Intercellular Adhesion Molecule-1 Gene Polymorphisms in Behçet's Disease

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ABSTRACT. ***Objective.*** Intercellular adhesion molecule 1 (ICAM-1) is strongly expressed in vascular endothelial cells and perivascular inflammatory infiltrates in immunopathologic studies of Behçet's disease (BD) lesions. ICAM-1 genes may contribute to the inflammatory events responsible for the vessel damage in BD. We examined potential associations of ICAM-1 gene polymorphisms with BD susceptibility.

Methods. Case patients were 74 consecutive Italian patients with BD who were followed at the Bologna, Ferrara, Milano, Potenza, Prato, Reggio Emilia, and Trento rheumatology, ophthalmology, and neurology units over a 3 year period (1997–99) who satisfied the International Study Group criteria for BD; 228 healthy Italian blood donors from the same geographic areas were selected as control groups. All BD patients and controls were genotyped by polymerase chain reaction and allele-specific oligonucleotide techniques for ICAM-1 polymorphisms at codon 241 (exon 4) and codon 469 (exon 6).

Results. The frequency of R241 was significantly higher in BD patients than in controls (20.3% vs 5.7%; $p = 0.001$, $p_{corr} = 0.002$, OR 4.2, 95% CI 1.9–9.3). The distribution of E/K 469 genotype was similar in patients and controls. Comparing patients with different clinical features, we found only a trend to higher frequency of R241 in patients with articular manifestations (21.4% vs 12.5%; $p = 0.08$).

Conclusion. Our findings show that G/R 241 polymorphism of ICAM-1 is associated with BD susceptibility. (J Rheumatol 2001;28:1283–7)

Key Indexing Terms:
BEHÇET'S DISEASE INTERCELLULAR ADHESION MOLECULE-1 POLYMORPHISM

Behçet's disease (BD) is a multisystem inflammatory disease of unknown cause characterized by recurrent oral aphthous ulcers, genital ulcers, uveitis, and skin lesions. Vasculitis is the pathological lesion common to most of the clinical manifestations of BD[1,2]. Susceptibility to BD is strongly associated with the presence of the HLA-B51 allele.

Cell adhesion molecule expression was evaluated in skin pathergy lesions and in conjunctiva of patients with ocular involvement in BD. Intercellular adhesion molecule-1 (ICAM-1) was strongly expressed in vascular endothelial cells[3,4]. Elevated serum soluble (s)ICAM-1 levels were found in patients with active BD[5,6]. These data suggest an important role for this adhesion molecule in the inflammatory events responsible for vessel damage in BD.

ICAM-1 is a cell surface glycoprotein that structurally belongs to the immunoglobulin gene superfamily. It is expressed in various cell lineages and is one of the cell surface ligands for lymphocyte function associated antigen-1 (LFA-1) and macrophage-1 antigen (Mac-1), which are members of the leukocyte integrin family. The interaction of leukocyte integrins with endothelial ICAM-1 leads to firm leukocyte adherence, transendothelial migration, and cell activation[7]. The upregulation of ICAM-1 on endothelial cells by proinflammatory cytokines is a central event in regulating leukocyte localization at inflammatory sites and can play an important role also in the regulation and amplification of the inflammatory response observed in BD.

Two ICAM-1 single base polymorphisms, which determine an amino acid substitution in the ICAM-1 protein in codons 241 and 469, have been described[8]. G/R 241 polymorphism occurs in immunoglobulin domain 3, which has been shown to be of importance in binding to the leukocyte

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integrin Mac-1[9]. While the pathogenetic role of these polymorphisms is unknown, it has been found in association with giant cell arteritis (GCA)/polymyalgia rheumatica[10], rheumatoid arthritis[11], inflammatory bowel disease[12], and chronic renal allograft failure[13].

To assess the role of genetic variation in the ICAM-1 molecule in susceptibility to BD, we evaluated the association between ICAM-1 polymorphisms and BD in a consecutive series of 74 Italian patients observed over a 3 year period in 7 Italian rheumatology centers.

MATERIALS AND METHODS

Study population. Case patients were consecutive BD patients who were followed at the Bologna, Ferrara, Milano, Potenza, Prato, Reggio Emilia, and Trento rheumatology, ophthalmology, and neurology units over a 3 year period (1997–99) who satisfied the International Study Group criteria for BD[14]. A total of 74 patients with BD were identified and they represented the cohort of patients studied. The control group consisted of 228 healthy subjects who were unrelated blood donor volunteers. All the study subjects were Caucasians residing in Italy for at least one generation. No ethnic differences were present between patients and controls; none were of Jewish background. Informed consent was obtained from patients and controls before inclusion in the study.

HLA class I typing. Serological HLA class I typing was performed by a standard microlymphocytotoxicity technique, using peripheral blood lymphocytes; 56 of 74 patients were typed for HLA-B51 allele. The control group consisted of 130 healthy blood donors.

Molecular analysis of ICAM 1 polymorphism. Genomic DNA was isolated from 500 μl whole blood collected in edetic acid. To detect the substitution of arginine for glycine responsible for the ICAM-1 polymorphism at position 241 (R/G) in exon 4 of the *ICAM-1* gene, we developed an allele-specific polymerase chain reaction (ASPCR) method. We designated allele-specific primers 5'-CGTGGTCTGTTCCCTGGACG-3', 5'-CGTGGTCTGTTCCCTGGACA-3' (nucleotide number 638 to 657) using published sequence information on the point mutation of the *ICAM-1* gene[15], and common primer 5'-GTCGTTGCCATAGGTGACTG-3'[8]. As an internal positive control, an additional primer pair for the glycoprotein *IIIa* gene[16] was used in all ASPCR with a pair of primers consisting of an allele-specific primer and a common primer, according to the method of Bein, *et al*[17]. The positive control primer pair amplified the 247 bp fragment of glycoprotein *IIIa* gene. The ASPCR was performed in a total volume of 50 μl that contained 0.2 μg genomic DNA, each primer pair consisting of 20 pmol allele-specific primer and 20 pmol common primer, 15 pmol each positive control primer, 200 μM each dNTP, 10 mM TRIS-HCl, pH 8.3, 50 nM KCl, 1.5 mM $MgCl_2$, and 1.5 units of AmpliTaq DNA polymerase (Perkin-Elmer Cetus, Norwalk, CT, USA). The PCR reaction was performed for 35 cycles, each consisting of a denaturation step at 95°C for 30 s, 68°C for 30 s, and 72°C for 30 s in a Perkin-Elmer GeneAmp PCR System 9600. The amplified PCR products were analyzed by 2% agarose gel electrophoresis followed by ethidium bromide staining and ultraviolet visualization.

The second amino acid polymorphism, which consists of a substitution of lysine for glutamic acid (K/E 469), was detected as described[8]. The amplified fragment was digested with 5 U *Bst* UI, which cut product from the E469 allele but not from the K469 allele, and then was subjected to an 8% (29:1) acrylamide/bis-acrylamide electrophoresis gel.

Statistical analysis. Statistical analysis was done using the SPSS statistical package (SPSS Inc., Chicago, IL, USA). The frequencies of the alleles and genotypes among patients and control groups were determined and were compared by chi-square test. Odds ratios were calculated together with 95% confidence intervals. Corrected p values were calculated by multiplying p by the number of alleles compared.

RESULTS

Table 1 shows the clinical and demographic characteristics of the 74 patients with BD.

The allele and genotype frequencies of G/R 241 and E/K 469 in patients and controls are shown in Table 2. The distribution of the G/R 241 genotype differed significantly between patients and controls ($p = 0.001$, $p_{corr} = 0.003$). Allele R was significantly more frequent in patients than in controls ($p = 0.0001$, $p_{corr} = 0.0002$).

The carriage rate of R241 was significantly higher in patients than in controls (20.3% vs 5.7%; $p = 0.001$, $p_{corr} = 0.002$, OR 4.2, 95% CI 1.9–9.3).

The distribution of allele and genotype frequencies of E/K 469 polymorphism did not differ significantly between patients and controls (Table 2).

We investigated ICAM-1 associations with BD, stratifying on HLA-B51. HLA-B51 allele was available from 56 patients. HLA-B51 was significantly higher in patients compared to controls (58.9% vs 19.2%; $p = 0.0001$, OR 6.0, 95% CI 3.0–12.0). The significant association with R241 was preserved in HLA-B51+ patients (18.2% vs 5.7%; $p = 0.02$, $p_{corr} = 0.04$). No significant association was observed in HLA-B51– patients, even though the frequency of R241 was higher compared to controls (13.0% vs 5.7%; p = NS). This analysis was limited by the low number of patients studied.

The association between R241 and clinical manifestations of BD defined in Table 1 was evaluated in the 74 BD patients. We found only a trend of higher frequency of R241 in patients with articular manifestations compared to those without (21.4% vs 12.5%; $p = 0.08$). No significant associations were found in other comparisons (data not shown).

DISCUSSION

BD is a polygenic disease whose multiple genetic factors, in combination with environmental risk factors such as infectious agents, are probably of importance in determining

Table 1. Demographic and clinical features of 74 Italian patients with BD.

	N (%)
Female/male	30/44 (41/69)
Mean age at disease onset ± SD, yrs	32 ± 12
Mean disease duration ± SD, yrs	12 ± 8
Oral ulcers	74 (100)
Papulopustular lesions	59 (79.7)
Eye lesions	53 (71.6)
Genital ulcers	43 (58.1)
Arthritis	34 (45.9)
Positive pathergy test*	8 (36.3)
Erythema nodosum	26 (35.1)
Central nervous system involvement	20 (27.0)
Deep vein thrombosis	17 (23.0)
Epididymitis	3 (4.1)

*Pathergy test was made in 22 patients.

Table 2. Allele and genotype frequencies of G/R 241 and E/K 469 ICAM-1 polymorphisms in patients and controls.

Variable	Healthy Controls, n = 228 (%)	Behçet's Disease, n = 74 (%)	p	OR (95% CI)
Alleles				
R	14/456 (3.1)	17/148 (11.5)	0.0001*	4.1 (2.0–8.5)
G	442/456 (96.9)	131/148 (88.5)		0.2 (0.1–0.5)
E	200/456 (43.9)	65/148 (43.9)	NS	1.0 (0.7–1.4)
K	256/456 (56.1)	83/148 (56.1)		1.0 (0.7–1.5)
Genotypes				
RR	1/228 (0.4)	2/74 (2.7)		
GR	12/228 (5.3)	13/74 (17.6)	0.001**	
GG	215/228 (94.3)	59/74 (79.7)		
EE	34/228 (14.9)	14/74 (18.9)		
EK	132/228 (57.9)	37/74 (50.0)	NS	
KK	62/228 (27.2)	23/74 (31.1)		

*P_{corr} = 0.0002; **P_{corr} = 0.003.

susceptibility. Although the pathological mechanism is not well understood, vasculitis is common and accounts for most of the lesions observed in BD. To date, the strongest genetic association identified in BD has been with HLA-B51 allele[1,2].

We found that polymorphism of ICAM-1 gene at codon 241 is associated with susceptibility to BD. The frequency of R241 allele was significantly higher in patients with BD than in healthy controls, suggesting that this allele is associated with an increased risk of developing BD.

ICAM-1 seems to play a pivotal role in BD pathogenesis. ICAM-1 is strongly expressed in vascular endothelial cells and perivascular inflammatory infiltrates in the immunopathologic studies of BD lesions[3,4]. ICAM-1 expression was enhanced in human dermal microvascular endothelial cells after treatment with serum from patients with BD[18,19]. Elevated serum soluble (s)ICAM-1 levels were found in patients with active BD[5,6]. This adhesion molecule plays an important role in the adhesion and migration of leukocytes, which are basic steps in the inflammatory events responsible for the vessel damage in BD.

An association between HLA-B51 and BD was also observed in our Italian population. We investigated ICAM-1 polymorphism associations with BD, stratifying on HLA-B51. The association between BD and the R241 seemed to be dependent on the status of the HLA-B51 allele. The R241 allele was significantly associated with BD only in HLA-B51+ patients. This should be interpreted with caution, however, since the sample size of HLA-B51– patients after stratification was small.

Recently, Verity, *et al* also studied ICAM-1 gene polymorphisms in BD[20]. Unlike our study, these authors found no associations with R/G 241 polymorphism, but only a weak association between ICAM-1 E469 allele and BD (p_{corr} = 0.046, OR 2.1).

The different results of these 2 population based association studies need to be interpreted. The diagnosis of BD was made using the same criteria[14], and the selection of controls seems to be adequate in both studies. However, the 2 studies were limited by small sample size, although originating from multiple research centers. BD is a rare disease and it is difficult to have a large sample of patients. The association found by Verity, *et al* with E469 allele was weak, with a corrected p value at the limit of significance[20]. Furthermore, E/K 469 polymorphism is unlikely to influence ICAM-1 ligand binding because this polymorphism occurs in Ig domain 5 of ICAM-1, which is not involved in this function. However, G/R 241 polymorphism could have a possible functional significance. This polymorphism occurs in Ig domain 3, which has been shown to be of importance in binding to the Mac-1 form of leukocyte integrin[9]. Further, the common allele (G241) that codifies for glycine is present in the same position in species other than humans[8]. This suggests that the nonconservative substitution of arginine at position 241 might have functional significance, affecting the adhesive function of ICAM-1.

The R241 association observed in BD was also described in GCA[10]. Similarly to BD, strong expression of ICAM-1 has also been found in GCA lesions, particularly in granulomatous inflammatory infiltrate[21] and adventitial microvessels and neovessels in the temporal artery[22]. In this association case-control study we evaluated not only healthy controls and patients with GCA but also 2 other control groups of the same ethnic background and from the same geographic areas[10]. One group was made up of patients with nonarteritic central retinal artery occlusion, the second of

cataract surgery patients. R241 frequency in these 2 groups was similar to that in the healthy controls.

We also considered the possibility that the frequency of the G/R 241 polymorphism might be underestimated in our healthy controls. Even if there are only a few studies on ICAM-1 gene polymorphisms, there is clearly heterogeneity in the distribution of R241 allele across the world (Table 3). The frequency of this allele is higher in Northern European caucasoid populations[13,23-26] compared to that observed in Mediterranean countries[10,20]. R241 allele is absent in the Japanese[27] and very rare in Palestinians and Jordanians[20]. The frequency of R241 in the Italian population, similar to that reported in the Jewish population[12], was much lower than that observed in northern European populations (although there were no Jewish subjects among our controls) and it was at least 2 times higher than in Palestinians and Jordanians[20]. Variations in the geographic distribution of R241 allele are probably related to the different ethnic backgrounds of the populations studied. The p values at the limit of significance, the unclear functional significance, and the nonreplication in our study could compromise the weak association between E469 allele and BD observed by Verity, *et al*[20]. Furthermore, we evaluated if there was a different selection of patients with BD in the 2 studies and if this might have been responsible for the discrepancies. As Verity, *et al* are ophthalmologists, 73% of BD patients they studied had ocular involvement[20]. However, a similar percentage of patients with eye lesions was also included in our study. The results of our study are supported by the small p value and the plausible functional significance of the association between R251 allele and BD. Other association studies are needed to see if our results can be replicated.

However, even if a causal association with a candidate single nucleotide polymorphism should be reproducible in ethnically diverse populations, its absence does not necessarily negate it. The same mutation can cause a major disease phenotype in one strain of mouse, but no phenotype in a genetically distinct strain. Thus, ethnic factors (genetic and otherwise) differentiating populations can modify the expression of a gene and lead to different levels of associations[28].

Our study shows a significant association of a polymorphism in the functionally important ICAM-1 domain 3 with BD. Future functional studies are needed to determine the abnormality present in ICAM-1 R241.

Table 3. Frequency of R241 allele in control population from different geographic areas.

Author	Geographic Area	Frequency of the R 241 allele, %
Mycko, *et al*[24]	Poland	18.0
Hirv, *et al*[23]	Estonia	14.3
Wenzel, *et al*[25]	Germany	11.5
Gengik, *et al*[26]	Germany	11.3
Yang, *et al*[12]	USA, non-Jews	10.2
	USA, Jews	2.3
McLaren, *et al*[13]	United Kingdom	9.4
Salvarani, *et al*[10]	Italy	3.1
Verity, *et al*[20]	Palestinian and Jordanian descent	1.5
Nishimura, *et al*[27]	Japan	0.0

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