The Predictive Value of HLA-DR Matching and Cytokine Gene Polymorphisms in Renal Allograft Acute Rejection: A Living-unrelated Donor (LURD) Study

Nader Tajik[1*], Tohid Kazemi[1], Aliakbar Delbandi[1], Ahad Ghods[2], Alireza Salek Moghaddam[1]

[1]Division of Immunogenetics, Department of Immunology, Iran University of Medical Sciences, Tehran, Iran,
[2]Transplantation Unit, Hashemi Nejad Kidney Hospital, Iran University of Medical Sciences, Tehran, Iran

ABSTRACT

Background: In addition to Human Leukocyte Antigens (HLA) compatibility, gene polymorphisms in cytokines might also be important in the quality of allogeneic immune response. **Objective:** To evaluate the influence of HLA-DR matching and a number of cytokine gene polymorphisms on acute rejection after living-unrelated donor (LURD) kidney transplantation. **Methods:** A total of 42 renal transplants performed at Hashemi Nejad Kidney Hospital (Tehran/Iran) and followed up for 3 months post-transplantation were included. Using PCR-SSP, HLA-DR alleles (DR1-18) of recipients and donors and gene polymorphisms in TNF-α, TGF-β1, IL-10, IL-6, and IFN-γ of recipients were determined. **Results:** Acute rejection was observed in 11(26.2%) of renal recipients. The frequency of one and two HLA-DR mismatches in rejector group was 2(18.2%) and 9(81.8%) and in non-rejector group was 13(41.9%) and 17(54.8%), respectively. HLA-DR incompatibility was not significantly higher in rejector (1.82±0.40) compared with non-rejector (1.52±0.57) recipients (P=0.069) and more than half of non-rejectors had completely mismatched HLA-DR antigens with donors. Polymorphisms associated with the mentioned cytokines had no correlation with acute rejection. **Conclusion:** The predictive value of HLA-DR mismatching for acute rejection is not as prominent in LURD kidney transplantation as in the cadaveric one. In addition, we failed to demonstrate an association between combined cytokine genotypes and HLA-DR matching with acute rejection. Further and more detailed immunogenetic investigations are required in order to have a better prediction of the transplant outcome.

Keywords: Living-unrelated Donor Kidney Transplantation, Acute Rejection, HLA, Cytokine, Gene Polymorphism

*Corresponding author: Nader Tajik, Ph.D., Director of Immunogenetic Laboratory, Immunology department, Iran University of Medical Sciences, Shahid Hemmat Highway, 14496, Tehran, Iran, Phone: (+) 98 912 3250344, Fax: (+) 98 21 88058719, e-mail: nadertajik@iums.ac.ir

INTRODUCTION

Successful clinical transplantation depends in part on the immune processes that mediate rejection of the engrafted organ. Allograft acute rejection is the clinical manifestation of an uncontrolled alloimmune response directed against an allograft, involving interactions between multiple cell types and a complex variety of factors including Human Leukocyte Antigens (HLA), immunoregulatory mediators such as cytokines and growth factors, and the adequacy of immunosuppression. There is a correlation between HLA matching especially of HLA-DR type and decreased incidence of clinical acute rejection episodes (1). In addition to recognition of specific non-self alloantigens, allograft rejection is stimulated by recognition of pre-transplantation damage mostly ischemic and reperfusion injury leading to the up-regulation of co-stimulatory, adhesion, and MHC class II molecules of donor tissue, followed by activation of specific T cells and production of cytokines and chemokines essential for evolution of an alloreactive immune response (2).

Despite reduction in acute rejection incidence, it is still one of the major obstacles in achieving long-term allograft survival. It has been found in several studies that both the frequency and severity of acute rejection episodes may be a predisposing factor to reduced renal function and subsequent graft loss (3-5). Variations in both rejection rate and long-term outcome between patients with comparable immunosuppression and matching status have not been fully explained.

Cytokines are important mediators in the regulation of immune response. A number of cytokine gene polymorphisms have been identified and their effects on the rate of acute rejection have been investigated.

Tumor necrosis factor-α (TNF-α) is a pleotropic cytokine produced mainly by macrophages and T cells. It mediates cellular immune response and has a pro-inflammatory nature. TNF-α stimulates macrophage function, increases HLA class II antigen expression, and is implicated in acute rejection. The polymorphism at position -308 in the human TNF-α promoter region results in an A to G transition and a subsequent 6- to 7-fold increase in transcriptional activity. High producer genotypes were associated with acute rejection of kidney transplants (6).

Transforming growth factor β1 (TGF-β1) is of particular interest because it promotes deposition of extracellular matrix and is shown to exert influence in many cellular growth and regulation events. TGF-β1 gene polymorphisms, located at positions +869 (T→C) and +915 (G→C) at codons 10 and 25, respectively, are in the signal sequence. High producer genotypes of TGF-β1 in donors (7) and recipients (8) were associated with kidney allograft rejection.

Interleukin-10 (IL-10) is an important component of the anti-inflammatory cytokine network, suppressing gene expression and synthesis of pro-inflammatory cytokines. In the proximal promoter region of IL-10 three different inheritable haplotypes involving three biallelic polymorphisms at -1082 (G→A), -819 (C→T), and -592 (C→A) have been identified. In cardiac transplantation IL-10 high producer genotype has been associated with reduced incidence of cellular rejection (9). However, in renal transplantation, in combination with TNF-α high producer genotypes, it was associated with increased acute vascular rejection (10).

Interleukin-6 (IL-6) is a multifunctional cytokine and mediates inflammatory and stress-induced responses. Within the promoter region of the IL-6 gene at position -174, a C→G substitution is associated with a different transcription rate. A polymor-

phism at position -174 of the donor IL-6 gene was associated with the incidence and severity of acute rejection (11).

Interferon- γ (IFN-γ) is a cytokine essential in the development and propagation of TH1-type immune response. A polymorphic dinucleotide (CA) marker has been detected within the first intron of the IFN-γ gene at position +874 from the transcription start site. The CA microsatellite contains a SNP (T→A) at the 5′ end of the repeat, causing high, intermediate, and low producer genotypes. The high producer genotype was associated with acute rejection of kidney transplants (1).

In the present study, we sought to determine the impact of HLA-DR matching and recipient cytokine gene polymorphisms on acute rejection after renal transplantation in a well-controlled living-unrelated donor (LURD) program.

MATERIALS AND METHODS

Graft outcome and acute rejection episode(s) in the first three months after transplantation was determined in a total of 42 living-unrelated donor renal transplant recipients at Shahid Hashemi Nejad Hospital (Tehran/Iran). Rejection episodes were identified by an expert nephrology team based on clinical diagnostic criteria. HLA-DR matching and cytokine gene polymorphisms were compared between rejector and non-rejector groups. The immunosuppressive protocol was triple therapy of cyclosporine, azathioprine or mycophenolate mofetile, and prednisolone.

DNA Extraction and Genotyping. Genomic DNA was obtained from fresh whole blood using a simple saturated NaCl based extraction technique (12). DNA quality and concentration was assessed by spectrophotometry at 260 and 280 nm. For HLA-DR low resolution typing and detection of gene polymorphisms in TNF-α (-308 A/G), TGF-β1 (+869 T/C, and +915 G/C), IL-10 (-1082 G/A, -819 C/T, and -592 C/A), IL-6 (-174 C/G), and IFN-γ (+874 T/A), PCR-SSP based genotyping trays of One Lambda company (CA, USA) were used.

Statistical Analysis. Association of rejection episodes with HLA-DR matching and high, intermediate, or low producer genotypes of each cytokine in rejector and non-rejector groups were determined using student's t test and contingency 2×2 table of Fisher's exact test, respectively. P-values less than 0.05 were statistically significant.

RESULTS

Forty two living-unrelated donor kidney allograft recipients, including 29 (69%) men and 13 (31%) women were included. All recipients were engrafted for the first time and were not sensitized to HLA antigens (panel reactive antibody and WBC cross match negative). The majority of donors were younger than 35 years and organ donation was done under tight control of governmental health authorities. Acute rejection episode(s) observed in 11 (26.2%) of recipients in the early post transplant period.

HLA-DR Matching and Acute Rejection. Frequency of HLA-DR mismatches in donor-recipient pairs is shown in table 1. HLA-DR incompatibility was not significantly higher in rejector (1.82±0.40) than non-rejector (1.52±0.57) recipients (P=0.069). Furthermore, statistical analysis showed no significant association between HLA-DR mismatching and acute rejection (P=0.57). Interestingly, more than

half of the non-rejector recipients had completely mismatched HLA-DR antigens with their donors.

Table 1. Frequency of HLA-DR mismatches in rejector and non-rejector groups

HLA-DR Mismatches(MM)	Rejectors (N=11)	Non-rejectors (N=31)
0 MM	0 (0.00%)	1 (3.23%)
1 MM	2 (18.18%)	13 (41.93%)
2 MM	9 (81.82%)	17 (54.84%)

Cytokine Genotypes and Acute Rejection. Considering cytokine genotypes, kidney recipients were grouped into high, intermediate, and low producer genotypes (Table 2). TNF-α low producer genotype (G/G), IL-10 (GCC/ACC) and IFN-γ (T/A) intermediate producer genotypes, and TGF-β1 (T/C G/G) and IL-6 (G/G) high producer genotypes were dominant genotypes. No TNF-α high producer genotype (A/A) and TGF-β low producer genotypes (C/C G/C, C/C C/C, T/T C/C, and T/C C/C) were observed. No association was found between each cytokine genotype and acute rejection.

Table 2. Frequency of cytokine genotypes in rejector and non-rejector groups

Cytokine	Genotype (Phenotype)	Rejectors (N=11)	Non-rejectors (N=31)
TNF-α[a]	G/G (L)	8 (72.72%)	24 (77.42%)
	G/A (H)	3 (27.28%)	7 (22.58%)
	A/A (H)	0 (0.00%)	0 (0.00%)
TGF-β1[b]	T/T G/G (H)	1 (9.09%)	8 (25.80%)
	T/C G/G (H)	6 (54.54%)	11 (35.48%)
	T/C G/C (I)	2 (18.18%)	6 (19.35%)
	C/C G/G (I)	1 (9.09%)	5 (16.13%)
	T/T G/C (I)	1 (9.09%)	1 (3.23%)
	C/C G/C (L)	0 (0.00%)	0 (0.00%)
	C/C C/C (L)	0 (0.00%)	0 (0.00%)
	T/T C/C (L)	0 (0.00%)	0 (0.00%)
	T/C C/C (L)	0 (0.00%)	0 (0.00%)
IL-10[c]	GCC/GCC (H)	3 (27.27%)	4 (12.90%)
	GCC/ACC (I)	2 (18.18%)	12 (38.70%)
	GCC/ATA (I)	3 (27.27%)	7 (22.58%)
	ACC/ACC (L)	1 (9.09%)	2 (6.45%)
	ACC/ATA (L)	1 (9.09%)	4 (12.90%)
	ATA/ATA (L)	1 (9.09%)	2 (6.45%)
IL-6[d]	G/G (H)	7 (63.64%)	18 (58.06%)
	G/C (H)	3(27.27%)	12 (38.70%)
	C/C (L)	1 (9.09%)	1 (3.23%)
IFN-γ[e]	T/T (H)	3 (27.27%)	5 (16.13%)
	T/A (I)	6 (54.54%)	20 (64.52%)
	A/A (L)	2 (18.18%)	6 (19.35%)

H: High, I: Intermediate, L: Low

[a] P-value = 1.000, [b] P-value = 1.000, [c] P-value = 0.504, [d] P-value = 0.460, [e] P-value = 0.717

Association between Combination of HLA-DR Matching and Cytokine Genotypes and Acute Rejection. No significant association was found between the occurrence of acute rejection episode(s) with the combination of HLA-DR matching and each cytokine genotype (Table 3).

Table 3. Frequency of cytokine genotypes and HLA-DR mismatches in rejector and non-rejector groups

		Rejectors (N=11)			Nonrejectors (N=31)		
		0 MM	1 MM	2 MM	0 MM	1 MM	2 MM
	Production	0	2	9	1	13	17
TNF-α	L	0 (0.00%)	0 (0.00%)	8 (88.89%)	1(100%)	11(84.62%)	12(70.59%)
	H	0 (0.00%)	2 (100%)	1 (11.11%)	0 (0.00%)	2 (15.38%)	5 (29.41%)
TGF-β1	L	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
	I	0 (0.00%)	1(50%)	3 (33.33%)	0 (0.00%)	7 (53.85%)	5 (29.41%)
	H	0 (0.00%)	1 (50%)	6 (66.67%)	1 (100%)	6 (46.15%)	12(70.59%)
IL-10	L	0 (0.00%)	0 (0.00%)	3 (33.33%)	0 (0.00%)	5 (38.46%)	3 (17.65%)
	I	0 (0.00%)	1 (50%)	4 (44.44%)	1 (100%)	7 (53.85%)	11(64.70%)
	H	0 (0.00%)	1 (50%)	2 (22.22%)	0 (0.00%)	1 (7.69%)	3 (17.65%)
IL-6	L	0 (0.00%)	0 (0.00%)	1 (11.11%)	0 (0.00%)	1 (7.69%)	0 (0.00%)
	H	0(0.00%)	2 (100%)	8 (88.89%)	1 (100%)	12(92.31%)	17(100%)
IFN-γ	L	0 (0.00%)	0 (0.00%)	2 (22.22%)	0 (0.00%)	3 (23.08%)	3 (17.65%)
	I	0 (0.00%)	2 (100%)	4 (44.44%)	1 (100%)	6 (46.15%)	13(76.47%)
	H	0 (0.00%)	0(0.00%)	3 (33.33%)	0 (0.00%)	4 (30.77%)	1 (5.88%)

H: High, I: Intermediate, L: Low

DISCUSSION

Currently, there are some identified variables that are considered in organ allocation algorithms and standardized therapeutic protocols which may be useful to improve long-term renal allograft survival. These factors include the degree of HLA matching especially in DR locus (1, 13), ischemic-reperfusion injury, and the immunosuppressive regimen (14, 15). Recently the recipient immune responsiveness is also considered (16). Inter-individual differences in immune responsiveness can be attributable to genetic variability in the coding and regulatory regions of immune system mediators especially cytokines (17). In spite of several studies, the exact role of cytokine gene polymorphism in transplant outcome remains controversial (18), with some groups showing a correlation (19, 10) and others not (13).

One must keep in mind that the immune response against graft tissue takes place under an immunosuppressive situation which can influence most aspects of the immune response including cellular activation and gene expression, making the therapeutic regimen a significant variable. For instance, in the study of Marshall et al. (13) cyclosporine, azathioprine, and prednisone were used. They didn't find any association between cytokine gene polymorphisms and the occurrence or severity of acute rejection. On the other hand, monotherapy regimen with cyclosporine was used in the study of Sankaran et al. (10). They found that TNF-α high producer/IL-10 high producer genotypes have the worst prognosis, but TNF-α low producer/IL-10 low producer genotypes are protective, suggesting that cyclosporine alone does not completely block cytokine production.

Recognition phase is an important step in the development of immune response. This is a fact that cytokine gene expression polymorphisms control the production of cytokines only when the source cells are activated. The most important antigens in alloantigen-dependent recognition pathway are HLA antigens and it has been suggested that the impact of cytokine genotype may be influenced by the degree of immune activation initiated by an HLA class II mismatch (13). In addition, recipient immune system is also stimulated via alloantigen-independent recognition pathways, especially by ischemic-reperfusion injury which besides parenchymal damage, increases the immunogenecity of allograft. This type of injury is important in cadaveric

renal transplantation, and is not crucial in live donors (2). Therefore, the source of kidney is important and studies on cadaveric kidney transplantation yields different results from living ones.

As the heart and the lung are considered to be more immunogenic than kidney, it is conceivable that the impact of some of these polymorphisms is only manifested when the immune system is maximally activated (13). In other words, controversial findings may result from tissue-specific effects of particular organs or tissues.

Our population study was LURD allograft kidney recipients and cyclosporine, azathioprine/mycophenolate, and prednisolone were used. There was no association between HLA-DR matching and cytokine gene polymorphisms, and the occurrence of acute rejection. It is noteworthy that in more than 50% of non-rejector recipients full mismatched HLA-DR status was observed. As transplanted organs taken from live donors have undergone minimal pre-transplantation stresses, alloantigen-independent stimulation was reduced; therefore, triple immunosuppressive regimen could overcome alloantigen-dependent stimulation of the recipient immune system.

Although our sample size was limited and clinical criteria were used to prove acute rejection; however, our findings are informative as LURD kidney transplantation is not widely used throughout the world, and there is tendency toward using this method in developed countries (20).

In conclusion**,** the predictive value of HLA-DR mismatching for acute rejection is not as prominent in LURD kidney transplantation as in the cadaveric one, however, there is no doubt about the influence of HLA compatibility on allograft survival. In addition, we failed to demonstrate an association between combined cytokine genotypes and HLA-DR matching with acute rejection. Further and more detailed immunogenetic investigations are required in order to have a better prediction of the transplant outcome.

REFERENCES

1 Tinckam K, Rush D, Hutchinson I, Dembinski I, Pravica V, Jeffery J, et al. The relative importance of cytokine gene polymorphisms in the development of early and late acute rejection and six-month allograft pathology. Transplantation 2005; 79: 836-841.

2 Wilhelm MJ, Pratschke J, Laskowski I, Tilney NL. Ischemia and reperfusion injury. Transplantation Reviews. 2003; 17: 140-157.

3 Leggat JE Jr, Ojo AO, Leichtman AB, Port FK, Wolfe RA, Turenne MN,et al. Long-term renal allograft survival: prognostic implication of the timing of acute rejection episodes. Transplantation 1997; 63: 1268-1272.

4 Ferguson R. Acute rejection episodes-best predictor of long-term primary cadaveric renal transplant survival. Clin Transplant 1994;8:328-321.

5 Matas AJ, Gillingham KJ, Payne WD, Najarian JS. The impact of an acute rejection episode on long-term renal allograft survival (t1/2). Transplantation 1994; 57: 857-859.

6 Hahn AB, Kasten-Jolly JC, Constantino DM, Graffunder E, Singh TP, Shen GK, et al. TNF-α, IL-6, IFN-γ, and IL-10 gene expression polymorphisms and the IL-4 receptor α-chain variant Q574R: effects on renal allograft outcome. Transplantation 2001; 72: 660-665.

7 Hoffmann S, Park J, Jacobson LM, Muehrer RJ, Lorentzen D, Kleiner D, et al. Donor genomics influence graft events: the effect of donor polymorphisms on acute rejection and chronic allograft nephropathy. Kidney International 2004; 66: 1686-1693.

8 McDaniel DO, Barber WH, Nguyan C, Rhodes SW, May WL, McDaniel LS, et al. Combined analysis of cytokine genotype polymorphism and the level of expression with allograft function in African-American renal transplant patients. Transplant Immunology 2003; 11: 107-119.

9 Turner D, Grant SC, Yonan N, Sheldon S, Dyer PA, Sinnott PJ, et al. Cytokine gene polymorphism and heart transplant rejection. Transplantation 1997; 64: 776-779.

10 Sankaran D, Asderakis A, Ashraf S, Roberts ISD, Short CD, Dyer PA, et al. Cytokine gene polymorphisms predict acute graft rejection following renal transplantation. Kidney International 1999; 56: 281-288.

11 Marshall SE, McLaren AJ, McKinney EF, Bird TG, Haldar NA, Bunce M, et al. Donor cytokine genotype influences the development of acute rejection after renal transplantation. Transplantation 2001; 71: 469-476.

12 Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988; 16:1215.
13 Marshall SE, McLaren AJ, Haldar NA, Bunce M, Morris J, Welsh KI. The impact of recipient cytokine genotype on acute rejection after renal transplantation. Transplantation 2000; 70: 1485-1491.
14 Hutchinson IV, Pravica V, Perrey C, Sinnott, P. Cytokine gene polymorphisms and relevance to forms of rejection. Transplantation Proceedings 1999; 31: 734-736.
15 Ochsner S, Guo Z, Binswanger U, Knoflach A. TGF-β1 gene expression in stable renal transplant recipients: influence of TGF-β1 gene polymorphism and immunosuppression. Transplantation Proceedings 2002; 34: 2901-2903.
16 Akalin E, Murphy B. Gene polymorphisms and transplantation. Current Opinion in Immunology 2001; 13: 572-576.
17 Bidwell J, Keen L, Gallagher G, Kimberly R, Huizinga T, McDermott MF, et al. Cytokine gene polymorphism in human disease: on-line databases. Genes and Immunity 1999; 1: 3-19.
18 Lee H, Clark B, Gooi HC, Stoves J, Newstead CG. Influence of recipient and donor IL-1alpha, IL-4, and TNFalpha genotypes on the incidence of acute renal allograft rejection. J Clin Pathol. 2004;57:101-3.
19 George S, Turner D, Reynard M, Navarrete C, Rizvi I, Fernando ON, et al. Significance of cytokine gene polymorphism in renal transplantation. Transplantation Proceedings 2001; 33: 483-484.
20 Ghods AJ. Changing ethics in renal transplantation: presentation of Iran model. Transplant Proceedings 2004; 36: 11-13.