J. clin Path., 1977, **30**, 250-253

Polymorphonuclear leucocyte function in Behçet's disease

J. D. SOBEL, S. HAIM, N. OBEDEANU, T. MESHULAM, AND D. MERZBACH

From the Departments of Dermatology and Microbiology, Rambam Medical Centre and Aba Khoushy School of Medicine, Haifa, Israel

SUMMARY Polymorphonuclear leucocyte function was investigated in 19 patients with active Behçet's disease. Spontaneous free leucocyte migration was found to be significantly reduced, yet after stimulation the leucocyte's chemotactic activity was considerably increased ($P < 0.05$) when compared to control leucocytes. Control leucocytes migrated more rapidly when incubated in serum taken from patients with Behçet's disease ($P < 0.005$). The enhanced chemotactic activity in Behçet's disease appears to be due to both serum and intrinsic leucocyte factors. Spontaneous nitroblue tetrazolium reduction was found to be normal, although after stimulation leucocyte nitroblue tetrazolium reduction was lower than in the control group ($P < 0.025$), as was leucocyte oxygen utilisation. It is suggested that the hyperreactive cellular inflammatory response that characterises Behçet's disease may be due to increased chemotactic activity and minor alterations in functional metabolic activity of leucocytes.

The aetiology of Behçet's disease remains enigmatic. Current concepts favour an immune mechanism to explain the systemic inflammatory lesions (Chajek and Fainaru, 1975).

Recently, Matsumura and Mizushima (1975) reported enhanced chemotactic activity of polymorphonuclear leucocytes (PMN) in Behçet's disease, and colchicine, a strong inhibitor of chemotaxis, was shown to exert a favourable effect on symptomatology. This prompted us to investigate leucocyte function in patients with Behçet's disease by means of the nitroblue tetrazolium (NBT) test, spontaneous and stimulated, leucocyte oxygen consumption, and *in vitro* free leucocyte migration and chemotactic response.

Subjects and methods

Leucocyte function was investigated in 19 patients with active Behçet's disease. There were 15 men and 4 women, ranging in age from 21 to 61 years, and the duration of their disease varied from 3 to 41 years. The diagnosis of Behçet's disease in these patients was based upon the presence of at least two of the three cardinal and one of the other symptoms recognised as part of the clinical syndrome. Nine subjects had received steroid medication, which had been stopped at least one week before investigation.

Received for publication 2 August 1976

Venous blood was drawn in plastic syringes and immediately mixed with 6% clinical dextran solution in saline (Macrodex, Pharmacia, Uppsala, Sweden) and 20 units/ml heparin. After 45-60 min sedimentation at room teperature, the leucocyte-rich plasma was separated and centrifuged at 500 × *g* for 5 min. The supernatant was again centrifuged at 3000 rpm for 20 min, yielding the plasma necessary for preparation of chemotactic factors. Residual erythrocytes in the leucocyte pellet were hypotonically lysed and the osmotic pressure was restored with 3·5% sodium chloride solution. After two more washings with either Ringer's solution containing 0·1% glucose or Medium 199 containing 10% fetal calf serum, total WBC counts and differential counts were performed. The leucocyte suspensions were finally adjusted to obtain 2×10^7 phagocytes/ml for oxygen consumption tests and $1\text{-}5 \times 10^8$ phagocytes/ml for chemotaxis experiments. Before each experiment leucocyte viability was assessed by the trypan blue extrusion test. An average of 95% cells appeared to be viable by this test.

Oxygen consumption experiments were made with a Clark type electrode (Yellow Springs Instrument Co, Model 53, Yellow Springs, Ohio). After a 10 min equilibration at 37°C of identical leucocyte suspensions contained in two chambers, an overnight

culture of *Staphylococcus albus* was added to only one chamber (bacteria: phagocyte ratio, 2:1). Parallel recordings of depletion in oxygen saturation were made simultaneously with two electrodes for 30 min, one curve indicating the resting leucocyte oxygen consumption and the other the phagocytosis-induced oxygen consumption burst. The net consumption due to phagocytosis of *Staph. albus* could be assessed at any moment by the difference between the two curves. Bacterial endogenous respiration was practically zero. Results were expressed in μl of oxygen consumed per 10^7 cells per hour.

Chemotaxis determinations were made on agarose plates, according to the method of Cutler (1974). This method proved to be useful in the simultaneous assessment of free and chemotactic leucocyte migration (Nelson *et al.*, 1975). The following chemotactic stimuli were used: (*a*) human serum incubated with *Escherichia coli* 0127:B8 lipopolysaccharide (Difco Laboratories, Detroit, Mich) (Gallin *et al.*, 1973); (*b*) *E. coli* culture filtrate (Hill *et al.*, 1974); (*c*) a mixture of dextran-diluted plasma incubated with human aggregated γ-globulin (Frei *et al.*, 1974).

The plates were incubated in a humid CO_2 incubator for 2½ hours at 37°C. The final evaluation of leucocyte migration was made by projecting the Giemsa-stained migration areas on a screen, copying on weighing paper, cutting out, and weighing. Every single mixture, as well as free leucocyte migration, was assayed at least three times and at most six times, and average results were calculated. The results are expressed in milligrams.

NBT reduction tests were performed according to our standardised method (Merzbach and Obedeanu, 1975). Spontaneous and endotoxin-stimulated tests were performed for each patient in two parallel incubation mixtures and the results were averaged. The lipopolysaccharide used for stimulation was the same as that used in chemotactic experiments.

Statistical evaluation of results, as well as the Student's significance *t* tests, were performed according to Hill (1961).

Results

Spontaneous NBT reduction was normal in the vast majority of patients with a range of 0-16% and mean 4·95 ± 4·68%. The mean level in 37 healthy controls was 4·13 ± 3·0% and the normal range 0-11% (Table 1). After stimulation the mean NBT reduction in controls was 46·35 ± 10·01%, significantly higher than in patients with Behçet's disease, 38·2 ± 17·47% ($P < 0.025$). In 5 of the 19 patients tested the stimulated NBT reduction was below the lower limit of normal for this laboratory.

Table 1 *NBT reduction*

	Patients (19)	*Controls (37)*	P *value*
Spontaneous	4·95 ± 4·68	4·13 ± 3·0	NS
Stimulated	38·21 ± 17·47	46·35 ± 10·01	<0·025

Results recorded as mean ± SD of individual tests expressed as % formazan-positive neutrophils
Number of individuals studied in each group in parentheses
NS = not significant

Resting oxygen consumption was 10·47 ± 5·01 μl $O_2/10^7$ phagocytes/hour in 19 patients, significantly lower than the corresponding value of controls, 14·51 ± 2·47 ($P < 0.025$). The oxygen consumption in the patient group was 22·45 ± 14·23 μl $O_2/10^7$ phagocytes/hour during phagocytosis of *Staph. albus*, as opposed to 31·59 ± 8·66 μl $O_2/10^7$ phagocytes/hour in the control group ($P < 0.10$) (Table 2).

Table 2 *Leucocyte oxygen consumption in resting state and after stimulation by phagocytosis of* Staphylococcus albus

	Patients (19)	*Controls (8)*	P *value*
Resting	10·47 ± 5·01	14·51 ± 2·47	<0·025
Stimulated	22·45 ± 14·23	31·59 ± 8·66	<0·05

Results recorded as mean ± SD of individual tests expressed as μl $O_2/10^7$ phagocytes/hour
Number of individuals studied in each group in parentheses

The free leucocyte migration in 12 Behçet's disease patients was 6·32 ± 0·52 mg, significantly less than in controls, 7·4 ± 1·37 mg ($P < 0.05$). In response to the chemotactic stimulus *E. coli* culture filtrate, leucocyte migration was similar in both groups, 8·5 ± 2·54 mg compared to 8·0 ± 1·68 mg ($P > 0.1$). Using *E. coli* 0127:B8 lipopolysaccharide and normal control serum, leucocytes from Behçet's patients had significantly increased migration compared to controls ($P < 0.05$).

No difference in migration was observed when patients' leucocytes were incubated in Behçet's serum (Table 3) although control leucocytes migrated more efficiently in the presence of Behçet's serum as compared to normal serum ($P < 0.005$). Using aggregated

Table 3 *Chemotaxis results with* E. coli 0127:B8 *lipopolysaccharide*

Serum	*Behçet's leucocytes (12)*	*Normal leucocytes (4)*	P *value*
Behçet	9·92 ± 1·71	9·86 ± 1·44	NS
Normal	9·08 ± 1·75	7·25 ± 0·99	<0·05
P value	NS	<0·005	

Results recorded as mean ± SD of individual tests expressed in milligrams
Number of individuals studied in each group in parentheses
NS = not significant

γ-globulin incubated with plasma as the chemotactic stimulus, Behçet's leucocytes migrated normally and behaved similarly to control leucocytes (Table 4). No difference was seen between normal plasma and plasma obtained from Behçet's patients.

Table 4 *Chemotaxis results with aggregated γ-globulin*

Plasma	*Behçet's leucocytes (12)*	*Normal leucocytes (4)*	P *value*
Behçet	8·44 ± 2·17	8·72 ± 0·90	NS
Normal	7·9 ± 1·07	8·15 ± 0·91	NS
P value	NS	NS	

Results recorded as mean ± SD of individual tests expressed in milligrams
Number of individuals studied in each group in parentheses
NS = not significant

Discussion

A unique feature of Behçet's disease is cutaneous hypersensitivity (pathergy) characterised by the formation of an indurated erythematous skin lesion within 12-48 hours after non-specific skin trauma such as a simple needle prick. Histologically, the appearance is essentially that of an inflammatory reaction. By 24 hours the cellular infiltrate is composed predominantly of mononuclear cells, mainly lymphoplasmocytic, and in addition, as we have recently confirmed, an increased number of mast cells (Haim *et al.*, 1976). The time sequence of the reaction is somewhat late for an immediate hypersensitivity reaction and early for a delayed hypersensitivity reaction. The striking feature of pathergy is rapid cellular inflammatory response to minimal stimulation.

Accordingly, the finding of high chemotactic activity of PMN in Behçet's disease by Matsumura and Mizushima (1975) suggested a new approach to the pathogenesis of Behçet's lesion and, in particular, of pathergy.

In the present study, free spontaneous migration of leucocytes in patients with Behçet's disease was diminished when compared to normal healthy control leucocytes ($P < 0.05$). However, when stimulated by chemotactic factors, the Behçet's leucocytes not only migrated normally but, in the presence of normal control serum and *E. coli* lipopolysaccharide, chemotactic activity was superior to that of control leucocytes ($P < 0.05$). The *E. coli* lipopolysaccharide acts by activating the chemotactic properties of the complement system.

Of further interest is the striking enhancement of leucocyte migration in the control group by the addition of Behçet's serum. These findings suggest enhanced chemotactic activity in Behçet's disease due to a serum chemotactic factor as well as an intrinsic leucocyte component. Why increased chemotaxis was observed in serum and not in plasma is unclear and requires further investigation.

The complement system plays an important role in normal chemotaxis, and it may be relevant that in most cases of Behçet's disease there is evidence of complement activation with increased total haemolytic complement activity as well as strikingly increased levels of C_9 (Kawachi-Takahashi *et al.*, 1974; Kogure *et al.*, 1971).

Spontaneous NBT reduction was normal in this series as opposed to high values described elsewhere (Okuda *et al.*, 1974). In fact, a lower than normal reduction response occurred after stimulation of the leucocytes with endotoxin. This observation, together with the finding of diminished oxygen consumption by Behçet's leucocytes, may suggest a minor functional metabolic abnormality of leucocytes in Behçet's disease.

Patients with Behçet's disease have no increased susceptibility to infection and therefore any such leucocyte metabolic defect does not impair the inflammatory reaction to trauma or tissue invasion. On the contrary, pathergy, as well as mucocutaneous lesions represent an exaggerated cellular inflammatory response to tissue prick. Increased chemotactic activity of PMN in Behçet's disease together with alterations in leucocyte function may be part of the complex pathogenesis of this chronic inflammatory disease which as yet defies explanation.

References

Chajek, T. and Fainaru, M. (1975). Behçet's disease. Report of 41 cases and a review of the literature. *Medicine (Baltimore)*, **54**, 179-196.

Cutler, J. E. (1974). A simple *in vitro* method for studies on chemotaxis. *Proceedings of the Society for Experimental Biology and Medicine*, **147**, 471-474.

Frei, P. C., Baisero, M. H., and Ochsner, M. (1974). Chemotaxis of human polymorphonuclears *in vitro*. II. Technical study. *Journal of Immunological Methods*, **5**, 375-386.

Gallin, J. I., Clark, R. A., and Kimball, H. R. (1973). Granulocyte chemotaxis: An improved *in vitro* assay employing ^{51}Cr-labeled granulocytes. *Journal of Immunology*, **110**, 233-240.

Haim, S., Sobel, J. D., Friedman-Birnbaum, R., and Lichtig, C. (1976). Histological and direct immunofluorescence study on cutaneous hyperreactivity in Behçet's disease. *British Journal of Dermatology* (In press).

Hill, A. B. (1961). *Principles of Medical Statistics*, 7th edition. *Lancet*, London.

Hill, H. R., Warwick, W. J., Dettloff, J., and Quie, P. G. (1974). Neutrophil granulocyte function in patients with pulmonary infection. *Journal of Pediatrics*, **84**, 55-58.

Kawachi-Takahashi, S., Takahashi, M., Kogure, M., and Kawashima, T. (1974). Elevation of serum C_9 level associated with Behçet's disease. *Japanese Journal of Experimental Medicine*, **44**, 485-487.

Kogure, M., Shimada, K., and Hara, H. (1971). Complement titer in patients with Behçet's disease (Japanese). *Acta Societatis Ophthalmologicae Japonicae*, **75**, 1260-1268.

Matsumura, N. and Mizushima, Y. (1975). Leucocyte movement and colchinine treatment in Behçet's disease (Letter). *Lancet*, **2**, 813.

Merzbach, D. and Obedeanu, N. (1975). Standardisation of the nitroblue-tetrazolium test. *Journal of Medical Microbiology*, **8**, 375-384.

Nelson, R. D., Quie, P. G., and Simmons, R. L. (1975). Chemotaxis under agarose: A new and simple method for measuring chemotaxis and spontaneous migration of human polymorphonuclear leukocytes and monocytes. *Journal of Immunology*, **115**, 1650-1656.

Okuda, K., Tanakoro, J., and Sekido, M. (1974). The NBT test in Behçet's syndrome (Letter). *New England Journal of Medicine*, **290**, 916-917.