

Cytokine Gene Polymorphisms in Renal Transplant Recipients

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Objective: Acute rejection remains an important cause of graft loss after renal transplantation, and cytokines are key mediators in the induction and effector phases of all immune and inflammatory responses. However, the influence of gene polymorphisms on the functional immune response of transplant recipient outcomes remains controversial.

Materials and Methods: The amplification refractory mutation system polymerase chain reaction was used to detect the interleukin-10 (IL-10) (-1082 G/A), tumor necrosis factor-alpha (TNF- α) (-308 G/A), and interferon-gamma (IFN- γ) (+874 T/A) single nucleotide polymorphisms in 100 of the first adult kidney recipients at our institution who were receiving cyclosporine-based immunosuppressive therapy. The diagnosis of acute rejection was based on clinical and histologic findings according to the Banff criteria.

Results: The results of multivariate analyses showed no significant association between episodes of acute rejection and single nucleotide polymorphisms in IL-10, TNF- α genes, or dinucleotide repeat polymorphisms in the IFN- γ gene.

Conclusions: Our results demonstrate that cytokine gene polymorphisms did not influence the early outcome of kidney transplantation.

Key words: Cytokine, Polymorphism, Rejection, Kidney transplantation

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Despite advances in immunosuppressive therapy and improvements in posttransplant management, immunologic pathways have continued to play essential roles in renal allograft dysfunction. Alloantigen-dependent and alloantigen-independent immune processes are mediated by both cytokines and chemokines [1]. The variability in immune responsiveness in some instances is related to the differential production of cytokines, which in turn is associated with single nucleotide polymorphisms in the gene promoter region (tumor necrosis factor-alpha [TNF- α] and interleukin-10 [IL-10]) and the gene intron (interferon-gamma [IFN- γ]). Cytokines, chemokines, and their receptors have been shown to be highly polymorphic (Table 1) [2-5].

Table 1. Cytokine producer phenotype classification

Genotype	Low Producer	Intermediate Producer	High Producer
INF (+874)	A/A	A/T	T/T
TNF (-308)	G/G	G/A	A/A
IL-10 (-1082)	A/A	A/G	G/G

INF, Interferon; TNF, tumor necrosis factor; IL-10, interleukin-10.

The most consistent correlates of chronic allograft nephropathy are acute rejection episodes. The identification of variables that can initiate rejection episodes or modulate their severity may be useful in improving long-term allograft survival.

Variables identified to date include the degree of donor-recipient human leukocyte antigen (HLA) disparity; ischemic-reperfusion injury (I/R), which is manifested by delayed graft function (DGF); and the adequacy of baseline immunosuppression [6-8].

Materials and Methods

One hundred recipients of cadaveric or living-related renal transplants were enrolled in this study, which was performed at the Transplantation Center of Nemazi Hospital in Shiraz, Iran. The average age of the patients was 36.92 ± 13.16 years (range, 13-73 years). All patients received an immunosuppressive

regimen consisting of steroids, cyclosporin A (CsA), and either mycophenolate mofetil or azathioprine. The steroid regimen consisted of intravenously administered methylprednisolone 500 mg at the time of surgery followed by intravenously administered methylprednisolone 1 g/d for the next 3 days, at which time the medication regimen was changed to oral prednisolone 30 mg/d, which was progressively tapered to 15 to 20 mg/d by the end of the first postsurgical month. The initial dosage of CsA, which was first administered during surgery, was 3 to 5 mg/kg/d. All patients received mycophenolate mofetil 1 g twice daily and azathioprine 2.5 mg/kg/d (which was administered as a single daily dose) for the first postsurgical month. Those dosages were changed as needed after that time according to the results of clinical and laboratory testing. An increase in the serum creatinine level that was $\geq 10\%$ from the baseline value and that could not be attributed to a cause such as urinary flow obstruction, a toxic reaction to CsA, or a urinary tract infection was defined as clinical rejection. Episodes of acute clinical rejection were recorded and treated with steroid pulse therapy. If the patient had no clinical response to that treatment, a kidney biopsy was performed. Biopsy specimens were graded according to the Banff criteria [9].

For genotype determination, deoxyribonucleic acid (DNA) was extracted from a peripheral blood sample by means of a commercial extraction kit (DNG plus DNA Extraction Kit, Sinagene Company, Tehran, Iran) and was stored at -20°C until the analysis was performed. Amplification refractory mutation system polymerase chain reaction was used to detect IL-10(-1082), TNF- α (-308), and IFN- γ (+874) single nucleotide polymorphisms as mentioned previously, according to the methods described by Perrey and coworkers [10] and Pravica and coworkers [11]. Products were separated on 2.5% agarose gel containing 0.5% ethidium bromide. DNA bands were then visualized with ultraviolet light on a transilluminator and were photographed for subsequent analyses. Patients were classified into the predicted high-producer (H), intermediate-producer (I), or low-producer (L) genotype group.

Analyses were performed with SPSS software (Statistical Package for the Social Sciences, version 13, SSPS Inc, Chicago, Ill, USA). Values are expressed as the mean $\pm$ SD. The clinical endpoint was defined as the number of acute clinical rejection episodes during the first postsurgical month. The parameters assessed were the recipient's age, the cytokine producer genotype, the DGF (ie, need for dialysis), and the mean CsA trough level at the end of the first postsurgical month. IFN- γ , IL-10, and TNF- α genotypes were

modeled linearly across categories (H>I>L producer genotypes). All possible subset multiple linear regression analyses were used to define the relative roles of predictive variants and their respective interactions. Backward stepwise logistic regression modeling was applied to clinical and pathologic rejections, and the results were considered to be significant when the *P* value was less than .05. The study was performed in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the Shiraz University of Medical Sciences. Written informed consent was obtained from all subjects.

Results

Pretransplant demographic data are shown in Table 2. Most of the organ donors (81%) were living. Overall, HLA matching was poor (which is typical for our center). The cytokine genotypes that correlated with L, I, or H producer genotypes are shown in Table 2. Because low producers of IFN- γ and IL-10 were so rare, those genotypes were modeled as a dichotomous variable (H vs I and L taken together). For TNF- α , the high producer genotype was also rare, and the TNF- α genotype was also a model for a dichotomous variable (low vs I and H). Posttransplant demographic data are shown in Table 3. DGF occurred in 10% of the patients. The mean CsA trough level for 1 month after surgery was 135 ± 18 $\mu\text{g/L}$ (range, 145-350 $\mu\text{g/L}$). Acute clinical rejection occurred most frequently in the early posttransplant period (ie, the first 2 weeks after surgery). Steroid-resistant rejection occurred in 2% of the patients studied.

Table 2. Pretransplant demographic characteristics

Recipient age (y)	36.92 $\pm$ 13.16		
Donor source (living/cadaveric)	81% /19%		
Donor age (y)	36.0 $\pm$ 1.4		
Cold ischemic time (min)	45 $\pm$ 12		
Genotype	Low Producer	Intermediate Producer	High Producer
INF	2	68	30
TNF	6	93	1
IL-10	2	92	6

INF, Interferon; TNF, tumor necrosis factor; IL-10, interleukin-10.

Table 3. Posttransplant demographic characteristics

Delayed graft function (%)	16
Clinical rejection (%)	12
Rejection in kidney biopsy (%)	5
Mean CsA levels ($\mu\text{g/L}$)	
1 month after transplantation	135 $\pm$ 18

CsA, Cyclosporin A.

IFN- γ Microsatellite polymorphism

In all, 32% of the patients were homozygous for A/A and T/T alleles, and 68% were heterozygous. Because low producers of IFN- γ were so rare, those genotypes were modeled as a dichotomous variable (H vs I and L taken together). Univariate and multivariate regression analyses showed no association between this polymorphism and DGF (OR = 0.184, CI = 0.063-0.540) or acute rejection (OR = 0.0777, CI = 0.130-1.517).

TNF α (-308) G/A polymorphism

In all, 7% of the patients were homozygous for G/G and A/A alleles, and 93% were heterozygous. Because the high producer genotype was also an infrequently occurring TNF α genotype, it served as a model of a dichotomous variable (low vs I and H together I plus H). Univariate and multivariate regression analyses showed no association between that polymorphism and DGF (OR = 0.162, CI = 0.017-0.554) or acute rejection (OR = 0.0931, CI = 0.058-4.897).

IL-10 (-1082) polymorphism

In all, 8% of patients were homozygous for A/A and G/G alleles, and 92% were heterozygous. Because a low producer of IL-10 was identified so infrequently, that genotype was modeled as a dichotomous variable (H vs I and L taken together). Univariate and multivariate regression analyses showed no association between this polymorphism and delayed graft function (OR = 0.178, CI = 0.021-0.487) or acute rejection (OR = 0.0707, CI = (0.59-2.781).

Discussion

Despite advances in immunosuppressive therapy, acute rejection remains an important cause of transplant injury. The aim of this study was to identify, for use in pretransplantation patient assessments, cytokine gene polymorphisms in recipients as non-HLA risk factors for acute rejection or delayed graft function.

For the past decade, the influence of cytokine gene polymorphisms has been studied in patients with autoimmune disorders and those who have undergone solid-organ transplantations, and the results of those investigations are inconsistent. Marked interindividual and intraindividual variations in the production of IL-10, TNF- α , IL-2, IL-4, IFN- γ , and other cytokines have been reported [12-14], but attempts to define the effects of particular genotypes on cytokine production, both in vitro and in vivo, have produced inconsistent results [12-14]. The TNF- α

(-308) G/A polymorphism is situated in the 5' flanking region of the TNF- α gene [15]. Contrary to prior reports of a significant association between the TNF- α (-308) polymorphism and the incidence of acute rejection of liver [1] and kidney [17-19] transplants, our study revealed no such effect. The observations reported here are consistent with those of recent studies that have demonstrated no significant association between acute rejection and the TNF- α (-308) polymorphism [20, 21].

Our analysis of the frequency of the IL-10 (-1082) SNP in patients who experienced acute rejection and those who did not revealed no difference in the allelic distributions between those groups. That result conflicts with the previously reported association of the IL-10 (-1082) polymorphism and the risk of acute rejection in patients who have undergone renal [22, 23], heart [16], or liver [24] transplantation but is consistent with the findings of other recent studies that show a lack of association between that polymorphism and acute renal rejection [14, 21].

The CA dinucleotide that repeats in the first intron of the IFN- γ gene has been reported to regulate the production of this important immunoregulatory cytokine [5]. However, in contrast to prior reports [14], our analyses of the frequency of the IFN- γ dinucleotide repeats did not reveal significant differences in the distribution of the alleles between the patients who experienced rejection and those who did not in our study.

DGF is linked to several known clinical risk factors, including donor age, recipient sensitization status, and cold ischemia time [25]. We found no significant evidence of an association between DGF and cytokine gene polymorphisms, although the kidney is known to secrete cytokines that contribute to DGF [26].

These data, which show no significant association between the TNF- α (-308), IL-10 (-1082), and IFN- γ polymorphisms and graft function and rejection, do not provide a consistent picture of the genetic variations in graft outcome. Some important factors, such as patient ethnicity, transplant type, and immunosuppressive treatment, may explain the discrepancy between the results of our study and the reports emphasizing positive gene effects. Our study examined only Iranian patients. Ethnicity correlates closely with genetic background [27], and both the frequency and biologic implications of gene polymorphisms that influence interpretation and reproducibility differ according to the patient's ethnic background [28]. It is logical, then, that immunosuppression may reduce the importance of functional gene polymorphisms. In summary, our study did not confirm the

previously reported effects of key immune response gene polymorphisms on early outcome in patients who have undergone renal transplantation.

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