Tumour necrosis factor-alpha gene promoter region –308 and –376 G → A polymorphisms in Behçet's disease

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Received on June 26, 2002; accepted in revised form on October 4, 2002.

Clin Exp Rheumatol 2003; 21 (Suppl. 30): S15-S18.

Key words: Tumour necrosis factor, Behçet's disease, single nucleotide polymorphisms, promoter region, cytokine polymorphisms, linkage disequilibrium.

ABSTRACT

Objective. *Contribution of HLA-B51 to the genetic susceptibility for Behçet's disease is well documented and recent studies suggest involvement of other genes. Tumour necrosis factor (TNF) genes are located in the vicinity of the HLA-B locus. Polymorphisms in the promoter region of TNF-α gene has been found to be associated with altered TNF secretion, and it may have a prominent role in the increased inflammatory responses of Behçet's disease.*

Methods. *The study group consisted of 99 Behçet's disease patients and 96 healthy matched controls. All patients fulfilled the International Study Group criteria for Behçet's disease. The TNF-α –308 and –376 promoter alleles were assigned by the digestion of each amplified PCR product with* NcoI *and* TasI *enzymes, respectively.*

Results. *No significant difference was observed in the distribution of TNF-α promoter region polymorphisms between patients with Behçet's disease and controls. There was no association between the presence of uncommon –308A and –376A alleles and the manifestations or severity of Behçet's disease either. The TNF-α –308A allele and HLA-B*50 was found to be associated in this series of Turkish patients and controls.*

Conclusion. *The role of TNF-α promoter region -308 and -376 polymorphisms in the pathogenesis of Behçet's disease is not supported by this data. The overexpression of TNF-α in Behçet's disease may be caused by other polymorphisms in the TNF gene or by post-transcriptional mechanisms.*

Introduction

Behçet's disease (BD) is a systemic inflammatory disorder mainly characterised by recurrent attacks of oral and genital ulcerations, skin lesions, uveitis. The etiopathogenesis of BD is unknown, although it has long been postulated that immunological abnormalities, which are possibly induced by microbial pathogens in genetically susceptible individuals, may be important in its pathogenesis (1).

The most widely reported susceptibility marker for BD is the major histocompatibility complex (MHC) class I molecule, HLA-B51. The presence of this HLA allele confers a 1.5- to 16-fold increased risk for BD in different populations (2). Although the HLA-B locus has recently been linked to BD using the transmission disequilibrium test, it is still unclear whether HLA-B51 has a direct role in the pathogenesis or whether this association reflects linkage disequilibrium with a putative susceptibility gene (3).

Overexpression of pro-inflammatory cytokines from various cellular sources seems to be responsible for the enhanced inflammatory reaction in BD. Increased tumour necrosis factor-alpha (TNF-α) release has been implicated in the pathogenesis and activity of BD (4, 5). *In vitro* studies have also shown that spontaneous secretion of TNF-α, interleukin 6 (IL-6) and IL-8 from mononuclear cells is higher in patients with active BD (6).

TNF-α is a prominent inducer of inflammation and higher levels of TNF-α are frequently related to the severity of inflammatory responses. Several studies have suggested that individual differences in TNF-α production can be related to the polymorphisms in the TNF-α gene (7-11). An effect of the polymorphism at position –308 on transcriptional activity has been shown with reporter gene assays(8). The –376 polymorphism of TNF-α affects OCT-1 binding and a less common allele (A) has been shown to cause a modest, but significant increase in basal TNF-α expression in human monocytes (11). Associations of TNF-α polymorphisms

with different diseases have previously been reported (9, 12, 13).

The TNF-α locus lies in the class III region of the MHC, appoximately 250 kilobases (kb) centromeric of the HLA-B locus, on the short arm of chromosome 6. The association of BD with HLA-B alleles may also have some implications for altered TNF-α production related to genetic variation of the TNF genes on the haplotype carrying the HLA-B susceptibility allele (8). We studied the allelic distribution of two TNF-α gene promoter region polymorphisms at positions –308 and –376, which are known to influence TNF-α production in patients with BD, and also investigated the TNF-α promoter region haplotypes in relation to HLA-B alleles in the Turkish population.

Table I. The distrubution of the TNF-α –308 and –376 allele and genotype frequencies in Behçet's disease and healthy control groups.

Position		Behçet's disease n = 99	Healthy controls n = 96
	Alleles		
	G	176	160
	A	22	32
-308	Genotypes		
	GG	79	67
	GA	18	26
	AA	2	3
	Alleles		
	G	195	187
	A	3	5
-376	Genotypes		
	GG	96	91
	GA	3	5
	AA	0	0

Materials and methods

Patients

We studied 99 patients (60 male and 39 female) with BD and 96 healthy controls with a similar ethnic origin. All patients were followed at the Division of Rheumatology at the Istanbul Faculty of Medicine and met the International Study Group Criteria for the diagnosis of BD (14). The BD group consisted of patients with a disease duration longer than 5 years (5 - 40, mean 14.4 years), and their mean age was 42.5 years (range 16 - 66). All patients and controls were previously genotyped for HLA-B alleles by molecular methods (15). HLA-B*51 was present in 64 out of 99 patients. For the investigation of the effect of the TNF-α polymorphisms on the severity of BD and also individually on the severity of eye disease, the patients were classified into mild + moderate and severe disease severity groups and mild and severe eye disease groups as described before (15).

Genotyping

Genomic DNA was extracted from EDTA anti-coagulated venous blood by conventional salting-out procedure. The single nucleotide polymorphisms at the –308 and –376 positions of the TNF-α promoter region were determined by polymerase chain reaction-restriction fragment length polymorphism. Genotyping for the –308 polymorphism was carried out as described previously by Wilson *et al.* (7). For the determination of the biallelic polymorphism at position –376, a 198-bp DNA segment was amplified with the primer pair of (forward) 5'-CTTCTGGGC-CACTGACTGAT-3' and (reverse) 5'-CTCTCCCTCAAGGACTCAGC-3'. The PCR conditions were as follows: Initial denaturation step of 2 min at 95ºC; 30 cycles of 45 sec at 95ºC, 45 sec at 60ºC and 45 sec at 72ºC; final extension step of 2 min at 72ºC. The amplified product was digested with *TasI* which cuts the –376A (adenine) allele into two fragments of 78-bp and 120-bp, whereas commonly the –376G (guanine) allele remains intact as a single 198-bp band. Confirmation of amplification and genotyping after restriction enzyme digestion were carried out by agarose gel electrophoresis.

Statistical analysis

Genotype and phenotype frequencies for both polymorphisms were compared between BD patients and controls using χ^2 or Fisher's exact tests, as appropriate. Estimated haplotype frequencies and linkage disequilibrium were calculated using the ARLEQUIN software.

Table II. The distribution of TNF-α –308 and –376 genotypes in Behçet's disease patients (n = 99) with different clinical manifestations and disease severity groups.

Manifestations and severity groups	n	-308 GG	-308 GA	-308 AA	-376 GG	-376 GA
Oral ulcer	99	79	18	2	96	3
Genital ulcer	86	68	16	2	84	2
Uveitis	53	43	10	-	51	2
Venous involvement	27	22	4	1	27	-
Arterial involvement	10	7	2	1	9	-
Neurologic involvement	11	6	5	-	11	-
Arthritis	57	47	9	1	57	-
Mild disease severity (mild and moderate)	64	51	12	1	62	2
Severe disease	35	27	7	1	34	1
Uveitis (mild)	32	24	8	-	32	-
Uveitis (severe)	21	19	2	-	19	2

Results

The distribution of the TNF-α promoter region –308 and –376 alleles and genotypes in the BD and control groups is shown in Table I. No difference in the TNF-α –308 and –376 genotypes was detected between both groups. The frequency of the TNF-α -308A (A2) al-

lele, associated with a higher TNF secretion, was not increased in the patient group.

Regarding the possible effects of different TNF-α alleles on the manifestations and severity of BD, comparison of subgroups of patients with different TNF-α genotypes showed no significant difference either (Table II).

The comparison of the patients with or without BD-associated HLA-B alleles, namely B*51 and B*2702, also did not reveal any association with the TNF-α promoter region polymorphisms. On the other hand, 15 out of 16 (94%) B*50-positive individuals were also positive for the -308A allele compared to 34/179 (20%) HLA-B*50-negative individuals ($p < 0.0001$). All 5 BD patients and 10/11 controls bearing HLA-B*50 were positive for the TNF-α –308A allele and a significant linkage disequilibrium between these two alleles could be demonstrated in both groups ($p < 0.001$ in both).

Haplotype analysis

The haplotype frequencies estimated with the ARLEQUIN program are given in Table III. Both common alleles were frequently together in both groups: frequency of the TNF-α -308G/-376G haplotype was 0.791 in BD patients and 0.868 in the controls. The haplotypic distribution in the disease severity groups did not show any significant difference, whereas the comparison between patients with severe and mild uveitis has implicated a possible decrease in –308A / -376G frequency in severe cases (2/21 versus 8/32).

Table III. The TNF-α promoter region haplotype frequencies in Behçet's disease patients and healthy controls estimated using the ARLEQUIN program.

	Haplotypes		
	–308G / –376A	–308G / –376G	–308A / –376G
Behçet's disease	0.015	0.868	0.116
Mild and moderate cases	0,015	0,882	0,101
Severe cases	0,014	0,857	0,128
Uveitis	–308G / –376A	–308G / –376G	–308A / –376G
Mild	0	0.875	0.125
Severe	0,047	0,904	0,047
Healthy controls	0.026	0.791	0.182

Discussion

An active inflammatory response is an important feature of BD. High serum levels of TNF-α as well as the increased expression of TNF-α have been documented in BD (4-6). TNF-α is critically involved in the pathogenesis of several chronic inflammatory diseases, and therefore it is considered to be an appropriate target for interfering with the inflammatory responses. Blocking of TNF-α action by biological agents has been established as an effective treatment in various inflammatory diseases. Recent reports suggest that monoclonal antibodies against TNF-α are effective for sight-threatening ocular inflammation in BD, as well (16).

Alterations of the TNF expression related to polymorphic alleles of the TNF genes may implicate a pathogenetic role in the increased activity of this cytokine in BD. The TNF-α gene –308 and –376 single nucleotide polymorphisms screened in this study did not differ between BD and controls. However, extending these studies may reveal evidence of the possible effects of different haplotypes on "clinical differences" as implicated by the finding of a decreased frequency of the TNF –308A-378G haplotype in severe eye involvement. Similarly, Verity *et al*. did not find an association of the TNF-2 allele with BD in Jordanian patients; however, they identified an association between the TNFB*2 allele and severe uveitis, resulting in vision loss (17).

The regulation of TNF expression is very complex, and includes both transcriptional and post-transcriptional mechanisms. Other regulatory regions of the TNF-α gene or linked genes may play a role in cytokine production, and the binding of inducible cytoplasmic factors may result in mRNA instability and translational blockade, leading to the dysfunction or overpresentation of cytokines in diseases like BD (10, 11).

In conclusion, the findings of this study have not revealed any association between TNF-α -308 and –376 polymorphisms and BD. Other regulatory mechanisms effective on TNF-α expression need to be studied to clarify the role of the TNF-related genetic contribution to the pathogenesis of BD.

Acknowledgments

The authors are grateful to Dr. D. Kwiatkowski and Dr. A.G. Wilson for providing TNF-α promoter region genotyped control DNA samples.

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