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Analysis of polymorphism at site -174 G/C of interleukin-6 promoter region in multiple myeloma

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Abstract

It is well established that interleukin-6 (IL-6) is an essential growth factor for multiple myeloma (MM) and patients with increased IL-6 levels have a poor prognosis. In healthy subjects, the presence of the C allele at a polymorphic site (-174 G/C) of the IL-6 gene is related to low IL-6 levels. In view of the potential association of this particular polymorphism with IL-6 concentration, and the relevance of IL-6 in MM pathogenesis, the objective of the present study was to investigate the prevalence of IL-6 (-174 G/C) promoter polymorphism and its association with development of MM in Brazilian individuals. We investigated the prevalence of these alleles in 52 patients and 60 healthy subjects (matched by age, sex, and race) of a Brazilian population. Thirty patients were male (42.4%), 24 (46.2%) were white and the median age at diagnosis was 58.5 years (range: 28 to 84 years). To determine the IL-6 (-174 G/C) polymorphism, molecular analysis was performed by polymerase chain reaction followed by endonuclease restriction digestion. The genotype distributions observed in the group of patients were 4% CC, 42% GC and 54% GG. The C allele frequency was 0.25. These results were similar to the control group, suggesting no impact of this polymorphism on the susceptibility to MM.

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- Multiple myeloma
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Interleukin-6 (IL-6) is a pro-inflammatory cytokine produced by both myeloma cells (autocrine mechanism) and bone marrow myeloid and monocytic cells (paracrine mechanism), and is considered to be one of the most important cytokines for plasma cell proliferation and survival (1). Evidence that IL-6 is involved in the pathogenesis of multiple myeloma (MM) was based on experimental and clinical findings: a) IL-6 induces *in vitro* growth of myeloma cells freshly isolated from patients, b) myeloma cells spontaneously produced IL-6 and expressed its receptor, c) anti-IL-6 antibodies inhibited the growth of MM cells or cell lines *in vitro*, and d) treatment of MM patients with monoclonal antibodies to IL-6 had some antitumor effect (2). Thus, IL-6 is considered to be an essential growth factor for MM and increased serum IL-6 levels have been associated with a poor prognosis (1,3).

Regulation of cytokine levels is under

genetic control through genetic polymorphisms in their coding and promoter sequences. It seems that the single-nucleotide polymorphism in the promoter region of the IL-6 gene at position -174 may regulate the plasma concentrations of IL-6 *in vivo* (4-6). The presence of lysine (allele C) at the guanine site (allele G) is associated with low levels of plasma IL-6 in healthy subjects. The CC genotype is considered to be a low producer and GG a high producer of IL-6 (4,7,8). However, Endler et al. (6) did not find this association and Brull et al. (9) found the opposite, i.e., patients with the CC allele had higher plasma IL-6 levels. Furthermore, other haplotypes cooperate with the transcriptional regulation of IL-6 influencing its production (10).

Considering the relevant role of IL-6 in MM biology and the influence of genetic polymorphisms on IL-6 production, our objective was to determine the impact of IL-6 polymorphism on the susceptibility to MM.

Between November 2002 and November 2003, patients with MM or solitary plasmacytoma from the MM Outpatient Service of the Discipline of Hematology and Hemotherapy, Universidade Federal de São Paulo (UNIFESP/EPM), São Paulo, SP, Brazil, were invited to participate independently of the time when the diagnosis was made.

Table 1. Distribution of interleukin-6 (IL-6) (-174 G/C) genotypes in the multiple myeloma and control groups studied.

IL-6 (-174 G/C)	Genotype				Allele		
	GG	GC	CC	Total	G	C	Total
Patients							
Present study							
N	28	22	2	52	78	26	104
Frequency	54%	42%	4%		75%	25%	
Mazur et al. (13)							
N	11	31	12	54	53	55	108
Frequency	20%	58%	22%		49%	51%	
Zheng et al. (7)							
N	22	36	15	73	80	66	146
Frequency	30%	49%	21%		55%	45%	
Control							
Present study							
N	35	23	2	60	93	27	120
Frequency	58%	38%	3%		77%	23%	
Mazur et al. (13)							
N	16	28	6	50	60	40	100
Frequency	32%	56%	12%		60%	40%	
Zheng et al. (7)							
N	33	69	26	128	135	121	256
Frequency	26%	54%	20%		53%	47%	

The patients were diagnosed according to the criteria of Durie and Salmon (11) and most of the data were analyzed prospectively. Plasmacytoma patients were treated with radiotherapy and MM patients were initially treated with standard chemotherapy protocols, namely melphalan and prednisone or vincristine, doxorubicin and dexamethasone.

Healthy individuals matched for age, gender and ethnic characteristics were used as controls. The study was approved by the Ethics Committee of Universidade Federal de São Paulo and all subjects gave written informed consent to participate.

Genomic DNA was extracted from EDTA-treated whole blood by standard proteinase K digestion and the phenol/chloroform method (12). A 300-bp fragment containing the -174 site according to the sequence of the IL-6 promoter region was amplified by PCR using the following primers: sense 5'-TTGTCAAGACATGCCAAA GTG-3' and anti-sense 5'-TCAGACATC TCCAGTCCTATA-3' (7). Digestion with the *Nla*III restriction enzyme yielded three fragments when allele C was present (54, 111, and 135 bp) and two fragments were obtained when allele G was present (54 and 246 bp). Genotype distribution and allelic frequency and their association with other variables were analyzed using the chi-square and Fisher test, respectively.

We studied 52 patients, 30 (42.4%) males. Twenty-four (46.2%) were white and 28 (53.8%) were African-Brazilians. The median age at diagnosis was 58.5 years (range: 28 to 84 years). The control group consisted of 36 (60%) male and 24 (40%) female healthy individuals; 33 (55%) of them were

African-Brazilians, and median age was 59.3 years (range: 31 to 85 years). The Pearson chi-square test showed that the two populations were statistically similar regarding gender and race and the Fisher test demonstrate age equivalence.

Six patients had solitary plasmacytoma at diagnosis and only one progressed to MM. Clinical stage at MM diagnosis was IIA for 3 (5.7%), IIIB for 14 (27%), and IIIA for 28 (53.8%) (data for 1 patient were incomplete). Of the 42 cases analyzed, 15 (35.7%) had the IgG kappa isotype, 6 (14.3%) were IgG lambda, 5 (11.9%) IgA kappa, and 4 (9.6%) IgA lambda. Twelve (28.5%) patients presented light chain disease (8 kappa and 4 lambda).

Genomic analysis did not reveal a difference between MM patients and controls in allelic frequency at the -174 position of the IL-6 gene promoter. The C allele frequency was 0.25 in the MM group and 0.23 in the control group ($P > 0.05$). Although, genotype frequency did not differ significantly between our groups, Table 1 shows the results obtained in the present study compared to those reported by other investigators. Our results are similar to the results reported by Zheng et al. (7) and Mazur et al. (13). The G allele frequency was higher in the Brazilian population (75% among patients and 77% among controls) than in the European population (7,13). This observation could be explained by the different ethnic background of the Brazilian population.

Despite the small number of patients enrolled, the similarity of the allelic frequency of the IL-6 polymorphism observed in Brazilian patients and controls suggests that the polymorphism of this allele has no impact on the susceptibility to MM.

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