Interleukin-10 Gene Polymorphism in Bone Marrow Transplant Recipients

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Abstract

Objectives: **Graft-versus-host disease is the main complication after hematopoietic stem cell transplant, occurring even after donor and recipient human leukocyte antigen matching, apparently because of donor/recipient minor histocompatibility antigen mismatches and cytokine polymorphisms. Interleukin-10 suppresses several activities of the immune response by inhibiting T helper 1 and T helper 2 cells. These properties suggest that interleukin-10 could act as a suppressive mediator and prevent graft-versus-host disease. This study evaluates the association between the interleukin-10 promoter gene polymorphism and transplant outcomes among 18 recipients of cytokine-mobilized peripheral blood stem cells from human leukocyte antigen-matched sibling donors.**

Materials and Methods: **We analyzed 3 single-nucleotide polymorphisms in the proximal region of the interleukin-10 promoter gene (-1082/-819/-592) by the amplification refractory mutation system and polymerase chain reaction-restriction fragment length polymorphism methods. Eighteen donors and their recipients who had undergone an allogeneic peripheral blood stem cell transplant at the Bone Marrow Transplant Center in Nemazi Hospital (Shiraz, Southern Iran) between September 2005 and September 2006 were enrolled.**

Results: **The GCC haplotype (1082*G/819*C/592*C) was predominant in both the donor and the recipient, but no significant correlations were present between the GCC haplotype in either the donor or the recipient and the risk of acute graft-versus-host disease (*P* = .56).**

Conclusions: **The interleukin-10 promoter gene polymorphism was found not to be associated with acute graft-versus-host disease in patients after an allogeneic peripheral blood stem cell transplant from human leukocyte antigen-matched sibling donors. Additional studies with larger samples are necessary to further define the influence of interleukin-10 on the immune response after bone marrow transplant.**

Key words: *IL-10, Hematopoietic cell, PCR, Recipient, Donor*

In this study, the genotype of IL10 (−1082 G/A, −819 C/A, −592 C/T) of 18 patients who had undergone a hematopoietic stem cell transplant from HLA-identical siblings donors was determined to detect any genetic polymorphisms that correlated with clinical course, including the incidence and severity of graft-versus-host disease.

Materials and Methods

Patients

The genomic DNA from 18 patients and 18 HLA-identical sibling donors was analyzed. Patients had received a transplant for hematologic disorders (Table 1) between September 2005 and September 2006 at the Bone Marrow Transplantation Centre of Nemazi Hospital, affiliated with Shiraz University of Medical Sciences in Shiraz, Iran. HLA-A and B typing was done by serology or molecular typing. HLA matching was

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also checked by family studies where possible. Written informed consent was obtained from all patients and donors allowing analyses of the clinical data and testing for all mutations and polymorphisms mentioned in this article. The study was conducted according to the guidelines of the 1975 Declaration of Helsinki, and the study protocol was approved before its beginning by the ethics committee of Shiraz University. The commonly used conditioning regimen consisted of busulfan, cyclophosphamide, and antithymocyte globulin (n=11, 61%). Conditioning regimens for thalassemic patients were composed of cyclophosphamide and busulfan (n=7, 39%). Table 1 shows the patient characteristics, including any underlying diseases, donor and recipient ages, cytomegalovirus polymerase chain reaction (PCR) results, and type of graft-versus-host disease prophylaxis.

Graft-versus-host disease prophylaxis

Prophylaxis for graft-versus-host disease was accomplished with a course of methotrexate and cyclosporine administrated intravenously as 15 mg/ m^2 on day 1, then 10 mg/m^2 on days 3 and 6. Cyclosporine was continuously infused at 1 to 2 mg/kg/day from 7 days before the transplant until resumption of oral intake to reach the target therapeutic C0 level of 180 μg/L.

Evaluation of graft-versus-host disease

Acute graft-versus-host disease was diagnosed and graded according to previously published criteria by Glucksberg and associates (12). We considered acute graft-versus-host disease as absent, mild (grade 0-1), or clinically significant (grades 2-4) to do the statistical analyses.

Genetic polymorphism analyses

Peripheral blood from recipients, donors, and controls was collected in EDTA, and genomic DNA was analyzed using an amplification refractory mutation system and PCR-restriction fragment length polymorphism analysis. DNA was extracted with a commercial kit (Sinagene Company, Tehran, Iran).

Three primers were used for allele-specific PCR (13, 14): the 3′ primer (5′-CAGTGCCAACTGAGA ATTTGG 3′) was combined with either the 5′ primer (1082G:5′-CTACTAAGGCTTCTTTGGGAG-3′) complementary to the IL-10-1082 G allele or the 5′ primer (1082A: 5′-ACTACTAAGGCTTCTTTGGG AA-3′) complementary to the IL-10-1082 A allele. Primers 1082G and 1082A differed only in their 3′ terminal nucleotide. DNA (1 μg) was amplified through 30 cycles (95°C for 30 seconds, 60°C for 1 minute, and 72°C for 1 minute) with sense and antisense primers.

The primer sequences used for -819 polymorphism were 5'- TCATTCTATGTGCTGGAG ATG G-3' and 5'- TGGGGGAAGTGGGTAAGAGT-3'; and -592 polymorphism, 5'-CCTAGGTCACAGTG AC GTGG- 3' and 5'-GGTGAGCACTACCT GACTA GC-3'. The products obtained in these 3 reactions were 209 and 412 base pairs (15).

Amplifications were done on a PCR system master cycle (Eppendorf AG, Hamburg, Germany) in a 25-μL reaction mixture containing 1 μg template DNA, 10 mM Tris HCl (pH 9.0), 40 mM KCl, 1.5 mM $MgCl_2$, 250 μM each of dNTP, 20 pM of each primer, and 1 unit of Taq polymerase (Fermantase, Germany). The following 3 cycling conditions were used: first, 94°C for 7 minutes; second, 30 cycles at 94°C for 45 seconds, 60°C for 45 seconds, and 72°C for 60 seconds; and third, 72°C for 10 minutes. A 10-μL aliquot of each PCR product was digested for 1 hour at 55°C with Mae III (Roche Diagnostics Corp., Penzberg, Germany) for the -819 polymorphism or for

Table 1. Transplant characteristics.

Variable	Demographic information	Number cases (n=18)	%
Diagnosis	Acute leukemia	5	27
	Lymphoma	4	22
	Metastatic carcinoma	2	12
	Thalasemia	7	39
Age	R (< 50)	18	100
	D (< 50)	18	100
CMV Statues	R (Negative)	18	100
	D (Negative)	18	100
ABO Compatibility	Matched	10	55
	Mismatched	8	45
Gender Compatibility	Matched	8	45
	Mismatched	10	55
aGvHD Prophylaxis	CSA + MMF	0	0
	CSA + MTX	18	100
HSC Source	PB	17	92
	BM	1	8
aGvHD	Grade 0 - I	12	67
	Grade II - IV	6	33

Abbreviations: ABO, blood group system; aGVHD, acute graft-versus-host disease; BM, bone marrow; CMV, cytomegalovirus; CSA, cyclosporine; D, donor; HSC, hematopoietic stem cell; MMF, mycophenolate mofetil; MTX, methotrexate; PB, peripheral blood; R, recipient.

1 hour at 37°C with Rsa I (Roche Diagnostics) for the -592 polymorphism (15). Electrophoresis was done with 3% agarose, stained with ethidium bromide, and visualized by ultraviolet transillumination and alleles identified by size. Few PCR products were sequenced with a genetic analyzer (Applied Biosystems, Foster City, CA, USA).

Statistical analyses

The chi-square test was used to determine the Hardy-Weinberg equilibrium and the linkage disequilibrium for pairs of polymorphisms at different positions of the same gene or at linked loci. Associations between clinical and genotypic risk factors were analyzed using 2 × 2 contingency tables. SPSS software (Statistical Product and Services Solutions, version 13.0, SPSS Inc, Chicago, IL, USA) was used to analyze the data, and values for *P* less than .05 were considered statistically significant.

Results

Table 1 shows the distribution of known risk factors for graft-versus-host disease, including age at transplant, presence or absence of sex mismatch between the donor and the recipient, use or nonuse of total-body irradiation in the conditioning regimen. Allele and genotype frequencies of each polymorphism are listed in Tables 2 and 3. They were estimated by direct gene counting and compared using the chi-square test. There was no significant deviation from the Hardy-Weinberg equilibrium.

Table 2. IL-10 allele and genotype frequencies.

	SNP Positive	Allele	R allele Frequency (%) n=18	D allele Frequency (%) n=18	Genotype	R genotype Frequency (%) n=18	D genotype Frequency (%) n=18
	-1082	A	18 (100%)	9 (50%)	A/A	2 (11)	4 (24)
		G	16 (88%)	14 (77%)	A/G	16 (89)	7 (38)
					G/G	——	7 (38)
IL-10	-819	C	17 (94%)	17 (94%)	C/C	6 (33)	13 (72)
		T	12 (66%)	5 (27%)	C/T	10 (56)	4 (22)
					T/T	2(11)	1 (6)
	-592	C	18 (100%)	14 (77%)	A/A	0	4 (24)
		A	15 (83%)	15 (83%)	A/C	15 (83)	11 (49)
					C/C	3 (27)	3 (27)

Abbreviations: D, donor; R, recipient; SNP, single nucleotide polymorphism.

Table 3. IL-10 genotype / haplotype frequencies in donors and recipients referred to absent or mild (grade 0-I) and clinically significant (II-IV) acute GVHD.

SNP Positive	Genotype /haplotype	Recipients genotype frequency		Donors genotype frequency	
		aGvHD 0-I (%) (n=9)	aGvHD II-IV (%) (n=9)	aGvHD 0-I (%) (n=9)	aGvHD II-IV (%) (n=9)
1082	G/G + A/G	8 (88)	8 (88)	9 (100)	5 (55)
	A/A	1 (12)	1 (12)	0	4 (45)
819	C/T + T/T	7 (77)	5 (55)	4 (33)	1 (16)
	C/C	2 (23)	4 (45)	8 (67)	5 (84)
592	C/A + A/A	8 (88)	7 (77)	9 (81)	6 (85)
	C/C	1 (12)	2 (23)	2 (19)	1 (12)
1082	GCC/GCC	0	0	1 (5)	1 (5)
	GCC/ACC	1 (5)	2 (10)	0	0
819	GCC/ATA	9 (45)	3 (15)	7 (36)	4 (20)
	ACC/ACC	1 (5)	0	3 (15)	1 (5)
592	ACC/ATA	1 (5)	1 (5)	1 (5)	0
	ATA/ATA	0	0	0	0
1089 819 592	GCC Presence	9 (81)	5 (71)	7 (63)	4 (57)
	Others	2 (19)	2 (29)	4 (37)	3 (42)

Abbreviation: SNP, single nucleotide polymorphism.

Incidence and severity of acute graft-versus-host disease

Half of the recipients (n=9) developed clinically acute graft-versus-host disease (grades II-IV). There was no significant association between grades of acute graft-versus-host disease and donor or recipient age or sex mismatch after hematopoietic stem cells transplant (data not shown). Associations between donor-recipient genetic polymorphisms of 10-IL the cytokine with the severity of acute graft-versus-host disease were determined for each polymorphism as an independent risk factor.

Association between recipient and donor IL-10 gene polymorphisms (genotypes and haplotypes) and acute graft-versus-host disease

No significant association was found for clinically significant acute graft-versus-host disease and IL-10 genotypes (Table 3). In the recipient group, the majority of recipients had the -1082G allele (n=16, 89%) with no association with a lower risk of acute graft-versus-host disease. The -819T allele (n=12, 66%) was more frequent than the -819C allele (n=6, 33.3%) with no association with acute graft-versus-host disease. The -592A allele was more common than the -592C allele with similar results.

In the donor group, the majority of donors had the -1082G allele (n=16, 89%) with no association with a lower risk of acute graft-versus-host disease. The -819C allele (n=15, 83%) was more frequent than the -819T allele, with no association with acute graft-versus-host disease. And the -592A allele (n=15, 83%) was more common than the -592C allele with similar results (Tables 2 and 3).

We then evaluated the correlation between IL-10 haplotypes (defined by polymorphisms at positions −1082, −819, and −592) and the severity of acute graft-versus-host disease with the GCC haplotype as the highest producer of IL-10 and the ACC and ATA haplotypes as the lowest (Table 3). Among 75% of recipients carrying GCC haplotype, 55% developed absent or mild acute graft-versus-host disease when compared with 25% of the recipients who developed significant acute graft-versus-host disease (Table 3). Furthermore, when the donors and the recipients were grouped, frequency of the GCC haplotype was 63% in patients with grades 0 though I acute graft-versus-host disease compared with 37% in patients with grades II though IV acute graft-versus-host disease (Table 4). It is clear that the GCC haplotype was more common in our donors and recipients, with a lower risk of clinically significant graft-versus-host disease.

Table 4. IL-10 genotype / haplotype frequencies in both donors and recipients referred to absent or mild (grade 0-I) and clinically significant (II-IV) acute GvHD.

Cytokine gene	SNP Positive	Genotype haplotype	Donors/Recipient Frequencies Genotype	
			aGvHD 0-I (%) n=9	aGvHD II-IV (%) n=9
	-1082	GCC Present in		
		Both R and D	7 (63)	4 (37)
IL-10	-819			
		Others	3 (42)	4 (58)
	-592			

Abbreviations: aGvHD, acute graft-versus-host disease; SNP, single nucleotide polymorphism.

Discussion

Hematopoietic stem cell transplant can be a lifesaving procedure for patients with otherwise fatal diseases. However, mature T cells in the graft can initiate immune reactions that cause graft-versus-host disease. Despite recent advances in supportive care, a severe grade of graft-versus-host disease remains a serious complication of transplant and contributes to transplant-related mortality (16). To optimize the transplant outcome, matching of the donor and recipient for HLA and minor histocompatibility antigens is important because mismatches increase the risk of graft-versus-host disease, and this occurs in 20% to 40% of recipients. Cytokines and other regulators of the immune response may have an important role in the pathogenesis of graft-versus-host disease. Certain genes also may affect the outcome by modulating the intensity of inflammation and tissue injury associated with the alloimmune reaction and other transplant-related complications (16). Nucleotide variations in the genes encoding these molecules may affect the transcription or translation of the genes or the secretion or function of the corresponding proteins. Cytokine gene promoter polymorphisms resulting in different production and minor histocompatibility antigen mismatches may significantly influence transplant outcome, especially the development of graft-versus-host disease (17).

Three single nucleotide polymorphisms in the promoter region at positions −1082, −819, and −592 influence IL-10 production in vitro (18). Positivity of the IL-10 A allele at position −1082 (A/G) is associated with low IL-10 production (19). Other studies

concerning IL-10 at position −592 have shown conflicting results, some suggesting that the common C allele is associated with higher IL-10 production and others outlining an association between the A allele and higher IL-10 production (20, 21, 17). Furthermore, the IL-10 haplotype resulting from alleles at positions −1082, −819, and −592 may influence the production of this cytokine, with the GCC haplotype correlated with high and the ACC and the ATA correlated with low and intermediate production, respectively, although this finding is not consistent (21, 22).

The main source of IL-10 is monocytes. In the case of acute graft-versus-host disease, monocytes originate from the donor and hence, a donor's low producer genotype is expected to increase the risk of acute graft-versus-host disease. However, other cells may produce IL-10, including mast cells and keratinocytes. The latter clearly originate from the recipient. Therefore, both the donor and the recipient genotypes may influence the occurrence of graft-versus-host disease (22, 23).

GCC was the most common haplotype among our recipients. In comparison, the minority of recipients (25%) developed high-grade graft-versus-host disease compared with those with clinically significant graft-versus-host disease (55%). When the donors and recipients were grouped, the frequency of the GCC haplotype was greater than other haplotypes (61% in GCC vs 39% in others). An association between the absence of the donor-recipient IL-10 GCC haplotype (as a high producer of the cytokine) and the development of acute graft-versus-host disease was not shown in our study; thus, we cannot make any recommendations about IL-10 genotype protection in acute graft-versus-host disease.

A favorable IL-10 genotype also could have a downside for patients with hematologic cancers: Graft-versus-host disease is closely associated with a beneficial graft-versus-leukemia effect, which occurs when donor T cells recognize foreign histo-compatibility antigens on cancer cells and kill them. If increased production of IL-10 induces tolerance to these antigens in donor T cells, they may fail to eradicate residual cancer (1). So, a good genotype for graft-versus-host disease is not a good one for a graft-versus-leukemia effect.

Thus, IL-10 acts in 2 different ways. The limitation of our study may be the low number of patients we analyzed. Further studies with a larger samples are recommend to define the influence of IL-10 on the immune response after bone marrow transplant.

References

1. Bertinetto FE, Dall'Omo AM, Mazzola GA, et al. Role of non-HLA genetic polymorphisms in graft-versus-host disease after haematopoietic stem cell transplantation. Int J Immunogenet. 2006;33(5):375-384.
2. Kim DH, Lee NY, Sohn SK, et al. IL-10 promoter gene polymorphism associated with the occurrence of chronic GVHD and its clinical course during systemic immunosuppressive treatment for chronic GVHD after allogeneic peripheral blood stem cell transplantation. Transplantation. 2005;79(11):1615-1622.
3. Lin MT, Storer B, Martin PJ, et al. Relation of an interleukin-10 promoter polymorphism to graft-versus-host disease and survival after hematopoietic-cell transplantation. N Engl J Med. 2003;349(23):2201-2210.
4. Hutchinson IV, Turner DM, Sankaran D, Awad MR, Sinnott PJ. Influence of cytokine genotypes on allograft rejection. Transplant Proc. 1998;30(3):862-863.
5. de Waal Malefyt R, Abrams J, Bennett B, Figdor CG, de Vries JE. Interleukin 10(IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. J Exp Med. 1991;174(5):1209-1220.
6. Rennick DM, Fort MM, Davidson NJ. Studies with IL-10-/- mice: an overview. J Leukoc Biol. 1997;61(4):389-396.
7. Cooke KR, Ferrara JL. A protective gene for graft-versus-host disease. N Engl J Med. 2003;349(23):2183-2184.
8. Kim JM, Brannan CI, Copeland NG, Jenkins NA, Khan TA, Moore KW. Structure of the mouse IL-10 gene and chromosomal localization of the mouse and human genes. J Immunol. 1992;148(11):3618-3623.
9. Turner D, Grant SC, Yonan N, et al. Cytokine gene polymorphism and heart transplant rejection. Transplantation. 1997;64(5):776-779.
10. 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.
11. Asderakis A, Sankaran D, Dyer P, et al. Association of polymorphisms in the human interferon-gamma and interleukin-10 gene with acute and chronic kidney transplant outcome: the cytokine effect on transplantation. Transplantation. 2001;71(5):674-677.
12. Glucksberg H, Storb R, Fefer A, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. Transplantation. 1974;18(4):295-304.
13. Perrey C, Turner SJ, Pravica V, Howell WM, Hutchinson IV. ARMS-PCR methodologies to determine IL-10, TNF-alpha, TNF-beta and TGF-beta 1 gene polymorphisms. Transpl Immunol. 1999;7(2):127-128.
14. Azarpira N, Aghdaie MH, Geramizadeh B, et al. Cytokine gene polymorphisms in renal transplant recipients. Exp Clin Transplant. 2006;4(2):528-531.
15. Kim DH, Lee NY, Sohn SK, et al. IL-10 promoter gene polymorphism associated with the occurrence of chronic GVHD and its clinical course during systemic immunosuppressive treatment for chronic GVHD after allogeneic peripheral blood stem cell transplantation. Transplantation. 2005;79(11):1615-1622.
16. Miura Y, Thoburn CJ, Bright EC, Chen W, Nakao S, Hess AD. Cytokine and chemokine profiles in autologous graft-versus-host disease (GVHD): interleukin 10 and interferon gamma may be critical mediators for the development of autologous GVHD. Blood. 2002;100(7):2650-3658.
17. Mullighan C, Heatley S, Doherty K, et al. Non-HLA immunogenetic polymorphisms and the risk of complications after allogeneic hemopoietic stem-cell transplantation. Transplantation. 2004;77(4):587-596.
18. Dickinson AM, Middleton PG, Rocha V, Gluckman E, Holler E; Eurobank members. Genetic polymorphisms predicting the outcome of bone marrow transplants. Br J Haematol. 2004;127(5):479-490.
19. Holler E, Kolb HJ, Möller A, et al. Increased serum levels of tumor necrosis factor alpha precede major complications of bone marrow transplantation. Blood. 1990;75(4):1011-1016.

20. Middleton PG, Taylor PR, Jackson G, Proctor SJ, Dickinson AM. Cytokine gene polymorphisms associating with severe acute graft-versus-host disease in HLA-identical sibling transplants. Blood. 1998;92(10):3943-3948.
21. Cavet J, Middleton PG, Segall M, Noreen H, Davies SM, Dickinson AM. Recipient tumor necrosis factor-alpha and interleukin-10 gene polymorphisms associate with early mortality and acute graft-versus-host disease severity in HLA-matched sibling bone marrow transplants. Blood. 1999;94(11):3941-3946.
22. Lin MT, Gooley T, Hansen JA, et al. Absence of statistically significant correlation between disparity for the minor histocompatibility antigen-HA-1 and outcome after allogeneic hematopoietic cell transplantation. Blood. 2001;98(10):3172-3173.
23. Socié G, Loiseau P, Tamouza R, et al. Both genetic and clinical factors predict the development of graft-versus-host disease after allogeneic hematopoietic stem cell transplantation. Transplantation. 2001;72(4):699-706.