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Determination of cytokine gene polymorphisms in Turkish patients with multiple myeloma

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Cytokines play a major role on multiple myeloma (MM) pathogenesis. Polymorphisms occurring in cytokine genes are effective on plasma level and/or function of the protein. The aim of our study was to determine the cytokine gene polymorphism (CGP) in patients with MM. Eighty patients with MM and 150 healthy individuals were included in the study. CGPs were typed by PCR method using sequence-specific primers (SSP) with Heidelberg kit. TGF-beta CG/CC, TNF-alpha AG/AA, IL-2 GG/TT and IL-4 GCC/TCC haplotypes in the patient group and TNF-alpha GG/GG haplotype in the control group were found to be significantly frequent. When the cytokines that have more than one polymorphic site were investigated one by one CC genotype of IL-4 gene at position -590 was found to be significantly frequent in the patient group. For TGF-beta, CC genotype at codon 10 and GC genotype at codon 25; for TNF-alpha, AA genotype at position -308 and GA genotype at position -238; for IL-2 gene, GT genotype at position +166 were found to be significantly frequent in the patient group. We believe that further studies regarding cytokine polymorphisms in MM may clarify the role of cytokines and contribute to identify novel therapeutic targets.

Key words: Multiple myeloma, cytokine, cytokine gene polymorphism

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Introduction

The occurrence of multiple myeloma (MM) among siblings, spouses, and family members of MM patients suggests that genetic factors and common environmental exposures such as radiation and benzene play a role in the development of this cancer.[1] There are also well-documented reports of familial clusters of two or more first-degree relatives.[2,3] Another convincing finding in favor of genetic predisposition is the significantly high incidence of MM among African Americans. The discovery of chromosome abnormalities,[4] oncogenes[5,6] and specific human leukocyte antigens (HLA)[7,8] among MM patients adds further support for a genetic predisposition for this cancer.

In view of pathogenesis, some cytokines are involved as having part. Interleukin-6 (IL-6) is essential for the survival and growth of myeloma cells.

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There are some evidences that IL-6 is produced by bone cells and stromal cells after stimulation by myeloma cells.[9] The expression of adhesion molecules on MM cells is imputed in permitting to home in the bone marrow. The IL-6 system (IL-6, soluble IL-6 receptor α, IL-1β) plays an important role in the pathogenesis of bone lesions because it activates osteoclasts in the vicinity of myeloma cells and therefore provokes bone reabsorption.[10]

Some of the cytokines such as IL-1β, TGF-β, TNF-a are known to be upregulated as a result of the interaction of myeloma cells with bone marrow stromal cells.[11] Several reports showed that TNF-α is also a survival factor for myeloma cell lines.[12,13]

We aimed in this study to determine the cytokine gene polymorphisms in MM patients and compare the findings with an ethnically matched control group.

Materials and Methods

Patient and control groups

Eighty patients (F/M: 37/43) diagnosed with multiple myeloma and treated in Istanbul University, Istanbul Medical Faculty, Division of Internal Medicine Department of Hematology and 150 healthy individuals were included in this study. Mean age of the patient group was 57.38 (24-79). The number of patients under 40 years of age was three. This study was approved by the local ethics committee and written informed consent according to Helsinki declaration was obtained from all patients and healthy controls.

Cytokine gene polymorphisms typing method

DNA was extracted from peripheral blood by a standard method.[14] All patients and controls were typed at Department of Medical Biology, Istanbul Medical Faculty. Typing was performed by sequence specific primer (PCR-SSP) method using "Heidelberg kit from the Institute of Immunology, Department of Transplantation Immunology, University of Heidelberg, Heidelberg, Germany". PCR was performed on a 9700 thermal cycler (PE Biosystems, CA) (Table 1). PCR products were visualized in agarose gels under UV illumination following ethidium bromide staining and documented by photography.

Statistical methods

Statistical analyses were performed by using Fisher's exact test and Pearson chi-square test. Allele frequencies and expected Hardy Weinberg proportions (HWP) for each SNP were determined in control groups.

Results

The study included 80 patients with multiple myeloma diagnosis and 150 healthy individuals (control group). The most frequent cytokine gene polymorphisms observed in patient and control groups are shown in Table 2.

Most of the SNPs in the control group were in concordance with Hardy-Weinberg equilibrium while some of the SNPs such as the ones in IL-1Rα, TNF-α -308, IL-2 +166, IL-6 -174 and 565, IL-10 -1082 were in discordance with HW equilibrium.

When the patient and the control groups were compared, TGF-β CG/CC, TNF-α AG/AA, IL-2 GG/TT and IL-4 GCC/TCC haplotypes in the patient group and TNF-α GG/GG haplotype in the control goup were found to be significantly frequent. Cytokines such as IL-10, IL-1β, IL-2, IL-6, IL-4, TGF-β and TNF-α with polymorphic loci at more than one position throughout the gene were evaluated for each position in the patient and the control groups independently and there was a significant difference for IL-10, IL-1β and IL-6 cytokine genes between these groups. CC genotype of IL-4 gene at position -590 was found to be significantly frequent in the patient group while TC genotype at the same position was found to be statisitically significant in the control group. For TGF-β, CC genotype at codon 10 and GC genotype at codon 25 were significantly frequent in the patient group while in the control group, CT and GG genotypes were found to be significantly frequent for codon 10 and 25, respectively. Similarly, AA genotype at position -308 and GA genotype at

Table 1
Content of the kit

No of wells	Cytokine	Specifity of allele	No of wells	Cytokine	Specifity of allele
1	IL-1α	-889 T	25	TNF-a	-308/-238 GA
2	IL-1α	-889 C	26	TNF-a	-308/-238 AA
3	IL-1β	-511 C	27	IL-2	-330/-166 TG
4	IL-1β	-511 T	28	IL-2	-330/-166 GG
5	IL-1β	+3962 T	29	IL-2	-330/-166 GT
6	IL-1β	+3962 C	30	IL-2	-330/-166 TT
7	IL-1R	1970 C	31	IL-4	-1098/-590 TT
8	IL-1R	1970 T	32	IL-4	-1098/-590 TC
9	IL-1Rα	11100 T	33	IL-4	-1098/-590 GT
10	IL-1Rα	11100 C	34	IL-4	-1098/-590 GC
11	IL-4RA	+1902 G	35	IL-4	-590/-33 TT
12	IL-4RA	+1902 A	36	IL-4	-590/-33 TC
13	IL-12	-1188 C	37	IL-4	-590/-33 CT
14	IL-12	-1188 A	38	IL-4	-590/-33 CC
15	IFN-γ	5644 A	39	IL-6	-174/565 GG
16	IFN-γ	5644 T	40	IL-6	-174/565 CG
17	TGF-β1	cod10/cod25 CG	41	IL-6	-174/565 GA
18	TGF-β1	cod10/cod25 CC	42	IL-6	-174/565 CA
19	TGF-β1	cod10/cod25 TG	43	IL-10	-1082/-819 GC*C
20	TGF-β1	cod10/cod25 TC	44	IL-10	-1082/-819 G*CC
21	TGF-β1	cod10 CG/CC	45	IL-10	-1082/-819 AC*C
22	TGF-β1	cod10 TG/TC	46	IL-10	-1082/-819 AT*A
23	TNF-α	-308/-238 GG	47	IL-10	-1082/-592 A*CC
24	TNF-α	-308/-238 AG	48	IL-10	-1082/-592 A*TA

position -238 of TNF-α gene were found to be significantly frequent in the patient group whereas in the control group GG at position -308 and GG at position -238 were the significantly frequent genotypes. Regarding IL-2 gene, GT and TT genotypes at position +166 were found to be significantly frequent in the patient and control groups, respectively (Fisher's exact test, $p<0.05$).

In our study, we could reach the clinical data concerning the grade and type of the disease in

Table 2
The most frequent alleles in patient and control groups

Cytokine	Genotype	Patients n=80	Frequency %	Genotype	Controls n=150	Frequency %
IL-1α	CC	49	61.3	CC	92	61.3
IL-1β	CT/CC	30	37.5	CT/CC	53	35.3
IL-1R	CC	35	43.8	CT	65	43.3
IL-1RA	TT	48	60	TT	74	49.3
IL-4Rα	AA	53	66.3	AA	98	65.3
IL-12	AA	44	55	AA	67	44.7
IFN-γ	AT	39	48.8	AT	68	45.3
TGF-β	TG/CG	24	30	TG/CG	64	42.7
TNF-α	GG/GG	39	48.8	GG/GG	101	67.3
IL-2	GG/TG	23	28.8	GG/TG	40	26.7
IL-4	TCC/TCC	38	47.5	TCC/TCC	71	47.3
IL-6	GG/GG	43	53.8	GG/GG	90	60
IL-10	GCC/ACC	22	27.5	GCC/ACC	36	24

terms of paraprotein secretion only in 63 of the patients. Therefore, the following statistical calculations were performed for only this group of patients. Of 63 patients, 4 (6%) had Grade I, 25 (40%) had Grade II, 33 (52%) had Grade III disease and one (2%) was diagnosed to have MGUS (monoclonal gammopathy of undetermined significance). Table 3 shows the comparison of the disease grade and cytokine gene polymorphisms in patients.

Seventeen (26.9%) out of 63 patients had disease type of IgG kappa, 10 (15.8%) had IgG lambda, 5 (7.9%) had IgA kappa, 11 (17.5%) had IgA lambda, 1 (1.6%) had IgD kappa, 2 (3.2%) had IgD lambda, 12 (19.1%) had kappa light chain, 1 (1.6%) had lambda light chain and 4 (6.4%) were diagnosed to have non-secretory disease type. Table 3 shows the comparison of disease type and cytokine gene polymorphisms in patients.

The immunoglobuline subtypes in patients were as follows: IgG in 27 (42.9%), IgA in 16 (25.4%) and IgD in 3 (4.8%) out of 63 patients. The most frequent haplotypes in patients according to the immunoglobuline subtypes are shown in Table 4.

When 63 patients were divided into 2 groups according to disease type concerning the light chain, 35 (55.6%) had kappa and 24 (38.1%) had lambda light chain (Table 4).

Discussion

Cytokines and their receptor genes are very polymorphic. The majority of such polymorphisms identified in cytokines are either single nucleotide (SNPs) or dinucleotide (microsatellite) polymorphisms. SNPs in the promotor region of the gene may influence the rate or extent of cytokine secretion. They may affect the biological activity of the encoded cytokine. A number of cytokines and cytokine receptors have been directly linked to the development of human cancers. Genetic polymorphism of proinflammatory IL-1β and TNF genes have been shown to be associated with several cancers. The overexpression of IL-2 and IL-2R has been associated with adult T cell leukemia.[15] Polymorphisms in IL-1β and its endogenous receptor antagonist are reported to be associated with risk of *Helicobacter pylori*-related gastric cancer.[16]

Table 3
Comparison of disease grade and disease type with CGP

		Cytokine	Genotype	p	OR	CI
Grade	I	TGF-β	TG/TG*	0.0008	54.529	2.685-1107.6
	II	IL-6	GG/GG**	0.03	0.3069	0.106-0.8884
		IL-10	GCC/GCC*	0.04	4.537	1.046-19.683
Disease type	IgG κ	IL-1α	CC*	0.04	14.278	1.082-16.918
		IL-4Rα	AA*	0.006	11.259	1.373-92.344
			GA**	0.02	0.1066	0.01296-0.8773
		IL-10	ATA/ACC*	0.0002	19.556	3.545-107.88
	IgG λ	IL-2	TG/TT*	0.02	6.400	1.338-30.618
		IL-10	GCC/GCC*	0.04	5.222	1.137-23.982
	IgA λ	IL-1α	CC**	0.004	0.0987	0.01912-0.5101
			TC*	0.03	4.317	1.100-16.945
		TGF-β	TG/CC*	0.03	9.375	1.348-65.179
	IgD κ	IL-1β	TT/CC**	<0.0001	0.1545	0.0608-0.3926
	IgD λ	IL-4	TCC/TTT*	0.02	31.471	1.387-714.26
	κ light C.	TGF-β	TG/CG*	0.03	4.550	1.218-16.995
	NS	TNF-α	AG/AA*	0.04	10.615	1.016-110.86

* significantly high in the patient group
** significantly low in the patient group

Table 4
The most frequent haplotypes in patients according to the Ig subtypes and light chain type

		Cytokine	Genotype	p	OR	CI
Ig subtype	IgA	IL-1α	CC**	0.008	0.1928	0.0564-0.6587
			TT*	0.014	24.630	1.196-507.21
		IL-10	ATA/ATA*	0.04	10.615	1.016-110.86
	IgG	TGF-β	CG/CG*	0.03	5.950	1.124-31.485
		IL-10	ATA/ACC*	0.01	7.158	1.377-37.214
Light Chain	Kappa	IL-1α	CC*	0.01	3.852	1.327-11.180
			TC**	0.03	0.2963	0.104-0.8747
		IL-1R	CC**	0.03	0.30	0.1038-0.8672
		IL-2	TG/TT**	0.007	0.0735	0.0085-0.6322
		IL-4	TCC/TCC*	0.04	3.333	1.156-9.609
			TCC/TTT**	0.003	0.0620	0.0072-0.5285
	Lambda	IL-1α	CC**	0.007	0.2069	0.0691-0.6188
			TC*	0.01	3.939	1.317-11.782
		IL-4Rα	AA**	0.02	0.2581	0.0845-0.7876
			GA*	0.02	3.868	1.229-12.172
		IL-2	TG/TT*	0.01	19.00	2.191-164.73
		IL-4	TCC/TTT*	0.004	9.250	1.764-48.511

* significantly high in the patient group
** significantly low in the patient group

Among multiple myeloma as a B cell lymhpoproliferative disease it has been shown that most cytokines have a role in normal B cell lympogenesis. Soluble CD23 and IL-1α may induce centrocytic cells to differentiate into plasmoblastic cells. IL-2, IL-4, IL-10 are also to induce partial differentiation of B cell into plasma cell.[17] In multiple myeloma IL-6, IL-11, Granulocyte Macrophage Colony Stimulating Factor (GM-CSF), Granulocyte Colony Stimulating Factor (G-CSF) and Tumor Necrosis Factor (TNF) contribute to myeloma cell proliferation.[18,19] IL-6 and gp130 IL-6 transducer-activating cytokines have been shown to be the major survival and proliferation factors for malignant plasma cells.[20,21] Recent studies demonstrated that four cytokines Ciliary NeuroTrophic Factor (CNTF), IL-11, Leukemia Inhibitory Factor (LIF) and Oncostatine M (OSM), which are also reported to be growth factors for myeloma cells using the same transducer chain (gp130) with IL-6. In addition, IL-1β, IL-6, IL-11, TNF-a and Lymphotoxin-a can stimulate osteoclastogenesis in myeloma.[22]

TNF-α is a critical cytokine produced early in the inflammatory reaction process. It can be produced virtually by all cells on activation. TNF-α has been detected in multiple solid and hematologic malignancies.[23] Early studies showed that TNF-α secretion from myeloma bone marrow cells was significantly greater than that from normal bone marrow cells.[24] TNF-α is the potent inducer of the production of IL-6, a major growth factor for myeloma cells and may therefore indirectly stimulate plasma-cell growth.[25] Polymorphisms in TNF-α gene at position –308 are the most widely studied ones and G→A substitution at this position was reported to encode for a high responder genotype.[26] In this study when we looked at the distribution of high and low producer haplotypes of TNF-α, we found that the high producer AG/AA haplotype in the patient group and the low producer GG/GG haplotype in the control group were significantly frequent. The high producer haplotype found in our study corresponds with data published by Davies et al.[27]

IL-10 production has been reported in various B cell malignancies including acute lymphoblastic leukemia and Burkitt's lymphoma. The significance of IL-10 in the development of B cell malignancies is not clear. Polymorphisms in IL-10 regulatory sites

were found at position –1082 (G→A), -819 (C→T) and –592 (C→A). GCC haplotype was found to be associated with high production of the cytokine while on the contrary ATA haplotype was associated with low production of IL-10.[28] In our study, we could not establish any association between the control and the patient groups for IL-10.

TGF-β is known to suppress normal hematopoiesis including B cell functions. This protein is secreted at higher levels in bone marrow stromal cells from myeloma patients. TGF-β has differential effects on malignant versus normal plasma cells and also stimulates IL-6 secretion by stromal cells and may be responsible for the enhanced IL-6 release by adhesion of myeloma cells to stromal cells.[29] In our study, we compared the TGF-β polymorphism in patients with myeloma and healthy controls. CG/CC haplotype (low producer) was found to be significantly frequent in patient group.

Recently, a number of studies have suggested that IL-6 plays a key role in the pathogenesis of MM.[30,31] The cytokine IL-6 is thought to be involved in survival, proliferation and progression of MM. Our results show that IL- 6 GG/GG haplotype (high producer) was significantly low in Grade II patients. This is in a contrast with previous studies however Brown et al. suggested that for the vast majority of patients with myeloma, especially in the early stages of disease, IL-6 and its receptor may not play a crucial role, in correspondence with our findings.[32]

Polymorphism of cytokines and cytokine receptors can alter the expression pattern of these genes resulting in abnormally high and low cytokine production. The association of the polymorphism of IL-1β, IL-2 and IL-4 genes with MM has been shown only in this study.

In recent years, some of the cytokines have been used extensively in anticancer therapies among the biological effect and antitumor activity that links innate and adaptive immunity. This study presented here is the only study that has evaluated the effect of cytokine gene polymorphisms in MM. We believe that further studies regarding cytokine polymorphisms in MM may clarify the role of cytokines and contribute to identify novel therapeutic targets. By defining high risk patient as patients with cytogenetic abnormalities, therapy algorithm may be designed to assess the suitable patients for immunomodulatory treatments and anti-cytokine treatment strategies.

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