

Th1 and Th2 Cytokine Gene Polymorphism in Iranian Patients with Chronic Myelogenous Leukemia

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ABSTRACT

Background: It has been hypothesized that genetic factors other than histocompatibility disparity may play a role in predisposition to developing Chronic Myelogenous Leukemia (CML). In this regard, Th1 and Th2 cytokines and their gene polymorphism seems to be important. Overall expression and secretion of cytokines is dependent, at least in part, on genetic polymorphism (nucleotide variations) within the promoter region or other regulatory sequences of cytokine genes. The majority of polymorphisms described are single nucleotide polymorphism (SNPs). The objective of this study was to analyze the genetic profile of Th1 and Th2 cytokines in 30 Iranian patients with CML and 40 healthy subjects. **Methods:** In the patients and control subjects, the allelic and genotype frequencies were determined for the cytokine genes. All typing were performed by PCR-SSP assay. Allele and genotype frequencies were calculated and compared with those of normal controls. **Results:** The results showed that the most frequent alleles in our patients were TGF- β TG/TG, IL-4 T at position -1089, C at position -590, T at position -33 and IL-10 A at position -1082. Whereas the following alleles - TGF- β CG/CG and IL-10 C at position -592 – were seen in much lower frequencies. **Conclusion:** In conclusion, it could be suggested that the frequency of high producing TGF- β alleles and low producing IL-4 and IL-10 alleles in the CML patients is higher than the normal subjects.

Keywords: cytokine gene polymorphism, CML

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INTRODUCTION

Chronic Myelogenous Leukemia (CML) is a hematologic malignancy that is believed to arise in hematopoietic stem cells with lymphoid and myeloid differentiation potential. CML is characterized biologically by the deregulated over growth of the marrow by a multi lineage clone of cells, all of which are marked by the presence of a novel BCR-ABL fusion gene. The formation of this new gene usually involves a reciprocal translocation between the long arms of chromosomes 9 and 22, leading to the formation of a unique chromosome identified as the Philadelphia chromosome (Ph). The transcription of the BCR-ABL gene leads to the synthesis of a new protein that has been shown to have transforming properties in a variety of model systems (1). Treatment with IFN- α not only reduces the WBC count but also reduces the neoplastic, ph⁺ cells. Possible mechanisms of action by IFN- α include the reduction of tumor burden, through the down regulation of BCR-ABL mRNA and P210 (BCR-ABL) protein, and regulation of tumor necrosis factor related apoptosis inducing ligand TRAIL mediated T cell cytotoxicity. Patients with CML present in the chronic phase are characterized by a relatively indolent, benign stage with the differentiation of precursors into functional, mature hematopoietic cells (2). It has been hypothesized that genetic factors other than histocompatibility (MHC) disparity may play a role in predisposition to developing CML. In this regard Th1 and Th2 cytokines and their gene polymorphism seem to be important. Overall expression and secretion of cytokines is dependent, at least in part, on genetic polymorphism (nucleotide variations) within the promoter region or other regulatory sequences of cytokine genes. The majority of polymorphisms described are single nucleotide polymorphism (SNPs) (3). The objective of this study was to analyze the genetic profile of Th1 and Th2 cytokines gene polymorphisms in 30 Iranian patients with CML and 40 healthy subjects.

MATERIAL AND METHODS

30 Iranian patients with CML and 40 healthy subjects were randomly selected. DNA was isolated from whole blood collected with EDTA as anticoagulant, using a "Salting out" method (4). All cytokine typings were performed by polymerase chain reaction with sequence specific primer (PCR-SSP) assay, which uses identical amplification and detection conditions, enabling rapid and cost-efficient analysis of polymorphisms. Amplification was carried out using a PCR Techne Flexigene apparatus (Roche) under the following conditions: initial denaturation for 2 min at 94°C; denaturation

Table 1: Allele and genotype frequencies

Cytokine	Position	Allele	Genotype	CML		Control		P-value
				F	%	F	%	
IL-1	-889	C	CC	10	33.33	19	47.5	0.0001
			TC	14	46.66	16	40	
IL-1	-511	C	TT	5	16.66	5	12.5	
			CC	7	23.33	10	25	
IL-1	+3962	T	CT	18	60	22	55	
			TT	5	16.66	8	20	
			CC	11	36.66	15	37.5	
			CT	18	60	25	62.5	
IL-1R	pst11970	C	TT	1	3.33	0	1	
			CC	13	43.33	16	40	
			CT	11	36.66	21	52.5	
IL-1RA	mspa111100	T	TT	6	20	3	7.5	
			CC	1	3.33	0	1	
			CT	7	23.33	17	42.5	
IL-4RA	+1902	A	TT	22	73.33	23	57.5	
			AA	16	53.33	29	72.5	
			AG	7	23.33	6	15	
IL-12	-1188	G	GG	3	10	5	12.5	
			AA	17	56.66	24	60	
			CA	11	36.66	13	32.5	
IFN- γ	UTR5644	C	CC	2	6.66	3	7.5	
			AA	9	30	21	52.5	
			AT	12	40	1	2.5	
TGF- β	codon 10/25	CT/GC	TT	7	23.33	18	45	
			TG/TG	11	36.66	5	12.5	
			CG/TG	14	46.66	17	42.5	
			CC/TG	4	13.33	1	2.5	
			CG/CG	0	0	15	37.5	
			TG/TC	0	0	0	0	
			CG/CC	1	3.33	0	0	
			CC/CC	0	0	0	0	
			TC/TC	0	0	0	0	
			TC/CC	0	0	2	5	
TNF- α	-308	A	AA	0	0	1	2.5	
			GA	8	26.66	13	32.5	
			GG	22	73.33	26	65	
IL-2	-238	G	AA	0	0	0	0	
			GA	8	26.66	11	27.5	
			GG	22	73.33	29	72.5	
IL-2	-330	T	GG	2	6.66	1	2.5	
			GT	24	80	23	57.5	
			TT	4	13.33	16	40	
			GG	22	73.33	21	52.5	
IL-4	+166	G	GT	7	23.33	19	47.5	
			TT	1	3.33	0	0	
			GG	0	0	0	0	
IL-4	-1098	T	GT	10	33.33	22	55	
			TT	20	66.66	18	45	
			CC	13	43.33	5	12.5	
			CT	17	56.66	35	87.5	
IL-4	-590	C	TT	0	0	0	0	
			CC	20	66.66	22	55	
			CT	5	16.66	18	45	
			CC	20	66.66	22	55	
IL-6	-33	T	CT	5	16.66	18	45	
			TT	5	16.66	0	0	
			CC	0	0	3	7.5	
IL-6	-174	G	CG	15	50	21	52.5	
			GG	15	50	16	40	
			AA	0	0	0	0	
IL-10	nt565	A	GA	13	43.33	20	50	
			GG	17	56.66	20	50	
			GCC/GCC	0	0	0	0	
			GCC/ACC	4	13.33	21	52.5	
			GCC/ATA	9	30	13	32.5	
			ACC/ACC	7	23.33	0	0	
			ACC/ATA	5	16.66	6	15	
IL-10	-1082,-819,-590	G,C,A,T	ATA/ATA	5	16.66	0	0	
			GCC/GCC	0	0	0	0	
			GCC/ACC	4	13.33	21	52.5	
			GCC/ATA	9	30	13	32.5	
			ACC/ACC	7	23.33	0	0	

for 10s at 94°C; annealing and extension for 1 min (10 cycles) at 65°C, denaturation for 10s at 94°C; annealing for 50s at 61°C; extension for 30s at 72°C (20 cycles). The presence or absence of PCR products was visualized by 2% agarose gel electrophoresis. After electrophoresis, the gel was placed on a UV transilluminator and a picture was taken for interpretation and documentation. Each of the primer mixes contained a control primer pair that amplified either a part of the β -globin gene or a part of the C-reactive protein (CRP) gene. The β -globin control primers produced a 89-bp fragment, while the primer pairs amplifying the CRP gene produced a 440-bp amplicon. The allele and genotype frequencies of the following cytokine genes were determined: IL-1 α (T/C -889), IL-1 β (C/T -511, T/C +3962), IL-12 (C/A-1188), IFN- γ (A/T UTR 5644), TGF- β (C/T codon 10, G/C codon 25), TNF- α (G/A -308, G/A -238), IL-2 (T/G -330, G/T +166), IL-4 (T/G -1089, T/C -590, T/C-33), IL-6 (G/C-174, G/A nt565), IL-10 (G/A-1082, C/T -

Table 2. cytokine production by different genotypes

Cytokine	Position	Genotype	Production
TNF- α G/A A/A	-308 High High	G/G	Low
TGF- β CG/TG CC/TG CG/CG TG/TC CG/CC CC/CC TC/TC TC/CC	codon10,25 High intermediate intermediate intermediate Low Low Low Low	TG/TG	High
IL-10 GCC/GCC GCC/ACC GCC/ATA ACC/ACC ACC/ATA ATA/ATA	-1082, -819,-590 High intermediate intermediate Low Low Low		
IL-6 G/C C/C	-174 High Low	G/G	High
IFN- γ T/A A/A	Intron 1 intermediate Low	T/T	High

819, C/A-592), IL-1R (C/T pst11970), IL-1RA (T/C mspa111100), IL-4RA (G/A +1902). Allele frequencies were estimated by direct gene counting. Allele frequencies of various genotypes were compared using

the chi-square test. The Odds ratio and P value were calculated for each allele in the patients and control groups.

RESULTS

Allelic and genotype frequencies are reported in Table 1. The most frequent alleles in our patients were TGF- β TG/TG (37% vs.12.5%), IL-4 T at position -1089 (67% vs. 45%), C at position -590 (43.3% vs.12.5%), T at position -33 (16.66% vs.1%) and IL-10 A at position -1082 (53.33% vs.15%). Whereas the frequency of the following alleles -TGF- β CG/CG (1% vs. 37.5%) and IL-10 C at position -592 (33.33% vs.52.5%) were very low.

DISCUSSION

For some of the cytokines studied, the relationship between genotype and *in vivo* production is known (Table 2) (5,6,7). In healthy subjects a certain level of production (high, intermediate, low) has been found more frequently for some cytokines. A possible reason for a high frequency of a genotype associated with a given rate of production is selection over the course of evolution, increasing the frequency of the most advantageous genotype. In this study, the C allele of IL-4 at position -590 was increased among patients. The -590 (C to T) polymorphism of the IL-4 gene is associated with high levels of IL-4 secretion and also is associated with high IgE production in asthmatic families (8).

TNF- α (low production more frequent in healthy individuals) shows a wide spectrum of biological activities. TNF- α is a potent proinflammatory cytokine. It causes cytolysis of many tumor cell lines *in vitro* and is a powerful promoter of angiogenesis *in vivo*. Although TNF- α is required for normal immune responses, its over expression may have severe pathological consequences. The most common SNP is seen at -308 and involves a G/A substitution (9). In our patients, the G allele at -308 was increased but the differences were not significant (73% vs 65%). It has been shown that an "A" at position -308 is associated with increased transcription and production of TNF- α (10,11). Patients with either renal or cardiac transplants exhibiting high producing TNF- α genotype were reported to be at increased risk for rejection (12,13,14,15).

TGF- β (high production more frequent in healthy individuals) as a multifactorial cytokine is the strongest known growth inhibitor of normal and transformed cells. Two SNPs in the first exon of TGF- β have been described at position +869 (T/C) and a +915 (G/C). These polymorphisms result in the change of codon 10 from leucine (T) to proline (C) and in the

change of codon 25 from arginine (G) to proline (C)(16). In vitro studies have shown that the presence of leucine or arginine leads to a higher production of TGF- β (17,18). As indicated in Table 2, the homozygous genotype CC, either at codon 10 or at codon 25 is strongly associated with low production, while the homozygous genotype GG at codon 25 is associated with high production. If the "high producer" GG genotype has a real selection advantage, this might explain the significant excess of GG homozygotes observed in our patients, as reported in table 1. When both GG and CC genotypes are present together, an intermediate production is observed, indicating an interaction, or dose effect, of the two alleles.

IL-6 (High production were more frequent in healthy individuals) is a pleiotropic cytokine with a central role in host defense. It has been shown that IL-6 derived from APC cells is able to initiate the polarization of native CD4⁺ T cells to effector T_H2 cells, thereby antagonizing differentiation to T_H1 cells (19). Recently, a biallelic polymorphism within the promoter region of the IL-6 gene at position -174 G to C has been detected, and the C allele was found to be associated with lower *in vitro* and *in vivo* production of IL-6. Even though the relevance of the recipient's genotype for acute rejections is still controversial, the -174 G allele has recently been associated with renoprotection (19). There were no significant differences in the G or C alleles in our patients and the control groups.

IL-10 (intermediate production more frequent in healthy individuals), as an anti-inflammatory cytokine, induces the secretion of IgG, IgA and IgM. This effect is synergized by IL-4 while the synthesis of immunoglobulins induced by IL-10 is antagonized by TGF- β . IL-10 is also considered to be a cytotoxic T cell differentiation factor. Three SNPs in the promoter region of IL-10 are most commonly observed including: Positions -1082 (G/A), -819 (C/T) and -592 (C/A) upstream from the transcriptional start site (12). The presence of an "A" at -1082 and -592 has been related to low IL-10 production in cultured cells (18,20). This low producer allele has been correlated with increased incidence of rejection in heart and kidney grafts (12,19). In our study, IL-10 haplotype ACC was increased significantly in CML patients, an allele which is associated with low production of IL-10.

IFN- γ (intermediate production more frequent in healthy individuals) gene has a (CA)_n repeat element within the first intron. IFN- γ intron-1 microsatellite polymorphism has association with a variety of autoimmune and alloimmune disease states, including lung transplant fibrosis and renal transplant rejection (21). This microsatellite has two common alleles, 2 and 3, which exhibit significant differences in IFN- γ production *in vitro*; allele 2 is associated with greater IFN- γ production than other alleles from mitogen-stimulated peripheral blood mononuclear cells. We could not find any significant relationship between IFN- γ genotype and CML predisposition

in our patients.

In conclusion it could be suggested that production of TGF- β in the CML patients is high and production of IL-4 and IL-10 is lower than the normal subjects. Presence of TGF- β TG/TG haplotypes (high production), IL-10 GCC/ACC haplotypes (intermediate production), IL-10 ACC/ACC haplotypes (low production) and IL-4 C allele at -590 (low production) were critical in drawing this conclusion. The genetic study of cytokines is likely to provide relevant information on their polymorphisms and a possible relationship between these polymorphisms and natural selections. As a further step, study of the associations between cytokine genotype and immunological phenomena may explain basic biological events and indicate clinical ways of predicting, preventing or managing harmful situations in disease. Also, it may help in increasing donor pools by haplo-identical transplants.

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