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Polymorphonuclear function in Behçet's syndrome

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SUMMARY Three aspects of polymorphonuclear leucocyte (PMN) function were studied in 19 patients with Behçet's syndrome (BS). By 2 different techniques directed motility was found to be increased. This increase was largely due to the subgroup of patients with ocular involvement. Counts of absolute numbers of cells migrating highlighted this finding. No difference was found in the phagocytic or adherent properties of PMN in Behçet's syndrome. Increased PMN motility in Behçet's syndrome may contribute to the expression of the syndrome. It remains to be tested whether altered PMN motility in this syndrome is genetically linked.

Several groups of workers have found an increased chemotactic response of polymorphonuclear leucocytes (PMN) from patients with Behçet's syndrome.[1-4] However, recently, Abdulla and Lehner[5] have reported a reduced chemotactic response to inulin-activated serum and a normal chemotactic response to casein.

These differences may be due to variations in technique. Because of this we have studied the motility of PMN from patients with Behçet's syndrome using 2 methods. We have also measured the phagocytic and adherent properties of these cells.

Patients and methods

A total of 19 patients was studied (Table 1). Fourteen were male. Twelve patients were drawn from our own rheumatology department and 7 were attending a uveitis clinic at Moorfields Eye Hospital. Each patient was examined and a full history taken. All patients satisfied the criteria of Mason and Barnes[6] having 3 or 4 major criteria or 2–3 major together with 2 minor. There was some overlap between the clinical features of these 2 groups, as can be seen from the table. The majority of the 'arthritic' group (cases 1–12) had joint symptoms controlled by therapy but 3 (cases 1, 2, 9) had signs of an active arthropathy. It was difficult to assess the degree of inflammatory activity in the 'ocular' group (cases 13–19), though the majority appeared to be suffering continuing loss of vision, and all were judged to require immunosuppressives in the form of prednisolone and chlorambucil, and some had received antilymphocytic globulin. Controls were drawn from healthy laboratory and medical staff.

Cell preparation. 15 ml of blood was taken into heparinised universal containers (10 IU preservative-free heparin/ml blood). PMN for filter chemotaxis were prepared by the method of Dioguardi *et al.*[7] by lysis of red blood cells with 8·5% ammonium chloride and subsequent purification by low-speed centrifugation. PMN were purified for agarose plate motility studies and phagocytosis by a Hypaque-Ficoll separation procedure.[8] Cells were washed in culture medium 199 containing 1% human serum albumin (AB Kabi, Stockholm) with $NaHCO_3$ 350 μg/ml, penicillin 500 IU/ml, and streptomycin 500 μg/ml.

Adhesion was measured by a modification of Stecher's method.[9] 1 ml disposable syringes were packed with 20 mg of scrubbed nylon fibre (Fenwal Labs, USA) to the 1 ml mark. A 25 g disposable needle was fitted and the syringe warmed to 37°C prior to adding blood to the column. 300 μl of heparinised whole blood was introduced into the open end of the syringe. The reintroduced plunger was then advanced until the blood was seen at the beginning of the Luer fitting below the nylon fibre. The columns were incubated at 37°C for 10 minutes, after which the blood was expelled by gentle pressure on the plunger. The effluent blood was collected in a small test-tube. Total white cell counts were carried out with a Coulter electronic counter and differential white cell counts with Leishman stained smears. Adherence was expressed as the percentage of PMN retained in the column. All tests were performed in triplicate.

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Table 1 *Patient details*

Case	*Sex*	*Age*	*Mouth ulcers*	*Genital ulcers*	*Ocular lesions*	*Skin lesions*	*Arthritis*	*Venous thrombosis*	*Gastro-intestinal*	*Treatment*
1	M	61	+	+		+	+			Indomethacin
2	M	27	+		+	+	+		+	Indomethacin, salazopyrine
3	M	38	+	+	+	+	+			Naproxen
4	M	39	+	+	+	+	+			Prednisolone
5	M	58	+	+		+	+			Prednisolone
6	M	34	+	+		+	+	+		Prednisolone
7	F	33	+	+					+	Colchicine
8	M	57	+	+		+	+			Prednisolone, indomethacin
9	F	23	+	+		+	+			Indomethacin
10	F	48	+	+		+	+			—
11	F	66	+	+	+	+	+			—
12	M	45	+	+	+	+	+			—
13	M	33	+	+	+	+		+		Chlorambucil, prednisolone
14	M	40	+	+	+		+			,, ,,
15	M	37	+	+	+		+			,, ,,
16	M	29	+	+	+	+				,, ,,
17	M	26	+	+	+	+	+			,, ,, and Colcnicine
18	F	32	+	+	+	+	+			Chlorambucil, prednisolone
19	M	20	+	+	+					,, ,,

Filter motility. A modified Boyden chamber technique was used.[10] Purified PMN (3×10^6 cells) were spun by cytocentrifuge on to 3 μm cellulose acetate filters (Schleicher and Schull, Dassel, West Germany). These were placed in Sykes-Moore chambers. Random motility was assessed as the distance to the 2 cell leading front (in μm) after 20 minutes' incubation of the cells in Hanks's solution. Stimulated migration was measured in a similar manner but with casein-activated pooled AB serum as chemoattractant. Each experiment was carried out in duplicate, and 5 separate measurements were made on each filter.

Agarose plate motility studies employed the method described by Repo.[11] Agarose (Marine Colloids, USA) was dissolved in distilled water in a boiling water bath. The agarose solution was allowed to cool to 45°C before adding it to an equal volume of 10% medium 199 containing human serum albumin. Antibiotics and sodium bicarbonate were then added to give final concentrations of agarose 1%, albumin 1%, penicillin 500 IU/ml, streptomycin 500 μg/ml, $NaHCO_3$ 350 μg/ml.

Molten medium was decanted into tissue culture dishes (Sterilin Products, Teddington, UK) to a depth of 2 mm and allowed to harden by refrigeration at 4°C for 30 minutes. Two wells were cut 2·5 mm apart in the agarose with a 3 mm skin biopsy punch (Steifel Labs, UK), and 7 μl of zymosan-activated pooled human serum (prepared by adding 100 μl of zymosan suspension (10 mg/ml in saline) to 500 μl of serum) instilled into one well. $2\cdot5\times10^5$ purified PMN were placed into the second well. Plates were left at 37°C in a humidified incubator with 4% CO_2 for $2\frac{1}{2}$ hours. On completion of incubation the cells were fixed by flooding the plates with 100% methanol. The agarose gel was then peeled off (leaving the cells adherent to the plastic surface) and the plates allowed to dry. Subsequent staining of the cells with toluidine blue was carried out either the same day or later.

Migration distances were measured to the 'leading cell' with an electronic micrometer attached to the microscope stage. All experiments were carried out in quadruplicate. Studies of random motility were carried out in a similar manner, but on separate tissue culture plates from which chemotaxins were excluded.

The total number of PMN migrating within a 100 μm wide corridor was counted by projecting the stained image of the migrating cells on to graph paper and marking the position of each cell. All such studies were carried out in quadruplicate. Sixteen patients and 16 controls were studied in this way.

Phagocytosis was measured by a modification of Lehrer's method.[12] Heat-killed candida blastospores were washed 3 times and then opsonised by incubation with 20% pooled AB serum for 30 minutes. 500 μl of purified PMN were added to an equal volume of candida and incubated for one hour. The final concentration of PMN was 10 000/μl and candida 90 000 μl. Further phagocytosis was stopped by adding 0·1 M iodoacetate. The candida were stained with methylene blue and smears prepared. One hundred consecutive PMN were identified. Phagocytosis was assessed as the mean number of phagocytosed or attached candida blastospores per PMN (the 'phagocytic index'). Each experiment was carried out in duplicate.

Statistical analysis was by Student's *t* test.

Results

Adherence (Table 2). There was no significant difference between the adherence of Behçet's syndrome PMN and controls, nor between the arthritic and ocular subgroups of Behçet's syndrome and controls.

Phagocytosis (Table 3). Similarly there was no difference in phagocytosis between PMN from Behçet's syndrome and from controls, nor between the ocular and arthritic subgroups and controls.

Motility (filter method) (Fig. 1). PMN motility was measured in 9 patients with Behçet's syndrome. There was an overall increase in *directed migration* when compared with control subjects (Behçet's syndrome 143±31·4 μm; controls 118·8±23·7 μm, p=<0·05). *Random motility* was not significantly different in the Behçet's syndrome group (controls 71·5±12·9 μm, Behçet's syndrome 85·2±24·2 μm). There was no significant difference between the performance of cells from the arthritic and ocular subgroups of patients.

Motility (agarose plate method) (Fig. 2). There was an increase in *directed motility* of the Behçet's syndrome group overall (controls 1691±327 μm, Behçet's syndrome 1907±366 μm, p<0·05), accounted for mainly by the ocular subgroup (2124±153 μm, p=<0·005). *Random motility* was not altered in the Behçet's syndrome group (Behçet's syndrome 1000±162 μm, controls 889±218 μm).

Motility: absolute numbers of cells migrating under agarose. Assessment of motility as the absolute numbers of cells migrating towards zymosan-activated serum (Fig. 3) shows a significantly greater number of Behçet's syndrome cells (598±286) migrating than in controls (301±111, p=<0·0001). Again, the ocular subgroup (761±263) accounts for the greater part of this increase.

Table 2 *PMN adherence in Behçet's syndrome expressed as % PMN adherent to nylon fibre columns*

	Controls	*Behçet's syndrome (total)*	*Behçet's syndrome (ocular subgroup)*
Mean ± standard deviation	45·6±15·5	47·5±12·9*	50·8±6·3*
Number of subjects	12	19	7

*Not significantly different from control value.

Table 3 *Phagocytic index of PMN in Behçet's syndrome (mean number of candida blastospores adherent or phagocytosed per PMN)*

	Controls	*Behçet's syndrome (total)*	*Behçet's syndrome (ocular subgroup)*
Mean ± standard deviation	4·81±1·09	4·53±0·86*	4·85±0·65*
Number of subjects	19	19	7

*Not significantly different from control value.

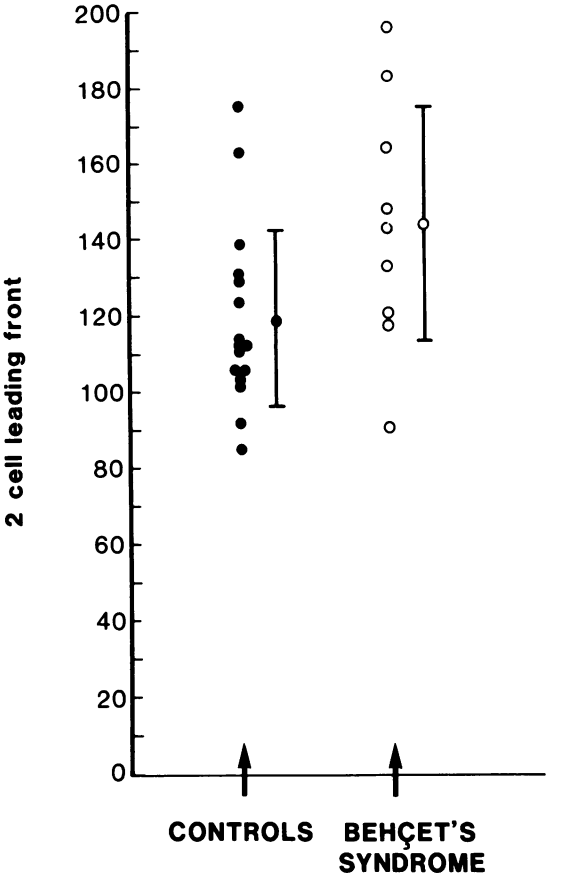

Fig. 1 *Directed motility of PMNs from controls and patients with Behçet's syndrome towards casein-activated AB serum (mean μm±1 standard deviation).*

Discussion

We have found that PMN from patients with Behçet's syndrome have an increased directed motility response to complement-derived chemotactic factors. This is in broad agreement with other reports,[1–4] though differing from those of Abdulla and Lehner.[5] Furthermore, this finding has been demonstrated by 2 different techniques and 2 methods of cell separation and purification. Since these experiments were carried out in a serum-free medium, this suggests that our findings point to a difference in the intrinsic property of the cells, though some latent effect of

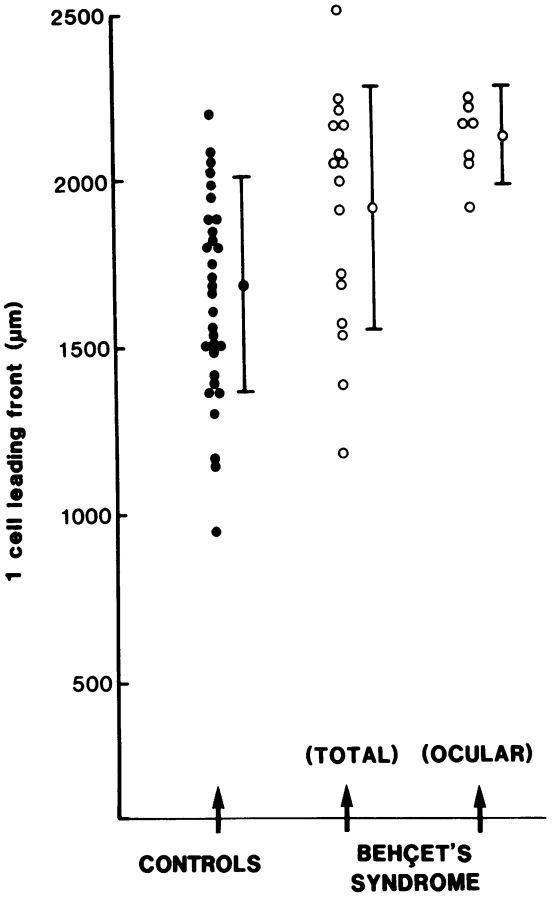

Fig. 2 *Motility response of PMNs from controls and patients with Behçet's syndrome towards zymosan-activated pooled serum (mean μm ± 1 standard deviation).*

serum on the cells cannot be excluded. (Indeed, Takeuchi and colleagues[4] have shown a stimulatory effect of Behçet's serum on normal cell migration due to the presence of a cytoplasmic fraction in Behçet's PMN.) The increased motility response in our study using the agarose technique appeared to be due largely to the ocular subgroup. This has not previously been reported. This may reflect the severity of the disease in these patients compared with the arthritic group. Alternatively, it is not possible to exclude some effect of drug treatment on PMN motility, since all of the ocular group were receiving immunosuppressives. However, 2 of the arthritic patients (nos. 7 and 9), taking only colchicine and indomethacin respectively, also showed increased motility. There is no agreement about the effects of steroids on the motility of PMN, though the majority of in-vitro studies suggest an inhibitory effect.[13-15]

When cell motility is assessed as the total numbers of cells migrating, the increased responsiveness of Behçet's PMN is highlighted and suggests that this method of assessing motility may be more sensitive than the leading front measurements. This is in agreement with the findings of Orr and Ward.[16] This aspect of cell motility is under continuing study by computer-linked image analysis of the migrating cells.

We found the phagocytic ability of Behçet's syndrome PMN to be unimpaired. Differences in phagocytic function of PMN have been reported by other workers, including increased lysosomal enzyme secretion, increased latex particle uptake, and decreased C3-coated zymosan uptake.[17] Our results and those of other workers suggest at most only minor functional differences in the phagocytic ability of these cells. In this respect it is pertinent to note that recurrent infection is not a feature of Behçet's syndrome.

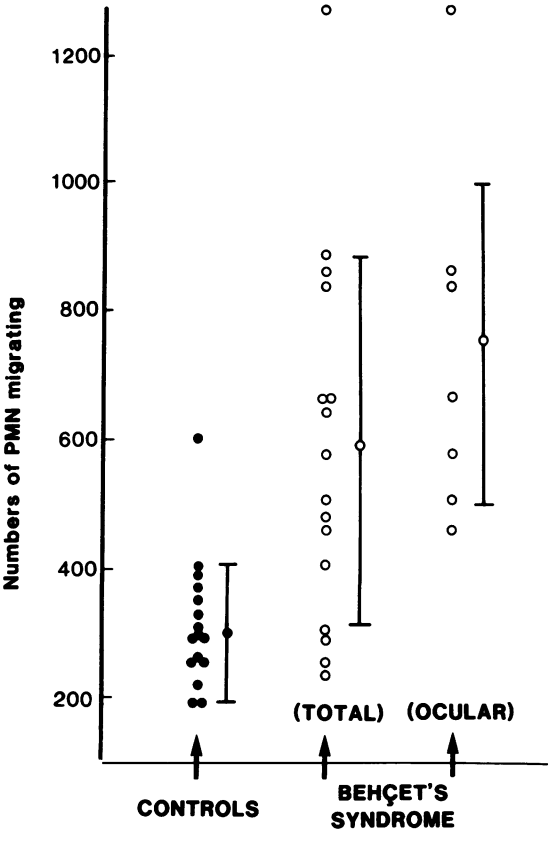

Fig. 3 *Numbers of PMNs migrating under agarose towards zymosan-activated serum (mean ± 1 standard deviation).*

Similarly, Behçet's syndrome PMN as a whole showed no alteration in adherence, neither did the ocular or arthritic subgroups. It has been shown[18] that steroids and nonsteroidal anti-inflammatory drugs impair PMN adhesion, and it is possible that drug effects may have obscured some changes in PMN adhesiveness in our study.

The question arises whether the changes in PMN motility can be related to the pathological changes in Behçet's syndrome. Increased inflammatory response to skin injury which characterises the so-called 'pathergy test' is largely accounted for by a polymorphonuclear infiltrate, at least in the first 24 hours. Subsequently a mononuclear infiltrate predominates.[19] This sequence may reflect the increased motility response of Behçet's syndrome PMN in response to cytotoxins, complement activation, or the bacterial contamination which may occur in this test. Similarly the predominant PMN infiltrate in the synovium and uveal tract[20 21] may also reflect this. It is possible that family studies of PMN motility may contribute to an understanding of the role of this cell type in the expression of Behçet's syndrome. Recently it has been reported[22] that the PMN of normal subjects carrying HLA B27 show an increased directed motility response in comparison with non-B27 subjects. It has been suggested that this may account for the increase in extra-articular features of B27-positive patients with yersinia arthritis compared with patients with yersinia arthritis but not carrying the B27 antigen. Since our study shows the increase in PMN motility in Behçet's syndrome to be due largely to the ocular subgroup, a group known to be associated with HLA B5,[23] it would be of interest to know if possession of this antigen per se influences PMN motility.

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