Interleukin-10 and interferon-gamma cytokine gene polymorphisms may be risk factors for chronic myelogenous leukemia

Bilkay BAŞTÜRK[1], Elif EVKE[2], Ahmet TUNALI[3], Sema KARAKUŞ[4]

[1] Department of Immunology, Baskent University School of Medicine, Ankara
[2] Research Laboratuary, Uludag University School of Medicine,
[3] Department of Hematology, Uludag University School of Medicine, Bursa
[4] Department of Hematology, Baskent University School of Medicine, Ankara, TURKEY

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ABSTRACT

We investigated the association of cytokine gene polymorphisms with the development of chronic myelogenous leukemia (CML) and whether there is an association between gene polymorphisms Th1 and Th2 or regulatory-type cytokines and CML. Thirty patients with CML and 60 healthy controls were enrolled in this study. All genotyping (TNF-α, TGF-β, IL-10, IL-6, and IFN-γ) studies were performed using sequence-specific primers PCR (PCR-SSP). Frequencies of IL-10 (-1082, -819, -592) GCC/ATA (p= 0.009) and IFN-γ; +874 T/A (p= 0.037) polymorphisms were significantly greater in the patients with CML. In contrast, significantly lower frequencies of the IFN-γ A/A (p= 0.004) genotype were observed in patients with CML compared with controls. The results suggest that the IL-10 GCC/ATA and IFN-γ T/A polymorphisms are potential risk factors, and that the IFN-γ A/A polymorphism is a protective factor, for CML in this study group.

Key Words: Cytokines, Chronic myelogenous leukemia, Gene polymorphism, PCR.

ÖZET

İnterlökin-10 ve interferon-gama sitokin gen polimorfizmleri kronik miyeloid lösemi gelişimi için risk oluşturabilir

Bu çalışmada, Th1, Th2 ve T-regülatuar tip sitokin gen polimorfizmleri ile kronik miyeloid lösemi (KML) gelişimi arasındaki ilişkiyi araştırdık. Bu amaçla çalışmaya 30 KML tanısı almış hasta ve 60 sağlıklı gönüllü alındı. Bütün gen tiplendirmeleri (TNF-α, TGF-β, IL-10, IL-6 ve IFN-γ) sekans spesifik primerler kullanılarak PCR yöntemi ile çalışıldı. Hasta grubunda IL-10 (-1082, -819, -592) GCC/ATA (p= 0.009) ve IFN-γ; +874 T/A (p= 0.037) polimorfizmleri istatistiksel olarak anlamlı derecede yüksek saptandı. Bunun aksine IFN-γ A/A (p= 0.004) genotipi sağlıklı kontrol grubu ile karşılaştırıldığında hasta grubunda düşük sıklıkta saptandı. Bu sonuçlar, çalıştığımız hasta grubu için, IL-10 GCC/ATA ve IFN-γ T/A polimorfizmlerinin KML gelişiminde bir risk faktörü, IFN-γ A/A polimorfizminin ise koruyucu bir faktör olabileceğini göstermektedir.

Anahtar Kelimeler: Sitokin, Kronik miyeloid lösemi, Gen polimorfizmi, PCR.

INTRODUCTION

Chronic myelogenous leukemia (CML) is a clonal proliferation of hematopoietic progenitor cells characterized by anemia, leukocytosis with basophilia, myeloid hyperplasia, thrombocytosis, and splenomegaly. Traditionally, CML is described as a phasic disease with most patients presenting in the chronic phase, which is followed by progression to an accelerated phase, and finally to blast crisis. Reciprocal translocation between chromosomes 9 and 22 [t(9;22)(q34;q11)], referred to as the Philadelphia chromosome, exists in 90% of patients and results in juxtaposition of the c-abl oncogene on chromosome 9 with the bcr region of chromosome 22 and resulting formation of a chimeric bcr/abl gene[1,2]. This chimeric gene expresses a 210 kDa protein that transforms activity for hematopoietic cells and is believed to play a major role in the pathogenesis of CML.

Cytokines play an important role in the regulation of normal immune function. In recent years, cytokines and their receptors have been shown to be highly polymorphic. Polymorphisms of these genes have been associated with several immune diseases. Current disease-association data are confusing and often contradictory. While single-locus analyses are the predominant form of cytokine polymorphism analysis, use of polymorphic haplotypes is becoming increasingly common. This may help to give a clearer picture of the association between cytokine polymorphisms and immune disfunction[3].

Cytokines help mediate many of the effector phases of immune and inflammatory responses. Synthesis profiles of cytokines can be considered as either T-helper-cell-type-1 (Th1) responses promoting cell-mediated immunity [interleukin (IL)-2 and interferon (IFN)-γ] or T-helper-cell-type-2 (Th2) responses promoting humoral immunity (IL-4, IL-5, IL-10, and IL-13)[4]. T-regulatory (Tr) cells also are involved in the regulation of both Th1- and Th2-type responses. Therefore, the balance among Th1, Th2, and Tr cells is extremely important for the development and progression of tumors. The in vitro maximal capacity of immune cells to produce different cytokines in response to mitogen stimulation has been shown to vary between individuals. Such differences can be attributed to several molecular mechanisms, including variations in transcription, translation, and secretion pathways[5]. Recently, an additional potential mechanism has been described involving conservative mutation within cytokine coding regions and nucleotide variations within more-pronounced regulatory regions[6-8]. These genetic polymorphisms have been shown to affect the overall expression and secretion of cytokines, both in vitro and sporadically in vivo[9]. Therefore, genetic polymorphisms affecting production levels of certain cytokines may be important determinants of disease risk, severity, or protection for several conditions in which the immune system plays significant roles (eg, malignancies). Eventually, many studies might report an association between cytokine gene polymorphisms and development of certain infectious diseases, allergies, autoimmune disorders, and cancer[10-16].

The aim of the present study was to investigate whether there is any association between cytokine gene polymorphism profile and risk of CML development. We used a panel of cytokines [tumour necrosis factor (TNF)-α, transforming growth factor (TGF)-β, IL-10, IL-6, and IFN-γ] that are known to be involved in tumor immunity.

MATERIALS and METHODS

Study Subjects

Thirty patients (14 men and 16 women, age range from 13 to 82, mean age is 46.16) who were Philadelphia positive, in chronic stages of CML, from the Department of Hematology, Uludag University Faculty of Medicine, Bursa, Turkey, were enrolled in this study. The control group was composed of geographically and racially matched adult healthy blood donor volunteers (n= 60). The study was approved by the Ethical Committee of Uludag University; all subjects gave written informed consent.

DNA Isolation and Cytokine Genotyping

Genomic DNA was extracted from whole ethylenediamine tetracetate (EDTA)-treated blood with a Machery Nagel DNA isolation kit (Duren, Germany) according to the manufacturer's instructions.

Single nucleotide polymorphisms were analyzed in five cytokines for genotype assignment. The presence of a G or A nucleotide in position -308 of the promoter region was analyzed for TNF-α. Two single nucleotide mutations in a coding region were surveyed for TGF-β1: codon +10 can be either T or C, and codon +25, either C or G. Three different polymorphisms were analyzed 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 in position -174 was examined for the IL-6 promoter. An additional coding sequence mutation (T vs A) at position +874 was analyzed for IFN-γ.

Cytokine genotypes were determined using a polymerase chain reaction (PCR) sequence-specific primer method by a commercially available kit (One Lambda Inc, Canoga Park, Calif, USA) in accordance with the manufacturer's instructions. DNA extractions and PCR amplifications were performed after completion of the follow-up period for challenge protocol and were performed by a technician blinded to the clinical results.

Statistical Analysis

Statistical analysis was performed by Epi Info Version 3.2.2 (Centers for Disease Control and Prevention, Bethesda, Md, USA). The distributions of cytokine genes polymorphisms were compared between patients with CML and healthy controls using the χ^2 or Fisher's exact test. P values smaller than 0.05 were considered significant. Odds ratios (OR) and 95% confidence intervals (CI) were calculated in cases when the χ^2 or Fisher's exact test results were significant.

RESULTS

The distribution of cytokine genotypes among the patients with CML and healthy control subjects and OR is summarized in Table 1. The cytokine genotyping results of patients with CML were compared with those of the healthy controls (Table 1). It was found that IL-10 (-1082, -819, -592) GCC/ATA (23% vs 5%, p= 0.009, respectively) and IFN-γ +874 T/A (63.3% vs 40.0%, p= 0.037) polymorphisms were significantly higher in patients with CML than they were in healthy controls. In contrast, significantly lower frequencies of the IFN-γ +874 A/A (13.3% vs 43.3%, p= 0.004, respectively) genotype were observed in patients with CML compared with controls (Table 2). The frequency of IL-10 (-1082, -819, -592) ATA/ATA (3.3% vs 15.0%) was lower in patients with CML than it was in controls; however, this result was not statistically significant. Allele frequencies for the other cytokine gene polymorphisms did not differ significantly between patients with CML and controls. Of thirty patients, five died during follow up period. Of which four patients died due to blast crisis and one due to pancreas cancer. When we compare the deceased patients and living patients, it was observed that all of the deceased patients have polymorphic regions which are compliant with high producing of TGF-β and IL-6 gene polymorphism structures.

DISCUSSION

In this study, we investigated the potential associations between cytokine gene polymorphisms and CML. Our data suggest that IL-10 (-1082, -819, -592) GCC/ATA and IFN-γ +874 T/A genotypes may increase the risk of developing CML. We also found that presence of the IFN-γ +874 A/A genotype might be a determinant in protecting against development of CML.

CML is a disorder of the hematopoietic stem cell that results in malignant expansion of myeloid cells. Leukemic cells proliferate under the influence of cytokines. The autocrine and paracrine effect of cytokines on leukemic cells may have a pathogenic role in the progression of leukemia. The role of T-cells in eradicating leukemic cells has be-

Table 1. Cytokine gene polymorphisms among CML patients and healthy controls

Cytokine gene	Genotype	Patients		Controls polymorphisms	
		n	%	n	%
TNF-α (-308)	G/G	19	63.3	39	65.0
	G/A	11	36.7	17	28.3
	A/A	0	0.0	4	6.7
TGF-β1 (codon 10-25)	T/T-G/G	12	40.0	17	28.3
	T/C-G/G	15	50.0	24	40.0
	T/C-G/C	0	0.0	3	5.0
	C/C-G/G	3	10.0	9	15.0
	T/T-G/C	0	0.0	1	1.7
	C/C-G/C	0	0.0	2	3.3
	C/C-C/C	0	0.0	0	0.0
	T/T-C/C	0	0.0	2	3.3
	T/C-C/C	0	0.0	1	1.7
IL-10 (-1082, -819, -592)	GCC/GCC	3	10.0	6	10.0
	GCC/ACC	6	20.0	13	21.7
	GCC/ATA*	7	23.3	3	5.0
	ACC/ACC	4	13.3	10	16.7
	ACC/ATA	9	30.0	19	31.7
	ATA/ATA	1	3.3	9	15.0
IL-6 (-174)	G/G	15	50.0	33	55.0
	G/C	11	36.7	18	30.0
	C/C	3	10.0	9	15.0
IFN-γ (+874)	T/T	7	23.3	10	16.7
	T/A*	19	63.3	24	40.0
	A/A*	4	13.3	26	43.3

* Statistically significant for the patients with CML vs controls.

Table 2. P values and odd ratios of potential risk and protective factors for CML

SNP*	p values	ORs	95% CI
Risk factors			
IL-10 GCC/ATA	0.009	5.78	1.19 < OR < 31.16
IFN-γ T/A	0.037	2.59	0.96 < OR < 7.09
Protective factors			
IFN-γ A/A	0.004	0.20	0.05 < OR < 0.71

* Single nucleotide polymorphism.

en well established for CML. Type-1 (T1) T-cell cytokines play a major role in this antileukemic immune effect[17].

IFN-γ is the signature cytokine of the Th1 subset of helper T-cells and plays a critical role in promoting host resistance to microbial infection. An additional role for IFN-γ in promoting protective antitumor responses has been identified[18]. It is a homodimeric protein produced by natural killer cells,

CD4+ Th1 cells, and CD8+ T cells. Reuben and coworkers have shown that the percentages of CD4+ and CD8+ T cells that synthesized Th1 cytokines IL-2, IFN-γ, and TNF-α from patients in chronic, in accelerated, and in blast crisis phases were significantly lower than those of patients in remission and normal controls[19]. Decreased type-1 cytokine production has been observed in the T cells of patients with untreated CML, and the frequency of IFN-γ-producing T cells in patients with CML resistant to or intolerant to previous IFN-α therapy was lower than it was in healthy individuals[20]. Our data demonstrate that the IFN-γ +874 T/A genotype seems to be a potential risk factor for CML.

IL-10 is an immunoregulatory cytokine produced by T regulatory, Th2, B, and monocytic cells. The molecular function of IL-10 in the pathogenesis of several diseases may be multifactorial. These effects include promotion of Th2 polarization, prevention of tumor antigen presentation by suppressing expression of MHC class I and II antigens, and regulation of the survival or death of immune cells via Fas/FasL-dependent apoptotic pathways[21].

IL-10 has been implicated in autoimmunity, tumorigenesis, and transplantation tolerance[22]. An increase of IL-10 has been shown to prevent spontaneous death of germinal-center B cells by induction of the bcl-2 protein, and in CML patients, inhibits the synthesis of IFN-γ and IL-1α in Th1 cells and CD8+ cells and strongly downregulates constitutive and IFN-γ– or IL-4–induced MHC class II antigen expression[23,24]. Induction of IL-10 correlates with a switch from Th1 to Th2 response[25]. Percentages of CD4+ and CD8+ T cells of patients in chronic and in blast crisis phases of CML preferentially synthesized the Th2 cytokine IL-10[19]. Treatment with IFN-α has proven to be an effective therapy in CML patients. However, it is unknown whether IFN-α can restore normal immune function for patients. One mechanism of action of IFN-α in vivo may involve decreasing endogenous IL-10 secretion, thereby reducing suppressive effects on T-cell reactivity and increasing IL-1β secretion, thus enhancing antigen presentation[26].

In our study, the IL-10 (-1082, -819, -592) GCC/ATA genotype correlated with an intermediate level of production of IL-10 and was found 23% in patients, whereas 5% of the healthy subjects had this genotype. These data suggest that the presence of the IL-10 (-1082, -819, -592) GCC/ATA genotype may be risk factor for CML. The frequency of the IL-10 (-1082, -819, -592) ATA/ATA (3.3% vs 15%) genotype correlated with a low level of production of IL-10 and was lower in patients with CML than it was in controls; however, this result was not statistically significant. The distribution tendency of the cytokine gene polymorphism in our patients was toward Th2.

In contrast, significantly lower frequencies of the IFN-γ +874 A/A genotype was observed in patients with CML compared with controls. The IFN-γ +874 A/A genotype seems to be a protective factor for CML. TGF-β and IL-6 which is known to be effective in malignant diseases, may be a prognostic factors for CML.

In conclusion, we have demonstrated that there is an association between certain cytokine gene polymorphisms and CML. These polymorphisms may be valuable predictor determinants for development of CML. The incidence of CML in Turkey is growing daily, and larger studies are therefore necessary to investigate the role of the polymorphisms involved in the oncogenesis of CML.

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Address for Correspondence:

Bilkay BAŞTÜRK, MD

Department of Immunology
Baskent University Faculty of Medicine
Ankara, TURKEY

e-mail: bilkayy@superonline.com