Changes of Neutrophil Migration Without Modification of *in Vitro* Metabolism and Adhesion in Behçet's Disease

ANTONIO CARLETTO, MARIA LUISA PACOR, DOMENICO BIASI, PAOLA CARAMASCHI, STEFANO ZEMINIAN, PAOLO BELLAVITE, and LISA MARIA BAMBARA

ABSTRACT. ***Objective.*** Increase of neutrophil chemotaxis in Behçet's disease (BD) has been described, but it is not clear whether there is a correlation with other variables of neutrophil function and whether these modifications correlate with disease activity.

Methods. We studied neutrophil functions in patients with BD in the acute phase in comparison with healthy control subjects and with the same patients during disease remission, with or without therapy. We investigated *in vivo* neutrophil migration by Senn's skin window technique and measured adhesion assay and superoxide production in circulating and migrating neutrophils after different stimuli.

Results. Neutrophil migration *in vivo* was $101.3 \pm 17.9 \times 10^6$ polymorphonuclear lymphocytes (PMN)/cm^2/24 h in patients with BD in the acute phase and $66.1 \pm 7.8 \times 10^6$ PMN/cm^2/24 h in controls ($p < 0.001$). No correlation was found between leukocyte counts and neutrophil migration. Neutrophil migration evaluated in the same patients in a phase of disease remission was $58.3 \pm 10.3 \times 10^6$ PMN/cm^2/24 h ($p < 0.001$ vs acute phase, not significant vs controls). The neutrophils of the exudate were normally primed to response to the chemotactic peptide fMLP. No differences between the 2 groups were found in superoxide production, adhesion under basal conditions, or in response to different stimuli by circulating and migrating neutrophils.

Conclusion. Abnormally high migration of neutrophils in the active phase of BD is the only consistent neutrophil dysfunction. Since this modification is reversed by therapy, the evaluation of *in vivo* neutrophil migration may be useful in diagnosing and monitoring disease activity. Blood neutrophils have normal responses to different stimuli, indicating they are not primed by the disease state. (*J Rheumatol 1997;***24:1332–6**)

Key Indexing Terms:
BEHÇET'S DISEASE SKIN INFLAMMATION SUPEROXIDE PRODUCTION
NEUTROPHIL ADHESION CHEMOTAXIS *IN VIVO* NEUTROPHIL MIGRATION

Behçet's disease (BD) is an autoimmune condition characterized by recurrent genital ulcers and oral aphthae, skin lesions, and ocular involvement including uveitis. The systemic character of the disease has been established (e.g., chorioretinitis, erythema nodosum, thrombophlebitis, arthritis, orchiepidimitis, arterial, central nervous system, gastrointestinal, pulmonary involvement)[1,2]. Its etiology is still unknown, although a genetic predisposition appears to play an important role, as suggested by the association with HLA-B51.

Neutrophil (polymorphonuclear leukocytes, PMN) infiltration is an initial characteristic described in skin and eye lesions in patients with BD; some authors have suggested hyperfunction of peripheral blood PMN plays a role in the pathogenesis of the disease. Enhanced PMN chemotaxis[3–7] and superoxide production[8,9] have been described. This PMN hyperfunction has been attributed to genetic factors[8], to the presence of a cytoplasmatic factor[6], and to overproduction of cytokines involved in the modulation of the immune responses[9].

However, the literature is not in agreement: Mege, *et al*[9] have shown increased basal superoxide production in patients with BD, but no differences in PMN stimulated by the chemotactic peptide fMLP; Pronai, *et al*[10] have reported increased superoxide production under basal conditions and after both phorbol myristate acetate (PMA) and zymosan; Takeno, *et al*[8] have described increased superoxide production after stimulus with fMLP, but not under basal conditions.

Another important function involved in chemotactic movement is adhesion; to the best of our knowledge, however, no studies on the adhesion function of BD neutrophils have been reported, with the exception of conflicting data

From the Institute of Patologia Medica, Institute of Clinica Medica, and Institute of Chimica and Microscopia Clinica, University of Verona, Verona, Italy.

A. Carletto, MD, Institute of Patologia Medica; M.L. Pacor, MD, Professor, Institute of Clinica Medica; D. Biasi, MD, Institute of Patologia Medica; P. Caramaschi, MD; S. Zeminian, MD, Institute of Clinica Medica; P. Bellavite, MD, MSc, Professor, Institute of Clinica and Microscopia Clinica; L.M. Bambara, MD, Professor, Institute of Patologia Medica.

Address reprint requests to Dr. A. Carletto, Institute of Patologia Medica, c/o Policlinico Borgo Roma, Via delle Menegone, 37134 Verona, Italy.

Submitted April 9, 1996 revision accepted December 30, 1996.

about membrane adhesion molecules[11,12]. These controversial results prompted us to re-evaluate neutrophil function in BD during various phases of disease, using both *in vivo* migration and *in vitro* superoxide release and adhesion as markers of cell priming, activation, and activity. *In vivo* PMN migration has been investigated by Senn's skin window technique. We studied the superoxide production and adhesion in cells from peripheral blood and in cells harvested from the skin exudate, because the major dysfunction so far described in neutrophils of BD is the enhanced migration in the exudate. We investigated patients with BD in the acute phase in comparison with healthy control subjects and in the same patients during disease remission, with or without therapy.

Using the skin window model to evaluate PMN migration and exudation in healthy subjects, we have previously shown that PMN that migrated *in vivo* into an inflammatory exudate are functionally primed to the response to fMLP but not PMA and zymosan[13]. Therefore, it was of interest to verify whether in patients with BD this characteristic stimulus-specificity of priming was maintained and whether the blood neutrophils were primed during the active phase of disease.

MATERIALS AND METHODS

Patients. Fifteen patients (10 men and 5 women, mean age 27.4 ± 5.3), 9 of whom were HLA-B51 positive and 6 HLA-B51 negative, were enrolled in the study. Twenty-five age and sex matched healthy subjects were considered as controls. These patients were consecutively admitted to our unit from January 1991 to December 1994. All participants provided informed consent.

The diagnosis followed the criteria proposed by the International Study Group for Behçet's Disease[2]. The patients were examined at the same time by the rheumatologist and the ophthalmologist. They underwent therapy with steroid (prednisone 10–20 mg/day) and cyclosporine (5 mg/kg/day). One year after, the disease was in remission in all cases: 10 patients were taking cyclosporine (at the same daily dosage) and steroid at low dose (prednisone to 10 mg/day); 5 patients were taking no drugs.

The disease was considered in remission when physical examination and ophthalmological evaluation revealed no typical lesions (oral and genital ulcers, skin lesions, joint involvement, thrombophlebitis, ocular involvement) and blood tests related to inflammation were normal.

The PMN functions were evaluated in acute phase of disease without therapy, and after one year, during disease remission.

Reagents. The chemotactic peptide fMLP, zymosan, and PMA were purchased from Sigma Chemical Company, St. Louis, MO, USA; cytochrome c from Boehringer, Mannheim, Germany; purified serum bovine albumin and human albumin from Behring Institut, Marburg, Germany. Percoll was from Pharmacia, Uppsala, Sweden. Sterile 96-well microtiter plates with flat bottom wells (Linbro type) were from Flow Laboratories. Hanks' balanced salt solution (HBSS) and reagents were of the highest purity. Zymosan, opsonized with pooled human sera (serum treated zymozan, STZ), as described by Metcalf, *et al*[11], was stored in aliquots at −20°C. We used sterile apyrogenic solutions and disposable plastic ware in all experiments to avoid contamination, a possible cause of artifactual activation or priming of the cells. Experiments were carried out, whenever possible, under a laminar flow hood. Reagents were prepared using pyrogen-free water or 0.9% NaCl solution.

Cell preparation. PMN were obtained from blood and from subjects' skin window exudates. Blood neutrophils were obtained from 40 ml of ethylene diamine tetraacetate-anticoagulated blood by centrifugation over Percoll gradients[13]. Cells were finally suspended in HBSS containing 5 mM glucose and 0.2% human serum albumin (HBSS + glucose + albumin, HGA) and kept at room temperature until use. A few minutes before use, × 100 concentrated solutions of $CaCl_2$ and $MgSO_4$ were added to the cell suspensions at the final concentration of 0.5 and 1 mM, respectively.

Exudate neutrophils were isolated according to the method described by Senn[14], with modifications[15,16]. The volar surface of the nondominant forearm was disinfected with ether and an abrasion of 1 cm^2 was obtained with a rotating sterile abrasive cylinder operated by a milling cutter (minidrill, Saint Julien en Genevois, France). The abrasion did not cause bleeding as only the epidermis was removed and the wet, transuding surface of derma was exposed. A bell shaped, sterile, disposable plastic skin chamber with circular adhesive base (FAR Italia, Verona, Italy) was put on the skin abrasion and fixed with a fenestrated sticking plaster. Atop the chamber is a 5 mm wide hole with a plug. One ml of autologous serum was then injected into the chamber and 24 h later the exudate was collected by aspiration. Exudate cells were then centrifuged at 1200 rpm, washed twice with phosphate buffered saline (PBS) and suspended in HGA and kept at room temperature until use. Before use, the cell suspensions were supplemented with $CaCl_2$ and $MgSO_4$ as described above.

Oxidative metabolism assay. Superoxide anion was measured by the superoxide dismutase inhibitable reduction of ferricytochrome c[17] in a microplate assay system. The microplate assay for O_2^- production was performed according to published methods[18], with the following modifications. The wells were coated with fetal bovine serum as described[18], then supplemented with 25 µl of 0.6 mM cytochrome c in HGA containing 0.5 mM $CaCl_2$ and 1 mM $MgSO_4$ (HGACM) and with 25 µl of the stimulants diluted in HGACM at a concentration exactly 4 times higher than that required in the assay. This was done because each agent is diluted 4 times in the final incubation mixture (see below). The plate was then brought to 37°C and 50 µl of the neutrophil suspensions (2×10^5 cells), prewarmed at 37°C, added to each well using a multichannel pipette, and the plates incubated at 37°C for the desired time. At the time indicated, the plates were rapidly transferred into a microplate reader (Reader 400, SLT Labs Instruments) and the reduction of cytochrome c was measured at 550 nm using 540 nm as reference wavelength, and using 0.037 optical density units as standard for 1 nmole of reduced cytochrome c[18].

Adhesion assay. For adhesion measurements, after cytochrome c reduction assay, the plates were transferred to an automatic washer (Easy Washer 2, SLT Labs Instruments) and subjected to 2 washing cycles with PBS at room temperature. Adherent cells were quantitated by measuring the membrane enzyme acid phosphatase and the percentage of adhesion was calculated on the basis of a standard curve obtained with known numbers of neutrophils[18].

Statistics. We used Wilcoxon's test for unpaired data to compare means obtained from patients in the active phase versus controls; and Wilcoxon's test for paired data to compare values obtained from patients in the active phase versus remission.

RESULTS

As reported in Figure 1, values obtained for PMN migration were 101.3 ± 17.9 × 10^6 PMN/cm^2/24 h in patients with BD in the acute phase and 66.1 ± 7.89 × 10^6 PMN/cm^2/24 h in controls ($p < 0.001$). Migration values in the same patients during remission were 58.3 ± 10.39 × 10^6 PMN/cm^2/24 h ($p < 0.001$ vs acute phase, NS vs controls). The number of cells that migrated into the exudate, independent of the number of circulating leukocytes, was 6482 ± 1187 and 6651 ± 1058 in the acute and remission phase, respectively ($n = 15$; $p = NS$).

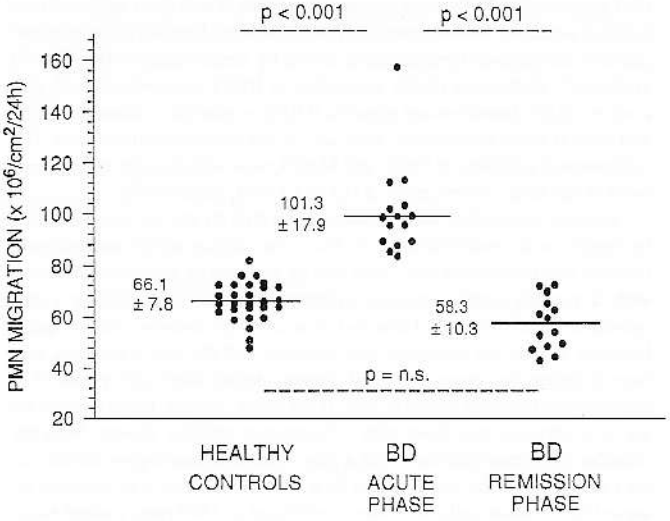

Figure 1. Values of PMN migration in healthy controls, in patients with BD in the acute phase and in the same patients in remission.

Table 1 gives data for superoxide production by circulating and migrating PMN of controls and of patients in the acute and remission phase, under basal conditions and after stimuli (fMLP, STZ, PMA). Exudate cells of both patient and control groups were primed when stimulated by fMLP but not by STZ or PMA. No difference between the 3 groups was found in superoxide production under basal conditions and in response to different stimuli by circulating and migrating PMN.

Table 2 gives results concerning adhesion of circulating and migrating PMN of controls and of patients in the acute and remission phase, under basal conditions and after stimuli (fMLP, STZ, PMA). Migrating PMN of both controls and patients showed increased adhesion compared with circulating PMN, and the difference between the 2 cell populations was particularly evident in the absence of stimulants (resting). Adhesion was increased by the various stimulants, but the priming effect of migration was very low or absent when the adhesion function was evaluated. There was no difference between the 3 subject groups.

Table 1. Superoxide production, under basal conditions and in response to different stimuli, by neutrophils from blood (B) and from skin window exudate (SW) in healthy control subjects (n = 28) and in patients (n = 15) with BD in the acute and remission phase. Values are nmoles $O_2^-/10^6$ PMN ± standard deviation. Incubations of resting cells, fMLP stimulated and PMA stimulated cells were carried out for 10 min; incubations of STZ stimulated cells were carried out for 40 min.

Stimulant		Controls	Patients with Active Disease	Patients in Remission
None	B	0.6 ± 0.6	0.7 ± 0.7	0.7 ± 0.5
	SW	0.9 ± 0.9	0.9 ± 0.8	0.8 ± 0.4
fMLP, 10^{-7} M				
	B	7.3 ± 3.1	6.6 ± 6.6	6.4 ± 2.0
	SW	20.1 ± 5.3	18.9 ± 3.5	18.7 ± 3.3
STZ, 0.1 mg/ml				
	B	8.3 ± 2.4	7.8 ± 2.5	7.8 ± 2.4
	SW	9.1 ± 2.3	8.9 ± 1.9	8.8 ± 1.7
PMA, 10 ng/ml				
	B	22.3 ± 4.5	22.1 ± 4.2	22 ± 4.0
	SW	21.8 ± 4.5	22.5 ± 4.4	22.6 ± 3.9

Table 2. Adhesion of neutrophils from blood (B) and from skin window exudate (SW) under basal conditions and after different stimuli in controls (n = 28) and in patients with BD (n = 15) in the acute and remission phase. Values are percentages of adherent PMN ± standard deviation. Incubations in all conditions were carried out for 40 min.

Stimulant		Controls	Patients with Active Disease	Patients in Remission
None	B	7.2 ± 2.0	7.3 ± 1.4	7.1 ± 1.6
	SW	16.4 ± 5.9	16.8 ± 3.5	17.1 ± 2.9
fMLP, 10^{-7} M				
	B	19.9 ± 9.5	24.2 ± 5.8	23.9 ± 5.0
	SW	28.1 ± 10.3	28.9 ± 6.2	29.6 ± 6.5
STZ, 0.1 mg/ml				
	B	23.9 ± 8.4	25.9 ± 4.8	24.9 ± 3.9
	SW	30.4 ± 10.1	33.8 ± 9.1	32.6 ± 7.5
PMA, 10 ng/ml				
	B	53.8 ± 13.7	54.2 ± 7.1	53.7 ± 5.2
	SW	52.6 ± 6.3	53.5 ± 4.7	53.9 ± 3.9

Control experiments showed that pharmacological treatments used in these patients did not affect superoxide production and adhesion *in vitro*. Indeed, peripheral blood neutrophils incubated 1 h with high, suprapharmacological doses of cyclosporine (up to 200 µg/ml) and prednisone (up to 20 µg/ml) exhibited a respiratory burst and an adhesion response to fMLP comparable with those of untreated cells (n = 6, data not shown).

DISCUSSION

Many authors who have studied PMN function in BD have hypothesized that PMN play an important role in the pathogenesis of the disease[3–6,19,20]. The enhancement of PMN chemotaxis has been confirmed; PMN accumulate in inflammatory lesions where they provoke leukocytoclastic or neutrophilic vasculitis[5,21,22].

Enhanced chemotactic activity may be important in the genesis of pathergy reactivity of the skin and mucous membranes to different triggers in the acute phase of disease; also, mild local injuries are sufficient to elicit rapid extravasation and local accumulation of leukocytes. These subsequently degenerate and release lysosomal enzymes into surrounding tissues[3].

Several authors investigated the oxidative metabolism of PMN and some found an increase in oxidative responses[8,10,23–25]. However, the data concerning this particular PMN function in BD are discordant and this prompted us to re-evaluate the problem using a well established multiwell plate method that allows simultaneous measurement of multiple variables in multiple subjects[10,16,18,26]. Unique aspects of our study are (a) the evaluation of PMN migration *in*

vivo; (b) availability for functional assays of PMN that have migrated to inflammatory exudate; (c) comparison of PMN functions among the same patients first investigated in the active phase of disease before therapy, and one year later, during remission.

Increased PMN migration *in vivo* observed in our patients with active BD is in agreement with the literature; we also observed its normalization in inactive disease, independently of the presence or absence of maintenance treatment. This supports the hypothesis that enhanced PMN migration is more probably due to overproduction of cytokines by skin in the acute phase of disease[9] than by genetic factors affecting neutrophil migration capacity[8]. Our small population of patients did not permit comparison of HLA-B51 positive and negative subjects, but it is our impression there are no differences.

Furthermore, we found no difference in superoxide production among patients in active versus inactive phases of disease. No difference was found in superoxide under basal conditions or after stimuli (fMLP, STZ, PMA) between patient and control subjects. This finding is significant because it indicates that circulating neutrophils are neither activated nor primed in patients with BD. This conclusion is strengthened if one considers (1) that our microplate test assay is particularly sensitive for detection of priming, as shown by consistent priming of the response to fMLP of exudate cells (in controls and patients); (2) our previous findings, obtained with the same method, of primed circulating neutrophils in other rheumatic diseases[16]; and (3) age related differences in the neutrophil oxidative responses to fMLP[27].

It is conceivable that some of the discrepancies regarding activation of oxidative metabolism in PMN of patients with BD might be due to the different methods of cell isolation; in fact, we used Percoll gradients (one step method), while others used the Ficoll procedure (2 step method), and we have observed that Ficoll occasionally causes cell activation. Moreover, we and other authors[27,28] have shown that the extent of oxidative activation in response to fMLP is markedly affected by the age of subjects; therefore, in this study our control subjects and patients were carefully matched for age.

There are few studies that deal with the question of adhesion: Ozgun and co-workers reported that sera obtained from patients with BD resulted in increased surface expression of CD11a, but not CD11b molecules in human normal PMN[11]. Inaba showed that L-selectin expression was not altered[12]. Although we did not measure expression of leukocyte integrins, our data do not indicate any differences in adhesion of resting and stimulated PMN between patients and controls or among patients in the active versus inactive phase of disease. Taken together, these data suggest that a possible increase of CD11a in BD PMN is not associated with activation of the functional properties of these membrane molecular moieties.

In accordance with previous investigations[10,26,29–33], our results show that exudate PMN are metabolically primed, being more responsive to fMLP stimulus than blood PMN, and present increased adherence in the absence of stimulants. Moreover, the metabolic state of circulating neutrophils and the extent of *in vivo* priming are not influenced by the disease or by disease activity.

Our investigation suggests that PMN dysfunction in BD is only indirectly related to enhanced migration into the inflammatory focus and that this dysfunction is not the consequence of a primary hyper-reactivity (priming) of circulatory cells. It is conceivable that the enhanced migration is related to local overproduction of chemokines in the active phase of disease[9], but further studies are necessary to confirm this hypothesis.

Finally, since the finding of increased migration in the patient group during active disease has proven to be highly significant and consistent, our study suggests that the evaluation of *in vivo* PMN migration may be useful in diagnosing and in monitoring disease activity.

REFERENCES

1. Chajek T, Fainaru M: Behçet's disease. Reports of 41 cases and review of the literature. *Medicine 1975;*54:179–96.
2. International Study Group for Behçet's Disease: Criteria for diagnosis of Behçet's disease. *Lancet 1990;*335:1078–80.
3. Djawari D, Hornstein OP, Schotz J: Enhancement of granulocyte chemotaxis in Behçet's disease. *Arch Dermatol Res 1981;*270:81–8.
4. Efthimiou J, Addison IE, Johnsonn BU: *In vivo* leukocyte migration in Behçet's syndrome. *Ann Rheum Dis 1989;*49:206–10.
5. Jorizzo JL, Hudson RD, Schalmstieg FC, *et al:* Behçet's syndrome: Immune regulation, circulating immune complex, neutrophils migration and colchicine therapy. *J Am Acad Dermatol 1984;*10:205–14.
6. Takeuki A, Kobayashi K, Mori M, *et al:* The mechanism of hyperchemotaxis in Behçet's disease. *J Rheumatol 1981;*8:40–4.
7. Matsumura N, Mizushima Y: Leukocyte movement and colchicine treatment in Behçet's disease (letter). *Lancet 1975;*2:813.
8. Takeno M, Karidone A, Yamashita N, *et al:* Excessive function of peripheral blood neutrophils from patients with Behçet's disease and from HLA-B51 transgenic mice. *Arthritis Rheum 1995;*38:426–33.
9. Mege JL, Dilsen N, Sanguedolce V, *et al:* Overproduction of monocyte derived tumor necrosis factor α, interleukin (IL) 6, IL-8 and increased neutrophil superoxide generation in Behçet's disease. A comparative study with familial Mediterranean fever and healthy subjects. *J Rheumatol 1993;*20:1544–9.
10. Pronai L, Ichikawa Y, Nakazawa H, *et al:* Enhanced superoxide generation and the decreased superoxide scavenging activity of peripheral blood leukocytes in Behçet's disease — effect of colchicine. *Clin Exp Rheumatol 1991;*9:227–33.
11. Ozgun S, Akoglu T, Direskeneli H, Yurdakull S: PMN adhesion to endothelial cells in patients with Behçet's disease (abstr). *Rev Med Int 1993;*(suppl 1)14:133S.
12. Inaba G, Kaku IIH, Ujihara H, *et al:* Leukocyte adhesion molecules in active ocular lesions of Behçet's disease (abstr). *Rev Med Int 1993;*(suppl 1)14:24S.
13. Metcalf JA, Gallin JI, Nauseef WM, Root RK: Laboratory manual of neutrophil function. New York: Raven Press, 1986.
14. Senn HJ, Jungi WF: Neutrophil migration in health and disease. *Semin Hematol 1975;*12:27–45.
15. Biasi D, Bambara LM, Carletto A, *et al:* Factor-specific changes in

oxidative burst response of human neutrophils in skin window exudates. *Inflammation 1993;*17:13–23.
16. Biasi D, Carletto A, Caramaschi P, *et al:* Neutrophil functions, spondylarthropathies and HLA-B27: A study of 43 patients. *Clin Exp Rheumatol 1995;*13:623–7.
17. Babior BM, Kipnes RS, Curnutte JT: Biological defense mechanisms. The production by leukocytes of superoxide, a potential bactericidal agent. *J Clin Invest 1973;*52:741–4.
18. Bellavite P, Chirumbolo S, Mansoldo C, Gandini G, Dri P: A simultaneous assay for oxidative metabolism and adhesion of human neutrophils. Evidence for correlations and dissociation of the two responses. *J Leukocyte Biol 1992;*51:329–33.
19. Djawari D, Hornstein OP, Lukner L: Skin window examination according to Rebuk and cutaneous pathergy test in patients with Behçet's disease. *Dermatol 1985;*170:265–70.
20. Mizushima Y: Recent research into Behçet's disease in Japan. *Int J Tiss React 1988;*10:59–65.
21. Haim S, Sobel JD, Friedmann-Birnbaum R, *et al:* Histological and direct immunofluorescence study of cutaneous hyperactivity in Behçet's disease. *Br J Dermatol 1976;*95:631–6.
22. Jorizzo JL, Solomon AR, Zanolli MD, *et al:* Neutrophilic vascular reactions. *J Am Acad Dermatol 1988;*19:983–1005.
23. Niwa Y, Miyake S, Sakane T, *et al:* Autooxidative damage in Behçet's disease — endothelial cell damage following the elevated oxygen radicals generated by stimulated neutrophils. *Clin Exp Immunol 1982;*49:247–55.
24. Fujita Y, Yamada MM, Asai K, *et al:* Generation of superoxide radical by PMN in Behçet's disease. *Jpn J Ophthalmol 1984;*3:221–6.
25. Sobel JD, Haim S, Obedeanu N, Meshulam T: Polymorphonuclear leukocyte function in Behçet's disease. *J Clin Pathol 1977;*30:250–3.
26. Biasi D, Bambara LM, Carletto A, *et al:* Molecular mechanisms of priming: Exudate human neutrophils are primed to fMET-LEU-PHE but have normal intracellular calcium and cAMP responses. *Bull Mol Biol Med 1993;*18:225–35.
27. Biasi D, Carletto A, Dell'Agnola C, *et al:* Neutrophil migration, oxidative metabolism and adhesion in young and elderly subjects. *Inflammation 1996;*20:673–81.
28. Tortorella C, Ottolenghi A, Pugliese P, Jirillo E, Antonaci S: Polymorphonuclear cells in elderly subjects. *Facts Res Gerontol 1994;*3:211–20.
29. Briheim G, Stendhal O, Coble BI, Dahlgren C: Exudate polymorphonuclear leukocytes isolated from chambers are primed for enhanced response to subsequent stimulation with the chemoattractant fMet-Leu-Phe and C3 opsonized yeast particles. *Inflammation 1988;*12:141–52.
30. Van Epps DE, Garcia ML: Enhancement of neutrophil function as a result of prior exposure to chemotactic factor. *J Clin Invest 1980;*66:167–75.
31. Bellavite P, Carletto A, Biasi D, *et al:* Studies of skin window exudate human neutrophils. Complex patterns of adherence to serum-coated surfaces in dependence on fMLP doses. *Inflammation 1994;*18:575–87.
32. Paty PB, Graeff RW, Mates SJ, Hunt TK: Superoxide production by wound neutrophils. Evidence for increased activity of the NADPH oxidase. *Arch Surg 1990;*125:65–9.
33. Nurcombe HL, Bucknall RC, Edwards SW: Neutrophils isolated from the synovial fluid of patients with rheumatoid arthritis: Priming and action *in vivo*. *Ann Rheum Dis 1991;*50:147–53.