migration of neutrophils preincubated for 10 minutes and assayed with added sodium ascorbate was expressed as a per cent of unstimulated migration (Goetzl and others, 1974). Statistical analyses used a standard two-sample t test.

Phagocytosis by human neutrophils of antibody-sensitized erythrocytes coated with C3b as a result of interaction with the first four components of complement was determined as described (Gigli and Nelson, 1968). Human neutrophils at a concentration of 3·4×109/ml were incubated with 2×107 target cells for 20 minutes at 37°C, and the per cent phagocytosis in duplicate tubes was calculated by subtracting the absorbancy units at 414 nm (AU 414) of 0·84% NH4Cl lysates of the unengaged erythrocytes in phagocytosis mixtures from the AU 414 of a lysate of total initial erythrocytes and dividing this difference by the latter AU 414 (Gigli and Nelson, 1968).

**Measurement of Hexose Monophosphate Shunt (Hmps) Activity**

The H MPS activity of layers of neutrophils adherent to plastic Petri dishes was measured by the rate of generation of 14CO2 from 1-13C-glucose as previously described (Goetzl and Austen, 1974). 3·4×106 neutrophils adhered to each Petri dish, and the counts per minute (cpm) of 14CO2 generated over 80 minutes at 37°C was standardized by dividing by the AU at 280 nm of a sodium lauryl sulphate solution of the adherent cells. H MPS activity was expressed as cpm/0·2 AU 280.

**Results**

The assessment of in vitro neutrophil random migration with the radioassay showed significantly depressed values relative to those of normal neutrophils only for the cells of patients with SLE (P<0·05), which was not appreciated (P=0·05) in the modified Boyden assay (Fig. 1). The radioassay was far more sensitive than the modified Boyden assay in unveiling a significant in vitro defect in chemotactic responsiveness of neutrophils from SLE patients to either C5a (P<0·01) (Fig. 2) or kallikrein. SLE patients with leucopenia had neutrophil migration values comparable to those for the SLE patients with normal leucocyte levels. Neutrophils from the...
patients with Felty's syndrome, rheumatoid arthritis (RA), or vasculitis exhibited random migration and chemotactic responsiveness to C5a and kallikrein that were indistinguishable from normal levels in both assays.

The ability of sodium ascorbate to stimulate random and chemotactic migration of human leucocytes by an action on the cells was used to confirm the migration defect in SLE. Ascorbate at a concentration of $2.5 \times 10^{-3} \text{ mol/l}$ failed in either assay to enhance the random migration (Fig. 3) or chemotactic responsiveness to C5a (Fig. 4) or to kallikrein of neutrophils from patients with SLE or Felty's syndrome. Ascorbate enhancement of random and chemotactic migration of neutrophils from normal subjects or patients with RA or vasculitis in an identical protocol averaged 55–95% of baseline migration.

Since the ascorbate enhancement of migration requires a concomitant stimulation of leucocyte HMPS activity (Goetzl and others, 1974b), the influence of increasing concentrations of ascorbate on both functions was examined in parallel studies (Fig. 5). A dose-related stimulation of random migration of neutrophils from two normal subjects was observed in modified Boyden chambers. Random migration of neutrophils from 2 patients with Felty's syndrome was first enhanced detectably at an ascorbate concentration of $5 \times 10^{-3} \text{ mol/l}$ and required an 8-fold higher ascorbate level for a rise to the study enhancement range (Figs. 3, 5). In contrast, ascorbate produced a similar dose-response stimulation of neutrophil HMPS activity for both normal controls and patients with Felty's syndrome (Fig. 5). Studies of neutrophil phagocytosis of antibody-sensitized C3b-coated erythrocytes
showed no significant differences between control subjects who had a range of 68.3–84.1% phagocytosis, patients with RA or vasculitis, with a range of 71.9–87.5% phagocytosis, and patients with Felty’s syndrome or SLE, who had a range of 64.7–82.6% phagocytosis.

Discussion

Neutrophil PMN leucocytes from patients with SLE exhibited a significant defect in random migration and chemotaxis in response to the purified stimuli, C5a and kallikrein, as assessed in a micropore filter radioassay (Figs. 1, 2). In contrast, the radioassay of random migration and chemotaxis of neutrophils from patients with Felty’s syndrome, RA, or vasculitis gave levels indistinguishable from those of neutrophils from normal subjects. Pre-treatment with sodium ascorbate of neutrophils from patients with SLE or Felty’s syndrome failed to stimulate their random migration or chemotactic responsiveness, whereas there was a 55–95% enhancement for neutrophils of control subjects and patients with RA or vasculitis (Figs. 3, 4). Thus, neutrophils of patients with RA or vasculitis were normal with respect to their capacity for phagocytosis of opsonized erythrocytes, random and chemotactic migration, and ascorbate enhancement of migration. Neutrophils of patients with SLE showed impaired baseline random and chemotactic migration and depressed responsiveness of migration to ascorbate, but normal phagocytosis. Patients with Felty’s syndrome were an intermediate group since both baseline migration and phagocytic capacity of neutrophils were normal, but ascorbate failed to enhance random or chemotactic migration.

The finding of normal neutrophil phagocytosis in RA agrees with previous studies (Brandt and Hedberg, 1969). A defect in phagocytosis by neutrophils from patients with SLE (Brandt and Hedberg, 1969; Orozco and others, 1970) has been attributed to a serum deficiency which can be reversed with normal serum (Orozco and others, 1970). The current erythropagocytosis assay, which yielded normal values for neutrophils in SLE, uses previously opsonized C3b-coated erythrocytes and thus would not detect defects other than those inherent to the neutrophils. Abnormally low chemotactic indices have been noted with modified Boyden assays for patients with RA and Felty’s syndrome, while most patients with SLE had a normal chemotactic index (Mowat and Baum, 1971; Zivkovic and Baum, 1972; Clark and others, 1974). The modified Boyden assay did not show a significant defect in neutrophil migration in any of the patient groups (Figs. 1, 2). The radioassay, which imposes a double micropore filter barrier, clearly unveiled the intrinsic leucotactic abnormality in SLE. Further differences in both methodology and patient populations exist between this study and previous investigations. The current assays used in vitro standardization of the concentration of neutrophil suspensions in contrast to the mathematical approach used to correct for differences in initial cell counts in some studies (Zivkovic and Baum, 1972). Finally, the majority of previously reported SLE patients were receiving moderate to high dose corticosteroid therapy at the time of migration studies (Zivkovic and Baum, 1972; Clark and others, 1974), which can be associated with improvement of the in vitro chemotactic index in SLE and other diseases (Mowat and Baum, 1971).

The strikingly decreased responsiveness to ascorbate of neutrophil migration in Felty’s syndrome and SLE was dose-related with moderate enhancement of migration at high concentrations of ascorbate (Fig. 5). The reduced responsiveness of neutrophil migration to ascorbate with full stimulation by ascorbate of HMPS activity in dose-response studies precludes a defect in ascorbate intracellular transport or metabolic utilization (Fig. 5). Stimulation of HMPS activity without enhancement of neutrophil migration has previously been observed with methylene blue (Goetzl and others, 1974b). HMPS stimulation by a chemotactic factor or ascorbate may be associated with inhibition of the neutrophil migration response to the same stimuli when the test cells have been deactivated by prior exposure to a chemotactic principle or previously treated with the neutrophil immobilizing factor (NIF) or the
basic tetrapeptide, Thr-Lys-Pro-Arg (Goetzl and others, 1973; Goetzl and Austen, 1974; Goetzl, 1975).

Deactivation of leucocytes by prior exposure to a chemotactic factor exhibits a dose-related leucocyte specificity that parallels the specificity of chemotactic stimulation by each principle (Goetzl, Wasserman, and Austen, 1974a; Goetzl, Schreiber, and Austen, 1976) and is dependent upon the stimulation and subsequent decay of a leucocyte esterase (Ward and Becker, 1968). NIF, a non-chemotactic 4000–5000 molecular weight basic peptide derived from leucocyte granules, inhibits the migration of polymorphonuclear leucocytes with no effect on phagocytosis or mononuclear leucocyte migration (Goetzl and Austen, 1972a; Goetzl and others, 1973). The leucocyte phagocytosis-stimulating basic tetrapeptide, Thr-Lys-Pro-Arg, is liberated by enzymatic digestion of a fraction of human γ-globulin and, like NIF, inhibits migration of PMN leucocytes without influencing mononuclear leucocyte migration (Nishioka and others, 1972; Goetzl, 1975).

Prior exposure of neutrophils to a chemotactic factor so as to induce deactivation, or to NIF or Thr-Lys-Pro-Arg tetrapeptide, results in inhibition of both random and chemotactic migration and blunts enhancement of migration by ascorbate without blocking the stimulation of HMPS activity. Thus, the isolated hyporesponsiveness to ascorbate of neutrophils in Felty’s syndrome and the more extensive defect in both baseline and ascorbate-stimulated migration in SLE may result from exposure of the cells to one or more humoral inhibitors elaborated in an area of inflammatory reaction (Goetzl, 1975). The defect in neutrophils in SLE could be caused by either chemotactic factor deactivation or by an encounter with NIF or the Thr-Lys-Pro-Arg tetrapeptide. The uniquely isolated lack of ascorbate responsiveness of neutrophil migration in Felty’s syndrome is not reminiscent of the effect of any one humoral inhibitor but may result from low levels of several inhibitors acting in concert.

Analysis of the precise relationship of the neutrophil migration defects in rheumatic diseases to individual inhibitors must await their further purification, and the development of techniques to measure their concentrations in serum and synovial fluid. Nonetheless, the presence of qualitative defects in neutrophil function, in addition to quantitative deficiencies, may contribute in part to impaired host resistance to infection.

Supported by grants AI-07722 and AI-10356 from the National Institutes of Health. E. J. G. is Director of the Laboratories for the Study of Immunological Diseases of the Howard Hughes Medical Institute.

References


BÖYUM, A. (1968) Scand. J. clin. Lab. Invest., 21, Suppl. 97, 31 (Isolation of leukocytes from human blood; further observations)


ESTES, D., AND CHRISTIAN, C. L. (1971) Medicine, 50, 85 (The natural history of systemic lupus erythematosus by prospective analysis)


Ascorbic acid stimulation of neutrophil migration

---, and Baum, J. (1971) J. clin. Invest., 50, 2541 (Chemotaxis of polymorphonuclear leukocytes from patients with rheumatoid arthritis)
Ruderman, M., Miller, L. M., and Pinals, R. S. (1968) Ibid., 11, 377 (Clinical and serologic observations on 27 patients with Felty’s syndrome)