ORIGINAL ARTICLE

The Relationship between Serum IL-17 and IL-23 Levels, and Other Disease Activity Parameters in Patients with Behçet's Disease

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ABSTRACT

Objective: *To investigate the relationship between disease activity and the involvement of Behçet's disease (BD) and serum levels of interleukin (IL)-17 and IL-23.*

Methods: *Sixty patients with BD and 20 healthy control group subjects were included in this study. The patients were divided into four groups according to clinical findings as follows: entero-Behçet, mucocutaneous-Behçet, neuro-Behçet and vascular-Behçet. The serum levels of the IL-17 and IL-23 levels were evaluated using enzyme-linked immunosorbent assay.*

Results: *Of the BD patients, 15 (25%) had active disease and 45 (75%) had inactive disease. The serum levels of IL-23 and IL-17 were statistically significantly higher in the patients with BD than in the control groups (*$p < 0.05$*). A significant relationship was also observed between the disease activity, and both the erythrocyte sedimentation rate and the C-reactive protein levels (*$p < 0.05$*). The mean serum levels of IL-17 and IL-23 in patients with active disease were 0.07 ± 0.25 pg/ml and 36.0 ± 30.5 pg/ml, respectively. There was no statistically significant relationship between the disease activity and the serum levels of IL-17 and IL-23 (*$p > 0.05$*). There were also statistically significant relationships between the disease activity and uveitis, retinal vasculitis or superficial thrombophlebitis.*

Conclusion: *No relationship was found between BD and serum levels of the IL-17 and the IL-23.*

Keywords: Behçet's disease, interleukin-17, interleukin-23.

INTRODUCTION

Behçet's disease (BD) is a complex, chronic, multisystemic inflammatory condition of unknown aetiology. It is characterized by recurrent oral and genital ulcers, relapsing uveitis, skin manifestations, and arthritis (1). Behçet's disease had been associated with a number of human leukocyte antigens (HLAs), including HLA-B51, and it has been suggested that severe forms of BD may be loosely associated with HLA positivity. Previous studies had found an association between HLA-B51 and BD manifestations, including posterior uveitis and the central nervous system involvement (2). Many reports had also suggested that autoimmunity might play an important role in pathogenesis (3). Peripheral blood mononuclear cells had been shown predominantly to produce T helper type 1 (Th1) cytokines—primarily interferon (IFN)-γ, tumour necrosis factor (TNF)-α and interleukin (IL)-12—in the patients with BD (4). However, the treatment of BD with anti-TNF-α and anti-IFN-γ drugs only partially reduces diseases activity, suggesting that other factors might be implicated in the disease's development (5).

T helper type 17 (Th17) cells represent a recently discovered T-cell population that characteristically produced large amounts of IL-17, IL-6 and TNF-α (6). Interleukin-17 is an important pro-inflammatory cytokine and its production is increased in inflammatory conditions such as rheumatoid arthritis (7),

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multiple sclerosis (8), inflammatory bowel disease (9, 10), asthma (11), bacterial and fungal infections (12), and BD-associated uveitis (13). Interleukin-23, a member of the IL-12-type family of cytokines, stabilizes Th17 cells and stimulates their production (14). Interleukin-23 had been shown to play a critical role in the development and the maintenance of particular inflammatory autoimmune diseases, including inflammatory bowel disease (9), collagen-induced arthritis and experimental autoimmune uveitis (13).

Both IL-17 and IL-23 are thought to play a major role in autoimmunity. The primary function of Th17 cells is to eliminate the pathogens that cannot effectively be cleared by Th1 or Th2 cells (12). T helper type 17 cells are rapidly recruited to the sites of inflammation, function as a bridge between natural and acquired immunity, and recruit other Th cells through the release of cytokines.

These previous reports suggest that the IL-23/IL-17 pathway may play an important role in the development of autoimmune inflammatory diseases. There have been few published studies investigating the association between the IL-23/IL-17 pathway and BD, however. The aim of this study, therefore, was to investigate the association between IL-23 and IL-17 levels, and various indices of BD.

MATERIALS AND METHODS

The study's sample comprised of 60 patients with BD (32 females, 28 males) who fulfilled three or more International Study Group criteria for BD (1), and 20 healthy subjects (9 females, 11 males). The study protocol was approved by the local Ethics Committee, and was in accordance with the Declaration of Helsinki 2008. The patients were divided into four groups, according to the clinical findings, as follows: mucocutaneous-Behçet, entero-Behçet, neuro-Behçet and vascular-Behçet.

Informed consent was obtained from all the subjects, and their serum samples were taken, centrifuged and stored at −20°C until the day of the study. The serum IL-17 and IL-23 levels were measured by enzyme-linked immunosorbent assay (ELISA) using a Platinum ELISA kit® (eBiosciences, San Diego, CA, USA). The minimum limit of detection for this assay was 0.5 pg/ml for IL-17 and 10 pg/ml for IL-23. All the analyses were conducted using SPSS 16.0 software (SPSS Inc., Chicago, IL, USA).

Inclusion criteria

- Diagnosis of BD according to the International Study Group criteria for Behçet's disease (1).

Exclusion criteria

- Any additional disease (including any malignancy or autoimmune diseases) or previous classification as positive for autoantibodies (including rheumatoid factor, anti-nuclear antibody and anti-neutrophil cytoplasmic antibody).
- Any major psychiatric disorders that could affect cooperation (especially treatment for psychosis or depression within the previous 30 days).

Statistical analyses

The statistical analyses were done using SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). The data were presented as means ± standard deviations. Kolmogorov–Smirnov and Shapiro–Wilk normality tests were done for the assessment of the normal distribution in the data sets. The Mann–Whitney *U* test was used for the non-normal variables. The Kruskal–Wallis non-parametric analysis of variance was used for the comparison of the three groups. Spearman's Rho coefficients were used for the correlation analyses. Statistical significance was set at $p < 0.05$.

RESULTS

Sixty BD patients (32 females, 28 males) and 20 healthy subjects (9 females, 11 males) were included in this study. Behçet's disease was active in 15 of the patients and inactive in 45 of them. The principal background characteristics of the participants are shown in Table 1. The serum IL-17 and IL-23 levels were found to be significantly higher in the BD group, relative to the healthy control group subjects.

Table 1: Main characteristics of the subjects.

	Patients (n = 60) (32 F, 28 M)	**Controls (n = 20) (9 F, 11 M)**	***p*-value**
Age (years) *	37.4 ± 9.8	34.6 ± 6.5	0.228
Disease duration (years) *	8.1 ± 7.4	–	
IL-23 in serum (pg/ml) **	29 (9–170)	22 (14–45)	0.011
IL-17 in serum (pg/ml) **	0.5 (0–2.5)	0.3 (0.05–1.1)	0.020
ESR* (mm/h)	25.7 ± 20.3	–	
CRP* (mg/L)	9.4 ± 18.4	–	

*The values are given in mean ± SD or n (%).
**The values are given in median (min–max).
CRP = C-reactive protein; ESR = erythrocyte sedimentation rate; IL = interleukin.

The features of the patients with BD and their clinical manifestations are outlined in Tables 2 and 3. There was no difference in IL-23 or IL-17 levels across the four different BD groups ($p > 0.05$). Of the BD patients, 5 (8.3%) were undergoing steroid treatment, 31 (51.7%) received azathioprine treatment, 22 (36.7%) received anti-TNF-α treatment, and 2 (3.3%) received cyclophosphamide treatment.

Table 2: The features of patients with Behcet's disease

	N (%)
Oral aphthae	48 (80)
Genital ulcer	12 (20)
Uveitis	17 (28.3)
Retinal vasculitis	7 (11.7)
Papulopustular eruptions	11 (18.3)
Erythema nodosum	15 (25)
Superficial thrombophlebitis	12 (20)
Pathergy positivity	14 (23.3)
Arthritis	3 (5)

Table 3: Manifestations of Behcet's disease

	N (%)
Mucocutaneous-Behcet	25 (41.6)
Vascular-Behcet	21 (35)
Entero-Behcet	8 (13.3)
Neuro-Behcet	6 (10)

The inflammatory marker profile of the patients in the BD group is provided in Table 4. The erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels were higher in the patients with active disease, relative to both the patients with inactive disease and the healthy control group subjects. A statistically significant relationship was also found between the disease activity and both the ESR and CRP levels ($p < 0.05$).

Table 4: Main characteristics of the inflammatory markers in BD

	Active BD (n = 15) (5 F, 10 M)	Inactive BD (n = 45) (27 F, 18 M)	*p*-value
ESR (mm/h)	46.5 ± 21.6	18.8 ± 14.4	0.000
CRP (mg/L)	27.8 ± 30.1	3.3 ± 3.8	0.000
IL-23 in serum (pg/ml)	36.0 ± 30.5	31.2 ± 15.7	0.620
IL-17 in serum (pg/ml)	0.7 ± 0.2	0.6 ± 0.1	0.083
Age (years)	36.4 ± 6.9	37.8 ± 10.7	0.649
Disease duration (years)	8.20 ± 7.2	8.11 ± 7.6	0.969

The values are given in mean ± SD or n (%).
BD = Behçet's disease; CRP = C-reactive protein; ESR = erythrocyte sedimentation rate; IL = interleukin.

The mean serum IL-17 and IL-23 levels in the patients with the active disease were 0.07 ± 0.2 pg/ml and 31.2 ± 15.7 pg/ml, respectively. There was no statistically significant relationship between the disease activity and the levels of IL-17 or IL-23 ($p = 0.083$ and $p = 0.620$, respectively).

There was also a statistically significant relationship between the disease activity and both the uveitis and the retinal vasculitis ($p = 0.021$ and $p = 0.008$, respectively). A similar significant relationship was found between the disease activity and the superficial thrombophlebitis ($p = 0.001$).

Using the logistic regression analysis, we determined that an increase in CRP of 1 unit was associated with a 1.4-fold increase in the disease activity. Similarly, the presence of uveitis was associated with a 16.23-fold increase in the disease activity (Table 5).

Table 5: The relation between CRP, uveitis and disease activity in patients with BD

	p	OR (%95 CI)
CRP	0.002***	1.457 (1.114–1.85)
Uveitis	0.039***	16.23 (1.15–228.19)

***Statistically significant with logistic regression analysis.
BD = Behçet's disease; CRP = C-reactive protein.

DISCUSSION

In this study, we sought to investigate the serum levels of IL-17, IL-23 and the CRP, and the ESR in patients with BD and in the healthy subjects. We found no relationship between the BD and the serum levels of IL-17 or IL-23. However, the levels of IL-23 tended to be higher in the patients with active disease.

T helper type 17 cells are one of the principal triggers of tissue inflammation, and had been implicated in many experimental autoimmune diseases and inflammatory conditions (12). Many recent trials have suggested that Th17 cells might be the 'sine qua non' of autoimmune and autoinflammatory diseases (6). Interleukin-23 regulates the maturation of self-reactive IL-17-producing T cells, and with the help of IL-17, IL-6, IL-8 and TNF-α, triggers chronic inflammation associated with neutrophil and macrophage activities (14, 15). In a previous study, IL-23 was shown to be effective in stimulating the proliferation of isolated IL-17-producing T cells (5). Chi *et al* (13) demonstrated that IL-23 and IL-17 were upregulated in patients with BD and active uveitis, compared with control group subjects and the BD patients without uveitis. In this study, we discovered that IL-17 and IL-23 were elevated in the serum

of the BD patients relative to the healthy control group subjects, but that cytokine levels did not alter according to the disease activity. One potential explanation for this observation was that the patients in our study were receiving immunosuppressive therapy.

Interleukin-17 activated fibroblasts, inducing them to secrete pro-inflammatory cytokines, such as IL-6 and IL-8, upregulated the surface expression of the intercellular adhesion molecule 1 (ICAM-1), and stimulated the endothelial cells to secrete IL-6. Interleukin-23, a heterodimeric cytokine comprising p19 and p40 subunits, was involved in the development of Th17 cells—a distinct T cell subtype characterized by IL-17 secretion (14). Recent early studies had focused on animal models of the experimental autoimmune encephalitis and rheumatoid arthritis, and had shown the beneficial effects of both IL-17 blockage and the inhibition of IL-17-producing cells (16, 17). Anti-IL-23-p19 treatment was similarly shown to be curative in mice with inflammatory bowel disease. By producing certain chemokines and cytokines, the Th17 cells trigger deleterious, and organ-specific inflammatory processes. Some studies of the patients with systemic sclerosis had suggested that, owing to their potent pro-inflammatory capacity, IL-17 and IL-23 might represent a key component of disease pathogenesis (17).

The acute phase response parameters (CRP and ESR) were associated with the disease activity in BD. Melikoglu and Topkarci (18) showed that erythema nodosum, superficial thrombophlebitis and joint involvement might be associated with the higher levels of ESR and CRP. In the present study, we found that CRP and ESR were more useful indicators of the disease activity than cytokine levels, given that a statistically significant relationship was found between the disease activity and the different levels of the vascular involvement (uveitis, retinal vasculitis and superficial thrombophlebitis).

The present study has numerous potential limitations. Most notably, it did not take into consideration the patients' treatment regimen; serum IL-17 and IL-23 levels in the patients with BD might also be affected by the immunosuppressive therapy. In order to eliminate this confounding factor, the role of IL-17 and IL-23 in the aetiopathogenesis of BD should be investigated in both the treated and the untreated patients. The second limitation of this study was that simultaneously in the same patient group, IL-17 and IL-23 should be studied in the plasma and the tissue.

In conclusion, this study showed that serum levels of IL-17, IL-23 and CRP, and ESR were increased in the BD patients compared with the control group subjects. For more accurate results, studies should be conducted in newly diagnosed and untreated BD patients. Further study is needed to investigate the role of these factors in detail.

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