Cytokine gene polymorphisms in Behçet's disease and their association with clinical and laboratory findings

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ABSTRACT

The association of the cytokine gene polymorphisms with the development of Behçet's Disease (BD) was investigated in this study. DNA samples were obtained from a Turkish population of 97 unrelated patients with BD, and 127 unrelated healthy control subjects.

All genotyping (IL-6, IL10, IFN-γ, TGF-β1 and TNF-α) experiments were performed using sequence-specific primers PCR. The frequency of TGF-β1 codon 25 GG genotype was found significantly lower in BD patients compared to healthy control subjects. The IL-10 -1082 GA genotype was more frequent whereas the AA genotype was less common in the BD group compared to the control group. The association between clinial findings and cytokine gene polymorphisms was further investigated in the patients with BD. The frequency of IFN-γ AA genotype was lower in the patients with genital ulcer. Additionally, it was found that the frequency of IL-6 -174 GG genotype was lower in the patients with Pathergy positivity. These results suggest that TGF-β1 and IL-10 gene polymorphisms may affect host susceptibility to BD. Also, to confirm the biological significance of our results, further studies should be performed on other population groups and in large number of cases.

Introduction

Behçet's disease (BD) is a systemic vasculitis characterized by inflammatory lesions of urogenital mucosa, eyes, skin, central nervous system and joints. The pathogenesis of BD is not clearly understood and remains to be elucidated. The disease is characterized by infiltration of lymphocytes and neutrophils into the affected organs. Cytokines play critical roles in the pathogenesis of BD, since they mediate many of the effector and regulatory functions of immune and inflammatory responses (1).

The synthesis profiles of cytokines are considered to cause one of two types of responses: T-helper cell type 1 (Th1) responses, which promote cell-mediated immunity (interleukin [IL]-2 and interferon [IFN]-γ); or Th2 responses, which promote humoral immunity (IL-4, IL-5 and IL-13) (2). T-regulatory (Tr) cells are also involved in the regulation of both Th1 and Th2 responses (3, 4). The adaptive, or inducible, Tr subset (Tr1) can develop in the presence of IL-10. Upon T cell receptor-mediated activation, human Tr1 cells have been shown to produce high amounts of IL-10, considerable levels of IFN-γ, TGF-β and IL-5 but no IL-4 and very low or no IL-2 (5, 6). Another subset of IL-17-producing effector T helper cells, called Th17 cells, has been discovered and characterized (7). These cells also produce IL-21 and IL-22. Interleukin-21 mediates the communication of the immune system cells, whereas IL-22 together with IL-17 induce a massive tissue reaction owing to the broad distribution of the IL-17 and IL-22 receptors (8).

There are several reports suggesting that host genetic factors, such as HLA-B51, play significant roles in susceptibility to BD (9). Therefore, the identification of other gene polymorphims responsible for susceptibility to BD should provide a significant contribution for understanding of the pathogenesis and may lead to the development of new diagnostic markers and treatment strategies. The *in vitro* maximal capacity of immune cells to produce different cytokines in response to mitogen stimulation has been shown to vary among individuals. Such differences can be attributed to several molecular mechanisms, including variations in transcription, translation, and secretion pathways (10, 11). An additional potential mechanism was

described involving conservative mutations within cytokine coding regions, and nucleotide variations within more pronounced regulatory regions (11-13). Genetic polymorphisms in several cytokine genes have been described and demonstrated to influence gene transcription, leading to interindividual variations in cytokine production (12-14). Therefore, it is reasonable to speculate that genetic polymorphisms that regulate the production of certain cytokines may be important determinants of susceptibility to BD and its some clinical and laboratory features.

The aim of this work was to determine whether there is any association between cytokine gene polymorphisms and susceptibility to BD in a Turkish population. We performed a study on Turkish patients suffering from BD and healthy control groups to determine the influence of single nucleotide polymorphisms (SNPs) in 5 cytokines (IL-6, IL-10, IFN-γ, TGF-β1 and TNF-α) on disease susceptibility.

Materials and methods

Patients and controls

Blood samples, collected in Ethylenediamine tetraacetate sterile tubes, were obtained from 97 Turkish patients affected by Behçet's disease. All patients were diagnosed according to the diagnostic criteria prepared by the international study group for BD.

A control group was composed of 127 healthy organ donors, matched for age, sex and ethnicity, and from the same geographical area as the patients. The study was approved by the Ethics Committee of Uludağ University, Bursa, Turkey, and all the subjects gave their written informed consent.

DNA isolation and cytokine genotyping

Genomic DNA was extracted from blood samples by using Puregene Genomic DNA isolation kit (Gentra Systems, Minneapolis, USA) according to the manufacturer's instructions.

Single nucleotid polymorphisms were analysed in 5 cytokines (IL-6, IL10, IFN-γ, TGF-β and TNF-α) for genotype assignment. A single nucleotide polymorphism at position -174 of the promoter region was analysed for the IL-6. Three different polymorphisms were examined for the IL-10 promoter region: position -1082 (G *vs* A), position -819 (C *vs*. T), and position -592 (A *vs*. C). The presence of a single nucleotide modification at position +874 (A *vs*. T) was examined for IFN-γ. Two single nucleotide polymorphisms in coding region were analysed for TGF-β: codon 10 can be either T or C, and codon 25, either C or G. A single nucleotide polymorphism at position -308 of the promoter region (either A or C) were surveyed for TNF-α.

All genotypes were determined with the use of PCR-sequence specific primers (PCR-SSP) method by a commercially

Table I. Genotype and allele frequencies of the cytokine gene polymorphisms in the patients with Behçet's disease and controls. *P_c<0.05 is considered significant.

Cytokine Gene	Allele frequency (%)		Genotype (%)		
TNF-α (-308)	G	A	G/G	G/A	A/A
Patients (n=94)	172 (88.7)	22 (11.3)	75 (77.3)	22 (22.7)	0 (0.0)
Controls (n=127)	217 (85.4)	37 (14.6)	95 (74.8)	27 (21.3)	5 (3.9)
TGF-β1 (cod. 10)	T	C	T/T	T/C	C/C
Patients (n=97)	100 (51.6)	94 (48.4)	28 (28.9)	44 (45.4)	25 (25.7)
Controls (n=127)	138 (54.3)	116 (45.7)	44 (34.6)	50 (39.4)	33 (26.0)
TGF-β1 (cod. 25)	G*	C	G/G*	G/C*	C/C
Patients (n=97)	164 (84.5)	30 (15.5)	70 (72.2)	24 (24.7)	3 (3.1)
Controls (n=127)	238 (93.7)	16 (6.3)	114 (89.7)	10 (7.9)	3 (2.4)
IL-10 (-1082)	G	A	G/G	G/A*	A/A*
Patients (n=96)	74 (38.5)	118 (61.5)	4 (4.2)	66 (68.7)	26 (27.1)
Controls (n=124)	78 (31.5)	170 (68.5)	10 (8.1)	58 (46.8)	56 (44.1)
IL-10 (-819)	C	T	C/C	C/T	T/T
Patients(n=96)	128 (66.7)	64 (33.3)	39 (40.6)	50 (52.1)	7 (7.3)
Controls(n=124)	175 (70.6)	73 (29.4)	64 (51.6)	47 (37.9)	13 (10.5)
IL-10 (-592)	C	A	C/C	C/A	A/A
Patients (n=96)	128 (66. 7)	64 (33.3)	39 (40.6)	50 (52.1)	7 (7.3)
Controls (n=124)	175 (70.6)	73 (29.4)	64 (51.6)	47 (37.9)	13 (10.5)
IL-6 (-174)	G	C	G/G	G/C	C/C
Patients (n=97)	147 (75.8)	47 (24.2)	54 (55.7)	39 (40.2)	4 (4.1)
Controls (n=122)	187 (76.6)	57 (23.4)	75 (61.5)	37 (30.3)	10 (8.2)
IFN-γ (+874)	T	A	T/T	T/A	A/A
Patients (n=97)	88 (45.4)	106 (54.6)	21 (21.6)	46 (47.4)	30 (31.0)
Controls (n=126)	108 (42.86)	144 (57.14)	22 (17.5)	64 (50.8)	40 (31.7)

n.s.: not statistically significant (p_c >0.05).

Table II. Distributions of combined polymorphisms at different positions of TGF-β1 and IL-10. *Pc<0.05 is considered significant.

Cytokine Gene Polymorphisms	Genotype	Patients n (%)	Controls n (%)
TGF-β1 (codon 10-25)	T/T-G/G	28 (28.9)	41 (32.3)
	T/C-G/G	23 (23.7)	45 (35.4)
	T/C-G/C*	21 (21.6)	4 (3.2)
	C/C-G/G	19 (19.6)	28 (22.0)
	T/T-G/C	0 (0.0)	1 (0.8)
	C/C-G/C	3 (3.1)	5 (3.9)
	C/C-C/C	3 (3.1)	0 (0.0)
	T/T-C/C	0 (0.0)	2 (1.6)
	T/C-C/C	0 (0.0)	1 (0.8)
IL-10 (-1082, -819, -592)	GCC/GCC	4 (4.2)	10 (8.1)
	GCC/ACC	29 (30.2)	38 (30.6)
	GCC/ATA*	37 (38.5)	20 (16.1)
	ACC/ACC	6 (6.3)	16 (12.9)
	ACC/ATA	13 (13.5)	27 (21.8)
	ATA/ATA	7 (7.3)	13 (10.5)

n.s.: not statistically significant (Pc >0.05).

available kit (One lambda, Inc., Canoga Park, CA, USA) in accordance with the manufacturer's instructions. This kit contains specific primers to detect the polymorphisms of several cytokines mentioned above. The DNA extractions and PCR amplifications were performed by a technician blinded to the study groups.

Statistical analysis

Statistical analysis was performed by Epi Info Software Version 3.2.2 (CDC, Atlanta GA, USA). The distribution of cytokine genes polymorphisms were compared between patients with BD and healthy controls by the χ^2 or Fisher's exact test. Odds ratios (OR) and 95% confidence intervals (CI) were also calculated in case of that χ^2 or Fisher's exact test was significant. Also, significant probability values obtained were corrected for multiple testing (Bonferroni correction; p_c). P_c values less than 0.05 were considered significant. The data were analysed for appropriateness between the observed and expected genotype values and their fit to Hardy-Weinberg equilibrium (Arlequin Software v. 2000, University of Geneva, Switzerland).

Results

Allele frequencies and genotype distributions of IL-6, IL-10, IFN-γ, TGF-β1 and TNF-α are shown in Tables I and II. In 7 of 8 positions of cytokine SNPs genotype frequencies in controls were in Hardy-Weinberg equilibrium, and TGF-β1 codon 25 did not meet the equilibrium criteria ($p<0.001$).

Following SNP analysis for TGF-β1 codon 25 position, the 'G' allele was more common than the 'C' allele in both BD and control groups. The frequencies of allele 'G' (84.5% *vs*. 93.7%, $p=0.0016$ [$p_c=0.0032$]; OR=0.37) and of the homozygous form (G/G) were found to be significantly lower in BD patients compared to healthy control subjects (72.3% *vs*. 89.7%, $p=0.0006$ [$p_c=0.0018$]; OR=0.30) (Table I). Moreover, as shown in Table II, TGF-β1 codon 10-25 T/C-G/C was found much more commonly among the BD patients in comparison to healthy controls (21.6% *vs*. 3.2%, $p=0.00001$ [$p_c=0.0001$]; OR=8.50).

After the analysis of three SNPs identified in the IL-10 promoter, located at positions -1082 (G to A), -819 (C to T) and -592 (C to A), only the 'A' allele at position -1082 was found more frequently in both BD and the control group without any statistically significant difference. However, the IL-10-1082 G/A genotype was more frequent whereas the A/A genotype was less common in the BD group compared to the control group (68.7% *vs*. 46.8%, $p=0.0011$ [$p_c=0.0033$] and 27.1% *vs*. 44.1, $p=0.0061$ [$Pc=0.0182$], respectively) (Table I). On the other hand significantly higher frequency of GCC/ATA genotype were observed in the patient group ($p=0.00017$ [$p_c=0.001$], OR=3.26) (Table II).

No statistically significant differences in the distribution of TNF-α, IL-6 and IFN-γ gene polymorphisms were observed between patients and controls (Table 1). Analysis of allele frequencies of TNF-α, IL-6 and IFN-γ genes also did not show any statistically significant differences between patients and controls (Table I).

In order to investigate the association between clinical findings and cytokine gene polymorphisms in the patients with BD, the patients were classified according to the clinical features of the disease, including genital ulcer, ocular involvement, papulopustular lesions, eythema nodosum, large vascular involvement, neurological involvement and arthralgia/arthritis. The frequency of IFN-γ A/A genotype was lower in the patients with genital ulcer (23.3% *vs*. 54.2%, $p=0.005$ [$p_c=0.0149$]; OR=0.26) (Table III). When the association between the diagnostic tests, including Pathergy positivity and HLA-B51 positivity, and cytokine gene polymorphisms was evaluated, it was found that the frequency of IL-6 -174 G/G genotype was significantly lower in the patients with Pathergy positivity (45.8% *vs*. 75.8%, $p=0.006$ [$p_c=0.0179$]; OR=0.27) (Table III).

Discussion

Although the aetiology of Behçet's disease is not yet known, it is thought that genetic predisposition and immune dysregulation seem to be critical factors in the pathogenesis. Most of the studies reported that the levels of T helper Type 1 cytokines; such as IL-1, IL-2, TNF-α, IFN-γ, IL-12, IL-18, were increased in sera of the patients with BD. Some studies have shown that the maximal capacity of cytokine production varies among individuals and correlate with single nucleotide polymorphism in various cytokine genes (15-17).

Wide variations have been documented in the frequencies of cytokine polymorphisms among different healthy populations, including the -1082 IL-10 polymorphism, which has been most widely investigated in healthy populations (18-21). We found a -1082 G allele in 31.5% of our healthy volunteers, a rate that is different from the prevalence rates reported in different regions of the world. The rates reported were 3.8% in Japanese, 13.0% in Koreans and 38% in Greek Cypriots (20-23). The implications of this heterogeneity underline the necessity of including a local control group when clinical studies are carried out.

Tumor necrosis factor (TNF) is a multifunctional proinflammatory cytokine, which plays critical roles in many inflammatory and autoimmune diseases, such as sepsis, inflammatory bowel disease (24), and rheumatoid arthritis (25). Also, TNF-α seems to be implicated in the pathogenesis and activity of BD, since some evidence indicates that the level of TNF-α is elevated in the sera of BD patients (26-29) and it is spontaneosuly oversecreted from monocytes in BD patients (30). Previous studies in the Turkish population demonstrated that the TNF-α -308 gene polymorphism was not significantly associated with BD (31, 32). In the present study no significant difference was observed in the TNF-α -308 gene polymorphism between BD patients and healthy controls. Studies form different geografical areas also did not demonstrate any significant association between TNF-α -308 gene polymorphism and the susceptibility BD (33-36). Also, similarly to our data, the -308 polymorphism was not significantly associated with the manifestations or severity of BD in other studies (31, 32, 35). However, in some other studies the SNP polymorphism at different position of TNF-α

Table III. Association of the most common clinical findings, the diagnostic test results, age of the disease onset and cytokine gene polymorphisms in the patients with BD. Since the size of samples are small, only Bonferroni correction of the *p*-values (p_c)<0.05 is considered significant (in bold).

	Genital ulcer		Ocular involvement		Papulopustular lesions		Erythema nodosum		Pathergy positivity*		HLA-B51 positivity **		Age of the disease onset (years)	
	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	≤18	>18
TNF-α -308 (n)	(73)	(24)	(53)	(43)	(52)	(45)	(50)	(47)	(59)	(33)	(58)	(20)	(26)	(71)
G/G	54 (74.0)	21 (87.5)	40 (75.5)	34 (79.1)	43 (82.7)	32 (71.1)	39 (78.0)	36 (76.6)	42 (71.2)	29 (87.9)	44 (75.9)	15 (75.0)	20 (76.9)	55 (77.5)
G/A	19 (26.0)	3 (12.5)	13 (24.5)	9 (20.9)	9 (17.3)	13 (28.9)	11 (22.0)	11 (23.4)	17 (28.8)	4 (12.2)	14 (24.1)	5 (25.0)	6 (23.1)	16 (22.5)
A/A	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
TGF-β1 cod. 10 (n)	(73)	(24)	(53)	(44)	(52)	(45)	(50)	(47)	(59)	(33)	(58)	(20)	(26)	(71)
T/T	22 (30.1)	6 (25.0)	17 (32.1)	11 (25.0)	10 (19.2)	18 (40.0)	14 (28.0)	14 (29.8)	16 (27.1)	8 (24.2)	16 (27.6)	5 (25.0)	7 (26.9)	21 (29.6)
T/C	30 (41.2)	14 (58.3)	22 (41.5)	22 (50.0)	24 (46.2)	20 (44.4)	22 (44.0)	22 (46.8)	26 (44.1)	17 (51.6)	29 (50.0)	11 (55.0)	13 (50.0)	31 (43.7)
C/C	21 (28.8)	4 (16.7)	14 (26.4)	11 (25.0)	18 (34.6)	7 (15.6)	14 (28.0)	11 (23.4)	17 (28.8)	8 (24.2)	13 (22.4)	4 (20.0)	6 (23.1)	19 (26.7)
TGF-β1 cod. 25 (n)	(73)	(24)	(53)	(44)	(52)	(45)	(50)	(47)	(59)	(33)	(58)	(20)	(26)	(71)
G/G	55 (75.3)	15 (62.5)	38 (71.7)	32 (72.7)	35 (67.3)	35 (77.8)	35 (70.0)	35 (74.5)	40 (67.8)	26 (78.8)	41 (70.7)	14 (70.0)	19 (73.1)	51 (71.9)
G/C	15 (20.6)	9 (37.5)	13 (24.5)	11 (25.0)	15 (28.9)	9 (20.0)	14 (28.0)	10 (21.3)	16 (27.1)	7 (21.2)	15 (25.9)	6 (30.0)	7 (26.9)	17 (23.9)
C/C	3 (4.1)	0 (0.0)	2 (3.8)	1 (2.3)	2 (3.8)	1 (2.2)	1 (2.0)	2 (4.2)	3 (5.1)	0 (0.0)	2 (3.4)	0 (0.0)	0 (0.0)	3 (4.2)
IL-10 -1082 (n)	(73)	(24)	(53)	(43)	(51)	(45)	(50)	(46)	(58)	(33)	(57)	(20)	(26)	(70)
G/G	4 (5.5)	1 (4.2)	2 (3.8)	2 (4.6)	3 (5.9)	1 (2.2)	3 (6.0)	1 (2.2)	2 (3.4)	2 (6.0)	0 (0.0)	2 (10.0)	0 (0.0)	4 (5.7)
G/A	48 (65.8)	18 (75.0)	40 (75.5)	26 (60.5)	39 (76.5)	27 (60.0)	34 (68.0)	32 (69.6)	40 (69.0)	22 (66.7)	41 (71.9)	13 (65.0)	21 (80.8)	45 (64.3)
A/A	21 (28.7)	5 (20.8)	11 (20.7)	15 (34.9)	9 (17.6)	17 (37.8)	13 (26.0)	13 (28.2)	16 (27.6)	9 (27.3)	16 (28.1)	5 (25.0)	5 (19.2)	21 (30.0)
IL-10 -819 (n)	(73)	(24)	(53)	(43)	(51)	(45)	(50)	(46)	(58)	(33)	(57)	(20)	(26)	(70)
T/T	6 (8.2)	1 (4.2)	0 (0.0)	7 (16.3)	2 (3.9)	5 (11.1)	3 (6.0)	4 (8.7)	4 (6.9)	3 (9.1)	4 (7.0)	1 (5.0)	0 (0.0)	7 (10.0)
T/C	41 (56.2)	15 (62.5)	30 (56.6)	20 (46.5)	24 (47.1)	26 (57.8)	30 (60.0)	20 (43.5)	31 (53.4)	16 (48.5)	33 (57.9)	10 (50.0)	16 (61.5)	34 (48.6)
C/C	26 (35.6)	8 (33.3)	23 (43.4)	16 (37.2)	25 (49.0)	14 (31.1)	17 (34.0)	22 (47.2)	23 (39.7)	14 (42.4)	20 (35.1)	9 (45.0)	10 (38.5)	29 (41.4)
IL-10 -592 (n)	(73)	(24)	(53)	(43)	(51)	(45)	(50)	(47)	(58)	(33)	(57)	(20)	(26)	(70)
C/C	30 (41.1)	10 (41.7)	23 (43.4)	16 (37.2)	25 (49.0)	14 31.1)	17 (34.0)	22 (47.8)	23 (39.7)	14 (42.4)	20 (35.1)	9 (45.0)	10 (38.5)	29 (41.4)
C/A	37 (50.7)	13 (54.2)	30 (56.6)	20 (46.5)	24 (47..1)	26 (57.8)	30 (60.0)	20 (43.5)	31 (53.4)	16 (48.5)	33 (57.9)	10 (50.0)	16 (61.5)	34 (48.6)
A/A	6 (8.2)	1 (4.1)	0 (0.0)	7 (16.3)	2 (3.9)	5 (11.1)	3 (6.0)	4 (8.7)	4 (6.9)	3 (9.1)	4 (7.0)	1 (5.0)	0 (0.0)	7 (10.0)
IL-6 -174 (n)	(73)	(24)	(53)	(44)	(52)	(45)	(50)	(47)	**(59)**	**(33)**	(58)	(20)	(26)	(71)
G/G	38 (52.0)	16 (66.7)	28 (52.8)	26 (59.1)	30 (57.7)	24 (53.3)	24 (48.0)	30 (63.8)	**27 (45.8)**	**25 (75.8)**	36 (62.1)	12 (60.0)	16 (61.5)	38 (53.5)
G/C	31 (42.5)	8 (33.3)	24 (45.3)	15 (34.1)	20 (38.5)	19 (42.2)	22 (44.0)	17 (36.2)	29 (49.1)	8 (24.2)	19 (32.8)	8 (40.0)	10 (38.5)	29 (40.9)
C/C	4 (5.5)	0 (0.0)	1 (1.9)	3 (6.8)	2 (3.8)	2 (4.5)	4 (8.0)	0 (0.0)	3 (5.1)	0 (0.0)	3 (5.1)	0 (0.0)	0 (0.0)	4 (5.6)
IFN-γ +874 (n)	(73)	(24)	(53)	(44)	(52)	(45)	(50)	(47)	(59)	(33)	(58)	(20)	(26)	(71)
T/T	17 (23.3)	4 (16.6)	10 (18.9)	11 (25.0)	12 (23.0)	9 (20.0)	10 (20.0)	11 (23.4)	10 (17.0)	29 (87.9)	13 (22.4)	7 (35.0)	4 (15.4)	17 (23.9)
T/A	39 (53.4)	7 (29.2)	29 (54.7)	17 (38.6)	24 (46.2)	22 (48.9)	27 (54.0)	19 (40.4)	29 (49.0)	4 (12.1)	28 (48.3)	7 (35.0)	10 (38.5)	36 (50.7)
A/A	**17 (23.3)**	**13 (54.2)**	14 (26.4)	16 (36.4)	16 (30.8)	14 (31.1)	13 (26.0)	17 (36.2)	20 (34.0)	0 (0.0)	17 (29.3)	6 (30.0)	12 (46.1)	18 (25.4)

*92 patients were evaluated; **78 patients were evaluated.

promoter region (-1031 C allele) has been found to be associated with susceptibility to BD (34, 37).

Interferons possess antiviral, antitumor and immunomodulatory properties. Interferon-γ has a critical role in modulating the IL-4, IL-10 and IL-12 cytokine network pathway. It is also considered as a proinflammatory cytokine because of its effects on TNF activity. In a previous study it was reported that the frequencies of IFN-γ +874 A allele and A/A genotype were higher in BD patients than in healthy controls, suggesting that individuals with this genotype may be more susceptible to the disease. In the same study no association was reported between clinical findings and IFN-γ +874 polymorphisms (38). In contrast, IFN-γ polymorphism at position +874 was not found to be associated with the susceptibility to BD in our study. However, it was observed that genital ulcer is rarely seen in the patients carrying IFN-γ +174 A/A genotype.

Interleukin-10 is a multifunctional cytokine first described as cytokine synthesis inhibitory factor, which inhibits IFN-γ cytokine production by Th1 cells in mice (39, 40). It inhibits monocyte/macrophage function during inflammation by downregulating the production of proinflammatory cytokines, such as IL-12 and TNF-α, and suppressing the surface expression of major histocompatability class II (41, 42). IL-10 can also inhibit $CD4^+$ T cell chemotaxis towards IL-8 (39, 43) and T cell apoptosis (44), by leading to Bcl-2 up-regulation. Interestingly, it can induce the proliferation of $CD8^+$ T cells (45). The suppressor effect of IL-10 on T cells has been recently shown to be directed to block CD28 signaling cascade and subsequent phosphatidylinositol 3-kinase activation in T cells (43). In our previous studies, it was also mentioned that IL-10 gene polymorphism is a potential host susceptibility factor in tuberculosis and brucellosis (22, 46). Since IL-10 has been demonstrated both in active lesions and sera of BD patients (47), determining the polymorphisms in IL-10 genes may be beneficial for predicting the disease susceptibility. In fact, Wallace *et al*. reported that carrying IL-10 -819 T allele was associated with the susceptibility to BD in the UK patients but not Middle Eastern patients (48). In our study, carrying IL-10 -819 T or C allele was not associated with the disease susceptibility whereas the presence of IL-10 -819 C/T seemed to be risk factor for the development of BD. Additionally, it was shown that IL-10 GCC/ATA haplotype, as well as IL-10 -1082 G/A and IL-10 -592 C/A genotypes were more common in patients when compared with healthy controls, suggesting that individuals with these genotypes may be more susceptible to the disease. We also demonstrated that IL-10 gene polymorphisms at position -1082, -819 and -592, do not seem to be associated with the clinical findings and laboratory features of BD.

Transforming growth factor-β1 is produced by mainly activated macrophages, in response to tissue injury (49). It is a potent immunosuppressive cytokine, which inhibits macrophage activation and modulates T cell function (50). Additionally, it plays a role in tissue fibrosis by leading to increase in the synthesis of extracellular matrix components (51, 52). To our knowledge, there is no report that demonstrates the association of TGF-β gene polymorphism with the development of BD. In our study, the frequency of TGF-β1 codon 10-25 T/C-G/C genotype was found to be higher than those of healthy controls.

IL-6 is pleitropic cytokine and an important mediator of inflammatory and immune responses. Increased IL6 plasma levels and enhanced IL6 mRNA expression have been found in patients with active Behçet's disease (29, 53). In one study, no evidence for genetic association conferred by the IL6 -174 polymorphism, whereas significant differences in the 3' flanking region of the IL-6 gene and allele frequencies were found between patients with Behçet's disease and controls (54). It was also mentioned that these differences were particularly apparent in the HLA-B51 negative subjects or female patients. Also, no relationship between IL-6 gene polymorphisms and disease susceptibility in BD was found in the present study. However, it was observed that the pathergy positive patients had lower IL-6 -174 G/G genotype frequencies than pathergy negative patients. This suggests that presence of IL-6 -174 G homozygote genotype may be a protective factor for the development of pathergy reaction.

In the light of these findings, our study demonstrates that IL-10 and TGF-β1 gene polymorphisms may be involved in the susceptibility to BD. In order to confirm the biological significance of our results on disease susceptibility and clinical findings, further studies should be performed in larger and different population groups.

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