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

Ali Akbar Amirzargar[1*], Morteza Bagheri[2], Ardeshir Ghavamzadeh[3], Kamran Alimoghadam[3], Farideh Khosravi[1], Mohammad Hossein Nicknam[1], Mandana Moheydin[3], Bita Ansaripour[1], Batul Moradi[1], Behrouz Nikbin[1]

[1]Immunogenetic Lab. Dep. of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran; [2]Department of Sciences, University of Khatam, Tehran, Iran; [3]Hematology- Oncology and BMT Research Center, Shariati Hospital, Tehran, Iran.

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

*Corresponding author: Dr. Ali Akbar Amirzargar, Dept. of Immunology, Immunogentic Laboratory, Medical School Tehran University of Medical Sciences, Tehran, Iran. Fax: +98 21 642 2337
e-mail: Amirzargar_Ali@yahoo.com

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 cytotoxity. 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 histocompatobility (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	
		T	TC	14	46.66	16	40	
			TT	5	16.66	5	12.5	
IL-1	-511	C	CC	7	23.33	10	25	
		T	CT	18	60	22	55	
			TT	5	16.66	8	20	
	+3962	C	CC	11	36.66	15	37.5	
		T	CT	18	60	25	62.5	
			TT	1	3.33	0	1	
IL-1R	pst11970	C	CC	13	43.33	16	40	
		T	CT	11	36.66	21	52.5	
			TT	6	20	3	7.5	
IL-1RA	mspa111100	C	CC	1	3.33	0	1	
		T	CT	7	23.33	17	42.5	
			TT	22	73.33	23	57.5	
IL-4RA	+1902	A	AA	16	53.33	29	72.5	
		G	AG	7	23.33	6	15	
			GG	3	10	5	12.5	
IL-12	-1188	A	AA	17	56.66	24	60	
		C	CA	11	36.66	13	32.5	
			CC	2	6.66	3	7.5	
IFN-γ	UTR5644	A	AA	9	30	21	52.5	
		T	AT	12	40	1	2.5	0.0001
			TT	7	23.33	18	45	
TGF-β	codon 10/25	CT/GC	TG/TG	11	36.66	5	12.5	0.001
			CG/TG	14	46.66	17	42.5	
			CC/TG	4	13.33	1	2.5	
			CG/CG	0	0	15	37.5	0.001
			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	
		G	GA	8	26.66	13	32.5	
			GG	22	73.33	26	65	
	-238	A	AA	0	0	0	0	
		G	GA	8	26.66	11	27.5	
			GG	22	73.33	29	72.5	
IL-2	-330	G	GG	2	6.66	1	2.5	0.08
		T	GG	24	80	23	57.5	
			GT	4	13.33	16	40	0.12
	+166	G	TT	22	73.33	21	52.5	0.06
		T	GG	7	23.33	19	47.5	
			GT	1	3.33	0	0	
IL-4	-1098	G	TT	0	0	0	0	
		T	GG	10	33.33	22	55	
			GT	20	66.66	18	45	0.02
	-590	C	TT	13	43.33	5	12.5	0.008
		T	CC	17	56.66	35	87.5	0.008
			CT	0	0	0	0	
	-33	C	TT	20	66.66	22	55	
		T	CC	5	16.66	18	45	0.02
			CT	5	16.66	0	0	0.04
IL-6	-174	C	TT	0	0	3	7.5	
		G	CC	15	50	21	52.5	
			CG	15	50	16	40	
	nt565	A	GG	0	0	0	0	
		G	AA	13	43.33	20	50	
			GA	17	56.66	20	50	
IL-10	-1082,-819,-590	G,C,A,T	GG	0	0	0	0	
			GCC/GCC	4	13.33	21	52.5	0.001
			GCC/ACC	9	30	13	32.5	
			GCC/ATA	7	23.33	0	0	0.001
			ACC/ACC	5	16.66	6	15	
			ACC/ATA	5	16.66	0	0	
			ATA/ATA					

for 10s at 94ºC; annealing and extension for 1 min (10 cycles) at 65ºC, denaturation for 10s at 94ºC; annealing for 50s at 61ºC; extension for 30s at 72ºC (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-1α (T/C -889), IL-1β(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 -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

Table 2. cytokine production by different genotypes

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

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 ot 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 relationsahip 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.

ACKNOWLEDGEMENTS

This work was supported in part by a grant from the Hematology-Oncology and BMT Research Center, Shariati Hospital, Tehran, Iran.

REFERENCES

1. Petzer AL, Eaves CJ, Barnett MJ, Eaves AC. Selective Expansion of Primitive Normal Hematopoietic Cells in Cytokine Supplemented Cultures of Purified Cells From Patients With Chronic Myeloid Leukemia. *Blood 1997;* **90(1)**:64-69.

2. Reuben JM, Lee BN, Johnson H, et al. Restoration of Th1 Cytokine Synthesis by T Cells of Patients with Chronic Myelogenous Leukemia in Cytogenetic and Hematologic Remission with Interferon-α. *Clin Cancer Res 2000;* **6(5)**:1671-1677.

3. Reynard MP, Turner D, Navarrete V. Allele frequencies of polymorphisms of the tumor necrosis factor-α, interleukin-10, interferon-γ and interleukin-2 genes in a North European Caucasoid group from the UK. *Eur J Immunogenet 2000*; **27(4)**:241-9.

4. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res 1988;* **16(3)**:1215.

5. Hoffmann SC, Stanley E, Cox ED, et al. Association of cytokine polymorphic inheritance and in vitro cytokine production in anti-CD3 / CD 28 stimulated peripheral blood lymphocytes. *Transplantation 2001*; **72(8)**:1444-50.

6. Melk A, Henne T, Kollmar T, et al. Cytokine single nucleotide polymorphisms and intrarenal gene expression in chronic allograft nephropathy in children. *Kideny Int 2003;* **64(1)**:314-320.

7. Capei, MUdeE, Fasano DME, Rendine S, Curtoni, ES. Genotyping for cytokine polymorphisms: allele frequencies in the Italian population. *Eur J Immunogenet 2003;* **30(1)**:5-10.

8. Noguchi E, Shibasaki M, Arinami T, et al. Association of asthma and the interleukin4 promoter gene in Japanese. *Clin Exp Allergy 1998;* **28(4)**:449-453.

9. Wilson AG, DI Giovine FS, Blakemor AI, Duff GW. Single base polymorphism in the human tumor necrosis factor alpha (TNF alpha) gene detectable by NcoI restriction of PCR product. *Hum Mol Genet 1992;* **1(5)**:353.

10. Wilson AG, Symons JA, Mc Dowell TL, et al. Effects of polymorphisms in the human tumor necrosis factor alpha promoter on transcriptional activation. *Proc Natl Acad Sci USA 1997*; **94(7)**:3195-3199.

11. Bouma G, Crusius JB, Oudkerk PM. et al. Secretion of tumor necrosis factor alpha and lymphotoxin alpha in relation to polymorphisms in the TNF genes and HLA-DR alleles. Relevance for inflammatory bowel disease. *Scand J Immunol 1996;* **43(4)**:456-463.
12. Turner D, Grant SC, Yonan N, et al. Cytokine gene polymorphism and heart transplantation rejection. *Transplantation 199*7; **64(5)**:776-779.
13. Hahn AB, Kasten Jolly JC, Constantino DM, et al. TNF-alpha, IL-6, IFN-gamma, and IL-10 gene expression polymorphisms and the IL-4 receptor alpha chain variant Q576R: Effects on renal allograft outcome. *Transplantation 2001*; **72(4)**:660-665.
14. Sankaran D, Asderakis A, Ashraf S, et al. Cytokine gene polymorphisms predict acute graft rejection following renal transplantation. *Kidney Int 1999;* **56(1)**:281-288.
15. Azzawi M, Hasleton PS, Turner DM, et al. Tumor necrosis factor alpha gene polymorphism and death due to acute cellular rejection in a subgroup of heart transplant recipients. *Hum Immunol* 2001; **62(2)**:140-142.
16. Derynck R, Rhee L, Chen EY, Van Tilburg A. Intron-exon structure of the human transforming growth factor beta precursor gene. *Nucleic Acids Res 1987;* **15(7)**:3188-3189.
17. Awad MR, El Gamel A, Hasleton P, et al. Genotype variation in the transforming growth factor beta 1 gene: association with Transforming growth factor beta production, fibrotic lung disease, and graft fibrosis after lung transplantation. *Transplantatio*n 1998; **66(8)**:1014-20.
18. Hutchinson IV, Turner D, Sancaran D, et al. Cytokine genotypes in allograft rejection: Guidelines for immunosuppression. *Transplant Proc 1998;* **30(8)**:3991-3992.
19. Muller-Steinhardt M, Hartel C, Muller B, et al. The interleukin-6 -174 promoter polymorphism is associated with long-term kidney allograft survival. *Kidney Int 2002;* **62(5)**:1824-1827.
20. Turner DM, Williams DM, Sancaran D, et al. An investigation of polymorphism in the interleukin-10 gene promoter. Eur *J Immunogenet 1997;* **24(1)**:1-8.
21. Hobbs K, Negri J, Klinnert M, et al. Interleukin-10 and transforming growth factor beta promoter polymorphisms in allergies and asthma. *Am J Respir Crit Care Med 1998;* **158(6)**:1958-1962.
22. Poole KL, Gibbs PJ, Evans PR, et al. Influence of patient and donor cytokine genotypes on renal allograft rejection: Evidence from a single center study. *Transpl Immunol 2001;* **8(4)**:259-265.
23. Cavet J, Dickinson AM, Norden J, et al. Interferon-γ and interleukin-6 gene polymorphisms associate with graft-versus-host disease in HLA-matched sibling bone marrow transplantation. *Blood* 2001; **98(5)**:1594-1600.