

**AIM** Behçet's disease (BD) is a systemic immunoinflammatory disorder and the aetiopathogenesis is to be specified. Cytokines play a role in immune response and in many inflammatory diseases. The aim of this case–control study is to investigate serum pro-inflammatory cytokine tumour necrosis factor (TNF)- $\alpha$ , interleukin-1beta (IL-1 $\beta$ ), soluble IL-2 receptor (sIL-2R), IL-6, and chemokine IL-8 levels in patients with BD. We also determined the end product of lipid peroxidation (malondialdehyde (MDA)) in BD patients as an index for oxidative stress.

**Methods:** A total of 37 patients (19 men, 18 women) with BD (active,  $n = 17$ ; inactive,  $n = 20$ ) and 20 age-matched and sex-matched healthy control subjects (11 men, nine women) included in this cross-sectional, blinded study. Serum TNF- $\alpha$ , IL-1 $\beta$ , sIL-2R, IL-6 and IL-8 levels were determined by a spectrophotometer technique using the immulite chemiluminescent immunometric assay. Lipid peroxidation was evaluated by Wasowicz *et al.* The levels of cytokines and lipid peroxidation in the active period were compared with the inactive period of the disease. Results are expressed as mean  $\pm$  standard error.

**Results:** IL-1 $\beta$  levels were below the detection limits of the assay ( $< 5$  pg/ml) in all samples. Mean levels of MDA ( $8.1 \pm 0.7$   $\mu$ mol/l), sIL-2R ( $800 \pm 38$  U/ml), IL-6 ( $12.6 \pm 1.1$  pg/ml), IL-8 ( $7.2 \pm 0.4$  pg/ml), and TNF- $\alpha$  ( $7.9 \pm 0.5$  pg/ml) in active BD patients were significantly higher than those in inactive patients ( $4.3 \pm 0.5$   $\mu$ mol/l,  $p < 0.01$ ;  $447 \pm 16$  U/ml,  $p < 0.001$ ;  $8.3 \pm 0.6$  pg/ml,  $p = 0.006$ ;  $5.3 \pm 0.1$  pg/ml,  $p < 0.001$ ; and  $5.1 \pm 0.2$  pg/ml,  $p < 0.001$ ; respectively) or control subjects ( $2.1 \pm 0.2$   $\mu$ mol/l,  $p < 0.001$ ;  $446 \pm 20$  U/ml,  $p < 0.001$ ;  $6.4 \pm 0.2$  pg/ml,  $p < 0.001$ ;  $5.4 \pm 0.1$  pg/ml,  $p < 0.001$ ; and  $4.7 \pm 0.1$  pg/ml,  $p < 0.001$ , respectively). On the contrary, only the mean IL-6 level was significantly different between inactive BD and control subjects ( $p = 0.02$ ). All acute phase reactants were significantly higher in active BD than in inactive period (for each,  $p < 0.01$ ).

**Conclusions:** High levels of sIL-2R, IL-6, IL-8 and TNF- $\alpha$  indicate the activation of immune system in BD. Serum sIL-2R, IL-6, IL-8 and TNF- $\alpha$  seem to be related to disease activity. Increased lipid peroxidation suggests oxidative stress in BD and therefore tissue damage in such patients. Amelioration of clinical manifestations would be envisaged by targeting these cytokines, chemokines and lipid peroxidation with pharmacological agents.

**Key words:** Behçet's disease, Chemokine, Cytokine, Immunoassay, Interleukin, Lipid peroxidation

## Serum levels of TNF- $\alpha$ , sIL-2R, IL-6, and IL-8 are increased and associated with elevated lipid peroxidation in patients with Behçet's disease

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## Introduction

Cytokines are low molecular weight polypeptides involved in the communication between cells.<sup>1</sup> Abnormal production of some cytokines such as tumour necrosis factor (TNF)- $\alpha$ , interleukin-1beta (IL-1 $\beta$ ), soluble IL-2 receptor (sIL-2R), IL-6, and chem-

okine IL-8 have been implicated in the pathogenesis of various inflammatory and autoimmune diseases.<sup>1,2</sup> Many *in vivo* studies have demonstrated that TNF- $\alpha$ , IL-1, IL-6, and IL-8 are all important components of the pro-inflammatory response and intraocular inflammation.<sup>2</sup> Oxidative stress is associated with many systemic inflammatory diseases, and free-radical

production leads to the formation of self-propagating lipid peroxidation.<sup>3</sup>

Beh et's disease (BD) is a systemic inflammatory disorder of young adults involving small blood vessels (veins and arteries) and is characterised by occlusion in both the deep venous and retinal circulations with unknown aetiology.<sup>4</sup> After its first description as oral and genital ulceration with hypopyon uveitis in 1937 by the Turkish physician Prof. Dr Hulusi Beh et,<sup>5</sup> the multisystem character of the disease has well been established.<sup>4</sup> The classical ocular involvement is iridocyclitis, anterior hypopyon uveitis, retinal vasculitis or panuveitis. In severe ocular disease, the visual prognosis is poor and blindness may occur despite immunosuppressive treatment. Although the aetiopathogenesis of the disease has not yet been clarified, several mechanisms such as genetics, infection, immunoglobulin, immune complexes and antibodies have all been suggested as the causes of BD.<sup>4,6</sup> It affects almost every tissue and organ in the body without exception, including the cornea,<sup>7</sup> inner ear<sup>8</sup> and the lung.<sup>9</sup> The intermittent nature and the lack of consistent response to therapy make the underlying aetiology difficult to define.

Our previous studies demonstrated that some molecules such as adrenomedullin,<sup>10</sup> leptin,<sup>11</sup> and homocysteine<sup>12</sup> might participate during the course of BD. Furthermore, our more recent study showed that nitric oxide (NO), one of the most abundant free radicals in the body, was significantly increased in BD and associated with disease activity, suggesting a new activity marker.<sup>13</sup> Since pro-inflammatory cytokines are known to be the potent inducer of leptin and, therefore, NO from the endothelial cells, it is important to study the levels of these cytokines as well as lipid peroxidation products as a cohort study during the course of BD. Similarly, homocysteine enhances NO synthesis from endothelial cells, induces the expression of chemoattractants by oxygen free radicals, and has important interrelations with cytokines.<sup>14</sup> Therefore, this study has attempted to overcome these limitations in our understanding by measuring the serum levels of pro-inflammatory cytokines such as TNF- $\alpha$ , IL-1 $\beta$ , sIL-2R, IL-6 and chemokine IL-8 as well as lipid peroxidation, linking to the state of disease activity. We wished to test the hypothesis that no one factor alone is predominant in the pathophysiology during the course of BD, but that a regular profile of levels of different molecules might be operating together, and that the profile might differ according to disease activity.

## Patients and methods

### Study population

Thirty-seven consecutive patients with BD (19 men, 18 women; mean age, 37.2 years) who attended to

the Department of Ophthalmology and/or the Department of Dermatology, and 20 age-matched and sex-matched healthy control subjects (11 men, nine women; mean age, 38.4 years) from a similar ethnic background were included in this study. Subjects with any disorder such as hepatic or renal diseases, diabetes, essential hypertension and pregnancies were excluded from the study. Since medications such as glucocorticosteroids reduce the transcription of the pro-inflammatory cytokines (IL-1, IL-2, IL-6, IL-8, and TNF- $\alpha$ ),<sup>15</sup> a detailed history of drug use was obtained in both groups and, as such, three patients using steroids and two patients using immunosuppressives were not included in the study. The diagnosis of BD was made according to the criteria for diagnosis of the International Study Group for Beh et's Disease<sup>16</sup> (Table 1). Informed consent was obtained from all subjects in both groups.

Nineteen patients (51.3%) had severe ocular involvement (anterior hypopyon uveitis/iridocyclitis, retinal vasculitis, cells in the vitreous, or panuveitis). In addition, oral lesions were present in all cases (100%). Thirty-four patients (91.8%) had various coetaneous lesions. Articular symptoms were present in 33 patients (89.1%) and genital ulceration in 31 (83.7%). Eighteen of 37 patients (48.6%) showed a positive pathergy test, nine patients (24.3%) complained of neurological symptoms and one of them (2.7%) had Neuro-Beh et's. Gastrointestinal system symptoms and signs were present in eight patients (21.6%).

Since there is no clinically acceptable scoring system and laboratory screening profile to define the severity of BD, active ( $n = 17$ ) and inactive ( $n = 20$ ) BD patients were determined by both clinical and laboratory findings. In clinical evaluation, worsening of clinical symptoms at the time of study and having at least three of the major symptoms (oral ulcers, genital ulcers, skin lesions and uveitis) were considered to be in the active period of the disease. The inactive patients had no any symptoms and signs of disease activity at least 3 months before admission. The diagnosis of uveitis was made according to the International Uveitis Study Group.<sup>17</sup> In laboratory investigations, the erythrocyte sedimentation rate (ESR), neutrophil count and acute phase reactants ( $\alpha_1$ -antitrypsin and  $\alpha_2$ -macroglobulin concentrations) were investigated.<sup>10-13</sup>

### Neutrophil count, ESR and acute-phase reactants analyses

This was a double-blind study. Therefore, both the physician taking the blood and the analyser were blinded to the group of the subject. In both groups, antecubital whole blood samples (total, 5 ml) were obtained by venipuncture from a peripheral vein, avoiding haemolysis, into plain tubes during resting

**Table 1.** Clinical findings of patients with active and inactive Behçet's disease: International Study Group for Behçet's disease criteria\* for the diagnosis of Behçet's disease

No.	Sex	A	D	Ou	Coetaneous lesions	Gu	Path.‡	Ocular lesions	Other findings
1†	M	38	8	+	Pseudofolliculitis, acneiform lesions	-	+	Right retinal vasculitis	Migraine headache
2	W	44	16	+	Erythema nodosum, pseudofolliculitis Acneiform lesions	+	+	-	Arthralgia at the knees ALP
3†	W	22	2	+	Pseudofolliculitis, acneiform lesions	-	+	-	Arthralgia at the ankles
4†	M	45	12	+	Pseudofolliculitis, acneiform lesions	+	-	Left anterior iridocyclitis	Arthritis at the knees
5	M	52	24	+	-	+	-	Bilateral panuveitis	Migraine headache Sacroiliitis
6†	W	52	21	+	Erythema nodosum, pseudofolliculitis	-	+	-	Neuro-Behçet's, arthralgia at the knees and ankles
7	W	35	6	+	Acneiform lesions	+	+	-	Arthralgia at the knees
8	M	34	7	+	Pseudofolliculitis, acneiform lesions	+	+	-	Arthralgia at the knees
9†	M	29	4	+	Acneiform lesions, thrombophlebitis	+	-	Right hypopyon uveitis	Arthritis at the knees
10	M	26	3	+	Pseudofolliculitis, acneiform lesions	+	-	Left retinal vasculitis	Arthritis at the ankles
11	W	44	10	+	Erythema nodosum, thrombophlebitis Pseudofolliculitis, acneiform lesions	+	+	-	Sacroiliitis ALP
12†	M	33	8	+	Pseudofolliculitis, acneiform lesions	+	-	Bilateral retinal vasculitis	Migraine headache
13	M	32	7	+	Thrombophlebitis, pseudofolliculitis	+	-	-	ALP
14	W	25	3	+	Pseudofolliculitis, acneiform lesions	+	+	-	Swelling in both knees
15†	W	45	12	+	-	+	+	Left hypopyon uveitis	Arthritis at the knees
16†	M	36	11	+	Pseudofolliculitis, acneiform lesions	+	+	Bilateral vitreal cells Right hypopyon uveitis	Arthralgia at the ankles ALP
17	W	26	2	+	Pseudofolliculitis, acneiform lesions	+	+	-	Migraine headache, Arthralgia at the knees
18	W	52	21	+	Erythema nodosum, thrombophlebitis	+	-	Bilateral retinal vasculitis	Arthralgia at the knees
19†	M	45	15	+	Erythema nodosum, thrombophlebitis Acneiform lesions	+	+	-	Melena Arthralgia at the right knee
20	M	45	17	+	Pseudofolliculitis	+	-	Bilateral retinal vasculitis	Arthralgia at the knees
21	M	34	8	+	Erythema nodosum	+	-	Right iridocyclitis	Arthritis at the ankles and fingers
22†	W	56	25	+	Erythema nodosum, thrombophlebitis Pseudofolliculitis, acneiform lesions	+	+	-	Sacroiliitis
23	W	25	3	+	Erythema nodosum, thrombophlebitis Pseudofolliculitis, acneiform lesions	+	-	-	Migraine headache
24	M	36	11	+	-	+	-	Bilateral retinal vasculitis	Arthralgia at the knees and fingers Left anterior uveitis
25	W	35	9	+	Thrombophlebitis, pseudofolliculitis Acneiform lesions	-	+	Bilateral iridocyclitis	Melena Arthralgia at the knees
26†	W	19	1	+	Pseudofolliculitis, acneiform lesions	+	+	-	Arthralgia at the knees and fingers
27†	M	33	9	+	Pseudofolliculitis, acneiform lesions	+	-	Bilateral retinal vasculitis	Arthralgia at the knees
28†	W	38	11	+	Pseudofolliculitis, acneiform lesions	+	-	-	Arthralgia at the fingers
29	W	36	7	+	Pseudofolliculitis	+	-	-	Migraine headache Arthralgia at the fingers
30†	M	31	5	+	Thrombophlebitis, pseudofolliculitis Acneiform lesions	+	-	Left retinal vasculitis	Arthralgia at the knees ALP
31	W	46	15	+	Erythema nodosum, thrombophlebitis Pseudofolliculitis	+	-	-	Ankylosing spondylitis ALP
32	M	26	3	+	Pseudofolliculitis, acneiform lesions	-	+	-	Arthralgia at the knees
33†	M	45	13	+	Erythema nodosum, pseudofolliculitis Acneiform lesions	+	-	Right iridocyclitis	Arthralgia at the knees
34†	W	41	15	+	Thrombophlebitis, pseudofolliculitis Acneiform lesions	+	-	Left anterior uveitis	Arthralgia at the knees and ankles
35	M	41	12	+	Pseudofolliculitis, acneiform lesions	+	+	Bilateral iridocyclitis	Migraine headache Arthralgia at the left knee
36	W	32	8	+	Pseudofolliculitis, acneiform lesions	-	+	-	Migraine headache Arthralgia at the right knee
37†	M	36	11	+	Erythema nodosum, pseudofolliculitis	+	-	Bilateral iridocyclitis	Arthralgia at the knees

A, Age (years); D, disease duration (years); Ou, recurrent oral aphthous ulceration; Gu, recurrent genital ulceration; Path., pathergy test; M, men; W, women, ALP, appendicitis-like pain.

\*Ou plus two of the following criteria (Gu, ocular lesions, coetaneous lesions, pathergy test positivity).

† Active patients with Behçet's disease.

‡ Observation of a pustular lesion by physician at 24–48 h after needle-stick.

position in the morning hours (08.00–10.00 h) after an overnight fast and 30 min of supine rest. None of the patients and healthy control subjects was receiving any topical or systemic medication on admission. Following centrifugation of the first half of the blood samples (2.5 ml) at  $800 \times g$  for 10 min, serum was separated and kept at  $-20^{\circ}\text{C}$  until the time of analysis.

The other half of the blood samples (2.5 ml), with ethylenediamine tetraacetic acid (1 mg/ml) anticoagulant was used for the neutrophil counting by an automated blood counter (Coulter-STKS, Luton, UK).  $\alpha_1$ -Antitrypsin and  $\alpha_2$ -macroglobulin concentrations were measured in the serum by a Behring nephelometer 100 analyser (Dade Behring, Messer Griesheim, Frankfurt, Germany). The ESR was determined by the classical Westergren method.

### Cytokine and chemokine analyses

Cytokine analysis was performed according to the Immulite® (Diagnostic Products Corporation, Los Angeles, CA, USA) chemiluminescent enzyme immuno-metric assay. The technique is based on a solid-phase (bead) two-site assay. The solid phase, a polystyrene bead, is coated with either a monoclonal specific antibody (TNF- $\alpha$ , IL-1 $\beta$ ) or an anti-ligand (sIL-2R, IL-6, IL-8). Patient serum and alkaline phosphatase-conjugated monoclonal antibody or ligand-labelled antibody are incubated for 30–60 min at  $37^{\circ}\text{C}$ . Unbound conjugate is then removed by a centrifugal wash (three times), after which a chemiluminescent substrate (a phosphate ester of adamantyl dioxetane) is added and the test unit is incubated for a further 10 min. The Immulite system automatically handles sample and reagent additions, the incubation and separation step, and measurement of the photon output via the temperature-controlled luminometer. It calculates test results for control and patient samples from the observed signal, using a stored master curve, and generates a printed report.<sup>18</sup> The values of the inter-assay imprecision study were similar to those from the intra-assay study with coefficient of variation (CV) ranging from 2 to 11.5%. CVs for the measured cytokines were usually around 5%. The linearity is satisfactory, with a regression coefficient higher than 0.99 ( $r^2$ ); slopes close to 1.0 were obtained. There is an excellent practicality of the system and good stability of the calibration curve (15 days). It is a good reliable method yielding a good precision along with a satisfactory detection limit.<sup>19</sup> The antibodies used in the Immulite® for TNF- $\alpha$ , IL-1 $\beta$ , sIL-2R, IL-6, and IL-8 are highly specific for each cytokine and chemokine, with no cross-reactivity to other cytokines that may be present in the serum samples.

For each new cytokine calibration, a master curve is constructed by the manufacturer using a material calibrated against the National Institute for Biological Standards and Control. For each new cytokine reagent

lot, an adjustment of the calibration slope is made by the user by measuring two serum matrix vials (low and high) designated as 'adjusters'. It should be mentioned that between-run and within-run imprecision data were similar, which is very important for stat measurement.

### Thiobarbituric acid-reactive substances analysis

The plasma malondialdehyde (MDA) level, referred to as thiobarbituric acid-reactive substances (TBARS), was measured according to the method described by Wasowicz *et al.*<sup>20</sup> In brief, 50 ml of sample or an adequate volume of MDA working standard solution was introduced into 10 ml glass tubes containing 1 ml of distilled water. After addition of 1 ml of the solution containing 29 mmol/l of TBARS in acetic acid and mixing, the samples were placed in a water bath and heated for 1 h at  $95$ – $100^{\circ}\text{C}$ . After the samples were cooled,  $25 \mu\text{l}$  of 5 mol/l HCl was added, and the reaction mixture was extracted by agitation for 5 min with 3.5 ml of *n*-butanol. The butanol phase was separated by centrifugation at  $1500 \times g$  for 10 min. The butanol extract was measured with a spectrofluorometer (F-4010 fluorescence spectrophotometer; Hitachi, Tokyo, Japan) at wavelengths of 525 nm for excitation. The calibration curve was prepared with MDA standards of 0–10  $\mu\text{mol/l}$ . Intra- and inter-assay CVs were 3.5 and 6%, respectively.

### Statistical analysis

Results were analysed statistically by using the analysis of variance or the Mann–Whitney *U*-test as indicated, and were expressed as mean  $\pm$  standard error (range).  $p < 0.05$  was considered a significant difference between the groups. Statistical analysis was performed with the Statistical Package for the Social Sciences for Windows (version 8.0; SPSS Inc., Chicago, IL, USA).

### Results

For IL-1 $\beta$ , all samples in both groups were below the detection limits of the assay ( $< 5 \text{ pg/ml}$ ). sIL-2R levels ranged from 347 to 1076 U/ml, with the highest values observed in the active BD patients. Mean sIL-2R concentration in active BD patients was  $800 \pm 38 \text{ U/ml}$  and the difference was significant when compared with inactive patients ( $447 \pm 16 \text{ U/ml}$ ,  $p < 0.001$ ) or healthy control subjects ( $446 \pm 20 \text{ U/ml}$ ,  $p < 0.001$ ) (Table 2). IL-6 concentrations ranged from 5 to 22.5 pg/ml, with the highest values observed in the active BD patients, and the mean values in active patients ( $12.6 \pm 1.1 \text{ pg/ml}$ ) were significantly higher than those in inactive patients ( $8.3 \pm 0.6 \text{ pg/ml}$ ,  $p = 0.006$ ) or control subjects ( $6.4 \pm 0.2 \text{ pg/ml}$ ,  $p < 0.001$ ). Mean IL-8 concentrations were found to be higher in patients with active BD ( $7.2 \pm 0.4 \text{ pg/ml}$ ) than in

**Table 2.** MDA, TNF- $\alpha$ , IL-1 $\beta$ , sIL-2R, IL-6 and IL-8 levels in patients with Behçet's disease and control subjects, and the relation with disease activity

Group	n	Age (years) (range)	Men/women	MDA ( $\mu$ mol/l) (mean $\pm$ SE)	IL-1 $\beta$ (pg/ml) (mean $\pm$ SE)	sIL-2R (U/ml) (mean $\pm$ SE [range])	IL-6 (pg/ml) (mean $\pm$ SE [range])	IL-8 (pg/ml) (mean $\pm$ SE [range])	TNF- $\alpha$ (pg/ml) (mean $\pm$ SE [range])
Active BD*	17	37.8 (19–56)	10/7	8.1 $\pm$ 0.7	< 5.0	800 $\pm$ 38 (465–1076)	12.6 $\pm$ 1.1 (6.0–22.5)	7.2 $\pm$ 0.4 (5.2–11.2)	7.9 $\pm$ 0.5 (4.9–12.2)
<i>p</i> value†				< 0.01	NS	< 0.001	0.006	< 0.001	< 0.001
Inactive BD	20	36.3 (25–52)	9/11	4.3 $\pm$ 0.5	< 5.0	447 $\pm$ 16 (347–563)	8.3 $\pm$ 0.6‡ (5.0–14.7)	5.3 $\pm$ 0.1 (5.0–6.5)	5.1 $\pm$ 0.2 (4.0–7.4)
<i>p</i> value‡				NS	NS	NS	0.02	NS	NS
Control subjects	20	38.4 (25–56)	11/9	2.1 $\pm$ 0.2	< 5.0	446 $\pm$ 20 (248–605)	6.4 $\pm$ 0.2 (5.0–8.5)	5.4 $\pm$ 0.1 (5.0–7.4)	4.7 $\pm$ 0.1 (4.0–7.1)

NS, not significant; SE, standard error.

\* Mean values for all parameters (except IL-1 $\beta$ ) were significantly (for each,  $p < 0.001$ ) higher than control subjects by analysis of variance.

† Statistical values between active and inactive BD subjects by analysis of variance.

‡ Statistical values between inactive patients and control subjects by analysis of variance.

patients with inactive BD ( $5.3 \pm 0.1$  pg/ml,  $p < 0.001$ ) and control subjects ( $5.4 \pm 0.1$  pg/ml,  $p < 0.001$ ). The highest TNF- $\alpha$  concentrations were observed in patients with active BD and the mean value ( $7.9 \pm 0.5$  pg/ml) was significantly higher than inactive BD ( $5.1 \pm 0.2$  pg/ml,  $p < 0.001$ ) or control subjects ( $4.7 \pm 0.1$  pg/ml,  $p < 0.001$ ). Mean MDA levels were also significantly higher in active BD ( $8.1 \pm 0.7$   $\mu$ mol/l) than in inactive BD ( $4.3 \pm 0.5$   $\mu$ mol/l,  $p < 0.01$ ) or control subjects ( $2.1 \pm 0.2$   $\mu$ mol/l,  $p < 0.001$ ). Mean MDA, sIL-2R, IL-8 and TNF- $\alpha$  levels in patients with inactive BD were not higher (for each,  $p > 0.05$ ) when compared with the control subjects, unlike IL-6 ( $p = 0.02$ ).

There were statistically significant (for each,  $p < 0.01$ ) differences for acute-phase reactants between active and inactive periods of the disease (Table 3).

## Discussion

The main pathology in BD is an inflammatory process of small arteries and veins and thrombosis as a result of vasculitis of the vaso vasorum.<sup>21</sup> The immune system is involved and activated in the course of disease, since increased immunoglobulins, immune

complexes, complement and acute phase proteins have all been reported.<sup>4,6</sup> Abnormalities of neutrophils, endothelial cells, or both, have been suggested to be responsible for many of the clinical manifestations of BD.<sup>22</sup> Activation of circulating T and B lymphocytes also occurs, and these immunoreactive cells infiltrate into the affected regions followed by a second phase of neutrophil chemotaxis.<sup>4,6</sup> Although there is no definitive treatment of BD, hydrocortisone, colchicine, cyclosporin and cyclophosphamide have been used to subside the symptoms and signs of the disease.<sup>4,6</sup> Given this immunological activity in BD, pro-inflammatory cytokines and mediators may be effected in the course of the disease.

IL-1 $\beta$  (17 kDa) regulates systemically the metabolic, immuno-inflammatory and reparative properties, and can be a mediator of some diseases.<sup>23</sup> It is secreted by monocytes and tissue macrophages, and is involved in the activation of T cells.<sup>24</sup> Control of IL-1 synthesis or its effects becomes a target of therapy in many diseases.<sup>25</sup> However, this study showed that IL-1 $\beta$  levels of BD patients and control subjects were below the detection limits of the assay (5 pg/ml) in all samples.

**Table 3.** Neutrophil count, erythrocyte sedimentation rate and acute-phase reactant levels in patients with active or inactive Behçet's disease compared with control subjects

	Active BD (n = 17)	Inactive BD (n = 20)	Controls (n = 20)
Neutrophil Count ( $10^3$ /ml)	6.2 $\pm$ 0.3*†	3.4 $\pm$ 0.1†	2.9 $\pm$ 0.1
Erythrocyte sedimentation rate (mm/1 h)	36.0 $\pm$ 1.6*†	20.4 $\pm$ 0.7†	9.9 $\pm$ 0.6
$\alpha_1$ -Antitrypsin (mg/dl)	231.7 $\pm$ 8.3*†	160.4 $\pm$ 4.0†	127.2 $\pm$ 3.2
$\alpha_2$ -Macroglobulin (mg/dl)	275.5 $\pm$ 6.1*†	214.9 $\pm$ 3.5†	159.7 $\pm$ 5.7

Data presented as mean  $\pm$  standard error.

\* Significantly different from the inactive period by analysis of variance (for each,  $p < 0.01$ ).

† Significantly different from the control subjects by Mann-Whitney *U*-test (for each,  $p < 0.01$ ).

The sIL-2R is a smaller (45 kDa) polypeptide than the membrane-bound IL-2R (55 kDa), and has a potential role in the modulation of immune responses by inhibiting IL-2.<sup>26</sup> Immunosuppressive agents such as hydrocortisone, cyclosporin and cyclophosphamide, which are widely used in the treatment of BD, downregulate the synthesis of IL-2.<sup>4</sup> Clinically, high levels of sIL-2R are believed to be associated with a potent activation of the immune system in many diseases such as atopic dermatitis and psoriasis.<sup>27</sup> In the present study, serum levels of sIL-2R were significantly higher in patients with BD when compared with control subjects. In addition, serum sIL-2R levels were significantly higher in active patients than in inactive patients with BD. We think that high serum levels of sIL-2R in the active period indicate the activation of the immune system in BD and may have a pathophysiological role in the course of the disease. Inactive patients showed no significant elevation of serum sIL-2R levels over control subjects.

IL-6 (26 kDa) is a multifunctional pro-inflammatory cytokine with its important role in the regulation of immune response.<sup>28</sup> It is produced by monocytes, epithelial cells and fibroblasts, and causes polyclonal B-cell activation, hypergammaglobulinaemia, and autoantibody production with T-cell activation.<sup>29</sup> The major effect of IL-6 described is the proliferation and differentiation of cells, as well as increasing secretion of acute-phase proteins by the liver.<sup>27</sup> It has also been reported that IL-6 induces uveitis *in vivo* in rats when injected intracamerally.<sup>30</sup> Abnormal IL-6 production has been implicated in some autoimmune diseases and chronic inflammatory reactions.<sup>31</sup> Our study showed that patients with BD had significantly elevated IL-6 levels compared with control subjects, with the highest values observed in the active period of the disease. In addition, even inactive patients with BD had significantly higher IL-6 levels when compared with control subjects. Since IL-6 is a key activator of the acute-phase response and implicated in liver synthesis of acute-phase reactants,<sup>31</sup> it is likely to play an important role in the course of BD.

IL-8, a chemokine secreted from many cells including endothelial cells, lymphocytes, monocytes, macrophages and retinal pigment epithelial (RPE) cells, is one of the main chemoattractants for polymorphonuclear neutrophils (PMNs) and can also directly activate the PMNs itself.<sup>32,33</sup> High levels of serum IL-8 have been detected in various diseases and suggested to be the marker of disease activity in sarcoidosis.<sup>34</sup> This study also showed that IL-8 levels were significantly higher in patients with BD over control subjects. In addition, IL-8 levels were significantly higher in active BD patients when compared with inactive subjects. Since IL-8 has pro-inflammatory properties in the inflammatory response with its potent effect on neutrophils by attraction of PMNs into the lesions (a characteristic finding of BD), we

think that IL-8 most probably participates during the course of BD. Considering the known properties of IL-8 as a chemoattractant and activator of neutrophils, the treatment of BD might be of immense value in the development of therapeutic regimens for managing the disease.

TNF (17 kDa) is a cytokine secreted by lymphocytes and reticuloendothelial cells in many acute and chronic inflammatory diseases.<sup>31</sup> TNF, like IL-6, is also a pro-inflammatory cytokine and is involved in the blood-retinal barrier breakdown by opening tight junctions of retinal vascular endothelial cells and RPE cells.<sup>35</sup> The present study showed that TNF- $\alpha$  levels in the serum of patients with BD were significantly higher in active disease than inactive BD patients or control subjects. It is probable that the disease activity is associated with the secretion of pro-inflammatory mediators by direct activation of circulating monocytes.<sup>31</sup>

Increased free-radical production by activated neutrophils has been demonstrated in BD.<sup>36</sup> The burst of activated, oxygen-derived free radicals is responsible for cell membrane peroxidation, resulting in tissue oedema with enzyme and protein degradation. Lipid peroxidation, in turn, leads to the subsequent formation of free fatty acids and arachidonic acid.<sup>3</sup> A vicious circle ensues, whereby the metabolism of these molecules leads to further free-radical formation and OH $\cdot$  generation, which may lead to further lipid peroxidation in cells and further oxidative damage.<sup>37</sup> This study clearly found that lipid peroxidation, measured as MDA levels, was increased in patients with BD, and that the levels were correlated with disease activity. Therefore, this may be responsible from the tissue damage in BD as well as endothelial dysfunction, which is the most characteristic feature of the disease.<sup>22,36</sup> Defence systems of cells against this free-radical-induced toxic lipid peroxidation consist of antioxidant molecules.<sup>38</sup> These molecules block the initiation of free-radical chain reactions, and therefore lipid peroxidation in cells.

Taken together, serum MDA, sIL-2R, IL-6, IL-8 and TNF- $\alpha$  levels were significantly higher in patients with active BD compared with inactive BD patients and healthy control subjects. We concluded that endothelial cells, PMNs and macrophages produced pro-inflammatory cytokines such as IL-1 $\beta$ , IL-6, and TNF, and these cytokines possibly induced inducible NO synthase and, thereby, increased NO production.<sup>39</sup> In fact, our another study demonstrated an increased total nitrite level (an indicator of NO production) in patients with BD when compared with control subjects with the highest level in the active BD patients.<sup>13</sup> This most abundant free radical possibly induces oxidative stress and leads to the formation of self-propagating lipid peroxidation. On the contrary, our further study found higher homocysteine levels in patients with BD,<sup>12</sup> which is known to be the potent

inducer for IL-6, IL-8 and TNF- $\alpha$ .<sup>14</sup> Therefore, homocysteine  $\rightarrow$  cytokine  $\rightarrow$  leptin  $\rightarrow$  nitric oxide  $\rightarrow$  lipid peroxidation relationships during the course of BD are open to further *in vivo* and *in vitro* studies.

In conclusion, we observed increased mean levels of MDA, sIL-2R, IL-6, IL-8 and TNF- $\alpha$  in patients with BD over control subjects. Furthermore, sIL-2R, IL-6, IL-8 and TNF- $\alpha$  levels in patients with active BD were significantly higher than those in inactive patients, suggesting that these cytokines to be related with disease activity. These pro-inflammatory cytokines may play a role during the course of the disease and may be responsible for tissue damage by lipid peroxidation. Understanding the role of these low molecular weight proteins in a wider context should help us to clarify the role of the chemokine and cytokines in the aetiopathogenesis of BD. Treatment of BD is presently difficult, partly because of the multisystem nature of the disease. Recent treatment modalities are encouraging, and it is probable that further treatment modalities that interfere with cell signalling processes may be the future direction for the clinical management of BD. In addition, amelioration of clinical manifestations may be envisaged by targeting lipid peroxidation with dietary or pharmacological antioxidants.

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