Effect of Donor Tumor Necrosis Factor-α and Interleukin-10 Genotypes on Delayed Graft Function and Acute Rejection in Kidney Transplantation

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Introduction. This study evaluated the influence of interleukin-10 (*IL10*) gene -1082G>A and tumor necrosis factor-α (*TNF*) gene -308G>A polymorphisms in the donor and recipients on the acute rejection (AR) episodes and delayed graft function (DGF) in kidney transplant recipients.
Materials and Methods. The *IL10* -1082G>A and *TNF* -308G>A polymorphisms were determined in 100 kidney allograft recipients and their donors using the polymerase chain reaction-amplification refractory mutation system polymerase chain reaction-restriction fragment length polymorphism methods. Transplantation outcomes were determined in terms of AR and DGF criteria.
Results. The A allele of the TNF polymorphism (high producer) in the donors was associated with DGF in the recipients (odd ratio, 3.1; 95% confidence interval, 1.2 to 8.1). There was also a significant association between the combination of donor's *IL10-TNF* genotypes and DGF (odd ratio, 4.8; 95% confidence interval, 1.4 to 17.1); the frequency of a combination of *IL10* AA or GA and *TNF* AA or GA was higher in the recipients with DGF. No association was found between the donors and recipients' *IL10* -1082G>A and *TNF* -308G>A polymorphisms and AR. No association was detected between recipients and donors' *IL10* polymorphisms or recipients' *TNF* polymorphisms and DGF.
Conclusions. This study showed that donors with high *TNF* production may have increased risk of DGF in their recipients. Routine screening of these gene polymorphisms may have a clinical role in identifying patients at risk of DGF.

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INTRODUCTION

Acute rejection (AR) is the major determinant for chronic allograft rejection and long-term graft survival, and the improvement of the transplantation results has been largely due to a progressive decrease in the incidence of AR.[1] Delayed graft function (DGF) is one of the risk factors for AR, as it has been shown that AR is more frequent in organs with DGF than in those that function immediately after transplantation.[2] Although AR is one of the most severe outcomes in kidney transplantation, DGF that is a measure

of graft functionality requires the most intense consideration.[2] The initial event leading to renal allograft injury and organ failure is reperfusion injury. The cause of reperfusion injury is re-establishment of blood flow to ischemic tissues that causes various intracellular events occur that lead to cellular dysfunction, apoptosis, and cell death.[2,3]

It has been revealed that tumor necrosis factor-α (TNF-α) and interleukin-10 (IL-10) play a key role in the pathogenesis of renal reperfusion injury.[4,5] Both cellular and humoral (antibody-mediated) effecter mechanisms can play roles in kidney transplant rejection. Cytokines are soluble proteins with a critical role for both normal innate and adaptive immune responses. In general, cytokines exert their effects by influencing gene activation that results in cellular activation, growth, differentiation, functional cell-surface molecule expression, and cellular effectors function. In this regard, cytokines can have dramatic effects on the regulation of immune responses and the pathogenesis of a variety of diseases including allograft rejection.[6,7]

A variety of cytokines, including TNF-α and IL-10, are produced in the graft during AR. Activated macrophages, which secrete substantial quantities of pro-inflammatory cytokines including TNF-α, bind to TNF receptors on endothelial or tubular cells, causing them to undergo apoptosis.[8] Interleukin-10 is a cytokine the primary function of which appears to be limiting and controlling inflammatory responses.[9] The deletion of genes for anti-inflammatory cytokines such as IL-10 and transforming growth factor-β accelerates graft rejection in mice.[9] Cytokines are polymorphic, and genetic variability exists among individuals in their production and functions.[6] This provides an excellent explanation for variation in susceptibility to disease and patterns of disease progression.[10,11]

In the present study, we tried to determine the relationship of *TNF* -308 G>A and *IL10* -1082G>A gene polymorphisms and human kidney transplant AR and DGF in recipient-donor pairs in Afzalipour Hospital in Kerman.

MATERIALS AND METHODS

Patients and Procedures

After obtaining informed consent, 100 consecutive recipient-donor pairs receiving a kidney transplant at a single centre (Afzalipour Hospital) were recruited in this prospective cohort study. Recipient and donor demographics were reported elsewhere by the authors.[12]

Genomic DNA was isolated from ethylenediaminetetraacetic acid whole blood using a rapid salting-out DNA extraction method. After measuring the quality and quantity of extracted DNA by determination of A_{260}/A_{280}, aliquots of DNA were stored in Tris-ethylenediaminetetraacetic acid buffer at -70°C until analysis of genotypes. Polymorphism of TNF-α -308G>A (M16441.1, GenBank) was determined using polymerase chain reaction (PCR)-restriction fragment length polymorphism as described by before.[13-15] Briefly, a 107-bp fragment of *TNF* gene was amplified by using the following primers:

sense: 5′-AGGCAATAGGTTTTGAGGGCCAT-3′
antisense: 5′-TCCTCCCTGCTCCGATTCCG- 3′

The PCR product was subsequently digested by 10 units of the restriction enzyme *NcoI* (Fermentase, Vilnius, Lithuania) for 3 hours at 37°C. The *TNF* -308G>A and *IL10* -1082G>A (NG-012088, GenBank) genotyping methods were based on the PCR-amplification refractory mutation system and were similar to those previously described.[16] The sequences of primers were as follow:

TNF sense (G-allele):
5′-ATAGGTTTTGAGGGGCATGG-3′
TNF sense (A-allele):
5′-ATAGGTTTTGAGGGGCATGA-3′
TNF antisense (common)
IL10 sense (G-allele):
5′-CTACTAAGGCTTCTTTGGGAG-3′
IL10 sense (A-allele):
5′-CTACTAAGGCTTCTTTGGGAA-3′
IL10 antisense (common):
5′-CAGTGCCAACTGAGAATTTGG-3′

The amplified fragments were 184 bp and 258 bp, respectively, for *TNF* and *IL10*. The samples were then analyzed by electrophoresis in 3% agarose gels and visualized by ethidium bromide staining.

Reliability and validity of the PCR and restriction fragment length polymorphism methods were assessed through reconducting the genotype assays using at least a 10% sample of our DNA samples. The polymorphisms of heterozygot, homozygot, and wild-type *TNF* were checked with both restriction fragment length polymorphism and amplification refractory mutation system methods. In addition, for *IL10*, the method was also assessed through reconducting the genotype assays using most of

DNA samples with mutations (both heterozygotes and homozygotes). The results for all re-assessments were 100% concordant.

Definitions

Delayed graft function was defined by stringent criteria on the basis of Boom definition and independent from the need of dialysis.[2] In this definition, to exclude patients who were on dialysis for reasons other than impaired graft function, the diagnose of DGF is made retrospectively if the serum creatinine level increased, remained unchanged, or decreased by less than 10% per day immediately after surgery during 3 consecutive days for more than 1 week. Acute rejection was defined as an increase in serum creatinine level of 20% from the postoperative baseline in the absence of other causes of graft dysfunction that responded to antirejection therapy.[17]

Statistical Analyses

All analyses were conducted using the SPSS software (Statistical Package for the Social Sciences, version 16.0, SPSS Inc, Chicago, Ill, USA). Testing deviation from the Hardy-Weinberg Equilibrium was performed using The Pearson chi-square test using the observed genotype frequencies obtained from the data and the expected genotype frequencies obtained using the Hardy-Weinberg Equilibrium.[17] According to the *TNF* and *IL10* polymorphisms, the study population was divided into 2 categories. The first category consisted of individuals who were wild type for *TNF* (-1308G>G) and *IL10* (-1082G>G), which were coded as reference genotype. The second category included persons who carried on mutant alleles.

The logistic regression model was used to determine the association of *TNF* and *IL10* genotypes with DGF and AR in a univariable model. Odds ratio (OR) and 95% confidence interval (95% CI) were used for estimating the risk of association between DGF and AR and a specific genotype. For all the tests, a *P* value less than .05 was considered significant.

RESULTS

TNF Polymorphism

Table 1 summarizes the frequency of recipient and donor *TNF* A allele in the patients with and without AR and DGF. It shows no significant differences in the distribution of the A allele in different groups with and without AR. There are significant associations between donor's *TNF* polymorphism and occurrence of DGF (OR, 3.1; 95% CI, 1.2 to 8.1). The frequency of *TNF* A allele was high in patients with DGF and low in patients with no DGF.

IL10 Polymorphism

As it is shown in Table 2, the frequency of recipient's *IL10* A allele is insignificantly higher in the patients with AR than nonrejectors (67% versus 33%). The insignificant OR of 1.75 confirms this result. The frequency of donor *IL10* A allele is equal in rejectors and nonrejectors. The frequency of recipient *IL10* A allele is not significantly different in the patients with and without DGF. Despite the high frequency of *IL10* A allele in patients with DGF, the OR was not significant.

Combination of Donor and Recipient's *TNF* Genotypes

Combinations of recipient and donor's *TNF* genotypes were analysed in order to determine if their interaction had a joint effect on AR and DGF. No associations were detected between combinations of recipient and donor's *TNF* genotype

Table 1. Recipient and Donor's *TNF* -308G>A Polymorphism by Acute Rejection and Delayed Graft Function*

	Acute Rejection				Delayed Graft Function			
TNF Genotype	**Yes**	**No**	**OR (95% CI)**	***P***	**Yes**	**No**	**OR (95% CI)**	***P***
Recipient								
AA/AG†	2 (17)	15 (16)			7 (23)	11 (14)		
GG‡	77 (84)	10 (83)	1.03 (0.2 to 5.2)	> .05	24 (77)	67 (86)	1.7 (0.6 to 5.1)	> .05
Donor								
AA/AG†	2 (20)	24 (30)			12 (48)	16 (23)		
GG‡	8 (80)	56 (70)	0.6 (0.1 to 2.9)	> .05	13 (52)	53 (77)	3.1 (1.2 to 8.1)	.04

**TNF* indicates tumor necrosis factor; OR, odds ratio; and CI, confidence interval.
†TNF-α high producer.
‡TNF-α low producer.

Table 2. Recipient and Donor's *IL10* -1082G>A Polymorphism by Acute Rejection and Delayed Graft Function*

	Acute Rejection				Delayed Graft Function			
IL10 Genotype	**Yes**	**No**	**OR (95% CI)**	***P***	**Yes**	**No**	**OR (95% CI)**	***P***
Recipient								
AA/AG†	8 (67)	49 (53)			18 (58)	43 (55)		
GG‡	4(33)	43 (47)	1.7 (0.5 to 6.2)	> .05	13 (42)	35 (45)	1.1 (0.5 to 2.6)	> .05
Donor								
AA/AG†	5 (50)	45 (56)			17 (68)	36 (52)		
GG‡	5 (50)	35 (44)	0.8 (0.2 to 2.9)	> .05	8 (32)	33 (48)	1.9 (0.7 to 5.1)	> .05

**TNF* indicates tumor necrosis factor gene; OR, odds ratio; and CI, confidence interval.
†IL-10 low producer.
‡IL-10 high producer.

and AR and DGF (Table 3).

Combination of Recipient's *IL10* and *TNF* Genotypes

The effect of ccombinations of recipient's *IL10* genotypes and *TNF* genotypes on the AR and DGF were analysed in order to determine if their interaction had a joint effect on AR and DGF. No associations were detected between combinations of recipient's *IL10* genotype and *TNF* genotypes and AR and DGF (Table 4).

Combination of Donor's *IL10* and *TNF* Genotypes

Table 5 summarizes the effect of combinations of donor's *IL10* genotypes and *TNF* genotypes on DGF. The frequency of a combination of donor's *IL10* AA or AG and *TNF* AA or AG (low *IL10*/high *TNF*) polymorphisms was higher in the patients with DGF.

Table 5. Combinations of Donor's *IL10* and *TNF* Genotypes by Delayed Graft Function*

	Delayed Graft Function			
Donor IL10/TNF Genotype	**Yes**	**No**	**OR (95% CI)**	***P***
GG/GG†	6 (24)	26 (37)	referent	
GA or AA/GA or AA‡	10 (40)	9 (13)	4.8 (1.3 to 17.1)	
GG/GA or AA§	2 (8)	7 (10)	1.2 (0.2 to 7.5)	
GA or AA/GG#	7 (28)	27 (39)	1.1 (0.3 to 3.8)	.04

**TNF* indicates tumor necrosis factor gene; *IL10,* interleukin-10 gene; OR, odds ratio; and CI, confidence interval.
†IL-10 high producer and TNF-α low producer.
‡IL-10 low producer and TNF-α high producer.
§IL-10 high producer and TNF-α high producer.
#IL-10 low producer and TNF-α low producer.

Table 3. Combinations of Recipient and Donor's *TNF* Genotypes by Acute Rejection and Delayed Graft Function*

	Acute Rejection			Delayed Graft Function		
Donor/Recipient TNF Genotype	**Yes**	**No**	***P***	**Yes**	**No**	***P***
GG/GG†	6 (54)	46 (58)	> .05	11 (44)	42 (61)	> .05
GA or AA/GG‡	2 (18)	19 (24)	> .05	8 (32)	15 (21)	> .05
GG/GA or AA§	2 (18)	8 (10)	> .05	2 (8)	9 (13)	> .05
GA or AA/GG#	1 (9)	7 (9)	> .05	4 (16)	3 (4)	> .05

**TNF* indicates tumor necrosis factor gene.
†Donor and recipient TNF-α low producer.
‡Donor TNF-α high producer and recipient TNF-α low producer.
§Donor TNF-α low producer and recipient TNF-α high producer.
#Donor and recipient TNF-α high producer.

Table 4. Combinations of Recipient's *IL10* and *TNF* Genotypes by Acute Rejection and Delayed Graft Function*

	Acute Rejection			Delayed Graft Function		
Recipient IL10/TNF Genotype	**Yes**	**No**	***P***	**Yes**	**No**	***P***
GG/GG†	4 (33)	37 (40)	> .05	11 (35.5)	31 (40)	> .05
GA or AA/GA or AA‡	2 (17)	9 (10)	> .05	5 (16)	7 (9)	> .05
GG/GA or AA§	0 (0)	6 (6.5)	> .05	2 (6.5)	4 (5)	> .05
GA or AA/GG#	6 (50)	40 (43.5)	> .05	13 (42)	36 (46)	> .05

**TNF* indicates tumor necrosis factor gene and *IL10,* interleukin-10 gene.
†IL-10 high producer and TNF-α low producer.
‡IL-10 low producer and TNF-α high producer.
§IL-10 high producer and TNF-α high producer.
#IL-10 low producer and TNF-α low producer.

DISCUSSION

In view of the pivotal role that cytokines play in the immune response, we evaluated the influence of genetic variants of *IL10* and *TNF* genes, two important pro- and anti-inflammatory cytokines, on kidney transplantation. Out results show that donor's *TNF* polymorphism was significantly associated with the frequency of DGF; the risk of DGF in allografts from high *TNF* producers was 3.1 times greater than low producers. There was also a significant association between the combination of donor's *IL10* and *TNF* genotypes and DGF; the frequency of donor's *IL10 AA or AG and TNF AA or AG* was higher in the recipients with DGF. There is evidence that these cytokines production is under genetic control and there is interindividual variation in the amount of cytokine production.[11,19,20] It is suggested that the utility of a cytokine genotype profile might provide a guideline in evaluating effective levels of the immunosuppressive agents.[11]

In this study, both recipients and their donors were recruited. There was no association between donor and recipient's *IL10* -1082G>A genotypes and occurrence of DGF (Table 2). The recipients' *TNF* -308G>A polymorphism was not also associated with the frequency of DGF in recipients (Table 1). The proportion of recipients with *TNF* high-producer genotype (A positive) with DGF (7 of 18) was not different from recipients with *TNF* low-producer genotype (A negative) with no DGF (24 of 91; OR, 1.7; 95% CI, 0.6 to 5.1). However, the frequency of DGF was higher in the recipients of whom the donors were high producers (A positive) of *TNF* (OR, 3.1; 95% CI, 1.2 to 8.1; Table 1). This result is contrary to the Israni and colleagues' report.[21] They in a prospective cohort study evaluated the association of some donor inflammation- and apoptosis-related genotypes including *TNF* and *IL10* with DGF. They found no association between *IL10* -1082G>A polymorphism and DGF. However, they found that the G allele of the *TNF* polymorphism (low producer) was associated with DGF in an adjusted analysis (OR, 1.85 compared with A allele; 95% CI, 1.16 to 2.96; $P < .01$).

The *TNF* SNP may contribute to DGF through several potential mechanisms. TNF-α, expressed in donor kidney tissue,[5] is a pro-inflammatory cytokine that upregulates cell adhesion molecules.[22] It also contributes to kidney injury because neutralizing antibodies against TNF-α decrease neutrophil infiltration and kidney injury in mice.[4,5] Greater levels of TNF-α expression occur in kidney allografts experiencing DGF than those without DGF.[23] Therefore, it is biologically plausible that a donor polymorphism that increases TNF-α activity could make a recipient more susceptible to DGF.

Our study could not find associations with donor's *IL10* gene polymorphisms and DGF and AR. In agreement with our results, no influence of *IL10* polymorphism on the presence of AR and DGF was observed by other authors.[12,24-26] However, the protective role of *IL10* genotypes on graft outcome in kidney and heart has been proved. The high-producer *IL10* genotype has been shown to be associated with protection of allograft in kidney and heart transplants.[27,28] Khan and colleagues[28] reported that kidney transplant recipients genetically predisposed to low expression of the regulatory cytokine IL-10 were more susceptible to high grades of interstitial fibrosis and tubular atrophy scores, graft inflammation, and high influx of inflammatory cells into the graft interstitium.

No association between the recipient and donor's *IL10* -1082G>A and *TNF* -308G>A genotypes and the incidence of AR was detected (Tables 1 and 2). The proportion of recipients with *IL10* low-producer genotype (A positive) with rejection (8 out of 57) was not different from recipients with *IL10* high-producer (A negative) with rejection (4 of 47; OR, 1.75; 95% CI, 0.5 to 6.3).

The role of *IL10* -1082G>A and *TNF* -308G>A polymorphisms in rejection episodes after kidney transplantation has been studied in several studies. Sankaran and coworkers[25] reported no association between donor and recipient's *IL10* and *TNF* polymorphisms and frequency of AR episodes when all patients were analyzed regardless of human leukocyte antigens-DR (HLA-DR) matching. Re-analysis of data while considering HLA-DR matching showed that a greater portion of recipients with the high-*IL10* producer genotype had multiple rejection episodes (OR, 2.80; 95% CI, 1.17 to 6.69). Meanwhile, the recipients with high-producer genotype (GA/AA) showed an association with increased rejection episodes only when HLA-DR-mismatched transplants were analyzed. In a study directed by McDaniel and colleagues,[29] the combination of cytokine polymorphism and the level of gene expression and AR episodes and DGF was analyzed. They found that low-*TNF* producer

and high-*IL10* producer (G/G) genotypes were significantly protective of the allograft. In relation to DGF, they found no correlation between the *IL10* and *TNF* genotypes and DGF. In another study conducted by Poole and colleagues,[30] the relationship of some polymorphisms including *IL10* and *TNF* with AR was evaluated in 120 consecutive first cadaveric recipient-donor pairs. There was an association (P = .06) between donor *IL10* -1082G>A and AR episodes when HLA-DR matching was considered.

The role of *IL10* -1082G>A polymorphism and transplant outcomes in Iranian patients has been reported in a few studies. Amirzargar and coworkers[31] showed no association between *IL10* A genotype and RE in a study on 100 recipients (OR, 1.12; P = .90). Azarpira and colleagues[32] reported no association between recipients and donors' *IL10* and *TNF* polymorphisms and AR episodes. Omrani and coworkers[33] obtained no association between *IL10* and *TNF* genes polymorphisms and long-term kidney allograft survival.

Combinations of recipient and donor's *IL10* and *TNF* genotypes were analysed in order to determine if their interaction had a joint effect on transplant outcomes. There was no association between different combinations of *TNF* and *IL10* genotypes and AR episodes. The effect of the combination of donor and recipient's genotypes on AR has been reported in some studies. Sankaran and associates[25] showed that a combination of *TNF* -308A (high) and *IL10* -1082G>G (high) was associated with multiple AR episodes in HLA-DR-mismatched kidneys (P = .01). Poole and colleagues[30] showed that the combination of recipient's *IL10* AA or AG and donor's *IL10* GG (recipient high producer-donor low producer), was significantly decreased in multiple rejectors.

This study involved a sample of donor-recipients which enabled us to study the combination of donor-recipient polymorphism on AR and DGF. Furthermore, the study was designed as cohort study enabling us to follow up the patients. Finally, DGF was defined using a robust definition not dependent on the need for dialysis therapy in the first week posttransplantation.[21] Our study should also be evaluated in light of its weaknesses. First, by using a relatively small sample size, it was possible that the association of single-nucleotide polymorphisms in these genes was not seen in our study. We were unable to pick up the role of rare genotypes like *TNF* AA for effects on DGF and AR. Second, lack of the HLA-DR matching of the patients prevented us from analyzing the role of cytokine polymorphism in combination with number of HLA matching.

CONCLUSIONS

High-production *TNF* donors may increase the risk of DGF in their recipients. Routine screening of these gene polymorphisms may have a clinical impact on identifying patients at risk of DGF, and therefore is suggestible. A larger sample size might be necessary to test more accurately the influence of *TNF* polymorphism on the association of donor and recipient genotypes with AR and DGF.

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CONFLICT OF INTEREST

None declared.

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