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Tumor necrosis factor α promoter polymorphisms at position −308 in Taiwanese chronic hepatitis C patients treated with interferon-alpha

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Abstract

A G-to-A polymorphic sequence at position −308 in the tumor necrosis factor alpha promoter (TNF308.2) might be associated with disease susceptibilities. To investigate the association between −308 TNF-α variants and pathogenesis of hepatitis C virus (HCV) infection and response to interferon-alpha (IFN-α) treatment for chronic hepatitis C (CHC), −308 TNF-α genotypes were determined in 100 unrelated Taiwanese CHC patients treated with IFN-α and in 100 unrelated healthy subjects. The distribution of −308 TNF-α genotypes did not differ between CHC patients and controls. Age, sex, HCV genotype, and the necroinflammatory activity of liver histopathology did not differ among CHC patients with different −308 TNF-α genotypes. Although pretreatment HCV RNA serum levels, aminotransferase and the rate of severe fibrosis decreased with the copy number of TNF308.2, the difference did not reach significance. We failed to demonstrate any association between −308 TNF-α promoter polymorphisms and response to IFN therapy, which was inversely correlated to liver cirrhosis, pretreatment serum HCV RNA levels and genotype 1b by using multivariate analysis. In conclusion, our findings suggest that −308 TNF-α promoter polymorphisms do not play a direct role in the susceptibility and pathogenesis of HCV infection, and in the response to interferon-alpha therapy for CHC.

Keywords: TNF-α promoter gene polymorphism; HCV; IFN

1. Introduction

Hepatitis C virus (HCV) is the major etiologic agent in parenterally transmitted non-A non-B hepatitis and frequently causes persistent infection leading to chronic liver disease and primary hepatocellular carcinoma (Alter et al., 1992). The mechanisms of the pathogenesis of the histological damage, in particular the immune-mediated lesions, in chronic hepatitis C (CHC) are still unclear (Gonzalez-Peralta et al., 1994). Treatment with interferon-alpha (IFN-α) was the first approved therapy but a sustained virological response could be achieved in only 20–35% of patients with IFN-monotherapy (Lauer and Walker, 2001; Yu et al., 2000). A number of factors have been indicated as predictors of the response to IFN-α therapy: infection with genotype other than 1b, lower levels of viremia and the absence of cirrhosis have been associated with a more favorable response (Lauer and Walker, 2001; Yu et al., 2000). IFN-α has direct antiviral effects and a number of immunomodulatory activities that can enhance antiviral immune responses (Peters, 1996). Response to IFN-α may also be determined by the host immune response (Koziel, 1999).

Tumor necrosis factor alpha (TNF-α), a prototype proinflammatory cytokine, exhibits a wide range of biological properties, including immune response to infectious agents and direct antiviral effects (Nokta et al., 1991; Rubin, 1992; Wong et al., 1988) and has been implicated as an important pathogenic mediator in a variety of liver condition (Bradham et al., 1998). Several studies showed that TNF-α plays a possible role in pathogenesis of acute and CHC, and

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Table 1
Diseases association of TNF-α promoter variants at position −308

Disease	Association	Reference
Cerebral malaria	TNF308.2 homozygotes	McGuire et al. (1994)
Mucocutaneous leishmaniasis	TNF308.2 homozygotes TNF308.2 heterozygotes	Cabrera et al. (1995)
Advanced primary biliary cirrhosis	TNF308.2 heterozygotes	Tanaka et al. (1999)
Severe *Plasmodium falciparum* malaria	TNF308.2 heterozygotes TNF308.2 homozygotes	Wattavidanage et al. (1999)
Typhoid fever	TNF308.2 heterozygotes	Dunstan et al. (2001)
Systemic lupus erythematosus	TNF308.2 heterozygotes TNF308.2 homozygotes	Rood et al. (2000)
Severe hepatitis C recurrence after liver transplantation	TNF308.2 heterozygotes TNF308.2 homozygote	Rosen et al. (1999)
Type C cirrhosis	TNF308.2 allele	Yee et al. (2000)
Chronic active hepatitis C	No association with TNF308.2	Hohler et al. (1998)
Interferon response for chronic hepatitis C	No association with TNF308.2	Yee et al. (2001)

Note: TNF308.2: A at −308.

also in IFN-α therapy (Tilg et al., 1992; Torre et al., 1994; Larrea et al., 1996; Kinnman et al., 2000). Recently, several genetic polymorphisms have been described in the human TNF-α promoter (Allen, 1999). Among them, the rare allele with G-to-A transition at position −308 (TNF308.2) is associated with high TNF-α levels in those with one or two TNF308.2 alleles (Galbraith et al., 1998; Wilson et al., 1997) and TNF308.2 heterozygotes and homozygotes have been related to poor prognosis in several diseases (Table 1). These observations suggest a role for the −308 TNF-α promoter polymorphism in altering TNF-α expression levels and possibly acting as a genetic susceptibility factor in certain immunogenetic-associated autoimmune and infectious diseases.

Little is known about the relationship between −308 TNF-α promoter polymorphisms and clinical manifestation of HCV infection. Previous evidence suggests that −308 TNF-α promoter polymorphisms might be involved in the pathogenesis and progression of CHC (Rosen et al., 1999; Yee et al., 2000). However, conflicting data have been reported on the association between TNF308.2 allele and chronic active hepatitis C (Hohler et al., 1998) or response to IFN therapy (Yee et al., 2001). To investigate the role of −308 TNF-α genotypes on susceptibility to HCV infection, the severity of chronic HCV infection and HCV response to IFN-α treatment, we determined the genotype frequencies of TNF-α promoter at position −308 in 100 unrelated Taiwanese CHC patients and in 100 unrelated healthy subjects as a control.

2. Materials and methods

2.1. Patients

One hundred consecutive unrelated Taiwanese patients undergoing IFN-α therapy for CHC at the Kaohsiung Medical University Hospital between 1996 and 1997 were enrolled in the study. These included 52 males and 48 females, aged between 18 and 65 years (mean 45.5 ± 11.7 years). All were positive for HCV antibodies and serum HCV RNA, and negative for hepatitis B surface antigen. Liver histology, assessed blindly by two pathologists showed chronic hepatitis of different severity in 85 patients and cirrhosis in 15. Disease activity grade and fibrosis stage were quantitatively scored according to the histological activity index (HAI) (Knodell et al., 1981). The present study was approved by the ethics committee of Kaohsiung Medical University Hospital. After they had given their informed consent, all patients were treated with recombinant IFN-α 2b given intramuscularly, 6 megaunits, thrice weekly for 24 weeks. The presence of HCV RNA in the serum was assessed every 3 months. Sustained response was defined as clearance of serum HCV RNA at the end of the therapy and 6 months after the cessation of therapy. All other patients were classified as non-responders. One hundred unrelated healthy subjects served as controls.

2.2. Detection/quantification of serum HCV RNA and genotyping

Detection of serum HCV RNA was performed using a standardized automated qualitative reverse transcription polymerase chain reaction assay (COBAS AMPLICOR HCV Test, version 2.0; Roche, Branchburg, NJ, USA). The detection limit was 50 IU/ml. HCV genotypes 1a, 1b, 2a, 2b and 3a were determined by amplification of the core region using genotype-specific primers described by Okamoto et al. (1993). Forty-three patients were infected by HCV type 1b, 35 by type 2a, 10 by type 2b, 7 by mixed types, and 5 had an indeterminate HCV type. Serum HCV RNA levels were measured by using the branched DNA assay (Quantiplex HCV RNA 2.0, Bayer, Emeryville, CA), performed strictly in accordance with the manufacturer's instructions. The quantification range was 0.2–120 million equivalents of HCV RNA per ml.

Table 2
Genotype distribution for TNF-α promoter variants at position −308 in control and chronic hepatitis C subjects

Genotype	Controls ($n = 100$)		Chronic hepatitis C ($n = 100$)		P
	n	%	n	%	
TNF308.1/308.1	73	73	74	74	NS
TNF308.1/308.2	25	25	23	23	NS
TNF308.2/308.2	2	2	3	3	NS

Note: TNF308.1: G at −308; TNF308.2: A at −308. NS: no significance.

2.3. −308 TNF-α promoter genotyping

Genomic DNA was purified from whole blood by the QIAamp blood kit (Qiagen, Valencia, CA, USA), and the quantity and purity was assessed by spectroscopic absorbance at 260 and 280 nm. The −308 TNF-α promoter polymorphisms were determined by method previously described by Wilson et al. (1992). Briefly, a 107-bp stretch of the TNF-α promoter was amplified by PCR using two primers (sense: 5′-AGGCAATAGGTTTTGAGGGCCAT-3′; antisense: 5′-TCCTCCCTGCTCCGATTCCG-3′). The PCR product was then digested with *Nco*I for 24 h at 37 °C and was analyzed on a 4% Metaphor™ agarose (FMC BioProducts, Rockland, ME). Homozygote TNF308.1 showed one fragment of 87 bp. Homozygote TNF308.2 showed a single 107-bp fragment. Heterozygote TNF308.1/308.2 showed fragments of 87 and 107 bp.

2.4. Laboratory tests

Second-generation HCV antibody and hepatitis B surface antigen were detected with commercially available enzyme-linked immunosorbent assay kits (Abbott, North Chicago, IL). Serum asparate aminotransferase (AST) and alanine aminotransferase (ALT) were measured on a multichannel autoanalyzer.

2.5. Statistical analyses

Frequency was compared between groups using the chi-square test with Yate's correction or Fisher's exact test. For all tests a $P \leq 0.05$ was considered to be significant. Group means were compared using the Student's t-test. Serum HCV RNA levels were expressed as the mean ± standard deviation after logarithmic transformation of original values. Stepwise logistic regression was used to analyze factors associated with response to IFN-α in CHC patients. All procedures were performed by using the package SAS statistical software (SAS Institute, Cary, NC).

3. Results

Genotype frequencies of −308 TNF-α promoter variants were listed in Table 2. No difference was found between controls and CHC patients. Age, sex, and distribution of HCV genotype did not differ among three groups of CHC patients with different TNF308 genotype (Table 3). Although serum levels of pretreatment HCV RNA, AST and ALT decreased with the copy number of TNF308.2 allele, this association did not reach significance. The genotype distribution for TNF308 was not related to the necroinflammatory activity of liver histopathology according to HAI score (Table 4). There was a trend toward an association between increased frequency of severe fibrosis (score 3 or 4) and TNF308.1 allele copy number ($P = 0.18$, chi-square with linear trend).

Thirty-six CHC patients achieved sustained response to IFN-α treatment. This was significantly inversely associated, in univariate analysis, with mean pretreatment serum HCV RNA levels and presence of cirrhosis but not with TNF308 genotype (Table 5). Further analysis by stepwise logistic regression model, confirmed this association for pretreatment serum HCV RNA levels, HCV genotype 1b and liver cirrhosis with odds ratio (95% confidence interval)

Table 3
Demographic, clinical and virological features associated with genotype distribution for TNF-α promoter variants at position −308 in 100 chronic hepatitis C patients

Factors	No.	TNF308 genotype			P
		TNF308.1/308.1 n (%) ($N = 74$)	TNF308.1/308.2 n (%) ($N = 23$)	TNF308.2/308.2 n (%) ($N = 3$)	
Sex (M/F)	52/48	42/32	9/14	1/2	NS
Age		45.7 ± 11.9	45.0 ± 12.0	43.3 ± 9.6	NS
Pretreatment serum HCV RNA levels (log equivalent/ml)		6.00 ± 0.69	5.90 ± 0.73	5.60 ± 0.53	NS
Pretreatment AST levels (IU/l)		73.0 ± 121.5	56.8 ± 32.4	36.7 ± 25.7	NS
Pretreatment ALT levels (IU/l)		92.5 ± 116.4	77.6 ± 55.0	51.0 ± 57.3	NS
HCV genotype					NS
1b	43	30 (69.8)	10 (23.3)	3 (7.0)	
Non-1b	57	44 (77.2)	13 (22.8)	0 (0)	

Note: TNF308.1: G at −308; TNF308.2: A at −308; HCV: hepatitis C virus; AST: asparate aminotransferase; ALT: alanine aminotransferase; NS: no significance; TNF: tumor necrosis factor.

Table 4
Relationship between genotype distribution for TNF-α promoter variants at position −308 and histological activity index score of 100 chronic hepatitis C patients

TNF308 genotype	No.	Histological activity index score				
		Necroinflammatory activity, *n* (%)			Fibrosis, *n* (%)*	
		Minimal (score 0–3) (*n* = 37)	Mild (score 4–8) (*n* = 59)	Moderate (score 9–12) (*n* = 4)	Mild (score 0–2) (*n* = 72)	Severe (score 3–4) (*n* = 28)
TNF308.1/308.1	74	28 (37.8)	42 (56.8)	4 (5.4)	51 (68.9)	23 (31.1)
TNF308.1/308.2	23	7 (30.4)	16 (69.9)	0 (0)	18 (78.3)	5 (21.7)
TNF308.2/308.2	3	2 (66.7)	1 (33.3)	0 (0)	3 (100)	0 (0)

Note: TNF: tumor necrosis factor; TNF308.1: G at −308; TNF308.2: A at −308.

Table 5
Factors associated with response to interferon-α in 100 chronic hepatitis C patients

Factors	No.	Non-responders *n* (%) (*N* = 64)	Sustained responders *n* (%) (*N* = 36)	*P*
Sex (M/F)	52/48	34/30	18/18	NS
Age		46.3 ± 9.8	44.1 ± 14.7	NS
Liver histology				
Non-cirrhotic	85	51 (60.0)	34 (40.0)	<0.05[a]
Cirrhotic	15	13 (86.7)	2 (13.3)	
HCV genotype				
1b	43	31 (72.1)	12 (27.9)	NS
Non-1b	57	33 (57.9)	24 (42.1)	
Pretreatment serum HCV RNA levels (log equivalent/ml)		6.14 ± 0.69	5.67 ± 0.60	0.001[a]
Pretreatment serum ALT levels (IU/l)		79.3 ± 64.4	103.0 ± 150.8	NS
TNF308 genotype				NS
TNF308.1/308.1	74	46 (62.2)	28 (37.8)	
TNF308.1/308.2	23	16 (69.6)	7 (30.4)	
TNF308.2/308.2	3	2 (66.7)	1 (33.3)	

Note: HCV: hepatitis C virus; ALT: alanine aminotransferase; NS: no significance; TNF: tumor necrosis factor; TNF308.1: G at −308; TNF308.2: A at −308.
[a] Statistical significance.

of 0.230 (0.100–0.529), 0.259 (0.085–0.788) and 0.140 (0.022–0.874), respectively.

4. Discussion

The association between the susceptibility to various diseases and −308 TNF-α promoter polymorphism suggests that this may be functionally relevant in vivo (Table 1). The results concerning the transcriptional changes due to the −308 TNF promoter polymorphism provide a possible explanation for the associations observed between TNF308.2 allele(s) and elevated TNF levels (Galbraith et al., 1998; Wilson et al., 1997). Previous data indicate that susceptibility to HCV infection and development of chronicity correlate with the strength and extent of T cell response and the frequency of TNF-α and IFN-gamma producing Th1-positive cell (Takaki et al., 2000). We hypothesized that TNF-α allele(s) associated with higher cytokine production (Wilson et al., 1997; Kwiatkowski et al., 1990; Castes et al., 1993; Galbraith et al., 1998) may correlate with self-limited disease. Nevertheless, the genotype distribution of −308 TNF promoter polymorphism between healthy controls and CHC patients in Taiwan did not differ in the present study, arguing against a possible correlation between TNF308 polymorphisms and viral persistence. However, this might be better investigated if one could enroll as a control group the patients who had recovered spontaneously from infection, a population difficult to find.

We also analyzed the possible association between −308 TNF-α genotypes and clinical manifestations of CHC. Although serum levels of pretreatment HCV RNA, AST and ALT decreased with the copy number of TNF308.2 allele, shown to have higher cytokine production (Wilson et al., 1997; Kwiatkowski et al., 1990; Castes et al., 1993; Galbraith et al., 1998), this association did not reach significance probably because of limited cases in the TNF308.2 heterozygote and homozygote groups. We were unable to identify any link between −308 TNF-α promoter polymorphisms and histological severity of chronic HCV infection. This is consistent with previous report by Hohler et al. (1998) but not with the findings of Rosen et al. (1999). In these

studies, a strong association between the presence within the donor organ of the TNF308.2 allele and increased susceptibility for severe HCV recurrence after transplant was observed in 56% of patients with at least one TNF308.2 allele developing severe HAI score (≥10) versus less than 10% of recipients of donor livers without TNF308.2 (Rosen et al., 1999). TNF308.2 was also observed to confer a 3.2-fold risk of cirrhosis for patients with chronic HCV infection (Yee et al., 2000). However, most patients with TNF308.2 homozygotes had mild necroinflammatory histological activity and fibrosis score in our series, although the difference did not reach significance due to limited cases. Since both a cohort effect and intrinsic properties of HCV genotypes are responsible for the pathogenesis of HCV infection (Yu et al., 2001), patients' age and infected HCV genotype might mask the effect of cytokine on the disease progression.

Looking at the possible role of −308 TNF-α genotypes in predicting HCV response to IFN therapy, we could confirm the associations of pretreatment serum HCV RNA levels, HCV genotype 1b and absence of cirrhosis with response to IFN-α treatment in CHC patients, previously reported by others (Lauer and Walker, 2001; Yu et al., 2000). High pretreatment serum levels of TNF-α may play a role in the resistance to IFN-α therapy (Larrea et al., 1996). This suggests that −308 TNF-α promoter polymorphisms, probably altering TNF-α expression, may act as a genetic susceptibility factor in antiviral therapy. However, our study failed to demonstrate any association between −308 TNF-α promoter variants and response to IFN therapy. These results are in agreement with the observation by Yee et al. that there was no correlation with −308 TNF-α promoter polymorphisms and response to IFN-α in combination with ribavirin therapy for CHC patients.

In conclusion, our findings suggest that −308 TNF-α promoter polymorphisms does not play a direct role in the susceptibility of HCV infection, in the pathogenesis of chronic HCV infection, and in the response to IFN-α therapy for CHC. TNF308 variants may not influence TNF expression (Brinkman et al., 1996) or that could be somehow regulated also by other nearby highly polymorphic major histocompatibility complex loci (D'Alfonso and Richiardi, 1994). The very low frequency of the TNF308.2 homozygotes might also contribute to the lack of clinical significance of TNF308 variants in CHC observed in the present study. Functional studies in a larger population characterizing TNF-α transcriptional activity in CHC patients carrying TNF308 variant allele(s) may help to clarify the role of −308 TNF-α promoter polymorphisms on susceptibility of HCV infection and the response to IFN-α therapy.

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