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IL-17A Has an Important Role in the Acute Attacks of Behçet's Disease

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TO THE EDITOR

Behçet's disease (BD) is a chronic, relapsing, systemic vasculitis of unknown etiology. Patients with BD exhibit elevated levels of proinflammatory cytokines and the affected organs show a significant neutrophile and lymphocyte infiltration. Current evidence suggest that the activated lymphocytes contribute to neutrophile and endothelial cell activation in these patients (Akman *et al.*, 2007; Alpsoy *et al.*, 2007). Recently, a new subset of Th cells, Th17 cells, in part characterized by their production of IL-17 has been identified and their roles in inflammation or immune regulation are under intensive investigation. IL-17 promotes inflammation by inducing various proinflammatory cytokines and chemokines; it modulates the secretion of IL-1, tumor necrosis factor-α, IL-6, IL-8, and prostaglandin E2. IL-17 also increases CXC chemokine secretion. Therefore, IL-17 causes neutrophil influx and regulates neutrophil-mediated inflammatory responses. Th17 cells have the most remarkable effects on neutrophil activity within T-cell sub-populations (Furuzawa-Carballeda *et al.*, 2007). It is well known that neutrophil activity is increased in BD. Again, IL-6, IL-1, and tumor necrosis factor-α, which have a role in the differentiation of naive T cells to Th17 cells, are found to be elevated in patients with BD (Fossiez *et al.*, 1996; Akman *et al.*, 2006). Serum G-CSF level was also reported significantly higher in active patients than in those inactive patients with BD (Kawakami *et al.*, 2004).

In a recent study, Chi *et al.* (2008) have shown elevated production of IL-17, IL-23, and IFN-γ by peripheral blood mononuclear cells (PBMC) besides increased frequencies of IL-17 and IFN-γ-producing T cells in BD patients with active uveitis. In this study, we particularly aimed at investigating the role of IL-17A in the activity and different organ involvements of the disease.

Forty-five patients (24 women, 21 men; mean age, 40 years) with BD, diagnosed according to the criteria of the International Study Group for BD (1990), and 33 age- and sex-matched healthy volunteers (17 women, 16 men; mean age, 36 years) were enrolled in the study. The study was approved by the Ethics Committee of Akdeniz University (Institutional approval number: 2007.02.0122007). Informed consent was obtained from all participants, and study was conducted according to the Declaration of Helsinki Principles. Control group had neither family history nor symptoms related to BD. Twenty-two patients (10 women, 12 men, mean age 41) with BD were at inactive stage, whereas 23 of them (14 women, 9 men, mean age 39 years) were at active stage. Any patient having any active sign of the disease at the time of blood drawn was accepted at active stage. All patients had oral ulcers, and in 16 of them this finding was at active stage. The same figure was 16 and 2 for genital ulcers, 13 and 1 for erythema nodosum, 23 and 6 for articular involvement, 18 and 8 for ocular symptoms. In addition, six patients had vascular, and two gastrointestinal involvement.

We examined serum levels of IL-17A by ELISA. *In vitro* IL-17A response of PBMC of patients with BD and controls after stimulation with *Streptococcus sanguis* (*S. sanguis*), *Escherichia coli* (*E. coli*), and phytohemaglutinin were evaulated by enzyme-linked immunosorbent spot method. The proportion of IL-17A-secreting cells (Th17 cells) in nine patients with active organ involvement and nine healthy

Abbreviations: BD, Behçet's disease; PBMC, peripheral blood mononuclear cells

volunteers was detected by flow cytometry with intracellular cytokine staining method.

Serum IL-17A levels of BD patients (20.7 ± 12.9) were significantly higher than healthy controls (11.8 ± 3.4) ($P = 0.011$). Active BD patients (28.7 ± 12.8) showed significantly higher levels of IL-17A compared with inactive patients (11.9 ± 2.7) and healthy controls ($P < 0.001$). Serum IL-17A levels were significantly higher in patients with active ocular disease (29.8 ± 18.2) compared with those of patients with inactive ocular disease (11.3 ± 2.9) and healthy controls ($P = 0.003$). Serum levels of IL-17A in BD patients with active stages of oral ulcers (29.4 ± 10.1 vs. 15.1 ± 11.4) ($P < 0.001$), genital ulcers (38.3 ± 15.1 vs. 17.4 ± 10.1) ($P < 0.001$), and articular symptoms (34.9 ± 17.9 vs. 17.9 ± 10.5) ($P = 0.002$) were also significantly higher than patients with inactive stages of these symptoms. Significant differences were also observed in patients with active papulopustular lesions (*n*:2) and erythema nodosum (*n*:1) compared with those of patients with inactive stages of these symptoms (48.4 ± 26.0 vs. 18.8 ± 10.8; 39.9 ± 17.9 vs. 19.9 ± 12.9 pg ml^{-1}, respectively). Because of the limited numbers of active patients, statistical analysis was not applied. Differently from the previously published data (Hamzaoui *et al.*, 2002; Chi *et al.*, 2008), our results showed that IL-17A has an important role in acute attacks of not only eye disease but also oral ulcers, genital ulcers, and articular symptoms. Serum IL-17A levels of BD patients with inactive stage were not different from healthy controls. This result can be explained by the fact that IL-17A is active only in acute inflammatory attacks of the disease.

Compared with healthy controls, a significant increase was observed in the number of IL-17A-producing cells obtained from BD patients after stimulation with *S. sangius* ($P = 0.003$), *E. coli* ($P < 0.001$), and phytohemaglutinin ($P = 0.008$) in enzyme-linked immunosorbent spot method (Table 1). We also stimulated PBMC of BD patients and healthy controls with 60 kDa heat-shock protein to evaluate IL-17A production. However, we did not observe any IL-17A response in both groups. We evaluated the response of IFN-γ, which was reported to inhibit Th17 differentiation. Interestingly, unlike IL-17A, both PBMC of BD patients and healthy controls produced IFN-γ after stimulation with 60 kDa heat-shock protein (data not presented). To our knowledge, enzyme-linked immunosorbent spot assay is previously unreported to detect IL-17A cytokine production by PBMC for BD and control groups in the literature. Our enzyme-linked immunosorbent spot findings confirm the ELISA results, and the IL-17A responses of BD patients are higher than healthy controls. Our results suggest that *S. sangius* and *E. coli* cause a higher response; but this is not specific to BD patients (Kibaroglu *et al.*, 2004; Shibata *et al.*, 2007). In the literature, we have not found any knowledge about the effects of heat-shock protein on IL-17 production. Our results suggest that heat-shock protein might affect Th1 rather than Th17-type immune response.

Although, an increase in the percentage of IL-17A(+) lymphocytes, CD4(+) IL-17A(+), and CD4(−) IL-17A(+) T cells after *S. sangius* stimulation of PBMC isolated from BD patients was obtained, the differences were not statistically significant. However, flow cytometry data showed a significant increase in the percentage of IL-17A(+) lymphocytes, CD4(+) IL-17A(+), and CD4(−) IL-17A(+) T cells in BD patients after stimulation with *E. coli* and phytohemaglutinin (Table 2). Our results, in general, are consistent with those observed by Chi *et al.* (2008) and emphasized the increased number of IL-17A-producing cells in BD patients. Our intracellular cytokine staining data indicate that an important part of the IL-17A-producing cells were CD4 (−) in BD patients (Table 2); unfortunately, we could not further analyze these CD4(−) IL-17A-producing cells, which might be gamma/delta T cells, CD8(+) lympho-

Table 1. Number of IL-17A-producing peripheral blood mononuclear cells isolated from Behçet's disease patients and healthy controls after stimulation with *S. sangius, E. coli,* and phytohemaglutinin (PHA)[1]

Study group stimulus	Patients with Behçet's disease (*n*: 33)	Healthy controls (*n*: 25)	*P*-value
S. sangius	157 ± 72.3	87 ± 68.0	0.003
E. coli	173 ± 55.1	98 ± 68.5	<0.001
PHA	188 ± 42.1	132 ± 74.0	0.008

[1]200,000 mononuclear cells were added to the wells and the final number of IL-17A-producing cells were given after subtracting the number of unstimulated wells from stimulated wells.

Table 2. The proportion of IL-17A(+), CD4(+) IL-17A(+), and CD4(−) IL-17A(+) T cells in Behçet's disease patients with active organ involvement and healthy controls after stimulation with *S. sangius, E. coli,* and phytohemaglutinin (PHA)

	Patients with Behçet's disease (%) (*n*: 9)			Healthy controls (%) (*n*: 9)		
	S. sangius	*E. coli*	PHA	*S. sangius*	*E. coli*	PHA
IL-17A(+) lymphocytes	2.14 ± 2.57	4.01 ± 2.88*	4.61 ± 2.72**	1.04 ± 0.38	1.37 ± 0.31	2.81 ± 0.44
CD4(+) IL-17A(+) T cells	0.25 ± 0.35	1.04 ± 0.77†	0.73 ± 0.43††	0.16 ± 0.12	0.16 ± 0.08	0.17 ± 0.16
CD4(-) IL-17A(+) T cells	0.45 ± 0.52	1.46 ± 1.16‡	1.05 ± 0.87‡‡	0.16 ± 0.12	0.16 ± 0.08	0.17 ± 0.16

A significant increase in the percentage of IL-17A(+) lymphocytes, CD4(+) IL-17A(+), and CD4(−) IL-17A(+) T cells in Behçet's disease patients after stimulation with *E. coli* (*$P = 0.004$; $^{\dagger}P = 0.002$; $^{\ddagger}P = 0.001$, respectively) and PHA (**$P = 0.006$; $^{\dagger\dagger}P = 0.001$; $^{\ddagger\ddagger}P = 0.001$, respectively) was observed.

cytes, or natural killer cells (Roark *et al.*, 2008).

Taken together, our results indicate that Th17 and IL-17 pathway are active in BD patients, and has an important role particularly in acute attacks of the disease. Neutrophil activity is increased in BD, and the affected organs show a significant neutrophile infiltration. Our data suggest that this result might be caused by increased IL-17A response in patients with BD. Treatment modalities attempting to evaluate novel approaches to eliminate the overactivity of IL-17A and/or IL-23–Th17 pathway may clarify the biological importance of IL-17A and Th17 cells in patients with BD.

Our study, however, had also some limitations. It is wise to keep in mind that the increased IL-17A serum levels is not clinical sign-specific and even not disease-specific; it indicates inflammation during the acute phases of the disease with involvement of neutrophils. It might have been of more scientific value to investigate longitudinal evaluation of IL-17A and other cytokine (IL-6, IL-8, tumor necrosis factor-α, and so on) serum levels in a group of patients with active and inactive periods of the disease.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Human β Defensin-2: Too Good to Be Dismissed in Atopic Dermatitis

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TO THE EDITOR

I read with interest the study by Harder *et al.* (2010), as they confirmed the previous finding that the human β defensin-2 (hBD-2) level in atopic dermatitis (AD) lesions is lower than that in psoriatic lesions, but higher than healthy skin (Ong *et al.*, 2002).

Based on the observation that psoriasis patients rarely suffer from skin infections (Henseler and Christophers, 1995), Harder *et al.* (1997) made their seminal discovery of hBD-2 in psoriatic lesions. This antimicrobial peptide (AMP) has since been proven to be the strongest and most psoriasis-specific protein (de Jongh *et al.*, 2005; Gambichler *et al.*, 2008). In addition, it has been shown to have immunomodulatory effects on innate and adaptive immunity (Yang *et al.*, 1999). Although hBD-2 has weak direct antimicrobial activity against *Staphylococcus aureus* (Harder *et al.*, 1997), the amount of hBD-2 present in psoriatic lesions significantly exceeds the amount required for both antimicrobial effects (2.5–25 μM) and immunomodulation (2.5–250 nM) (Jansen *et al.*, 2009). On the other hand, the amount of hBD-2 present in AD lesions, although higher than that in healthy skin, has not been proven to exceed these ranges. Furthermore, correlation between hBD-2 level and clinical infection in AD has not been studied. However, the findings of Harder *et al.*

Abbreviations: AD, atopic dermatitis; AMP, antimicrobial peptide; CCR6, chemokine receptor; hBD-2, human β defensin-2