

NEUTROPHIL CHEMOTAXIS IN PSORIASIS

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Abstract. Neutrophil chemotaxis was assessed in 69 psoriatic patients and 37 healthy human subjects. It was found to be significantly enhanced in 52 untreated patients. In 20 patients treated with an orally-administered phosphodiesterase inhibitor, Diphyllyne, neutrophil chemotaxis was normal. The enhanced chemotactic response of neutrophils from untreated patients with minimal skin lesions was at least equal to the response of those from patients with extensive skin lesions. Preincubation of normal human leukocytes with plasma derived from patients with widespread lesions markedly reduced their chemotactic activity. Plasma derived from patients with extensive skin lesions exhibited marked chemoattracting properties in comparison with plasma from healthy subjects. It is postulated that the basic intrinsic abnormality of neutrophil function in psoriasis could be caused by a decreased cyclic AMP/cyclic GMP ratio, similar to the decreased cyclic AMP/cyclic GMP ratio found in the lesional epidermis of this disease. Plasma factors which influence chemotaxis in psoriasis are related to the extent of the eruption and their effect is contrary to the effect of the basic intrinsic abnormality of psoriatic neutrophils.

Key words: Neutrophil chemotaxis; Psoriasis; Diphyllyne; Cyclic AMP; Cyclic GMP

One of the histologic findings in psoriasis is the infiltration of the epidermis by neutrophils, a feature which becomes much more prominent in pustular psoriasis (1). Moreover, soluble substances which were extracted from psoriatic scales were shown to exert a chemotactic attraction over peripheral blood leukocytes (2). These observations promoted us to undertake a detailed investigation of neutrophil chemotaxis in psoriasis. In a preliminary study (3), we have shown that leukocytes derived from the peripheral blood of patients with psoriasis vulgaris have enhanced chemotactic and phagocytic capacities in comparison with leukocytes from healthy subjects. This abnormality was considered to be a primary one as it was also found in patients with minimal or no skin involvement.

In the present paper, we wish to report on addi-

tional observations which have been made on patients with psoriasis vulgaris as well as patients with arthropathic and generalized pustular psoriasis. We have also investigated the effect of treatment with an orally-administered phosphodiesterase inhibitor, diphyllyne (4) on neutrophil chemotaxis in psoriasis. Further experiments were carried out to determine whether plasma factors influence chemotaxis and whether psoriatic plasma contains any chemotactic activity.

PATIENTS AND METHODS

Sixty-nine psoriatic patients and 37 healthy human volunteers (hospital and laboratory personnel) were studied. Thirty-three of the patients were male and 36 were female, while 17 of the control subjects were male and 20 were female. The ages of the patients ranged between 10 and 85 years (mean 32.5) while the ages of the controls were between 19 and 60 years (mean 30.3). There was no history of any preceding or present infection in any of the patients studied and none of them were receiving oral corticosteroids. Fifty-two of the patients were not receiving any form of systemic medication for at least one month prior to the time the study was conducted. Twenty patients were evaluated while on oral Diphyllyne (1.0 to 3.2 g per day). The duration of treatment ranged from 1 to 11 weeks at the time of the investigation. Seven patients were studied both prior to and during Diphyllyne treatment. Two patients were studied while being treated with methotrexate and another 2 while receiving hydroxyurea. Sixteen of the untreated patients had minimal skin lesions (less than 5% of the total body surface involved), 7 additional patients were completely free of clinical lesions and 8 patients had extensive skin involvement (more than 50% of the body surface covered with lesions). Two patients suffered from generalized pustular psoriasis, one had arthropathic psoriasis and one suffered from severe seropositive rheumatoid arthritis with psoriasis.

Chemotactic assay

The chemotactic assay was performed using a modification of the Boyden chamber method (5). Each experiment was performed in duplicate. PMN-rich buffy coats were obtained by sedimentation of heparinized peripheral

Table 1. The chemotactic capacity of neutrophils derived from psoriatic and healthy subjects

Subjects	No. of subjects	Mean chemotactic count ^a	P ^b	Mean chemotactic index ^c
1. Healthy subjects	37	104±10	—	100
2. Untreated psoriatics	52	191±11	0.001	173±13
(a) Patients in clinical remission	7	174±35	0.01	192±14
(b) Patients with minimal skin lesions	16	194±20	0.005	217±29
(c) Patients with extensive skin lesions	8	193±37	0.01	143±21
3. Diphylline-treated psoriatics	20	127±12	N.S.	102±10
4. Psoriatics on methotrexate	2	239±45	—	185±55
5. Psoriatics on hydroxyurea	2	123±28	—	127±30

^a Number of cells per high-power field (HPF) ± standard error of the mean (S.E.M.). ^b Compared with the mean chemotactic count of healthy subjects. ^c % of control (for same day) ± S.E.M.

blood. Neutrophils were obtained by centrifugation of the buffy coats followed by ammonium chloride lysis of erythrocytes (6). The cells were then washed twice with phosphate-buffered saline (PBS), resuspended in RPMI-1640 medium, and brought to a concentration of 2.5×10^6 polymorphs per ml for use in the upper compartment.

The chemotactic factor was prepared by incubating normal human serum with 1.5 mg/ml *Escherichia coli* lipopolysaccharide (Difco Labs, Detroit, Mich.) at 37°C for 90 min. The endotoxin-serum mixture was then heated at 56°C for 30 min and centrifuged at 3 000g for 30 min at 4°C. Aliquots of the activated serum were stored at -20°C. The chemotactic factor was diluted 1:5 in RPMI-1640 before being added to the lower well of Lucite chambers (0.2 ml/well). Polycarbonate membrane filters (5 µm pore size, Nucleopore Corp., Pleasanton, Calif.) were placed over the lower wells. The upper well insert (8 mm diameter) was screwed into place and filled with 0.2 ml of the cell suspension in medium. The chemotactic chambers were incubated at 37°C for 1 hr in humidified 5% CO₂ in air. Following incubation the cells in the upper well were removed and the filters were fixed in methanol, air-dried and stained with Giemsa. Ten randomly chosen high-power fields (HPF) were counted using a microgrid and the mean number of neutrophils per high-power field was determined. The result from each patient was also compared with that of the healthy subject tested in the same experiment and expressed as a percentage of the control value. This value was defined as the chemotactic index (CI).

In order to evaluate whether plasma factors affect chemotaxis in psoriasis, normal human leukocytes were preincubated with either plasma from psoriatic patients or normal human AB plasma at 37°C for 90 min in 5% CO₂ in air. The mixtures were then centrifuged and the leukocytes were resuspended in RPMI and their chemotactic capacity measured as described above.

Finally, plasma samples obtained from psoriatic and healthy subjects were added to the lower wells of chemotactic chambers in order to evaluate their chemoattracting properties. Leukocytes from 2 normal healthy subjects were used in the upper well inserts and the chemoattracting activity of each plasma sample was measured by determining the mean number of migrating neutrophils per HPF.

Statistical methods

Student's *t*-test (one-tailed) was used to compare mean values. Differences were considered to be significant when *P*-values were smaller than 0.01.

RESULTS

Untreated patients

Fifty-two patients were studied. In all but 7 patients, the chemotactic response was higher than in the controls. The mean Chemotactic Index (CI) was 73% higher in patients than in the controls (Table 1); this difference was highly significant ($p < 0.001$).

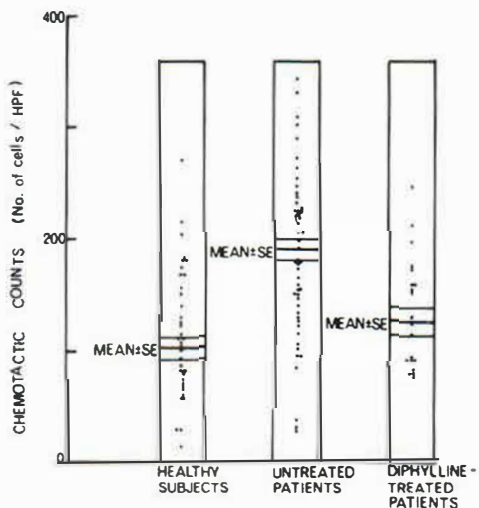


Fig. 1. Chemotaxis of neutrophils derived from healthy subjects, from untreated psoriatics and from psoriatics treated with oral Diphylline. Each dot represents the mean number of migrating cells per high-power field (HPF) in one individual.

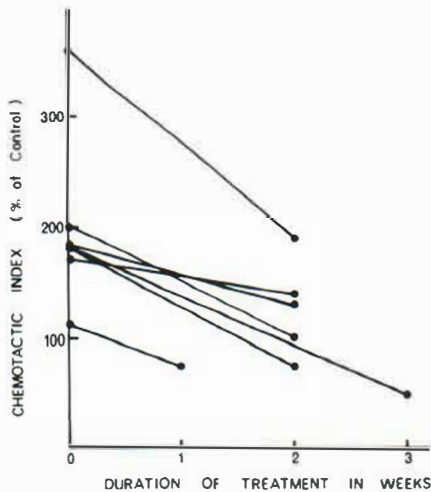


Fig. 2. Effect of oral Diphyllyne treatment on the chemotactic responses of neutrophils derived from 7 psoriatic patients.

The enhanced chemotactic activities of psoriatic neutrophils did not correlate with the degree of skin involvement. On the contrary, the mean CI was higher in patients with minimal skin lesions than in those with extensive lesions (Table I). However, this difference was not statistically significant. One patient had an extremely low CI (10% of control value); this was a patient with severe seropositive rheumatoid arthritis and psoriasis. On the other hand, a high CI (200% of control value) was found in a patient with typical seronegative psoriatic arthropathy. Two patients with generalized pustular psoriasis had chemotactic responses which were similar to those encountered in patients with psoriasis vulgaris.

Systemically treated patients

Twenty patients were investigated while being treated with oral Diphyllyne (Table I, Fig. 1). The chemotactic response of these patients' neutrophils did not differ from that of the controls ($p > 0.50$). Seven of these patients were studied both prior to and 1–3 weeks after the beginning of therapy (Fig. 2). In 3 patients, the CI remained high 3 weeks after Diphyllyne therapy was started; these patients showed no clinical improvement with this form of treatment. Two patients were investigated while on Methotrexate; their chemotactic responses were comparable to those of untreated patients. The 2 patients on Hydroxyurea had chemotactic indices

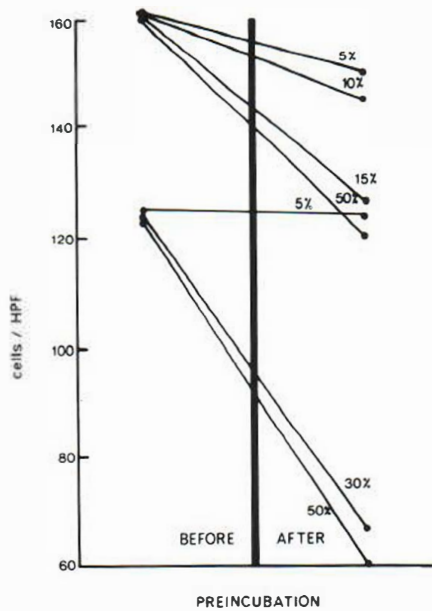


Fig. 3. Effect of preincubation with plasmas from psoriatic patients with different degrees of skin involvement on the chemotactic capacity of normal human leukocytes. (The number on each line indicates the percentage of body surface involved.)

which were higher than in the controls but lower than in the untreated group (Table I).

Preincubation of normal human leukocytes in psoriatic plasmas

The chemotactic activity of normal human neutrophils derived from 2 different donors was not affected by preincubation in undiluted normal human AB plasma, whereas preincubation in undiluted plasma obtained from some psoriatic patients resulted in a marked inhibition of their chemotactic capacity (Fig. 3). Plasma derived from patients with minimal skin lesions had little if any inhibitory effect while plasma which was obtained from patients with more extensive skin disease had a prominent inhibitory effect on normal human neutrophil chemotaxis (Fig. 3).

Chemoattracting property of psoriatic plasmas

Plasma from some of the patients had a marked chemoattracting capacity in comparison with plasma from healthy human subjects. Again, a correlation was found between the degree of skin involvement of the patients from whom the plasma samples

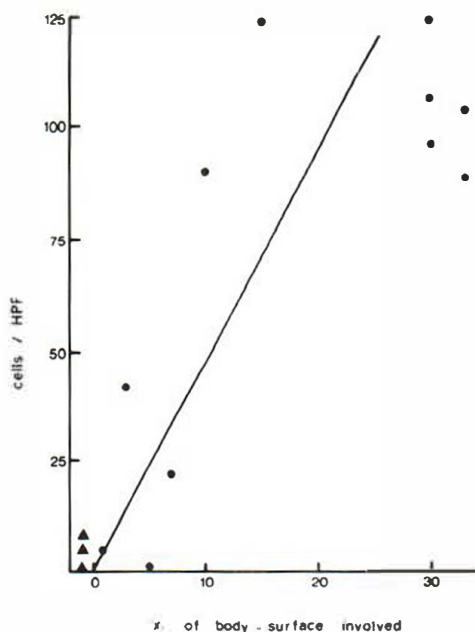


Fig. 4. The chemoattracting activity of plasmas derived from psoriatic patients with different degrees of skin involvement. Normal human leukocytes were placed in the upper wells of chemotactic chambers. In the lower wells either normal human plasma (▲) or psoriatic plasma (●) were used. The number of migrating cells/HPF was scored.

were obtained and the chemoattracting property of these plasma samples (Fig. 4).

DISCUSSION

This study confirms and expands on our previous findings (3) that the chemotactic activity of neutrophils from psoriatic patients is significantly enhanced in comparison with that of normal human neutrophils. This enhancement was not dependent on the extent of the eruption. We therefore believe that there is a primary abnormality of neutrophil function in patients with psoriasis. As it is known that increased cyclic AMP levels inhibit chemotaxis and phagocytosis (7–8), while increased cyclic GMP levels enhance chemotaxis (8–10), it is possible that the enhanced leukocyte chemotaxis in psoriasis might be caused by a decreased cyclic AMP/cyclic GMP ratio, similar to the decreased cyclic AMP/cyclic GMP ratio found in the lesional epidermis of this disease (11). Compatible with this hypothesis is the fact that successful treatment of the skin lesions with an orally administered phosphodiesterase in-

hibitor, Dipylline (4), reduced the chemotactic responses of psoriatic neutrophils to normal levels. Three patients whose skin condition did not improve with oral Dipylline had persistently elevated chemotactic indices. We did not measure the blood levels of Dipylline in our patients and it is possible that the blood levels achieved in these patients were inadequate. In contrast, 2 patients whose skin cleared completely with Methotrexate still had highly elevated chemotactic responses.

Preincubation with plasma derived from patients with widespread lesions reduced the chemotactic activity of normal human neutrophils. The presence of plasma factors which inhibit the cells' response to chemotactic mediators is thus correlated with the extent of skin involvement. This inhibition is probably caused by blocking of leukocyte membrane receptors required for interaction with chemotactic factors. Indeed, rinsing of the cells three times resulted in an almost complete abrogation of the inhibitory effect.

These findings are somehow analogous to those of Ginski et al. (12) who demonstrated the existence of a factor which inhibits E-rosette formation by normal lymphocytes in the sera of psoriatic patients during the active stage of the disease. Plasma derived from patients with widespread lesions exhibited marked chemoattracting activity in comparison with plasma from patients with minimal skin lesions or from healthy subjects. Psoriatic scales have been shown to contain soluble substances with marked chemoattracting activity for peripheral blood leukocytes (2). It seems logical to assume that in patients with widespread skin disease, significant amounts of these substances reach the circulation, thus becoming detectable in the plasma.

One of our patients who had psoriatic arthropathy had a highly elevated CI, while another patient, with rheumatoid arthritis and psoriasis, had a markedly reduced CI. Leukocyte chemotaxis is markedly reduced in rheumatoid arthritis due to the presence of immune complexes (13). Thus, assessment of neutrophil functions may be of value in differentiating psoriatic arthropathy from rheumatoid arthritis. Further studies are needed in order to establish whether assessment of chemotaxis can be used as a diagnostic aid in certain atypical cases of psoriasis such as in young children and infants or in the differentiation between psoriatic erythroderma and erythroderma due to other causes.

Finally, the incidence and density of bacterial

micro-organisms are much lower in psoriatic plaques than in atopic lesions; and most psoriatic patients do not have clinical pyoderma (14). These observations could be explained by the enhanced activities of neutrophils in psoriasis.

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