Study of relationship between IL-1Ra gene polymorphism and GVHD in HLA – identical sibling allogenic transplants

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Abstract

Introduction: The interleukin-1 (IL-1) gene family includes three members (IL-1α, IL-1β and IL-1Ra) that mediate immune and inflammatory responses. Interleukin-1 (IL-1) is an inflammatory cytokine involved in various autoimmune and inflammatory diseases. IL-1 receptor antagonist (IL-1Ra) is the naturally occurring antagonist to IL-1α and IL-1β. A variable number tandem repeat (VNTR) polymorphism in the IL-1Ra gene has been associated with increased IL-1Rα production and affects the severity of aGVHD.
Material and methods: Three hundred and fifty pairs (175 HSCT recipients and their donors) were analyzed by VNTR/PCR. Because of haematological disorders all patients were transplanted. All genotypes were screened blind to the clinical outcome of the transplants. GVHD was graded using Glucksberg criteria.
Results: The influence of different alleles on incidence of aGVHD was investigated with univariate analysis. None of them showed an association with aGVHD, but possession of allele 2 in donors was associated with less severe aGVHD, although the frequency of allele 2 in our study population was low. However, aGVHD correlated with recipient age, donor age and recipient disease, particularly thalassaemia.
Conclusions: No significant correlation was observed between the IL-1Ra polymorphism and incidence of aGVHD. In addition there was a powerful association between diagnosis, particularly thalassaemia, and GVHD (26 out of 30 thalassaemia patients). These findings may help to predict the risk/severity of GVHD, which may contribute to selecting strategies for treatment/prevention in thalassaemia patients.

Key words: BMT, cytokine, genotype.

Introduction

Haematopoietic stem cell transplantation has evolved as a central treatment modality in the management of different haematologic malignancies. Despite adequate posttransplantation immunosuppressive therapy, acute graft-versus-host disease (aGVHD) remains a major cause of morbidity and mortality in the haematopoietic stem cell transplantation setting, even in patients who receive human leukocyte antigen (HLA) identical sibling grafts [1].

Allogeneic haematopoietic stem-cell transplantation (SCT) is well established as a curative treatment for many haematological malignancies and some non-malignant disorders [2]. However, complications like aGVHD, infections and recurrence of malignancy (relapse) are still major obstacles to success [3–4]. GVHD is caused by alloreactive T-cells of donor origin attacking recipient tissues [5], but now it is clear that cytokines are closely involved in the development and maintenance of GVHD [6]. The pro-inflammatory cytokine interleukin-1 (IL-1) is a key molecule in the mediation and amplification of the inflammatory response. The IL-1 family consists of at least three polypeptides, some of which are structurally related [7]. There are three members of the IL-1 gene family: IL-1α, IL-1β, and IL-1 receptor antagonist (IL-1Ra). IL-1α and IL-1β are agonists and IL-1Ra is a specific receptor antagonist [7].

There are interindividual differences in production of the three IL-1 proteins, which are encoded by three genes on chromosome 2q. The intron-exone organization of the three IL-1 genes suggests duplication of a single gene through evolution [7]. The genes of the IL-1 family are also found to exhibit polymorphisms [8]. The polymorphism in intron 2 of the IL1Ra gene is caused by a variable copy number of an 86-bp sequence. IL2RN has 5 alleles, comprising 2 to 6 repeats of the 86-bp sequence [9]. It was stated that the 4-repeat and 2-repeat alleles are most common, while the other alleles occur at a combined frequency of less than 5% [10]. The IL-1Ra VNTR genotype has been shown in a few studies to be implicated in the pathology of a number of diseases, including severity of aGVHD and incidence of chronic GVHD [11–12], lupus erythematosus [10], ulcerative colitis [13], and alopecia areata [14].

Though the potential association of IL-1Ra alleles with GVHD has not been extensively investigated in Iran we know the ability to identify high and low responders to allograft by a simple genetic test. The aim of this study was to ascertain the influence of IL-1Ra gene polymorphisms on HSCT outcome.

In this study we examine a number of polymorphic sites present in the IL-1Ra genes, which are known to influence levels of IL-1 proteins in vitro and in vivo, and as risk factors for acute and chronic GVHD.

Material and methods

Study population

One hundred and seventy-five patients with a history of haematological malignancies and their donors were enrolled in protocols at Dr. Shariati Hospital in Tehran, Iran, between 2002 and 2005 after submission of their written informed consent. The patients, disease and transplant characteristics are described in Table I. All of the patients had a background of Asian origin. Patients included in this study received a first transplant from HLA-identical siblings and were followed up for clinical outcome up to July 2005. The trial was performed in accordance with the Declaration of Helsinki and subsequent revisions and approved by the ethics committee at Tehran University of Medical Sciences.

HLA typing

HLA matching was performed serologically for HLA-A and -B antigens using the microcytotoxicity method (NIH method), and HLA-DRB1 using molecular typing (Heidelberg sequence specific primer, Germany).

IL-1Ra gene polymorphism analysis

An IL-1Ra gene polymorphism study was performed on 175 recipients' and 175 donors' available DNA samples. The patients' follow-up was performed in order to evaluate the influence of genotype on the clinical course post transplant. All genotypes were determined blind to the clinical outcome of the transplants. DNA was extracted and purified from whole blood collected in 5% EDTA using the salting out method. Donor/recipient genotypes for the IL-1Ra gene polymorphism were analyzed by polymerase chain reaction with sequence specific

Table I. Patient, donor, disease and transplant characteristics

HSCT	175
Recipients median age, y (range)	21 (3-53)
Donor median age, y (range)	22(3-58)
Sex mismatch	82 (46.9%)
Ethnic group	Iranian mixed population
Conditioning regimen	Bu + Cy = 160 Bu + Cy +VP = 12 Cy = 3
Stem-cell source	
PBSC	165 (94.3%)
BM	10 (5.7%)
GVHD prophylaxis	MTX + CsA =100 CsA = 64 CsA + cort = 11
Malignant disease:	
AML	55 (31.4%)
ALL	38 (21.7%)
CML	27 (15.4%)
Thalassaemia	30 (17.1%)
Other malignant and non-malignant disease:	
(AA, HL, MDS, FA, CLL, Osteoporosis, Histocytosis, RCC)	25 (14.3%)

Bu – busulfan, Cy – cyclophosphamide, VP – etoposide, MTX – methotrexate, CSA – cyclosporin A, BM – bone marrow, PBSC – peripheral blood stem cells, AML – acute myeloid leukaemia, ALL – acute lymphoblastic leukaemia, CML – chronic myelogenous leukaemia, AA – aplastic anaemia, HL – Hodgkin's lymphoma, MDS – myelomonocytic leukaemia, CLL – chronic lymphocytic leukaemia, RCC – renal cell carcinoma

Table II. Distribution of IL-1Ra gene polymorphisms in the recipient and donor genotype with GVHD grade following transplant

Recipient Genotype	aGVHD grade 0-II	aGVHD grade III-IV	Donor Genotype	aGVHD grade 0-II	aGVHD grade III-IV
1/1	116	24	1/1	113	24
1/2	1	0	1/2	1	0
1/3	3	1	1/3	1	0
1/4	8	2	1/4	7	3
1/5	3	0	1/5	1	0
2/2	11	1	2/2	4	1
3/3	1	0	3/3	21	0
4/4	2	0	4/4	2	0

primers (MWG Germany). This polymorphism, located in intron 2 of the IL-1Ra gene, was analyzed as described [15], using PCR cycle conditions; 94°C for 30 s, 60°C for 1 min and 72°C for 1 min, with a 5 s auto extension step per cycle, for 30 cycles. All amplifications were performed according to the manufacturer's recommendations. The PCR products were then visualized by electrophoresis in 2% agarose gel. Then the correlation between donor and recipient genotype and GVHD grade for their respective transplant was assessed.

Statistical analysis

The purpose of this study was to find factors affecting severe aGVHD. Statistical analysis was conducted using SPSS 11.5 software package. Allele frequencies were determined. The variable donor's and recipient's age, transplantation from a female donor into a male recipient, source of stem cell, donor's and recipient's cytomegalovirus (CMV) serological status, disease status, and donor's and recipient's IL-1Ra gene polymorphisms were analyzed as risk factors for acute GVHD. Selected risk factors of post-transplant complications were considered in univariate analysis. Odds ratio and chi square test were used for univariate analysis. In the analysis, $p<0.05$ was considered as the level of significance.

Results

Clinical outcomes

One hundred and eighteen patients were available for this study. The frequency of the aGVHD grades in this cohort were grade 0 (n=67), grade I (n=31), grade II (n=48), grade III (n=24), grade IV (n=5) according to Glucksberg et al. [16]. In this study, only 13 patients died of GVHD. 77 patients had moderate to severe aGVHD (grade II to IV) and 98 patients had either no or mild aGVHD (grades 0 and I) which led to a 44% incidence of moderate to severe aGVHD in this patient population. The distributions of the genotypes for both recipients and donors and aGVHD outcome are shown in Table II.

Chronic GVHD [17] was assessable in those who survived at least 100 d post BMT (n=153). One hundred and fifty-three patients survived beyond day 100 post transplant, among whom 56 developed limited and 13 developed extensive chronic GVHD (according to Atkinson et al. [18]), which led to a 42.9% incidence of chronic GVHD.

Table III. Allele frequency in recipients and donors

Allele	Frequency In Recipients	Frequency In Donors
A1	90.3%	89.7%
A2	7.4%	6.9%
A3	1.7%	2.3%
A4	6.9%	9.7%
A5	1.7%	1.7%

Allele frequencies of the IL-1Ra gene polymorphism

Allele frequencies of the IL-1Ra VNTR polymorphism studied are shown in Table III. The influence of this polymorphism on incidence of acute and chronic GVHD was investigated in this study.

Effect of IL-1Ra gene polymorphism on GVHD

The influence of this polymorphism on the incidence of acute and chronic GVHD was investigated. GVHD was diagnosed and graded according to previously published criteria [16]. Table IV shows univariate analysis of association of IL-1Ra gene polymorphism with acute GVHD.

None of the polymorphisms showed an association with the presence of acute or chronic GVHD. However, considering all significant factors, acute GVHD turned out to be correlated with recipient age, donor age and recipient disease, particularly thalassaemia (Table V). Chronic GVHD was associated with PBSC as a source of cells ($p<0.03$, RR=4.92, 95%CI=0.906-59.09).

Discussion

Allogenic bone marrow transplantation is an established but complex therapy for patients with haematological malignant and non-malignant disease. Unfortunately, complications such as GVHD still cause significant morbidity and mortality after transplantation. GVHD occurs in 20–40% of recipients of HLA-matched sibling donor grafts, indicating that factors other than being HLA matched are important in the initiation of GVHD.

Genetic factors related to patients and donors, such as cytokine gene polymorphisms, have been reported to modify the incidence and severity of aGVHD [11]. IL-1Ra is an anti-inflammatory non--signalling molecule that competes for receptor binding with IL-1α, IL-1β. Differences in the ability of BMT donors and recipients to produce IL-1 might influence the occurrence of post-BMT complications.

A number of previous studies have suggested that polymorphism in cytokine genes influences susceptibility to post-BMT complications [11–12]. In this study we examined polymorphisms of the IL-1Ra gene that might influence outcome of BMT. These VNTR were selected for study because they have been shown to influence the result of BMT, and to be associated with acute or chronic inflammatory disease [11–12].

Initial studies in HLA-matched siblings transplant demonstrated an association of the IL-Ra VNTR (intron 2) where possession of the allele 2 in the donor genotype was associated with less severe acute GVHD. Cullup et al. (2001) studied the IL-1β and IL-1Ra polymorphism and showed modest evidence for an association between donor IL-1Ra genotype and incidence of acute GVHD [11].

In a larger study, Lin et al. (2000) reported little association between donor or recipient IL-β and IL-1Ra genotypes and frequency of GVHD [19].

In our study, no correlation between acute and chronic GVHD and patient and donor IL-1Ra gene polymorphism could be found, in accordance with other studies [19–20]. However, there are some differences between allele frequencies with other ethnic populations. For example, in comparison with the English population allele A1 is more frequent in the Iranian population, but allele A2 is rare, mainly due to ethnic background [9].

As IL-1Ra A2 allele has been described as a 'pro--inflammatory' haplotype [15], recipients and donors carrying these alleles were compared with those without and, again, no difference in GVHD frequencies was seen.

In our evaluation of risk factors for acute GVHD after allogeneic blood stem cell transplantation, we found that the incidence of GVHD was affected by recipient age, donor age and recipient disease, particularly thalassaemia.

We did not find any correlation between severe aGVHD risk with donor-recipient sex mismatch, in accordance with the results of Bortin et al. and Ramsay et al., who did not see sex mismatch as a significant parameter for development of GVHD [21–22] (in contrast with Bross et al. [23] and Ghavamzadeh et al. [24]) .

Table IV. Statistical analysis of risk factors for acute GVHD

P Value	Odds Ratio	Risk factor
0.018	1.033	Recipient age (years)
0.996	1.014	Recipient sex
0.960	0.979	Recipient CMV status (seropositive)
0.249	1.932	Recipient genotype (allele 2+) Recipient disease (diagnosis)
0.002	5.136	Thalassaemia
0.060	0.538	AML
0.930	0.967	ALL
0.281	0.637	CML
0.752	1.153	Others
0.008	1.040	Donor age (years)
0.123	0. 618	Donor sex
0.131	1.606	Gender mismatch
0.096	0.559	Donor male recipient female
0.960	1.022	Donor female recipient male
0.412	1.629 1.619	Donor genotype (allele 2+) Stem-cell source
0.457	0.618	PBSC
0.460		BM

Conclusions

These findings may help to predict the risk/severity of GVHD, which may contribute to selecting strategies for treatment/prevention in thalassaemia patients. Although we found a powerful association between thalassaemia and GVHD, further studies with more patients will be required to draw any conclusion. In summary, our results do not demonstrate an association between the incidence of aGVHD and donor and recipient polymorphism of IL-1RN, possibly because the frequency of allele number 2 was observed to be low in the population studied (Table II). Of the 350 individuals examined, we only observed 25 cases of allele 2. Hence, we are aware that the number of patients in our study does not allow a strong conclusion to be made regarding the relative impact of IL-1Ra genotype-associated GVHD risk. In certain populations, the frequency of this allele seems to be more prevalent [11] and it could be more important in predicting acute GVHD. Consequently, IL-1Ra gene polymorphisms and their association with transplant-related complications in Iran.

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