Original paper

Serum IL-17A in Behçet's disease

Nazan Emiroglu[1], Fatma Pelin Cengiz[1], Gul Bahar Erdem[2]

[1]Department of Dermatology, Bezmialem Vakif University, Istanbul, Turkey
Head of the Department: Prof. Nahide Onsun MD, PhD
[2]Department of Microbiology, Diskapi Yildirim Beyazit Training and Research Hospital, Ankara, Turkey
Head of the Department: Dr. U. Gul Bahar Erdem

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Abstract

Introduction: Behçet's disease is a chronic multisystem disease with spontaneous remissions and relapses. Several studies show that autoimmune mechanisms play an important role in the development of Behçet's disease. Activation of T cells and neutrophils is important in the pathogenesis of the disease. Interleukin 17 (IL-17) is a new cytokine that induces several types of cells to secrete proinflammatory cytokines in many inflammatory and autoimmune diseases.
Aim: This study evaluated the serum levels of IL-17A in active and stable Behçet's disease patients.
Material and methods: Seventy-six patients who had active clinic findings of Behçet's disease were enrolled in our study. Seventy age- and sex-matched controls were also enrolled. Serum IL-17 levels were studied in peripheral venous blood samples.
Results: No significant differences were found between active Behçet's disease patients and controls in terms of serum IL-17A ($p > 0.05$).
Conclusions: These results suggest that IL-17A serum levels do not play an important role in active Behçet's disease.

Key words: active, Behçet's disease, interleukin 17.

Introduction

Behçet's disease (BD) is a chronic multisystem disease with spontaneous remissions and relapses like many autoimmune diseases. Behçet's disease is primarily characterized by recurrent oral aphthous ulcers, genital ulcers, skin lesions, and uveitis [1]. Arthritis, arthralgia, gastrointestinal tract lesions, and vascular lesions in the large or small vessels may also be seen. Although the etiology and pathogenesis of BD are still poorly understood, infections or genetic structure have been presumed in the development of BD [2, 3]. Several reports show that autoimmune mechanisms play an important role in the development of BD. Activation of T cells and neutrophils has an important role in the pathogenesis of the disease. Especially, Thh1 response and proinflammatory cytokines such as interleukin (IL) 6, 8, 18 and tumor necrosis factor α (TNF-α) have been known to be an important step [4–10]. Interleukin 17 is a new discovered cytokine with a proinflammatory nature [11, 12]. An increased expression of IL-17 has been seen in some inflammatory rheumatic diseases such as systemic sclerosis [11], rheumatoid arthritis [13], and systemic lupus erythematous (SLE) [14], and osteoarthritis (OA) [15].

Aim

In this study, we determine the role of IL-17A in the attacks, different organ involvement of and stable period of BD.

Material and methods

Seventy-six active BD patients (56 females, 20 males) were enrolled in the study. They were diagnosed according to the criteria of the International Study Group for BD (1990). Seventy stable BD patients (36 females, 34 males) joined the study as a control group. Informed consent forms were received from all patients and the control group. The Ethics Committee approved the study. Patients who had at least one criterion like oral ulcers, genital ulcers, anterior uveitis, posterior or panuveitis, cutaneous manifestations, pathergy test positivity criteria were accepted to be active BD patients. Peripheral venous blood samples were received from active BD patients and control group after 8 h of fasting. After blood samples were kept on hold at room temperature for 30 min they were centrifuged at 4000 rpm for 15 min and separated from serum. Centrifuged blood samples were stored at –70°. All

Address for correspondence: Nazan Emiroglu PhD, Department of Dermatology, Bezmialem Vakif University, Dişkapi Yildirim Beyazit Education and Research Hospital, 06290 Ankara, Istanbul, Turkey, phone: +90 5057424786, e-mail: dr.nazanyilmaz@hotmail.com
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serum samples were moved to room temperature before the study. The levels of serum IL-17A were analyzed by Enzyme-Linked ImmunoSorbent Assay in Microbiology Elisa Laboratory.

Statistical analysis

All statistical calculations were performed using the Statistical Package for Social Sciences (SPSS) v15.0 for Microsoft Windows. The results are expressed as the mean ± standard deviation. Continuous variables were compared using Student's *t* test and Mann-Whitney *U*-test. Value of $p < 0.05$ was regarded as statistically significant.

Results

Seventy-six patients with active BD (56 females, 20 males) and 70 controls (36 females, 34 males) were included in the study. The mean age of patients was 39.31 ±17.07. The mean age of the control group was 43.71 ±19.68. The patient group and the control group corresponded to each other in terms of sex and age ($p > 0.05$) (Table 1). There was statistically no difference between serum IL-17A levels of active and inactive BD patients ($p > 0.05$) (Figure 1). We compared two groups according to clinical involvement, too. Fifty-six patients had oral ulcers. Twenty-two patients had genital ulcers. Six patients had blurred vision but not uveitis. Six patients had headache. Two patients had venous failure. Thirty-four patients had skin involvement (erythema nodosum, papulopustular eruption). Forty-four patients had arthralgia and arthritis. None had gastrointestinal involvement (Table 2). We did not observe an increase in serum IL-17A levels in patients who had oral ulcers compared with patients who do not have oral ulcers, patients who had genital ulcers compared with patients who do not have genital ulcers, patients who had skin involvement (erythema nodosum, papulopustular eruption) compared with patients who do not have any skin involvement, patients who had arthralgia/arthritis compared with patients who do not have arthralgia/arthritis, patients who were taking medications compared with patients who were not taking any medications. There were no patients with uveitis. And there were not enough patients with the gastrointestinal, central nervous system, vascular involvement for statistical assessment (Table 1).

Table 1. Demographic characteristics of patients with Behçet's disease (BD)

Parameter	Active BD	Inactive BD	Value of *p*
Age	39.3 ±17.07	43.71 ±19.68	> 0.05
Gender:			> 0.05
Female	56 (73.7%)	36 (51.4%)	
Male	20 (26.3%)	34 (48.6%)	
IL-17A levels [pg/ml]	21.2 ±15.1	19.1 ±5.9	> 0.05

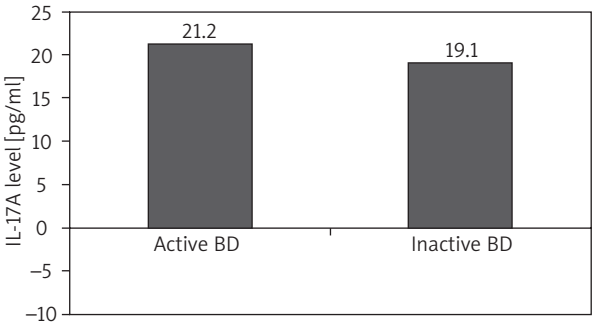

Figure 1. Serum IL-17A levels of Behçet's disease

Discussion

In this study we investigated serum IL-17A levels of active BD patients and stable BD patients. We analyzed serum IL-17A levels using ELISA method. We observed no significant increase in active BD patients compared with healthy controls. Recently, a few reports were published that serum IL-17A levels were higher in acute attacks of BD but we could not find a statistically significant difference between these groups. We classified patients according to organ involvement. We did not determine an increase in serum IL-17A levels in patients who had oral ulcers compared with patients who do not have oral ulcers, patients who had genital ulcers compared with patients who do not have genital ulcers, patients who had skin involvement (erythema nodosum, papulopustular eruption) compared with patients who do not have any skin involvement, patients who had arthralgia/arthritis compared with patients who do not have arthralgia/arthritis, patients who were taking medications compared with patients who were not taking any medication. There

Table 2. Clinical features of patients with Behçet's disease

Parameter	Number of patients	Percentage (%)
Oral ulcers	56	73.7
Genital ulcers	22	28.9
Active uveitis	–	–
Pathergy test	15	19.7
Skin involvement (folliculitis, erythema nodosum)	34	44.7
Vascular involvement	2	2.6
Articular (arthritis or arthralgia) involvement	44	57.9
Gastrointestinal involvement	–	–
Neurological involvement	6	7.9

were no patients with uveitis. There were not enough patients with the gastrointestinal, central nervous system, vascular involvement for statistical assessment.

Behçet disease is accepted as an induced vasculitis by immunological mechanism but its pathogenesis is not clear yet. Yazici suggests that BD is an autoimmune disease but does not have some findings like Raynaud phenomenon, serosal involvement, hypersensitivity, hemolytic anemia, seconder Sjögren syndrome that are seen in many autoimmune diseases [16]. Behçet disease and first-degree relatives usually have positive HLAB51. Whereas HLAB51 positiveness is not found with many autoimmune diseases. Behçet disease does not have B cell hyperactivity. These findings are evidence that BD is not an autoimmune disease [16].

Some authorities suggest BD is an autoinflammatory disease. But BD patients do not have a mutation like CARD, MEVF, NOD that are seen in many autoinflammatory diseases [17, 18]. Behçet disease attacks are more frequent than autoinflammatory disease attacks and its severity decreases with time. Eventually these findings demonstrate that BD is not an autoinflammatory disease [17, 18].

T cell response is accepted as an important step of BD pathogenesis. The causes of this response are not known. Innate and acquired immunity, neutrophils hyperactivity are important pathogenic pathways [19, 20]. Recent studies show that Th17 which is a major subgroup of T helpers may be important in the pathogenesis. Interleukin-17 is produced from Th17 cells that are T cell subtypes [21]. Th17 cells also produce IL-6, IL-21, IL-22 and TNF-α, too [22].

IL-23, IL-6, transforming growth factor β (TGF-β) lead to the differentiation of Th0 to Th17 cells. Th17 cells secrete IL-17A, IL-17F, TNF-α, IL-1 and lead to a proinflammatory reaction. Th17 probably participates in the inflammatory process of BD although few studies support this [23].

First IL-17-related studies focused on experimental autoimmune encephalitis (EAE) and rheumatoid arthritis (RA) animal models. The therapeutic effect of IL-17 blockade and inhibition of IL-17 producing cells on diseases was observed [24, 25]. Th17 cells that trigger the destructive organ-specific inflammatory process by producing chemokines and cytokines lead to the development of these diseases. In the literature, studies show that cytokines and IL-17 have potent proinflammatory capacity in the pathogenesis of BD.

Recent researchers have pointed out that Th17, a subgroup of Th, has an important role in BD pathogenesis. The first report of high IL-17 levels in BD was published by Kamel *et al.* [8, 23]. Hamzaoui e*t al.* investigated the cytokine profile in BD patients [8]. IL-4, IL-6, IL-12, interferon γ (IFN-γ) and IL-10 levels of BD patients are found higher than in healthy controls. They reported that active and inactive BD patients' IL-4, -10, -12 levels were similar. But IFN-γ and IL-6 levels were higher in the active BD patients. Th1/Th2 rate was elevated in active BD. Behçet disease patients' IL-17 and IL-18 levels were higher than in the healthy group. IL-6, IL-18, IL-17 were higher in the patients who had a severe involvement like a neurologic or pulmonary one [8].

Ekinci *et al.* analyzed the levels of IL-17A during BD acute attacks [26]. Serum IL-17A levels were elevated in the patients who had uveitis, oral and genital ulcers, erythema nodosum, papulopustular eruption. However, in our study we did not see any difference between acute attacks and the stable phase. Many reports, published until now, showed that IL-17 had a remarkable role in the pathogenesis of BD. Chi *et al.* demonstrated that IL-17 and IL-23 increased in the active BD with uveitis [27]. We did not have any BD patients with uveitis so we cannot comment on IL-17A levels of BD patients with uveitis.

In 2010, Ferrante *et al.* studied the gastrointestinal involvement in BD. Ankylosing spondylitis (AS), Crohn's disease, BD patients with gastrointestinal involvement were enrolled in the study and their serum levels of Th1 and Th17 cytokines were compared. The IL-23/17 pathway was active in AS and CD whereas Th1 but not Th17 response was seen in the gastrointestinal involvement of BD. In our study there were not enough patients with gastrointestinal involvement and we observed no upregulation in the serum IL-17A levels of active BD patients. This study propounded that the active Th1 pathway caused a defective Treg response. Our study supports this hypothesis [28].

Conclusions

Consequently, in our study we did not observe any upregulation in the serum IL-17A levels of active BD patients so this study suggests that new studies must be done for resolving the confusions about BD pathogenesis and immunologic background.

Conflict of interest

The authors declare no conflict of interest.

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