Relationship between cytokine gene polymorphisms and acute rejection following liver transplantation

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ABSTRACT. Acute rejection (AR) recurrence after liver transplantation (LT) is one of the major complications that leads to chronic graft dysfunction. It has been reported that the polymorphisms in some cytokine genes are associated with human liver allograft rejection. This study mainly investigated the associations between polymorphisms in the genes encoding interleukin-10 (*IL10*), transforming growth factor-β1 (*TGFB1*), and tumor necrosis factor-α (*TNF*), and the risk of AR recurrence. We enrolled 359 LT recipients; they were divided into two groups: an AR group (N = 165) and a non-AR group (N = 194) according to whether they experienced rejection within the first month following liver transplantation. After providing informed consent, blood was collected and DNA was extracted. The single nucleotide polymorphisms of *IL10* (-1082, -819, and -592), *TGFB1* (+869 and +915), and *TNF* (-308) were investigated according to the methods used in previous studies. A significant difference was observed in the

distribution of allelic frequencies at position +869 in *TGFB1* between the AR and non-AR groups (P = 0.000). However, no significant differences (P > 0.05) were found in the genotype distributions in *IL10*, *TNF*, and *TGFB1* between the AR and non-AR groups. Our study suggests that the +869 gene polymorphism of *TGFB1* is significantly associated with liver graft rejection, while the other gene polymorphisms investigated in *IL10*, *TNF*, and *TGFB1* are probably not risk factors for AR in LT recipients.

Key words: Cytokines; Liver transplantation; Acute rejection; Single nucleotide polymorphism

INTRODUCTION

Liver transplantation is considered an effective therapy for severe liver disease, but graft dysfunction occurs in up to 13% of patients during the first year following transplantation, and rises to 35% after 5 years (Keeffe, 1999; Yu et al., 2001). Graft rejection is one of the major immunological complications following liver transplantation (Zheng et al., 2006). It is well known that modulation of the immune response, both cellular and humoral, is primarily controlled by various cytokines. These cytokines influence cellular activation, differentiation, and function. Therefore, cytokines play an important role in the immunological events following transplantation, and are intimately implicated in graft rejection.

T helper subtype 1 (TH1)-type proinflammatory cytokines, such as interleukin-2 (IL-2), interferon-γ (IFN-γ), and tumor necrosis factor-α (TNF-α), are associated with graft rejection, whereas TH2-type cytokines such as IL-10 have been associated with graft tolerance (Platz et al., 1996; Shi et al., 2008; Zhang and Sun, 2010). Although some studies have shown an effect on rejection (Warlé et al., 2002; Rattanasiri et al., 2013), others have shown no association (Conti et al., 1998; Xie et al., 2008). The regulatory-type cytokines, such as transforming growth factor-β1 (TGF-β1), may influence both TH1- and TH2-type cytokines in the immune response (Liu et al., 2009), and some researchers have reported that they are upregulated in rejection (Eikmans et al., 2002), while others have reported no relationship (Eurich et al., 2011; Rattanasiri et al., 2013). To date, studies on the association between cytokine gene polymorphisms and graft rejection have focused mainly on the cytokines mentioned above, in which serum or plasma levels or messenger RNA expression levels were found to correlate with rejection.

In liver transplantation, it is unknown which cytokines may function as initiator or effector cytokines in human liver graft rejection. We hypothesized that polymorphisms in a large array of TH1-, TH2-, and regulatory-type cytokines may have a role in the development of acute rejection following liver transplantation. We investigated the following specific cytokine gene polymorphisms and their relationship with transplant outcome: *IL10* (-1082, -819, and -592), *TGFB1* (+869 and +915), and *TNF* (-308). The aim of this study was to determine whether associations exist between polymorphisms in TH1-, TH2-, or regulatory-type cytokine genes (*IL10*, *TNF*, and *TGFB1*), and acute liver graft rejection.

MATERIAL AND METHODS

Subjects

A total of 359 patients who had undergone liver transplantation in our hospital between

2013 and 2015 were enrolled in this study. In our patient group, rejection episodes usually took place within the first month following transplantation, whereas a few patients also experienced rejection later. Therefore, we defined rejectors as patients who experienced rejection within the first months following liver transplantation [acute rejection (AR), N = 165], whereas those who did not experience rejection within the first month were termed non-rejectors (non-AR, N = 194). We did not further segregate rejectors based on severity or number of rejection episodes. All participants provided written informed consent and the study was approved by the Ethics Committee of our hospital.

Polymorphism genotyping

Blood samples (5 mL) were taken from all subjects by venipuncture and stored in ethylenediaminetetraacetic acid (EDTA) tubes for total genomic DNA extraction using a QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). Sample concentrations were determined with ultraviolet spectrometry by measuring absorbance at 260 nm (A_{260}). Purity was determined by the A_{260}/A_{280} ratio. Samples were diluted to a standard concentration and stored at -20°C.

Gene polymorphisms for *IL10* (Edwards-Smith et al., 1999), *TNF* (Lympany et al., 1998), and *TGFB1* (Powell et al., 2000) were detected using polymerase chain reaction (PCR)-based methods, as described previously.

Statistical analysis

All single nucleotide polymorphism (SNP) data were evaluated for agreement with the Hardy-Weinberg equilibrium. Data are reported as median and range or means and standard deviation (SD). Data were analyzed using the chi-square test and the Fisher exact test where appropriate. Differences in the frequencies of cytokine gene polymorphisms between the AR and non-AR groups were compared using logistic regression analysis. All tests were two-tailed and 95% confidence intervals are provided. Statistical analysis was performed by using the SPSS version 20.0 software. A P value <0.05 was considered statistically significant.

RESULTS

TNF polymorphism

The results are shown in Table 1, which presents the genotype and allele frequencies of the G and A alleles of the *TNF* -308 G/A polymorphism in the AR and non-AR groups. No significant differences (P = 0.317) were identified in the genotype or allele frequencies in the G and A alleles of the -308 G/A polymorphism when the AR and non-AR groups were compared.

IL10 polymorphisms

The frequencies of the genotypes/alleles -1082 A/G, -819 C/T, and -592 C/A in *IL10* are presented in Table 2. No significant differences (P = 0.297, P = 0.801, and P = 0.515) were identified in the frequencies of the three polymorphisms when the AR and non-AR groups were compared.

Table 1. Genotype and allele frequencies of the tumor necrosis factor-α gene (*TNF*) promoter region polymorphism in the acute rejection (AR) group compared with the non-AR group.

Genotype	AR	Non-AR	Control *vs* patients		P value
	N (%)		OR	95%CI	
TNF -308					
GG	97 (58.79)	105 (54.12)			
GA	64 (38.79)	81 (41.75)	1.169	0.762-1.795	0.475
AA	4 (2.42)	8 (4.13)	1.848	0.539-6.331	0.329
Total	165	194			
Allele	(Frequency)				
G	258 (0.78)	291 (0.75)	1.042	0.843-1.691	0.317
A	72 (0.22)	97 (0.25)	0.837	0.668-1.140	

OR = odds ratio; 95%CI = confidence interval.

Table 2. Genotype and allele frequencies of the interleukin-10 gene (*IL10*) promoter region polymorphisms in the acute rejection (AR) group compared with the non-AR group.

Genotype	AR	Non-AR	Control *vs* patients		P value
	N (%)		OR	95%CI	
IL10 -1082					
AA	141 (85.45)	170 (87.63)			
AG	20 (12.12)	23 (11.86)	0.954	0.503-1.808	0.885
GG	4 (2.42)	1 (0.52)	0.207	0.023-1.876	0.162
Total	165	194			
Allele	(Frequency)				
A	302 (0.92)	363 (0.94)	0.978	0.938-1.020	0.297
G	28 (0.08)	25 (0.06)	1.317	0.784-2.213	
IL10 -819					
CC	93 (56.36)	116 (59.79)			
CT	60 (36.37)	64 (32.99)	0.855	0.548-1.335	0.491
TT	12 (7.27)	14 (7.22)	1.069	0.482-2.371	0.870
Total	165	194			
Allele	(Frequency)				
T	213 (0.75)	244 (0.76)	0.988	0.896-1.088	0.801
C	84 (0.25)	92 (0.24)	1.033	0.803-1.328	
IL10 -592					
CC	109 (66.06)	109 (56.19)			
CA	48 (29.09)	69 (35.57)	1.437	0.913-2.264	0.117
AA	8 (4.85)	16 (8.24)	2.000	0.822-4.867	0.127
Total	165	194			
Allele	(Frequency)				
C	205 (0.81)	287 (0.74)	1.030	0.942-1.126	0.515
A	64 (0.19)	101 (0.26)	0.914	0.696-1.199	

OR = odds ratio; 95%CI = confidence interval.

TGFB1 polymorphisms

Table 3 presents the genotype and allele frequencies of *TGFB1* +869 C/T and +915 G/C polymorphisms in the AR and non-AR groups. No significant differences (P = 0.260) were identified in the genotype or allele frequencies of C and T alleles in the +915 C/T polymorphism, while the genotype and allele frequencies in the +869 C/T polymorphism showed a statistically significant difference when the AR and non-AR groups were compared (P < 0.05).

Table 3. Genotype and allele frequencies of the transforming growth factor-β1 gene (*TGFB1*) promoter region polymorphisms in the acute rejection (AR) group compared with the non-AR group.

Genotype	AR	Non-AR	Control *vs* patients		P value
	N (%)		OR	95%CI	
TGFB1 +869					
CC	16 (9.70)	69 (35.57)			
CT	73 (44.24)	77 (39.69)	0.245	0.13-0.46	0.000*
TT	76 (46.06)	48 (24.74)	0.146	0.076-0.281	0.000*
Total	165	194			
Allele	(Frequency)				
C	105 (0.32)	215 (0.55)	0.574	0.479-0.688	0.000*
T	225 (0.68)	173 (0.45)	1.529	1.338-1.747	
TGFB1 +915					
GG	145 (87.88)	162 (83.51)			
CG	20 (12.12)	32 (16.49)	1.432	0.784-2.615	0.242
CC	0 (0)	0 (0)	0	0	0
Total	165	194			
Allele	(Frequency)				
G	310 (0.94)	356 (0.92)	1.024	0.983-1.066	0.260
C	20 (0.06)	32 (0.08)	0.735	0.429-1.260	

OR = odds ratio; 95%CI = confidence interval; *statistically significant.

DISCUSSION

Cytokines are potent immunomodulatory molecules that take part in inflammation and the immune response. This study investigated whether a genetic predisposition involving polymorphisms of TH1-, TH2-, or regulatory-type cytokine genes was associated with rejection following human liver transplantation. Several polymorphisms in cytokine genes have been investigated for their possible relationship with cytokine production (Platz et al., 1996; Warlé et al., 2002; Shi et al., 2008; Liu et al., 2009; Zhang and Sun, 2010; Rattanasiri et al., 2013) and graft rejection. Polymorphisms of *IL10*, *TNF*, and *TGFB1* have been reported to be closely linked to AR (Bathgate et al., 2000; Jonsson et al., 2001; Mas et al., 2004; Azarpira et al., 2013). However, the results are controversial (Eurich et al., 2011; Rattanasiri et al., 2013). This study showed significant correlation between the SNPs *IL10* -1082 and *TGFB1* +869 and AR, but there was no significant correlation between the SNPs of the other genes and AR.

Our results showed that the polymorphism in *TNF*, a TH1-type cytokine gene, did not correlate significantly with acute liver graft rejection, even though some studies on the relationship between *TNF* gene polymorphisms and graft rejection have shown that the -308 genotype is associated with liver allograft rejection. Other researchers have produced similarly inconsistent results; Jonsson et al. (2001) reported that the -308 polymorphism in the *TNF* gene promoter correlated with graft rejection, while Warlé et al. (2002) did not agree with the result.

IL-10 is a TH2-type anti-inflammatory cytokine that regulates immunoproliferation, the inflammatory response, and allograft tolerance. In this study, we found there was no association between the *IL10* -1082 allele and risk of AR. This result is partially in line with the results from other studies (Liu et al., 2011, 2012). In contrast, the authors of other studies (Mas et al., 2004; Liu et al., 2011) found that there was an association between the *IL10* SNP at position -1082 and graft rejection. The results from these studies and those from our research were strongly influenced by patient ethnicity. In the study reported by Mas et al. (2004), the *IL10* AA genotype in Caucasians at position -1082 occurred in 32.5% of patients, while in Han Chinese (Xie et al., 2008) it occurred in 88.2% of patients; the figure was 85.45% in our study.

Therefore, apparent discrepancies might be due to cytokine frequency differences among the different ethnic backgrounds.

TGF-β1 is a regulatory cytokine that can influence the Th1 and Th2 reactions of the immune response. In this study, we found that the *TGFB1* +869 polymorphism was significantly associated with liver graft rejection, which was consistent with the study by Warlé et al. (2002). It is possible that TGF-β1 itself contributes to the rejection reaction. Moreover, studies have shown that TGF-β1 is related to chronic rejection of liver, heart, kidney, and lung grafts (Suthanthiran et al., 2000; Warlé et al., 2002). In addition, the mRNA levels of *TGFB1* were elevated in acute rejection biopsy specimens from heart transplant recipients, and TGF-β1 protein expression levels increased in kidney biopsy specimens during AR (Shihab et al., 1995). Perhaps TGF-β1 itself is directly involved in AR through its role in apoptosis.

In conclusion, this study suggested that the *TGFB1* +869 cytokine polymorphism might play a role in AR in liver transplant patients, while the polymorphisms in the *IL10* and *TNF* genes did not have a major association with AR. Further studies with larger sample sizes are required to confirm this association and to explore the role of these polymorphisms in the regulation of cytokine gene expression.

Conflicts of interest

The authors declare no conflict of interest.

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