Cytokine Gene Polymorphism Might Affect the Outcome of Clinical Rejection in Cardiac Transplantation

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KEY WORDS: cytokine, genotype, polymorphism, rejection, transplantation

ABSTRACT

Introduction: Cardiac allograft rejection is associated with an individual's inflammatory cytokine gene polymorphism. It was hypothesized that possession of specific cytokine alleles might be influential in predisposing the recipient to allograft rejection.

Methods: DNA from nucleated peripheral blood cells of 65 cardiac transplantation patients and 77 controls were tested for IL-2, TNF-□, TGF-□1, IL-10, IL-6, and IFN-□ gene polymorphism by polymerase chain reaction. Genotype variation was analyzed in view of the recipient's clinical condition and histopathological assessment for rejection. Transplant rejection was determined by endomyocardial biopsy and scored 1A, 2, and 3A/3B.

Results: Overall, 57% of recipients at pre-transplantation suffered from ischemic-cardiomyopathy; whereas 43% had some form of non-ischemic-cardiomyopathy. There were fewer IL-10 high producer genotypes in recipients with 2 and 3A/3B rejection scores than in those with 1A scores (28.6% vs. 22.6% vs. 80%; $P<0.01$ respectively).

Table 1. Demographic and Clinical Characteristics of Patients

	African American	white
Ethnicity (%)	29	71
Gender (F %)	42	24
IsCM (%)	16	84
NIsCM (%)	46	54
IsCM (HTN or IDDM)	8	30
NIsCM (HTN or IDDM)	21	18
IsCM with NIDDM	0	3
NIsCM with NIDDM	7	0

IsCM indicates ischemic cardiomyopathy; NIsCM, non-ischemic cardiomyopathy; HTN, hypertension; IDDM, insulin-dependent diabetes mellitus; and NIDDM, non-insulin-dependent diabetes mellitus.

IFN-γ low producer genotype was increased in African American recipients compared to white recipients with grade 3A/3B rejection (P<0.006, RR=2.4). A majority of recipients carried either the TGF-β1 TT/GG or TC/GG high producer genotype, but the ratio between the genotypes were reversibly associated with 3A/3B rejection grades in African Americans as compared with whites.

Conclusions: The IL-10 high producer genotype appears to be an effective factor in protecting the recipients from rejection. The IFN-γ low producer genotype (A/A) was a greater risk factor for African American recipients as the intermediate producer genotype was for white recipients with 3A/3B rejections. Testing peripheral blood for genetic markers that controls the production levels of inflammatory cytokines might predict the outcome of allograft survival in cardiac transplant recipients.

INTRODUCTION

Cardiac transplantation still suffers from the consequence of coronary vasculopathy and allograft rejection. Pre-transplantation mechanical factors, such as ischemia and reperfusion, are the initial factors, facilitating the costimulatory environment of donor tissue by upregulating the donor major histocompatibility complex (MHC) class II antigens thus leading to activation of the immune response.[1-3] An activated vascular immune response to transplantation injury causes endothelial cell activation, increasing vasodilation, vascular permeability,[4] and production of cytokines and growth factors.[5] Cytokines and growth factors play a central role in inflammatory response and in specific immune modulation directed toward vascular repair and allograft survival.[6] Large amounts of transforming growth factor (TGF-β1) are released at the site of allograft, attracting neutrophils, monocytes, and T cells.[5,7] In addition, the proinflammatory cytokine, tumor necrosis factor-a (TNF-α), is consistently found at the site of inflammation.[8] The plasma concentration and the circulating TNF-α receptors have been shown elevated with the severity of the heart failure,[9-10] and during the episodes of acute rejection after cardiac transplantation.[11]

Evidence supports the role of cytokines in the inflammatory and immune responses that mediate allograft survival outcome. IL-10, which is released by monocytes, T and B cells inhibits the production of proinflammatory cytokines such as IL-1, TNF-α, IL-6, IL-8, IL-12, and Interferon-γ (IFN-γ).[12] In the transplantation setting, local IL-10 production therefore, might have inhibitory effects on the cells that are

Table 2. IL-6 and IFN-γ Genotype Distribution in Association with Pretransplantation Clinical Conditions.

Genotypes	Ischemic N=37 +/%	Non-Ischemic N=28 +/%	*P* value
IL-6			
G/G (high)	12/32	18/64	<0.01
G/C (high)	17/46	8/29	
C/C (low)	8/22	2/7	
IFN-γ			
T/T (high)	10/27	1/4	<0.02
T/A (intermediate)	16/43	10/36	
A/A (low)	11/30	17/61	<0.02

The majority of African American patients were in the non-IsCM group and the likelihood of association with the IL-6 might be influenced by the ethnicity of the genotype. There was an association between the IFN-γ high producer genotype with IsCM and low producer genotype with non-IsCM.

involved in cell-mediated inflammatory processes.[13] Long-term survival of heart transplantation has been reported to be associated with a marked reduction in the expression of IFN-γ and IL-2 and an increase in the level of IL-10 expression in allograft infiltrating leukocytes in the mouse.[14] In clinical studies, such an association has not been clearly observed. However, there is evidence that the amount of proinflammatory cytokine expression decreases after the initial anti-rejection therapy,[13] suggesting that an underlying mechanism of allograft rejection might be coordinated by the cytokine expression profile. Thus, an assessment of cytokine production level in transplant patients might have diagnostic value in determining the outcome of allograft survival. One mechanism that could affect the differences in cytokine production between individuals is polymorphism of cytokine genes.

There is evidence that cytokine production level is under genetic control. It was first demonstrated by Hutchinson et al that nucleic acid sequence polymorphism within promoter regions of the gene may affect the transcriptional activation of the gene, causing variation in the amounts of cytokine production.[15-19] Such variation provides a unique profile of high and low cytokine response for each individual.[20] In general terms, the high and low cytokine producer status is defined by the zygosity, being homozygote high/high, or low/low and heterozygote high/low. Thus, the zygosity status determines the inheritance of the cytokine production profile in transplant patients, and that might have impact on the outcome of allograft survival.

The majority of polymorphisms identified in cytokines are either single nucleotide (SNPs) or dinucleotide (microsatellite) polymorphisms.[16,20] The most widely studied polymorphism has been the TNF-α gene that involves a nucleotide G→A substitution at position –308 in the protein signal sequence, affecting production of TNF-α.[17] Such polymorphism generates a high responder genotype (G/A or A/A), and is associated with acute rejection of heart,[21-23] kidney,[24-25] and liver transplants.[26] Three single base-pair mutations have been described in the IL-10 promoter region at positions -1082(G→A), -819(C→T), and -592(C→A), with the GCC haplotype being associated with high produc-

Table 3. Frequency Distribution of Cytokine Genotypes and Association with Allograft Rejection

Cytokine	Genotype	Group 1A N=5	Group 2 N=7	Group 3A/3B N=53	*P* value
TNF-	High	2 (40%)	3 (42.8%)	14 (26.4%)	
	Low	3 (60%)	4 (57.1%)	39 (73.6%)	
TGF-1	High	4 (80%)	7 (100%)	39 (73.6%)	
	Intermediate	1 (20%)	0	10 (19%)	
	Low	0	0	4 (7.5%)	
IL-10	High	4 (80%)	2 (28.6%)	12 (22.6%)	*P*<0.01
	Intermediate	1 (20%)	4 (57.1%)	25 (47.2%)	
	Low	0	1 (14.3%)	16 (30.2%)	
IL-6	High	4 (80%)	4 (57.1%)	47 (88.7%)	
	Low	1 (20%)	3 (42.9%)	6 (11.3%)	
IFN-	High	1 (20%)	3 (42.9%)	8 (15.1%)	
	Intermediate	4 (80%)	1 (14.3%)	19 (35.8%)	*P*<0.07
	Low	0	3 (42.9%)	26 (49.1%)	*P*<0.05
IL-2	High	4 (80%)	2 (28.6%)	18 (34%)	*P*<0.06
	Low	1 (20%)	5 (71.2%)	35 (66%)	*P*<0.05

All African American recipients were in the group with rejection scores of 3A/3B. From this group 19 (36%) were African American and 34 (64%) were white recipients.

tion and ATA with low production of IL-10, respectively.[16] The high producer IL-10 genotype has been shown to be associated with protection of allograft in heart transplants.[21,27] TGF-1 gene polymorphisms, located in positions +869 (T C) and +915 (G C) at codons 10 and 25 respectively in the signal sequence, have variable effects on production of TGF-1in individuals with different clinical conditions.[28-31] In cardiac transplantation an association of TGF-1 gene polymorphisms with the outcome of allograft survival is not yet established. A polymorphic dinucleotide (CA) marker has been detected within the first intron of the IFN-at position +874 from the translation start site[32] causing high and low producer genotypes. High IFN-producer genotype is associated with acute rejection of the kidney,[33-34] the occurrence of infections,[35] and the development of fibrosis after lung transplantation.[36] Such association in cardiac transplantation remains unproven.

The clinical setting of heart transplantation provides an opportunity to evaluate the influence of cytokine genotypes in clinical condition, and the outcome of allograft survival. In this study, we have applied the cytokine genotyping approach to investigate the contribution of different cytokines in the occurrence of rejection episodes in African Americans and whites who had undergone cardiac transplantation. Our data might allow determining whether possession of a specific cytokine genotype could predict the outcome of allo-

Table 4. Effects of Combined Predicted Phenotypes in Association with Rejection Grades.

Predicted Phenotype	Group 1A N=5	Group 2 N=7	Group 3A/3B N=53
IL-10 high/IFN-γ low	0	0	6 (11.3%)
IL-10 high/IFN-γ intermediate*	4 (80%)	1 (14.3%)	4 (7.5%)
IL-10 high/IFN-γ high	0	1 (14.3%)	1 (1.8%)
IFN-γ high/IL-10 low	0	0	0
IFN-γ high/IL-10 intermediate	1 (20%)	2 (28.6%)	6 (11.3%)
IL-10 intermediate/IFN-γ intermediate	0	0	11 (21%)
IL-10 intermediate/IFN-γ low	0	2 (28.6%)	8 (15.1%)
IL-10 low/IFN-γ intermediate	0	0	6 (11.3%)
IL-10 low/IFN-γ low†	0	1 (14.3%)	10 (18.9%)

*There was a significant interaction between IL-10 high and IFN-γ intermediate producer genotypes associated with a stable graft function as compared with the recipients in the group 3A/3B (P<0.0008, RR=10.6).
†There was a trend of association between IL-10 low/ IFN-γ low producer genotype and the severity of rejection. However, it was not statistically significant.

graft function.

MATERIALS AND METHODS

Informed consent was obtained as part of protocol approved by the University of Mississippi Medical Center Review Board for obtaining blood samples from patients who had undergone cardiac transplantation. Blood samples from 19 African American, 46 whites, and 77 controls were studied. Demographic and clinical characterization of the patient population is given in Table 1. Pre-transplantation clinical evaluation demonstrated 16 % of allograft recipients that were characterized with ischemic cardiomyopathy (IsCM) were African American as compared to 84% white. In contrast, 46% of recipients with non-IsCM were African American as compared with 54% of white patients. Patients were classified into testing groups, based on endomyocardial biopsy and histopathological assessment for rejection scores: 1A, 2, 3A, and 3B according to an established criterion by the International Society for Heart and Lung Transplantation.[37]

Each blood sample for cytokine testing was collected in sterile ACD tubes and was tested within a 2 to 3 hours after blood was drawn. Genomic DNA was isolated from whole blood using modification of Blin and Staford method,[38] followed by phenol extraction and precipitation with 3M sodium acetate and ethanol. DNA was stored in TE buffer at 4°C until analysis of cytokine genotypes by PCR and cytogene technique (One Lambda, Inc., Canoga Park, Calif).

Six cytokines and growth factors were tested. These included IL-2, TNF-α, TGF-β1, IL-10, IL-6 and IFN-γ. For detection of IL-2 polymorphism 2 PCR primers was designed to amplify region –352 to -167 to detect substitution at position -330. We modified previously published primer pairs as follow: 5'-TCA CAT GTT CAG TGT AGT TCT AGG-3' (-352 to -331), and reverse and complimentary 5'-AGA CTG ACT GAA TGG ATG TAG GTG-3' to detect the presence or absence of an amplified DNA fragment in the subjects possessing the G or T genotype respectively. In addition, we used a forward primer 5'-TATTCACATGTTCAGTGTAGTTCT-3'[39] that contained a restriction site CTAG for the enzyme Bfa1 (New England Biolabs Inc., Beverly, Mass). Amplification was carried out on 50-100

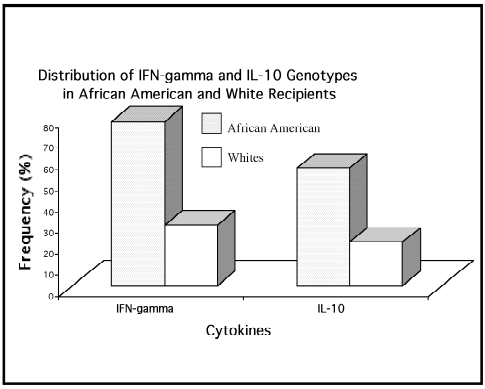

Figure 1. Differential presentation of frequency distribution of IFN-γ and IL-10 low producer genotypes in African American (indicated with dotted bars) and white (indicated with empty bars) patients with 3A/3B rejection grades. IFN-γ low producer genotype is 2.6 fold increased in African American patients as compared with white patients, *P* <0.001, RR=2.6. IL-10 low producer genotype is 3.2 fold increased in African American patients as compared with white patients, *P*<0.01, RR=3.2.

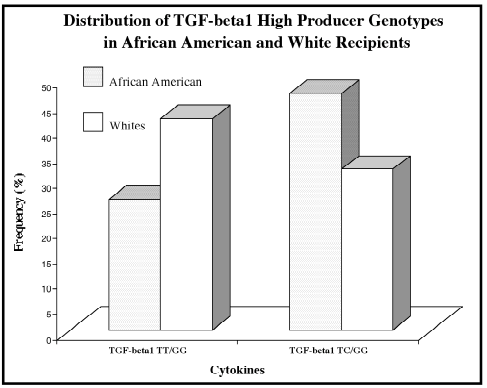

Figure 2. Frequency distribution of the TGF-β1 high producer genotypes in African American (indicated with dotted bars) and white (indicated with empty bars) patients with grades 3A/3B rejection. These genotypes are reversibly associated with rejection in African Americans as compared with whites. TGF/β1 TT/GG high producer genotype is significantly lower in African American patients as compared with white patients. TGF-β1 TC/GG high producer genotype is significantly higher in African American patients as compared with white patients, P<0.01, RR=0.7.

ng genomic DNA at 95°C for 5 minutes, followed by 35 cycles at 95°C for 10 seconds; 59°C for 50 seconds and 72°C for 30 seconds and a final extension cycle of 72°C for 4 minutes in a 9600 Perkin Elmer Thermal Cycler. The amplified DNA fragments were visualized by 2% agarose gel based on the presence or absence of the target DNA fragment. A second set of amplification product was digested with Bfa1 at 37°C overnight and the products were visualized on 4% NeSeive agarose gel, stained with ethidium bromide.

The remaining cytokines were tested using a commercial cytokine genotyping tray (One Lambda, Inc., Canoga Park, Calif). PCR was performed according to the manufacturer's instructions. One hundred ng genomic DNA was amplified at initial 1 cycle of 96°C for 130 seconds, followed by 63°C for 60 seconds, then 9 cycles of 96°C for 10 seconds, 63°C for 60 seconds; 20 cycles of 96°C for 10 seconds; 59°C for 50 seconds; followed by 72 for 30 seconds. The amplified DNA fragments were visualized by 2.5% agarose gel based on the presence or absence of the target DNA fragment. Each genotype of these cytokines is associated with high, intermediate or low production described previously.[20]

For statistical analysis, allele frequencies of the genotypes were compared using contingency 2 x 2 table of Fisher's exact test. A *P* value less than 0.05 was considered statistically significant. An INSTAT3 program was used to analyze the relation between grades of rejection and cytokine genotype.

RESULTS

Sixty-five patients with cardiovascular disease that had undergone cardiac transplantation were studied. There were 19 African American recipients, which consisted of 42% female (8 of 19) and 58% male (11 of 19). The white recipients consisted of 24% female (11 of 46) and 76% male (35 of 46). Demographic and clinical characterization is summarized in Table 1. Overall,

based on pretransplantation clinical diagnosis, the patients were stratified into 2 groups of those with ischemic cardiomyopathy (IsCM) (57%), and those with viral mediated or idiopathic heart failure (non-IsCM) (43%). In the initial evaluation for the association of cytokine genotypes, across the entire study population, we found that some genotypes were exclusively associated with the ethnicity. The IL-6 high producer genotype (G/G) was present in majority of African American recipients (18 out of 19) as compared with 12 out of 46 in white recipients ($P<0.001$). The IL-6 high producer genotype (G/G) was present in a higher frequency in non-IsCM patients (64%) as compared with IsCM patients (32%) ($P<0.01$, RR=0.5) (Table 2). Nonetheless, the finding was due to the fact that the majority of African American recipients were in the group that the non-IsCM was the diagnosis of their heart failure. Thus, the likelihood of association between the IL-6 and non-IsCM was reflected by to the ethnicity of the genotype.

The IFN-γ high producer genotype (T/T) was present with a higher frequency in patients with IsCM as compared to patients with non-IsCM (27% vs. 4%, $P<0.02$, RR=7.6). In contrast, as shown in Table 2, the IFN-γ low producer genotype (A/A) was observed with a lower frequency in IsCM patients as compared with non-IsCM (30% vs. 61%, $P<0.02$, 0.5). IFN-γ is a specific marker for inflammation and infection, and it is unclear to what extent the production level could identify the inflammation or infection. This data suggests that individuals with IFN-γ high producer genotypes (T/T) might be at a higher risk of developing an inflammatory response based cardiomyopathy, whereas patients with a low producer genotype might be protected from an inflammation induced heart failure. The IL-2, TNF-α, TGF-β1, and IL-10 genotypes were not significantly different between the 2 groups.

The association between frequency distribution of cytokine genotypes and allograft function after transplantation was evaluated based on endomyocardial biopsy and histopathological assessment for rejection grades described earlier. At least at 1-year post transplantation, there were 5 (8%) patients that pathologically did not develop a significant grade of rejection. All 5 patients were white and were graded in category 1A, which pathologically is considered minimal rejection. There were 7 (11%) patients, who at least once might have had a mild rejection and the remaining 53 (81%) patients were in the category in which at least once they experienced an episode of 3A/3B grade of rejection. All African American patients were in the 3A/3B-rejection group. In this study, we have not investigated the effects of cytokine genotypes on rejection rates. The study was designed to identify the individuals in whom the possession of a particular genotype might put them at the risk of developing at least once a 3A/3B grade of rejection. As shown in Table 3, the majority of patients with rejection grade 1A, possessed, the IL-10 GCC high producer genotype (80%). The frequency of IL-10 high producer genotype was drastically reduced in group 2 (28.6%) with mild rejection and group 3A/ 3B (22.6%), with considerably severe rejections ($P<0.01$), indicating, that the IL-10 high producer genotype might have a protective effect on the outcome of allograft function.

The IFN-γ T/T high producer genotype was present at a low frequency 15.1% (8 out of 53) in patients with considerably severe rejections (group 3A/ 3B), as compared with 42.9% in patients with mild rejection (group 2). Although, due to a small number of patients in group 2, the difference was not statistically significant, the relative risk (RR) value was 2.9, which is considered high.

The IFN-γ low producer genotype was absent in the patients with minimal rejection (group 1A), while it was present in 49.1% of patients in group 3A/3B ($P<0.05$). This observation is contrary to the literature[14,40-41] that classifies the IFN-γ as a proinflammatory cytokine, and thus the low production should be associated with more stable graft function. However, when recipients with rejection grade 3A/3B were separated based on the ethnicity, the IFN-γ high producer genotype (TT) was absent in the African American patients, but the low producer genotype (AA) was 2.6 fold increased as compared in whites ($P<0.001$, RR=2.6) (Figure 1). Similar differences in the frequency distribution of the IL-10 low producer genotype was observed in the African Americans as compared with the whites, in group 3A/3B, $p<0.01$, RR=3.2, demonstrated in Figure 1.

A great majority of recipients carried the TGF-β1, TT/GG or TC/GG high producer genotypes. The frequency distributions of the TC/GG genotypes were significantly higher in African American patients as compared with white patients, whereas the TT/GG genotypes were significantly elevated in whites as compared with African Americans (Figure 2). The African Americans showed a T/C allelic distribution of 38.5%/61.5% whereas whites had a distribution of 56%/44% ($P<0.01$, RR=0.7).

Patients were then tested for multiple genotype effects and the likelihood of allograft rejection according to the pathology grading described earlier. In this definition, we tested combined effects of IL-10 and IFN-γ genotypes. Based on our findings demonstrated in Table 4, there was a significant interaction between IL-10 high and IFN-γ intermediate producer genotypes, associated with a stable graft function. Four out of 5 recipients in group 1A had both the IL-10 high and IFN-γ intermediate producer genotypes. Whereas only 4 out of 53 recipients in group 3A/3B carry the same genotypes (group 1 vs. group 3: 80% vs. 7.5%, $P<0.0008$, RR=10.6). There was a trend of association toward the co-presence of the IFN-γ and IL-10 low producer genotypes and rejection episodes, but that was not statistically significant. The genotypes that predict low IFN-γ and low IL-10 production was absent in the group 1A, with stable graft function. Only 1 out of 7 (14.3%) in the group 2 (mild rejection), and 10 out of 53 (19%) in the group 3A/3B, with severe rejection episodes, carry both genotypes (Table 4). The data reported here represents only a few cytokine genes that are relevant to allograft rejection and the combined analysis of a greater number of genes might allow identification of the inflammatory markers associated with the outcome of cardiac transplantation.

DISCUSSION

In a study of 65 cardiac transplant patients, we have investigated whether the recipient's cytokine genotype profile might be predictive of the outcome of allograft function. There is emerging evidence suggesting that blood associated molecular markers that reflect systemic inflammation might play an essential role in the primary disease process that may require cardiac transplantation. Cytokines have been found to be useful markers of severity of injury in various clinical settings. Current studies suggest that in human allograft transplantation, pro-inflammatory Th1 type cytokines (eg, IL-2, IFN-γ, TNF-α) are considered to be responsible for allograft rejection, whereas, the Th2 type cytokines (eg, IL-4, IL-5 and IL-10) are involved in the process of allograft tolerance.[42-44] In addition, a Th3 category of cytokines, such as IL-10 and TGF-β1 with pleotrophic function, are responsible for immune regulation and the main-

tenance of immunologic balance in allograft recipients.[44] The rejection process that occurs in the allograft, in part, is cell mediated and is orchestrated by circulating lymphocytes. However, the driving force for recruiting the infiltrating mononuclear cells into the allograft is directly dependent upon 2 factors: a) pretransplantation mechanical factors, such as ischemia and reperfusion, which provides the costimulatory environment of donor tissue, and b) the compatibility of the immunogenetic markers between the donor-recipient pair. To identify the role that cytokines may play in allograft outcome, we have focused on individual cytokine production capacity in the context of cytokine genotype variation.

The data analysis presented here separates recipients based on high/intermediate/low producer genotypes previously identified in vitro.[15-16, 18-19] Variation in the production levels results from polymorphic nucleotides located within the regulatory regions of these genes, influencing a decrease or an increase production of the cytokines.[20] Our results indicated that the frequency distribution of the polymorphism within the promoter region for some cytokine genes were statistically different in African Americans as compared with whites. The IL-2 (T/T) high, IL-6 (G/G) high and the IFN-γ (A/A) low producer genotypes were found significantly increased in African Americans as compared with white recipients (Table 2). This observation is consistent with our earlier findings, testing the frequency distribution of the cytokine genotypes in African American renal transplant recipients in association with allograft rejection.[45] In addition, others,[46] also have shown similar findings for IL-6 and IL-2 but not for IFN-γ low producer genotype.

In a number of studies, cytokine gene polymorphism has been implicated as a risk factor affecting the outcome of cardiac transplantation.[21,27,31,47-49] Some of cytokine gene polymorphism has also been associated with number of diseases.[50] This suggests that possession of such polymorphism is more likely to identify a disease condition and severity of the outcome, or response to a treatment regimen.[34,46,51] It was evidenced from this study that the variations in the frequency of the genotypes were also influential in the overall pretransplantation clinical conditions of the recipients. We noted that the IL-6 high producer (G/G) and IFN-γ low producer (A/A) genotypes were both associated with non-ischemic cardiomyopathy as compared with ischemic group. Non-ischemic cardiomyopathy includes several types, and multiple factors have been linked to the occurrence of the disease.[52] However, in this study, the association between the IL-6 high and IFN-γ low producer genotypes and the non-ischemic condition was in part influenced by the prevalence of IL-6 high producer and IFN-γ low producer genotypes in African Americans. There was also statistically significant difference between the distribution of the TGF-β1 TT/GG and TC/GG high producer genotypes in African American and white recipients with 3A/3B grades rejections (P<0.01, RR=0.7) (Figure 2). However, in comparison with the control data obtained from our previous study[45] there was no significant difference between the distribution of the T/C alleles in African American patients and controls. Whereas comparing with the white control data reported by Cox et al[46] there was a significant difference between the distribution of the T/C alleles in white patients and controls (P<0.01, RR=1.6). This polymorphism has not been associated with any kind of cardiomyopathy or the incidence of rejection.

The association of IL-10 high producer genotype with stable graft function suggests that IL-10 could play a major role as an anti-inflammatory cytokine on the immune process involved in rejection. The fact, that IL-10 low producer genotype was absent in 1A, the group with a minimal rejection, and 32% of recipients with grade 3A/3B rejection carry this genotype (Table 4), shows a trend towards greater risk factor for rejection. In addition, it supports the notion that the low IL-10 producer genotype may have stronger Th1 responses, responsible for higher grades of rejection episodes. The effect of IL-10 polymorphism on the outcome of heart transplantation to an extent is controversial. Some investigators reported associations between the low IL-10 producer genotypes and a greater score of rejections.[21,27,48] Others found no association between frequency distribution of the IL-10 genotypes and the severity of rejection episodes.[47,51] Such observations support the pleotrophic roles of the IL-10 in rejection. As others have also noted,[21,51] low IL-10 production may allow increased inflammatory response, leading to rejection episodes, but the high producer IL-10, although potentially reducing the inflammation, could increase the levels of production of antibodies against the allograft. The recipient's initial immune response to the allograft along with HLA-incompatibility can exert a differential response to cytokine stimulation and can disturb the Th1/Th2 balance. Examining the cytokine gene polymorphisms and their implication in clinical setting may allow understanding of how cytokines affect the direction of the Th1/Th2 balance in transplantation.

A combined use of multiple genotypes to assess the recipient's immune response might more precisely predict the outcome of allograft function. Presence of both high IL-10 and intermediate IFN-g genotypes in the recipient conferred the greatest protection effect on the allograft survival. Unfortunately, our study population size was not sufficiently large to allow the impact of multivariate genotype evaluation, including the HLA-DR alleles. In addition, the prevalence of the IFN-□ A/A low producer genotype associated with ethnicity (African American 79% vs. white 21%) in this study, might have impact on the differential outcome of long-term heart transplantation in different populations.

The production of high levels of IL-10 has been associated with long-term transplantation tolerance. In murine model of transplantation, high levels of IL-10 expression on allograft infiltrating cells have been associated with long-term allograft survival.[14] IL-10 inhibits the synthesis of a number of cytokines, including IFN-□and IL-2,[53,54] the 2 major cytokines that are involved in alloreactivity and allograft rejection. However, in humans the inhibitory effect on IFN-□ is indirect and depends upon the synthesis of IL-12, a Th1 type cytokine[55] and IL-18,[56] whereas in mice the suppression of IFN-□production is regulated by IL-10 in association with CD40 ligand antibody.[57] Nevertheless, the levels of IL-10 production relative to IFN-□and IL-2 may determine whether the recipient's immune response is directed towards T cell proliferation and alloreactiviry or tolerance induction. It should be added that cytokine production upon stimulation by transplantation may vary under various immuno/physiological conditions. The process of rejection and alloreactivity is affected by many variables that are beyond the focus and the capability of the present data. We are currently, investigating functional effects of IL-10 and IFN-□gene polymorphisms in cardiac allograft vasculopathy and the effects in long-term graft survival. We believe that the knowledge of an indi-

viduals cytokine profile, could contribute to a better understanding of the recipient's immune response to allograft both before and after cardiac transplantation.

ACKNOWLEDGEMENTS

We thank the coordinators, nurses and staff of the Heart Transplantation Center, at the University of Mississippi Medical Center for their support to this study. Special thanks to Ms. Susan D. Tutor at the Heart Station for her efforts of arranging and obtaining the patients blood samples, and to Dr. Larry S. McDaniel, for his critical review of the manuscript. This work was supported in part by a grant awarded to D. Olga McDaniel through the University of Mississippi Medical Center, and through the Minority Institutional Research Training Program (NHLBIHL07635) awarded to Joseph A. Cameron.

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