Polymorphonuclear leucocyte function in Behçet’s disease

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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.

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 temperature, the leucocyte-rich plasma was separated and centrifuged at 500 x 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 x 10⁷ phagocytes/ml for oxygen consumption tests and 1-5 x 10⁸ phagocytes/ml for chemotaxis experiments. Before each experiment leucocyte viability was assessed by the trypan blue exclusion 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
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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 10⁷ 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) 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 CO₂ 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 Obideanu, 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](http://jcp.bmj.com/)

<table>
<thead>
<tr>
<th></th>
<th>Patients (19)</th>
<th>Controls (37)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneous</td>
<td>4-95 ± 4-68</td>
<td>4-13 ± 3-0</td>
<td>NS</td>
</tr>
<tr>
<td>Stimulated</td>
<td>38-21 ± 17-47</td>
<td>46-35 ± 10-01</td>
<td>&lt;0-025</td>
</tr>
</tbody>
</table>

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 0₂/10⁷ 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 0₂/10⁷ phagocytes/hour during phagocytosis of *Staph. albus*, as opposed to 31-59 ± 8-66 μl 0₂/10⁷ phagocytes/hour in the control group (p < 0.010) (Table 2).

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

<table>
<thead>
<tr>
<th></th>
<th>Patients (19)</th>
<th>Controls (3)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting</td>
<td>10-47 ± 5-01</td>
<td>14-51 ± 2-47</td>
<td>&lt;0-025</td>
</tr>
<tr>
<td>Stimulated</td>
<td>22-45 ± 14-23</td>
<td>31-59 ± 8-66</td>
<td>&lt;0-05</td>
</tr>
</tbody>
</table>

Results recorded as mean ± SD of individual tests expressed as μl 0₂/10⁷ 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

<table>
<thead>
<tr>
<th>Serum</th>
<th>Behçet's leucocytes (12)</th>
<th>Normal leucocytes (4)</th>
<th>p value</th>
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</thead>
<tbody>
<tr>
<td>Behçet</td>
<td>9-92 ± 1-71</td>
<td>9-86 ± 1-44</td>
<td>NS</td>
</tr>
<tr>
<td>Normal</td>
<td>9-08 ± 1-75</td>
<td>7-25 ± 0-99</td>
<td>&lt;0-05</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Plasma</th>
<th>Behçet's leucocytes (12)</th>
<th>Normal leucocytes (4)</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td>Behçet</td>
<td>8.44 ± 2.17</td>
<td>8.72 ± 0.90</td>
<td>NS</td>
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<tr>
<td>Normal</td>
<td>7.9 ± 1.07</td>
<td>8.15 ± 0.91</td>
<td>NS</td>
</tr>
<tr>
<td>P value</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

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 C9 (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


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