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Association of TGF-β1 Codon 25 (G915C) Polymorphism With Hepatitis C Virus Infection

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Cytokines play a key role in the regulation of immune responses. In hepatitis C virus infection (HCV), the production of abnormal cytokine levels appears to contribute to the progression of the disease, viral persistence, and affects response to therapy. Cytokine genes are polymorphic at specific sites, and certain polymorphisms located within coding/regulatory regions have been shown to affect the overall expression and secretion of cytokines. The aim of the present study was to identify potential markers of cytokines genes associated with the susceptibility to HCV infection. The cohort was composed of 128 individuals infected by HCV and 94 healthy controls. Genotyping was carried out by PCR-SSP. The distributions of the following polymorphisms were compared in these groups: TNF-α (−308G/A [rs1800629]), TGF-β1 (codon 10 T/C [rs1982073], codon 25 G/C [rs1800471]), IL-10 (−1082 A/G [rs 1800896]; −819T/C [rs1800871]; −592A/C [rs 1800872]), IL-6 (−174G/C [rs1800795]), and IFN-γ (+874T/A [rs2430561]). This study demonstrated a statistically significant difference in the frequency of TGF-β1 codon 25 polymorphism between healthy subjects and those infected with HCV. No associations were observed between polymorphisms of TNF-α, IFN-γ, IL-10, TGF-β1 codon 10, and IL-6 and HCV infection. These findings suggest that TGF-β1 codon 25 polymorphism could be a host genetic factor associated with susceptibility to HCV infection. ***J. Med. Virol. 80:58–64, 2008.*** © 2007 Wiley-Liss, Inc.

KEY WORDS: hepatitis C; genes; cytokines; polymorphism (genetics)

INTRODUCTION

Hepatitis C virus (HCV) is a major cause of chronic liver disease [Shepard et al., 2005]. An estimated 180 million people worldwide are carriers of HCV [Dai et al., 2006]. Approximately 80% of infected patients fail to clear the virus and progress to chronic hepatitis. Some of those with chronic HCV infection may progress to liver cirrhosis and eventually hepatocellular carcinoma [Lauer and Walker, 2001]. In addition to viral genotypes and environmental/behavioral factors (coinfections or excessive alcohol intake), host genetic factors are believed to influence the outcome of HCV infection [Yee, 2004].

Cytokines play an important role in defense against viral infection [Koziel, 1999]. In HCV infection, several studies have described associations between elevated blood levels of interleukin-6 (IL-6) and transforming growth factor-β (TGF-β) and development of disease pathology [Malaguarnera et al., 1997; Oyanagi et al., 1999; Tsushima et al., 1999; Lapinski, 2001]. The production of abnormal levels of interleukin-10 (IL-10) and tumour necrosis factor-α (TNF-α) has been associated with spontaneous elimination of HCV infection, fibrogenesis, and even resistance to interferon-α (IFN-α) therapy [Larrea et al., 1996; Kuzushita et al., 1997; Nelson et al., 1997]. Studies on liver tissue revealed that intrahepatic mRNA levels for interferon-γ (IFN-γ) were higher in chronic HCV infection and the level of its expression correlated with the degree of histologic injury as well as the likelihood of non-responsiveness to IFN-α therapy [Napoli et al., 1996].

Although immune responses are known to play a key role in both HCV clearance and the development of infection, the impact of host genetic factors and its effect on the clinical outcome of HCV infection have not been elucidated [Thursz, 2001; Yee, 2004; Richardson et al., 2005; Hwang et al., 2006].

There are significant differences between individuals in their ability to produce cytokines after in vitro stimulation of peripheral blood leukocytes [Turner

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et al., 1995; Awad et al., 1998; Bidwell et al., 1998; Pravica et al., 1999]. Such differences can be attributed to several molecular mechanisms including variations in transcription, translation, and secretion pathways [Bidwell et al., 1999, 2001]. The maximal capacity of cytokine production in individuals has a major genetic component. A potential mechanism was described involving polymorphisms within the regulatory regions or signal sequences of cytokine genes [Turner et al., 1997; Wilson et al., 1997; Fishman et al., 1998; Pravica et al., 2000]. These genetic polymorphisms were shown to affect the overall expression and secretion of cytokines both in vitro and sporadically in vivo systems. Associations between polymorphisms in cytokine genes and inflammation, allograft rejection, autoimmune, and infectious diseases have been reported [Awad et al., 1998; Fishman et al., 1998; Powell et al., 2000; Ben-Ari et al., 2003; Fernandez-Mestre et al., 2004; Bendicho et al., 2005; Laguila Visentainer et al., 2005]. The role of polymorphisms in TGF-β1, IFN-γ, IL-6, IL-10, and TNF-α in the pathogenesis of HCV infection has been investigated, but some of these results are contradictory [Hohler et al., 1998; Yee et al., 2000; Tambur et al., 2001; Rosen et al., 2002; Vidigal et al., 2002; Barrett et al., 2003; Abbas et al., 2005]. This discrepancy may be due to ethnic differences in the populations examined, leading to a differential distribution of these cytokines genes polymorphisms.

Genetic variability in cytokine genes may account for susceptibility to HCV infection. In particular, inheritance of allelic or genotype variations in the cytokines genes may predispose an individual to HCV infection in an ethnically homogeneous cohort, with a predominance of African descendents. To investigate this hypothesis, polymorphisms in the cytokine-encoding genes TNF-α (rs1800629), IL-10 (rs1800896; rs1800871; rs1800872), IL-6 (rs1800795), IFN-γ (rs2430561), and TGF-β1 (rs1 982073; rs1800471) were analyzed in patients with HCV infection and in healthy controls.

MATERIALS AND METHODS

Study Population

The study involved 128 Brazilian patients with HCV infection who attended the Osvaldo Cruz Foundation in order to confirm infection by detection of circulating HCV RNA between March and October 2004. Out of the patients examined, the mean age was 45.84 ± 10.61 years (range 22–64 years) and 94/128 were males. The HCV genotype distribution was as follows: 1 in 87 (68%) patients, 2 in 5 (3.9%) patients, and 3 in 36 (28.1%) patients. All patients did not have any of the following exclusion criteria: hepatitis B or HIV infection, or history of any disease to severely compromise immune function. The patients could not be stratified according to the stage of HCV infection. The control group was comprised of 94 healthy blood donors. All of the patients and the control group were residents of Salvador and the surrounding region in the State of Bahia and were ethnically homogeneous. All individuals enrolled at the study had a similar mixed genetic background of European, African, and American origin. Sera and whole blood were aliquoted within 2 hr and stored immediately at −70°C until use. Informed consent was obtained from all subjects and the Ethics Committee of the Osvaldo Cruz Foundation approved the study protocol.

HCV-RNA and HCV Genotyping

HCV-RNA detection and genotyping were performed as described previously [Chomczynski and Sacchi, 1987; Davidson et al., 1995].

DNA Extraction

Genomic DNA was isolated from fresh peripheral blood mononuclear cells using the QIAamp DNA blood protocol according to the manufacturer's instructions (Qiagen Ltd, Crawley, UK).

PCR Sequence-Specific Primer

PCR amplification of TNF-α, TGF-β, IL-10, IL-6, and IFN-γ alleles and internal control, the human β-globin gene, were carried out according to the manufacturer's recommendations (Cytokine Genotyping Tray, One Lambda, Canoga Park, CA). Briefly, after addition of the appropriate primer pairs, salts, buffer, and Taq polymerase, the samples were subjected to PCR. Amplification conditions were 1 cycle of 130 sec at 96°C, dropping to 63°C for an additional 60 sec; 9 cycles of 10 sec at 96°C e 60 sec at 63°C; and the final 20 cycles, which included a three-temperature ramp—denaturation for 10 sec at 96°C, annealing for 50 sec at 59°C, followed by an extension step of 30 sec at 72°C. The amplified products were analyzed by horizontal agarose gel electrophoresis and visualized by staining with ethidium bromide and exposure to ultraviolet light. Interpretation of PCR results was based on the presence or the absence of a specific amplified fragment.

Cytokine Gene Polymorphism

Single nucleotide polymorphisms were analyzed in five cytokine genes for genotype and phenotype assignment. Specifically, for TNF-α, the presence of a G or A nucleotide in position −308 of the promoter region generates three potential genotypes corresponding to two different phenotypes. The A/A and G/A genotypes are associated with the production of high levels of TNF-α while the G/G genotype is associated with low levels of TNF-α [Wilson et al., 1997]. For IL-6 promoter, we determined the nucleotide modification at position −174. Both the G/G and G/C genotypes correlate with the ability to produce high levels of IL-6, whereas C/C is associated with low IL-6 production [Fishman et al., 1998]. An additional polymorphism in the gene IFN-γ at position +874 (T/A) was identified. The homozygous T genotype is associated with the ability to produce high levels of IFN-γ, the heterozygous T/A genotype with intermediate levels, and the homozygous A genotype

with low levels [Pravica et al., 1999]. For TGF-β1, two single nucleotide polymorphisms (SNPs) in the coding region were analyzed: codon 10 (+869) T/C, and codon 25 (+915) C/G. Potentially, there are nine different combinations of these SNPs, which give rise to three different phenotypes: high, intermediate, and low producers of TGF-β1 [He et al., 1998]. Three different polymorphisms were analyzed in the IL-10 promoter region: position −1082 (G/A), position −819 (C/T), and position −592 (A/C). These produce three haplotypes GCC, ACC, and ATA corresponding to three different secretion phenotypes: high, intermediate, and low producers of IL-10 [Turner et al., 1997; Tagore et al., 1999].

Statistical Analysis

Statistical analysis was carried out using the Statistical Analysis System (SAS 8.0). Significant differences between the observed frequencies of alleles/genotypes/phenotypes in patients with HCV infection and in healthy blood donors were assessed using the Chi-Square test or Fisher's exact test. All reported *P*-values were two-sided, $P < 0$, 05 were considered significant. To verify Hardy–Weinberg expectations, the software program BioEstat 3.0 was used.

RESULTS

A total of 128 patients infected with HCV were typed for SNPs in five cytokine genes. Table I presents allelic frequencies among patients infected with HCV and healthy blood donors. Table II summarizes the frequency of cytokine genotypes in the two groups and Table III summarizes the cytokine phenotypes, deduced from genetic polymorphisms in the five cytokines in both groups.

There was a significant association between a SNP in the TGF-β1 gene and HCV infection. Statistically significant differences were found in allele frequency of TGF-β1 (codon 25 G) when HCV-infected patients were compared with the healthy control group ($P = 0.0005$; OR = 2.9; 95% CI = 1.6–5.6; Table I). The high-producing TGF-β1 (codon 25) genotype was associated with HCV infection (Table II). Among 128 HCV-infected patients, 113 (88.3%) had the GG genotype compared to 64 (68.1%) of 94 healthy controls ($P = 0.0002$; OR = 3.7; 95% CI = 1.8–7.4). An association between the high-producing TGF-β1 phenotypes (T/T G/G and T/C G/G) and HCV infection became apparent when both codons were combined (Table III). Among HCV-infected patients, 94 (73.4%) had the high phenotype compared to 49 (52.1%) healthy controls ($P = 0.0015$; OR = 2.6; 95% CI = 1.4–4.9).

No other statistically significant differences were found in the distribution of the two TGF-β1 polymorphisms analyzed as combined haplotypes between HCV patients and healthy controls (data not shown).

None of the remaining cytokines SNPs were associated with HCV infection. Similarly, no significant differences in the frequency of cytokines genes polymorphisms were associated with HCV genotype distribution (data not shown).

DISCUSSION

This study investigated the distribution of the following cytokine genes SNPs: TNF-α (−308G/A), TGF-β1 (codon 10 T/C, codon 25 G/C), IL-10 (−1082A/G; −819T/C; −592A/C), IL-6 (−174G/C), IFN-γ (+874T/A), in order to determine whether any of these genetic factors correlated with the occurrence of hepatitis C in a Brazilian population.

TABLE I. Allelic Frequencies of Cytokines Genes Among Patients With Chronic HCV Infection and Healthy Blood Donors

Gene	Allele	Chronic HCV patients (n = 128)	Healthy blood controls (n = 94)	Statistical analysis
TGF-β				
Codon 10	T	139 (54.3%)	91 (48.4%)	NS
	C	117 (45.0%)	97 (51.6%)	
Codon 25	G	240 (93.7%)	157 (83.5%)	0.0005
	C	16 (6.3%)	31 (16.5%)	
IFN-γ (+874)				
	T	91 (35.5%)	63 (33.5%)	NS
	A	165 (64.5%)	125 (66.5%)	
IL-6 (−174)				
	G	203 (79.3%)	150 (79.8%)	NS
	C	53 (20.7%)	38 (20.2%)	
IL-10				
(−1082)	A	167 (65.3%)	119 (63.3%)	NS
	G	89 (34.7%)	69 (36.7%)	
(−819)	C	160 (62.5%)	120 (63.8%)	NS
	T	96 (37.5%)	68 (36.2%)	
(−592)	C	160 (62.5%)	120 (63.8%)	NS
	A	96 (37.5%)	68 (36.2%)	
TNF-α (−308)				
	G	217 (84.8%)	167 (77.7%)	NS
	A	39 (15.2%)	21 (22.3%)	

TABLE II. Genotypic Frequencies of Cytokines Genes Among Patients With Chronic HCV Infection and Healthy Blood Donors

Gene	Genotype	Chronic HCV patients (n = 128)	Healthy blood controls (n = 94)	Statistical analysis
TGF-β (codon 10)	T/T	37 (28.9%)	21 (22.4%)	
	T/C	65 (50.8%)	49 (52.1%)	NS
	C/C	26 (20.3%)	24 (25.5%)	
TGF-β (codon 25)	G/G	113 (88.3%)	64 (68.1%)	
	G/C	14 (10.9%)	29 (30.8%)	0.0002
	C/C	1 (0.8%)	1 (1.1%)	
IFN-γ (+874)	T/T	20 (15.6%)	9 (9.6%)	
	T/A	51 (39.8%)	45 (47.8%)	NS
	A/A	57 (44.6%)	40 (42.6%)	
IL-6 (−174)	G/G	80 (62.5%)	60 (63.8%)	
	G/C	43 (33.6%)	30 (31.9%)	NS
	C/C	5 (3.9%)	4 (4.3%)	
IL-10 (−1082)	G/G	17 (13.3%)	13 (13.8%)	
	G/A	55 (43.0%)	43 (45.8%)	NS
	A/A	56 (43.7%)	38 (40.4%)	
IL-10 (−819)	C/C	50 (39.1%)	36 (38.3%)	
	C/T	60 (46.9%)	48 (51.1%)	NS
	T/T	18 (14.0%)	10 (10.6%)	
IL-10 (−592)	C/C	50 (39.1%)	36 (38.3%)	
	C/A	60 (46.9%)	48 (51.1%)	NS
	A/A	18 (14.0%)	10 (10.6%)	
TNF-α (−308)	G/G	94 (73.4%)	73 (77.7%)	
	G/A	29 (22.7%)	21 (22.3%)	NS
	A/A	5 (3.9%)	0	

The results showed that the frequency of allele G at TGF-β1 codon 25 was significantly higher in patients with hepatitis C than in healthy controls. The TGF-β1 gene polymorphism at codon 25 is significantly associated with TGF-β1 production in vitro. After in vitro stimulation of peripheral blood mononuclear cells, patients with the arginine/arginine homozygous genotype produce substantially more TGF-β1 protein than individuals with the arginine/proline genotype [Awad et al., 1998]. TGF-β1 is a polypeptide that is potentially linked with fibrogenesis in the liver and also has a number of suppressive effects on cells of the immune system, including inhibition of T-cell proliferation and differentiation and negative effects on macrophage activation and dentritic cells maturation [Schuppan et al., 2003]. The CD4+CD25+ regulatory T-lymphocytes have been implicated in suppressing T-cell immune responses by secretion of TGF-β1 and IL-10 [Nakamura et al., 2001; Zheng et al., 2004; Fahlen et al., 2005]. A characteristic of HCV infection is the impairment of HCV-specific effector T-cell responses, thus it is intriguing to hypothesize that T-regulatory cells may play a role in the long-term persistence of HCV infection.

TABLE III. Phenotypic Frequencies of Cytokines Genes Among Patients With Chronic HCV Infection and Healthy Blood Donors

Gene	Phenotype	Chronic HCV patients (n = 128)	Healthy blood controls (n = 94)	Statistical analysis
TGF-β				
	High	94 (73.4%)	49 (52.1%)	0.0015
	Intermediate	26 (20.3%)	36 (38.3%)	NS
	Low	8 (6.3%)	9 (9.6%)	NS
IFN-γ				
	High	20 (15.6%)	9 (9.6%)	NS
	Intermediate	51 (39.8%)	45 (47.9%)	NS
	Low	57 (44.6%)	40 (42.5%)	NS
IL-6				
	High	123 (96.1%)	90 (95.7%)	NS
	Low	5 (3.9%)	4 (4.3%)	NS
IL-10				
	High	17 (13.3%)	13 (13.8%)	NS
	Intermediate	55 (43.0%)	43 (45.8%)	NS
	Low	56 (43.7%)	38 (40.4%)	NS
TNF-α				
	High	34 (26.6%)	21 (22.3%)	NS
	Low	94 (73.4%)	73 (77.7%)	NS

Sugimoto et al. [2003] reported a higher frequency of CD4+CD25+ regulatory T-cells in individuals with chronic hepatitis C compared to healthy controls. Cabrera et al. [2004] reported that depletion of CD4+ CD25+ cells in vitro enhanced HCV-specific CD4+ and CD8+ T-cell proliferation. In the same study, cytokine analysis suggested CD4+CD25+ T-cells secrete TGF-β1 and IL-10 and the inhibitory role for TGF-β1 was confirmed by anti-TGF-β1. A positive correlation was also detected between CD4+CD25+ T-cell frequency and HCV RNA titer, supporting the association between T-regulatory cells and persistence of infection. Kanto et al. [1997] reported that TGF depletion in vitro increases cytotoxic T-lymphocytes. It was demonstrated that TGF-β1 and IL-10 producing CD4+CD25+ T-regulatory cells are able to induce other activated CD4+ cells to become T-regulatory cells [Zheng et al., 2004]. TGF-β1 seems to act as an effector cytokine involved in the immunosuppressive function of T-regulatory cells in vitro and in vivo. In addition, TGF-β1 signaling in peripheral T-regulatory cells seems to be essential for the regulation of peripheral T-regulatory cell numbers and for their immunosuppressive function in vivo. Others studies suggested that the suppression of proliferation of CD8+ T-cells is contact-dependent [Boettler et al., 2005; Rushbrook et al., 2005]. However, in both of these studies proliferation was not inhibited by anti-TGF-β antibody. These data suggested that assays of different CD8+ T-cells functions may identify distinct activities of membrane-bound and secreted TGF-β1 by CD4+CD25+ T-cells. Further investigations are required to elucidate potential differences in the activity of CD4+CD25+regulatory T-cells on HCV-specific CD4+ and CD8+T-cells.

NK cells produce IFN, and their proliferation and cytotoxicity are critical for viral clearance. Kimura et al. [2006] suggested that low TGF-β1 producers might have less suppression of NK cells and be more likely to resolve HCV infection. They reported that −509C SNP was significantly linked to higher HCV clearance rates and to lower transcriptional activity.

Theoretically, it is plausible that TGF-β1 suppressive effects on the immune response in high production phenotype individuals may be more pronounced and contributes to HCV infection. One possible mechanism is that the activation of T-regulatory cells is stronger in these subjects. This may favor suppressive effects on TCD4+ and TCD8+ cells and impairment of NK cells functions. Further studies are required to confirm this hypothesis.

It has been established that TNF-α is an important cytokine in the immune pathogenesis of HCV infection particularly with regard to the noncytolytic control of viral replication [Biron, 1994; Tough et al., 1996]. Hohler et al. [1998] have reported such an association with a polymorphism in the TNF-α promoter (−238A) and chronic active hepatitis C infection. The latter study investigated the role of two TNF polymorphisms, one at position −238 and the other at position −308. While the −238 polymorphism was associated with HCV outcome, no such correlation was observed with the −308 polymorphism. Similarly this study demonstrated no correlation between the −308 polymorphism and the susceptibility to HCV infection.

IL-6 is considered to be a Th2 cytokine that functions both in innate and adaptative immunity. Mononuclear phagocytes, vascular endothelial cells, fibroblasts, and other cells, in response to inflammatory stimuli and to other cytokines, notably IL-1 and TNF-α, synthesize IL-6 [Diehl and Rincon, 2002]. Barrett et al. [2003] demonstrated that a significant proportion of patients with persistent HCV infection had a high production genotype, whereas those who cleared the virus had a low production genotype. The current study could not confirm this observation.

IL-10, produced mainly by macrophages, is a potent immunosuppressive cytokine that downregulates the expression of major histocompatibility complex (MHC) class I and class II molecules, as well as the production of Th1 cytokines [Pestka et al., 2004; Vicari and Trinchieri, 2004]. Thus is reasonable to assume that hepatitis C patients who produce high levels of IL-10 have less ability to control infection. The −1082A SNP is associated with reduced IL-10 production in vitro [Turner et al., 1997]. Vidigal et al. [2002] reported an association between inheritance of the IL-10 −1082 G/G genotype and susceptibility to chronic HCV infection, as this genotype was found more commonly in patients infected chronically than in healthy controls. However, not all studies support the influence of these polymorphisms. Three polymorphic sites in the IL-10 gene promoter were studied in two independent DNA banks, each with appropriate controls [Constantini et al., 2002]. No significant difference in the distribution of any of the polymorphisms was reported in either study set. Similarly, no significant difference in the distribution of the IL-10 gene polymorphisms was found in these study groups.

Similarly, no differences in the frequency of genotypes associated with IFN-γ polymorphisms were observed between infected patients and healthy controls.

Ethnicity influences greatly the distribution of cytokine gene polymorphism and these polymorphisms located within the promoter or other regulatory regions affect gene transcription and cause inter-individual variation in cytokine production. Allele frequency variation is such that, within each cytokine, there is a tendency for at least one population to present itself as different from the other groups. The present study contributes to the knowledge of cytokine genotype distribution in an ethnically homogeneous cohort.

Firstly, the data concerning TGF-β1 codon 25 polymorphism identified in this study must be confirmed in groups of patients of different origins to exclude a false positive result. On the other hand, the C allele of TGF-β1 codon 25 polymorphism may be interpreted as a protective allele against HCV infection or liver fibrosis. Additionally, this polymorphism may be in linkage disequilibrium with other SNPs within or outside the TGF-β1 gene, only acting as a marker of an extended

haplotype. Considering that only one to three SNPs for each gene have been studied, a genetic association may have been missed for the others genes. Based on these finding, a further analysis of others immune response genes polymorphisms in large series is required to elucidate the consequence of gene variants in HCV infection. A previous study demonstrated that the TGF-β1 polymorphism in codon 25 is associated with the progression of fibrosis in chronic HCV infection [Powell et al., 2000]. This preliminary study is the first report of a Brazilian cohort with HCV infection and clinical data of patients, as a progression of liver disease, is not available. Since TGF-β1 is profibrogenic and data about liver fibrosis are not provided for the studied population, cohorts with moderate/severe liver disease need to be studied to determine the role of these cytokine gene polymorphisms in the progression of HCV infection.

In summary, the results of this study suggest that the presence of the TGF-β1 G/G genotype at codon 25 was associated with HCV infection. Further studies are required to confirm the role of cytokine genotypes in terms of susceptibility to HCV infection. The knowledge of the genetic markers may contribute to the understanding of the host's immune response to HCV and allows the development of new therapeutic strategies.

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REFERENCES

Abbas Z, Moatter T, Hussainy A, Jafri W. 2005. Effect of cytokine gene polymorphism on histological activity index, viral load and response to treatment in patients with chronic hepatitis C genotype 3. World J Gastroenterol 11:6656–6661.

Awad MR, El-Gamel A, Hasleton P, Turner DM, Sinnott PJ, Hutchinson IV. 1998. Genotypic variation in the transforming growth factor-beta1 gene: association with transforming growth factor-beta1 production, fibrotic lung disease, and graft fibrosis after lung transplantation. Transplantation 66:1014–1020.

Barrett S, Collins M, Kenny C, Ryan E, Keane CO, Crowe J. 2003. Polymorphisms in tumour necrosis factor-alpha, transforming growth factor-beta, interleukin-10, interleukin-6, interferon-gamma, and outcome of hepatitis C virus infection. J Med Virol 71:212–218.

Ben-Ari Z, Mor E, Papo O, Kfir B, Sulkes J, Tambur AR, Tur-Kaspa R, Klein T. 2003. Cytokine gene polymorphisms in patients infected with hepatitis B virus. Am J Gastroenterol 98:144–150.

Bendicho MT, Guedes JC, Silva NN, Santana GO, dos Santos RR, Lyra AC, Lyra LG, Meyer R, Lemaire DC. 2005. Polymorphism of cytokine genes (TGF-beta1, IFN-gamma, IL-6, IL-10, and TNF-alpha) in patients with chronic pancreatitis. Pancreas 30:333–336.

Bidwell JL, Wood NA, Morse HR, Olomolaiye OO, Laundy GJ. 1998. Human cytokine gene nucleotide sequence alignments, 1998. Eur J Immunogenet 25:83–265.

Bidwell J, Keen L, Gallagher G, Kimberly R, Huizinga T, McDermott MF, Oksenberg J, McNicholl J, Pociot F, Hardt C, D'Alfonso S. 1999. Cytokine gene polymorphism in human disease: On-line databases. Genes Immun 1:3–19.

Bidwell J, Keen L, Gallagher G, Kimberly R, Huizinga T, McDermott MF, Oksenberg J, McNicholl J, Pociot F, Hardt C, D'Alfonso S. 2001. Cytokine gene polymorphism in human disease: On-line databases, supplement 1. Genes Immun 2:61–70.

Biron CA. 1994. Cytokines in the generation of immune responses to, and resolution of, virus infection. Curr Opin Immunol 6:530–538.

Boettler T, Spangenberg HC, Neumann-Haefelin C, Panther E, Urbani S, Ferrari C, Blum HE, von Weizsacker F, Thimme R. 2005. T cells with a CD4+CD25+ regulatory phenotype suppress in vitro proliferation of virus-specific CD8+ T cells during chronic hepatitis C virus infection. J Virol 79:7860–7867.

Cabrera R, Tu Z, Xu Y, Firpi RJ, Rosen HR, Liu C, Nelson DR. 2004. An immunomodulatory role for CD4(+)CD25(+) regulatory T lymphocytes in hepatitis C virus infection. Hepatology 40:1062–1071.

Chomczynski P, Sacchi N. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156–159.

Constantini PK, Wawrzynowicz-Syczewska M, Clare M, Boron-Kaczmarska A, McFarlane IG, Cramp ME, Donaldson PT. 2002. Interleukin-1, interleukin-10 and tumour necrosis factor-alpha gene polymorphisms in hepatitis C virus infection: An investigation of the relationships with spontaneous viral clearance and response to alpha-interferon therapy. Liver 22:404–412.

Dai CY, Chuang WL, Chang WY, Chen SC, Lee LP, Hsieh MY, Hou NJ, Lin ZY, Huang JF, Wang LY, Yu ML. 2006. Tumor necrosis factor-alpha promoter polymorphism at position −308 predicts response to combination therapy in hepatitis C virus infection. J Infect Dis 193:98–101.

Davidson F, Simmonds P, Ferguson JC, Jarvis LM, Dow BC, Follett EA, Seed CR, Krusius T, Lin C, Medgyesi GA, Kiyo Kawa H, Olim G, Duraisamy G, Cuypers T, Saeed AA, Teo D, Conradie J, Kew MC, Lin M, Nuchaprayoon C, Ndimbie OK, Yap PL. 1995. Survey of major genotypes and subtypes of hepatitis C virus using RFLP of sequences amplified from the 5' non-coding region. J Gen Virol 76: 1197–1204.

Diehl S, Rincon M. 2002. The two faces of IL-6 on Th1/Th2 differentiation. Mol Immunol 39:531–536.

Fahlen L, Read S, Gorelik L, Hurst SD, Coffman RL, Flavell RA, Powrie F. 2005. T cells that cannot respond to TGF-beta escape control by CD4(+)CD25(+) regulatory T cells. J Exp Med 201:737–746.

Fernandez-Mestre MT, Gendzekhadze K, Rivas-Vetencourt P, Layrisse Z. 2004. TNF-alpha-308 A allele, a possible severity risk factor of hemorrhagic manifestation in dengue fever patients. Tissue Antigens 64:469–472.

Fishman D, Faulds G, Jeffery R, Mohamed-Ali V, Yudkin JS, Humphries S, Woo P. 1998. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. J Clin Invest 102:1369–1376.

He B, Xu C, Yang B, Landtblom AM, Fredrikson S, Hillert J. 1998. Linkage and association analysis of genes encoding cytokines and myelin proteins in multiple sclerosis. J Neuroimmunol 86: 13–19.

Hohler T, Kruger A, Gerken G, Schneider PM, Meyer zum Buschenfelde KH, Rittner C. 1998. Tumor necrosis factor alpha promoter polymorphism at position -238 is associated with chronic active hepatitis C infection. J Med Virol 54:173–177.

Hwang Y, Chen EY, Gu ZJ, Chuang WL, Yu ML, Lai MY, Chao YC, Lee CM, Wang JH, Dai CY, Shian-Jy Bey M, Liao YT, Chen PJ, Chen DS. 2006. Genetic predisposition of responsiveness to therapy for chronic hepatitis C. Pharmacogenomics 7:697–709.

Kanto T, Takehara T, Katayama K, Ito A, Mochizuki K, Kuzushita N, Tatsumi T, Sasaki Y, Kasahara A, Hayashi N, Hori M. 1997. Neutralization of transforming growth factor beta 1 augments hepatitis C virus-specific cytotoxic T lymphocyte induction in vitro. J Clin Immunol 17:462–471.

Kimura T, Saito T, Yoshimura M, Yixuan S, Baba M, Ji G, Muramatsu M, Kawata S. 2006. Association of transforming growth factor-beta 1 functional polymorphisms with natural clearance of hepatitis C virus. J Infect Dis 193:1371–1374.

Koziel MJ. 1999. Cytokines in viral hepatitis. Semin Liver Dis 19:157–169.

Kuzushita N, Hayashi N, Katayama K, Kanto T, Oshita M, Hagiwara H, Kasahara A, Fusamoto H, Kamada T. 1997. High levels of serum interleukin-10 are associated with a poor response to interferon treatment in patients with chronic hepatitis C. Scand J Gastroenterol 32:169–174.

Laguila Visentainer JE, Lieber SR, Lopes Persoli LB, Dutra Marques SB, Vigorito AC, Penteado Aranha FJ, de Brito Eid KA, Oliveira GB, Martins Miranda EC, Bragotto L, de Souza CA, 2005. Relationship between cytokine gene polymorphisms and graft-versus-host disease after allogeneic stem cell transplantation in a Brazilian population. Cytokine 32:171–177.

Lapinski TW. 2001. The levels of IL-1beta, IL-4 and IL-6 in the serum and the liver tissue of chronic HCV-infected patients. Arch Immunol Ther Exp (Warsz) 49:311–316.

Larrea E, Garcia N, Qian C, Civeira MP, Prieto J. 1996. Tumor necrosis factor alpha gene expression and the response to interferon in chronic hepatitis C. Hepatology 23:210–217.

Lauer GM, Walker BD. 2001. Hepatitis C virus infection. N Engl J Med 345:41–52.

Malaguarnera M, Di Fazio I, Romeo MA, Restuccia S, Laurino A, Trovato BA. 1997. Elevation of interleukin 6 levels in patients with chronic hepatitis due to hepatitis C virus. J Gastroenterol 32:211–215.

Nakamura K, Kitani A, Strober W. 2001. Cell contact-dependent immunosuppression by CD4(+)CD25(+) regulatory T cells is mediated by cell surface-bound transforming growth factor beta. J Exp Med 194:629–644.

Napoli J, Bishop GA, McGuinness PH, Painter DM, McCaughan GW. 1996. Progressive liver injury in chronic hepatitis C infection correlates with increased intrahepatic expression of Th1-associated cytokines. Hepatology 24:759–765.

Nelson DR, Lim HL, Marousis CG, Fang JW, Davis GL, Shen L, Urdea MS, Kolberg JA, Lau JY. 1997. Activation of tumor necrosis factor-alpha system in chronic hepatitis C virus infection. Dig Dis Sci 42: 2487–2494.

Oyanagi Y, Takahashi T, Matsui S, Takahashi S, Boku S, Takahashi K, Furukawa K, Arai F, Asakura H. 1999. Enhanced expression of interleukin-6 in chronic hepatitis C. Liver 19:464–472.

Pestka S, Krause CD, Sarkar D, Walter MR, Shi Y, Fisher PB. 2004. Interleukin-10 and related cytokines and receptors. Annu Rev Immunol 22:929–979.

Powell EE, Edwards-Smith CJ, Hay JL, Clouston AD, Crawford DH, Shorthouse C, Purdie DM, Jonsson JR. 2000. Host genetic factors influence disease progression in chronic hepatitis C. Hepatology 31:828–833.

Pravica V, Asderakis A, Perrey C, Hajeer A, Sinnott PJ, Hutchinson IV. 1999. In vitro production of IFN-gamma correlates with CA repeat polymorphism in the human IFN-gamma gene. Eur J Immunogenet 26:1–3.

Pravica V, Perrey C, Stevens A, Lee JH, Hutchinson IV. 2000. A single nucleotide polymorphism in the first intron of the human IFN-gamma gene: Absolute correlation with a polymorphic CA microsatellite marker of high IFN-gamma production. Hum Immunol 61: 863–866.

Richardson MM, Powell EE, Barrie HD, Clouston AD, Purdie DM, Jonsson JR. 2005. A combination of genetic polymorphisms increases the risk of progressive disease in chronic hepatitis C. J Med Genet 42:e45.

Rosen HR, McHutchison JG, Conrad AJ, Lentz JJ, Marousek G, Rose SL, Zaman A, Taylor K, Chou S. 2002. Tumor necrosis factor genetic polymorphisms and response to antiviral therapy in patients with chronic hepatitis C. Am J Gastroenterol 97:714–720.

Rushbrook SM, Ward SM, Unitt E, Vowler SL, Lucas M, Klenerman P, Alexander GJ. 2005. Regulatory T cells suppress in vitro proliferation of virus-specific CD8+ T cells during persistent hepatitis C virus infection. J Virol 79:7852–7859.

Schuppan D, Krebs A, Bauer M, Hahn EG. 2003. Hepatitis C and liver fibrosis. Cell Death Differ 10:S59–67.

Shepard CW, Finelli L, Alter MJ. 2005. Global epidemiology of hepatitis C virus infection. Lancet Infect Dis 5:558–567.

Sugimoto K, Ikeda F, Stadanlick J, Nunes FA, Alter HJ, Chang KM. 2003. Suppression of HCV-specific T cells without differential hierarchy demonstrated ex vivo in persistent HCV infection. Hepatology 38:1437–1448.

Tagore A, Gonsalkorale WM, Pravica V, Hajeer AH, McMahon R, Whorwell PJ, Sinnott PJ, Hutchinson IV. 1999. Interleukin-10 (IL-10) genotypes in inflammatory bowel disease. Tissue Antigens 54: 386–390.

Tambur AR, Ortegel JW, Ben-Ari Z, Shabtai E, Klein T, Michowiz R, Tur-Kaspa R, Mor E. 2001. Role of cytokine gene polymorphism in hepatitis C recurrence and allograft rejection among liver transplant recipients. Transplantation 71:1475–1480.

Thursz M. 2001. Genetic susceptibility in chronic viral hepatitis. Antiviral Res 52:113–116.

Tough DF, Borrow P, Sprent J. 1996. Induction of bystander T cell proliferation by viruses and type I interferon in vivo. Science 272: 1947–1950.

Tsushima H, Kawata S, Tamura S, Ito N, Shirai Y, Kiso S, Doi Y, Yamada A, Oshikawa O, Matsuzawa Y. 1999. Reduced plasma transforming growth factor-beta1 levels in patients with chronic hepatitis C after interferon-alpha therapy: Association with regression of hepatic fibrosis. J Hepatol 30:1–7.

Turner DM, Grant SC, Lamb WR, Brenchley PE, Dyer PA, Sinnott PJ, Hutchinson IV. 1995. A genetic marker of high TNF-alpha production in heart transplant recipients. Transplantation 60: 1113–1117.

Turner DM, Williams DM, Sankaran D, Lazarus M, Sinnott PJ, Hutchinson IV. 1997. An investigation of polymorphism in the interleukin-10 gene promoter. Eur J Immunogenet 24:1–8.

Vicari AP, Trinchieri G. 2004. Interleukin-10 in viral diseases and cancer: Exiting the labyrinth? Immunol Rev 202:223–236.

Vidigal PG, Germer JJ, Zein NN. 2002. Polymorphisms in the interleukin-10, tumor necrosis factor-alpha, and transforming growth factor-beta1 genes in chronic hepatitis C patients treated with interferon and ribavirin. J Hepatol 36:271–277.

Wilson AG, Symons JA, McDowell TL, McDevitt HO, Duff GW. 1997. Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. Proc Natl Acad Sci USA 94:3195–3199.

Yee LJ. 2004. Host genetic determinants in hepatitis C virus infection. Genes Immun 5:237–245.

Yee LJ, Tang J, Herrera J, Kaslow RA, van Leeuwen DJ. 2000. Tumor necrosis factor gene polymorphisms in patients with cirrhosis from chronic hepatitis C virus infection. Genes Immun 1:386–390.

Zheng SG, Wang JH, Gray JD, Soucier H, Horwitz DA. 2004. Natural and induced CD4+CD25+ cells educate CD4+CD25− cells to develop suppressive activity: The role of IL-2, TGF-beta, and IL-10. J Immunol 172:5213–5221.