INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Functional Gene Polymorphisms of Interleukin-10 are Associated with Liver Disease Progression in Japanese Patients with Hepatitis C Virus Infection

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Abstract

Objective The inter-individual difference in response to liver injury appears to be important in the progression of liver fibrosis. Interleukin 10 (IL-10) is an anti-inflammatory cytokine, and several functional gene polymorphisms have been found. The aim of this study was to examine the possible association of IL-10 polymorphisms with the progression of liver fibrosis in hepatitis C virus (HCV)-related chronic liver disease patients.

Methods We examined the IL-10 -1087 A/G and -824 T/C gene polymorphisms in 184 Japanese patients with HCV-related chronic liver disease: 94 chronic hepatitis (CH) and 90 with liver cirrhosis (LC).

Results There were no significant differences in the genotype distributions or allele frequencies of IL-10 -824 T/C and -1087 A/G between the CH and LC groups. However, among the cirrhotic patients, the lower transcriptional allele, -824 T homozygotes had significantly lower serum albumin and platelet counts, and a higher Child-Pugh score than the -824 C carriers, and the lower transcriptional allele, -1087 A homozygotes had a higher ICG-R 15 compared with -1087 G carriers. Haplotype analysis of IL-10 -1087/-824 showed no significant difference between the CH and LC groups, but the combinations of AT and AC haplotypes (AT/AT, AT/AC and AC/AC) had a significantly higher ICG-R 15 than the GC carriers.

Conclusion IL-10 lower transcriptional -824 T allele, -1087 A allele, and -1087/-824 haplotypes AT and AC are risk factors for the progression of liver fibrosis in HCV-related chronic liver disease.

Key words: interleukin-10, HCV, liver fibrosis, gene polymorphism, haplotype, liver function test

(Intern Med 50: 659-666, 2011)
(DOI: 10.2169/internalmedicine.50.4581)

Introduction

Hepatitis C virus (HCV)-related chronic liver disease is a major health problem and a key issue in antiviral research. Approximately 170 million people are infected with HCV worldwide (1). Chronic HCV infection commonly induces immune reactive inflammation, which results in continuous liver tissue damage and progression of liver fibrosis to cirrhosis. The mechanism of liver fibrosis is a highly dynamic process and the rate at which fibrosis develops shows substantial individual variation. Epidemic surveillance has shown that older age at infection, male gender, excessive alcohol consumption, obesity, and immune deficiency are all associated with a rapid progression to fibrosis (2). However, the promoting or protective factors of HCV-related liver fibrosis remain poorly understood. Recently, functional gene polymorphisms have been identified in inflammatory cytokines and some reports have shown possible relationships between these genotypes and the grade of tissue inflammation in HCV-related liver disease (3).

IL-10 is a multifunctional cytokine mainly regarded as one of the strong suppressors of pro-inflammatory and antiviral cytokine secretion, and occurs through various mechanisms including reduction of HLA class II expression and diminished production of IL-1α, IL-1β, IL-2, TNF-α, and IL-8 (4, 5). Administration of recombinant IL-10 reduces the immune response and fibrogenesis in patients with chronic

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Received for publication September 24, 2010; Accepted for publication December 6, 2010
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Table 1. Patient Profiles.

	CH	LC	p value
Age (years)	63.4 ± 10.9	66.2 ± 9.5	0.041 *
Gender (M/F)	54 / 40	45 / 45	0.31
Patelet (×10⁴/μL)	17.1 ± 8.9	10.2 ± 9.4	<0.0001*
Bilirubin (mg/dL)	0.75 ± 0.3	1.2 ± 0.7	<0.0001*
AST (IU/L)	56.2 ± 37.8	81.4 ± 42.1	<0.0001*
ALT (IU/L)	67.6 ± 60.2	69.6 ± 44.4	0.053
ALP (IU/L)	277.6 ± 104.2	382.9 ± 187.2	<0.0001*
γ-GTP (IU/L)	55.7 ± 49.9	77.3 ± 62.3	0.089
Albumin (g/dL)	4.0 ± 0.3	3.3 ± 0.5	<0.0001*
Prothronbin Time (%)	91.0 ± 17.3	71.9 ± 14.7	<0.0001*
ICG-R (%)	15.9 ± 6.7	31.6 ± 15.6	<0.0001*
Child-Pugh score	5.4 ± 0.8	6.5 ± 1.12	0.018*
Past history of IFN +	17 (18.9%)	8 (8.5%)	

*: $p < 0.05$
+: All of IFN treatments were resulted in non-responder
CH: chronic hepatitis, LC: liver cirrhosis

hepatitis C (6). As for the gene polymorphism of IL-10, three single nucleotide polymorphisms (SNPs) have been identified in the promoter region of -1087 A/G, -824 T/C and -597 A/C with respect to the transcription initiation site (7). It has been shown that -824 C allele and -1087 G allele have higher IL-10 transcriptional activities compared to -824 T and -1087 A alleles, respectively. The -597 A and C alleles are in tight genetic linkage disequilibrium with the position of -824 T and C alleles, respectively. Indeed, these SNPs at -1087, -824 and -597 consist of the haplotypes ATA, ACC and GCC, respectively (4). Of these haplotypes, the ATA and ACC haplotypes are associated with low levels of IL-10 production (8).

Previous reports have shown positive relationships between these IL-10 polymorphisms and various inflammatory and autoimmune diseases, such as systemic lupus erythematosus (9) and aggressive periodontitis (10). Some reports have examined IL-10 gene polymorphisms and viral chronic liver disease progression, but the results of these studies remain controversial.

In this study, we investigated the possible association of IL-10 polymorphisms with the progression of liver fibrosis in Japanese HCV-related chronic liver disease patients.

Methods

Subjects

Patients with HCV-related chronic liver disease (n=184) were enrolled in this study. CH and LC patient group profiles are listed in Table 1. All patients were Japanese and were positive for serum HCV-RNA. Patients were excluded if they had chronic hepatitis B infection, alcoholism, autoimmune liver disease, or primary biliary cirrhosis. The diagnosis of the stage of liver disease (CH or LC) was confirmed by laboratory tests and/or histological examination. Cirrhosis was diagnosed based on the clinical features, laboratory data, imaging tests (computer tomography) and/or histological features. Of the 184 patients, 94 patients were diagnosed with chronic hepatitis (mean age in years; 63.4± 10.9, 54 males and 40 females) and the other 90 were diagnosed with liver cirrhosis (mean age in years; 66.2±9.5, 45 males and 45 females). This study was approved by the Committee for the Ethics of Medical Experiments on Human Subjects of the Medical Faculty of Tottori University, and written informed consent was obtained from each subject before blood was collected.

DNA extraction

Genomic DNA was extracted from peripheral white blood cells using a DNA extraction kit (DNA Quick II: Dainippon Pharmaceutical, Osaka, Japan) according to the manufacturer's instructions.

Analysis of the IL-10 -824 T/C polymorphism

The IL-10 -824T/C polymorphism was analyzed by PCR, followed by restriction fragment length polymorphism (RFLP) (9). The PCR was carried out in a final volume of 50 μL: 2 μL (5 ng) of genomic DNA, 0.5 μL of 0.02 μM forward primer (5´-ATCCAAGACAACACTACTAA-3), 0.5 μL of 0.02 μM reverse primer (5´-TAAATATCCTCAAAGT TCC-3´), 4.0 μL of dNTPs, 25 mM $MgCl_2$, Taq polymerase (Takara Shuzo, Kusatsu, Japan), and 34.5 μL of sterile H_2O. The conditions for amplification were as follows: 95℃ for 3 min; 30 cycles of 95℃ for 30 s, 56℃ for 30 s, and 72℃ for 1 min; and 72℃ for 3 m. RFLP for IL-10 -824T/C was

Table 2. IL-10 -824/-1087 Genotype/allele Frequencies between CH and LC Patients.

-824T/C	Genotype				Allele frequency		
	T/T	T/C	C/C	p value	T	C	p value
CH	47%	41%	12%	0.276	0.67	0.33	0.424
LC	48%	47%	5%		0.71	0.29	

-1087A/G	Genotype				Allele frequency		
	A/A	A/G	G/G	p value	A	G	p value
CH	84%	15%	1%	0.283	0.92	0.08	0.121
LC	91%	9%	0%		0.96	0.04	

IL = Interleukin, CH = chronic hepatitis, LC = liver cirrhosis

carried out. In brief, a mixture of 12 μL of the PCR product, 12.5 μL of 2× buffer (40 mM Tris-HCl, 550 mM NaCl, 12 mM $MgCl_2$, 14 mM 2-Mercaptoethanol, pH 8.2) (Roche, Penzberg, Germany), and 0.5 μL of Mae III were incubated at 55℃ overnight. The mixture was loaded onto a polyacrylamide gel (total concentration 10%, concentration of cross-linker 3%, 1× TBE buffer: 89 mM Tris, 89 mM borate, and 2.2 mM EDTA). Electrophoresis was performed at 200 V for 70 min at 20℃ using 1× TBE buffer. Bands were visualized by silver staining.

Analysis of the IL-10 -1087 A/G polymorphism

The IL-10 -1087A/G polymorphism was analyzed by the allele-specific primer-polymerase chain reaction (ASP-PCR) method according Sugimoto et al (11). The PCR condition was as follows: 95℃ for 10 min; 35 cycles of 95℃ for 15 s, 61℃ for 5 s, and 72℃ for 18 s. The PCR amplicon was obtained only when the allele-specific primer matched its corresponding SNP sequence. Real-time SYBR-green PCR (Roche) was performed to detect the allele-specific amplicons.

Statistical analysis

Values were expressed as the mean ± SD. Differences in genotype distributions were determined with the χ^2 test. The differences of data between the 2 groups were assessed using the t test. A p value of <0.05 was regarded as statistically significant.

Results

Genotype distributions and allelic frequencies of IL-10 -824T/C and -1087A/G are shown in Table 2. The frequency of each genotype was in Hardy-Weinberg equilibrium. The IL-10 genotype distributions of our HCV-positive patients were similar to those previously reported in Japanese populations (11, 12).

There were no significant differences in the genotype distributions or allele frequencies of IL-10 -824 T/C and -1087 A/G between chronic hepatitis and liver cirrhosis. However, when examining the cirrhotic patients, the lower transcriptional allele, -824 T homozygote had significantly lower serum albumin and platelet counts, and a higher Child-Pugh score than the -824 C carriers, and the lower transcriptional allele, -1087 A homozygotes had a higher ICG-R 15 compared with -1087 G carriers (Table 3). IL-10 -1087 A/G and -824 T/C are in linkage disequilibrium and they are responsible for three different haplotypes: AT, AC and GC. Haplotype analysis of IL-10 -1087/-824 showed no significant difference between CH and LC (Table 4), but the combinations of -1087/-824 AT and AC haplotypes (AT/AT, AT/AC and AC/AC) showed significantly higher ICG-R 15 than the GC carriers (Table 5).

In addition, we examined the liver fibrosis progression speed between IL-10 haplotypes using the fibrosis indexes, which are based on the common clinical serum tests. We selected 52 CH patients who were followed up for 5 years; they were separated into progressive and non-progressive liver fibrosis groups according to the change in the FibroIndex (13) and Forns index (14) in that 5-year period. The progressive cases will show the index subtraction of the index value of enrolled point from that of 5 years later >0. Conversely, non-progressive cases will show the index subtraction ≤0.

The formulas of FibroIndex and Forns index used are as follows:

FibroIndex =1.738 -0.064 (platelet count $\times 10^4/mm^3$) + 0.005 (AST IU/L) + 0.463 (g-globulin g/dL)

Forns index =7.811 - 3.131 ln (platelet count) + 0.781 ln (GGT) +3.467 ln (age) - 0.014 (cholesterol)

In both fibrosis indexes, there were no significant differences in the ratio of progressive/non-progressive patients or in the index value changes between IL-10 haplotypes (Table 6, 7).

Discussion

Our results of the IL-10 genotype distributions and allele

Table 3. Clinical Findings according to IL-10 -824T/C and -1087G/A Genotypes in LC Patients

	IL-10 -824 T/C			IL-10 -1087 A/G		
	T homo	C carrier	p value	A homo	G carrier	p value
Gender (M/F)	20 / 23	25 / 22	0.527	39 / 42	5 / 3	0.439
Age (years)	66.6 ± 8	65.9 ± 10	0.711	66 ± 9.8	66 ± 9.4	0.957
Bilirubin (mg/dL)	1.4 ± 0.7	1.1 ± 0.7	0.188	1.2 ± 0.7	0.8 ± 0.3	0.112
Albumin (g/dL)	***3.1 ± 0.5***	***3.4 ± 0.6***	***0.006****	3.3 ± 0.5	3.5 ± 0.6	0.317
Prothrombin time (%)	69.0 ± 14.6	74.9 ± 15.3	0.069	70.8 ± 13.4	80.3 ± 22.7	0.078
Platelet ($\times 10^4/\mu L$)	***8.4 ± 4.4***	***10.7 ± 4.5***	***0.014****	10.3 ± 10.0	11.7 ± 5.7	0.703
ICG-R (%)	32.6 ± 14.4	29.6 ± 16.8	0.397	***31.8 ± 15.0***	***18.2 ± 5.5***	***0.029****
Child-Pugh score	***7.0 ± 1.3***	***6.0 ± 0.8***	***0.002****	6.5 ± 1.2	5.7 ± 0.6	0.216

*: $p < 0.05$

Table 4. IL-10 -1087/-824 Haplotypes Distribution and Frequency between CH and LC Patients.

	IL-10 Haplotype distribution (%)						
	AT/ AT	AT/ AC	AC/ AC	AT/ GC	AC/ GC	GC/ GC	
CH	40	26	8	11	2	1	
LC	41	32	4	7	1	0	
Transcriptional activity	Low			Intermediate / High			p value
CH	74			14			0.199
LC	77			8			

	IL-10 Haplotype frequency			
	AT	AC	GC	p value
CH	0.66	0.25	0.09	0.333
LC	0.71	0.24	0.05	
Transcriptional activity	Low		High	
CH	0.91		0.09	0.154
LC	0.95		0.05	

frequencies bear a good resemblance to those of former reports studied in Japanese populations (11, 12). On the other hand, some studies in Caucasians have reported higher IL-1 -1087 G and -824 C allele frequencies than those in Japanese or Eastern Asian populations (4, 8). These differences are due to the ethnic characteristics of the different populations.

In this study, we found no significant difference in the IL-10 genotypes or allele frequencies between chronic hepatitis and liver cirrhosis. We also found no relationship between the IL-10 haplotypes and the progression speed of liver fibrosis in CH patients observed for 5 years. Similar to our results, Abe et al (12) reported that the genotype frequencies at -824T/C and -1087A/G are similar between HCV-related CH and LC groups, and other reports have examined various ethnic groups also showing that genotype distributions of IL-10 -824T/C and -1087A/G are not significantly different between HCV-related chronic liver disease patients and healthy control groups (15-17).

In cirrhotic patients, we found that the patients with low transcriptional IL-10 genotypes (-1087 A/A and -824 T/T) and haplotypes (-1087/-824 AT and AC, respectively) show a relatively rapid progression toward liver failure. Some previous reports have revealed the significant relationship between liver function tests and liver fibrosis in chronic liver disease patients. Kusaka et al have shown that liver fibrosis progression has a significantly positive correlation with indocyanine green test and has negative correlations with serum albumin, cholinesterase, prothrombin time (18). Taken together, the liver reserve function could be associated with the progression of liver fibrosis. Knapp et al (4) also reported that the IL-10 -1087A/A genotype and the haplotype

Table 5. Clinical Findings according to IL-10 -1087/-824 Haplotype in HCV-related Liver Disease

	CH			LC		
	Low	Intermediate / High	p value	Low	Intermediate	p value
Gender (M/F)	22 / 26	8 / 5	0.315	38 / 39	5 / 3	0.713
Age (years)	63.5 ± 9.8	59.1 ± 14.0	0.191	65.9 ± 9.9	66.1 ± 9.4	0.967
Bilirubin (mg/dL)	0.76 ± 0.3	0.93 ± 0.2	0.107	1.2 ± 0.7	0.8 ± 0.3	0.108
Albumin (g/dL)	3.3 ± 0.5	3.5 ± 0.6	0.339	3.3 ± 0.5	3.5 ± 0.6	0.339
Prothrombin time (%)	89.5 ± 23.7	100.5 ± 13.2	0.223	70.8 ± 13.6	80.3 ± 22.7	0.083
Platelet ($\times 10^4/\mu L$)	39.6 ± 56.2	23.4 ± 27.0	0.320	9.3 ± 4.5	11.7 ± 5.7	0.179
ICG-R (%)	15.4 ± 5.0	12.3 ± 7.7	0.354	***31.6 ± 15.0***	***18.2 ± 5.5***	***0.033****
Child-Pugh score	-	-		6.6 ± 1.2	5.7 ± 0.6	0.203

*= p < 0.05

AC/AC and AT/AT homozygotes are more frequent among patients with rapid fibrosis. The present results suggest that IL-10 gene polymorphisms can affect the progression of the disease only when it is at the advanced liver fibrosis stage. Previous reports have shown a positive correlation between the grade of histological hepatic inflammation and the stage of hepatic fibrosis in HCV-related liver disease patients (17). Namely, pro- and anti-inflammatory cytokines play a principal role in the cirrhotic liver and the functional differences between the gene polymorphisms must be amplified to be detectable.

Some in vitro studies have revealed significant relationships between IL-10 expression levels and its promoter genotypes and haplotypes. For example, Edwards-Smith et al examined the production levels of IL-10 in peripheral blood mononuclear cells and showed that IL-10 promoter -1087, -824 and -597 haplotypes, GCC, ACC, and ATA are associated with high, intermediate, and low IL-10 production, respectively (8). And there are several lines of evidence that the IL-10 -1087A/G and -824T/C genotypes and the -1087/-824 haplotypes are relevant in their genetic capacity for IL-10 production, and -1087A, -824T, -1087/-824 AT haplotypes are associated with low IL-10 production, respectively, following in vitro stimulation of peripheral blood mononuclear cells (19). Patients with chronic HCV infection have an activated T-cell response cytokine pattern. CD4+ T cells have been implicated in both the damage of liver tissue and the perpetuation of chronic HCV infection. CD4+ cell responses are polarized into T-helper type 1 (Th1) and Th2 types. Th1 cells secrete IL-2, IL-4, IL-8, tumor necrosis factor α (TNF-α), and interferon gamma (IFN-γ), which are required for host antiviral immune responses (20). Th2 cells produce IL-5, IL-13, and IL-10, which facilitate antibody production and inhibit the Th1 response by reducing MHC-II expression, and inhibiting IL-1 α/β, IL-2, IL-8, and TNF-α production (5).

The significance of serum levels of IL-10 in patients with HCV-related liver disease is still debatable (21, 22). For example, Chen et al (23) examined the relationship between IL-10 gene polymorphisms and reported no impact of IL-10 gene polymorphisms on serum levels. Not only the circulating or ubiquitous immune-related cells but also the liver tissue-specific cells such as hepatocytes, sinusoidal endothelial cells, hepatic stellate cells, and Kupffer cells also secrete IL-10 and some of their IL-10 production levels seem to be sufficient enough to affect the grade of liver tissue inflammation (24, 25). Furthermore, a recent in vitro study showed that direct HCV interference on dendritic cell maturation resulted in the induction of IL-10 expression (26). All of this evidence leads to the hypothesis that the hepatic microenvironmental IL-10 level is extremely important for assessing its influence on liver tissue inflammation and fibrosis.

In contrast to HCV-related hepatitis, chronic HBV-infected patients with the low IL-10 production haplotype (AT) tended to be asymptomatic HBV carriers, even after adjusting for the seropositivity of the antibody to hepatitis B e antigen (27). The suppression of HBV replication by the Th1-related anti-viral immune system plays a major part in the protective effect of chronic liver disease B progression (28). Indeed, it has been reported that the serum HBV viral load is in proportion to the activity and progression of chronic hepatitis B infection (28-30).

Some former reports have revealed that an IL-10 high production genotype/haplotype is related to reduced complete HCV clearance by IFN treatment (31-33). On the other hand, several reports that have examined various ethnic groups found no relationship between IL-10 gene polymorphisms and sustained response to IFN treatment (34-36). In the present study, 25 patients had received IFN therapy before they enrolled in this study and all of these cases have resulted in being non-responders (NR) (Table 1). IL-10 -1087 and -824 genotype distributions and allele frequencies in the NR group were A/A 89%, A/G 8% and G/G 4%, with A 0.94 and G 0.06, and T/T 48%, T/C 39% and C/C 13%, with T 0.69 and C 0.31, respectively. The -1087/-824 haplotype frequencies of NR were AT 0.66, AC 0.30 and GC 0.04. There were no significant differences in the genotype distributions, allele or haplotype frequencies between the NR and the patient group who had no past history of IFN treatment. The details of the discrepancy in the results between IL-10 gene polymorphisms and IFN anti-viral response are still unknown. Abbas et al (37) suggested that the

Table 6. The 5-year Changes of FibroIndex between IL-10 -1087/-824 Haplotypes in CH Patients.

IL-10 haplotypes	Low (AT/AT, AT/AC, AC/AC) (n=39)	Intermediate / High (AT/GC, AC/GC, GC/GC) (n=8)	p value
FibroIndex difference (Mean ± SD)	0.007 ± 0.33	0.115 ± 0.55	0.463

IL-10 haplotypes		Low	Intermediate / High	p value
Category of Liver fibrosis progression	Non-progressive	19 (49%)	4 (50%)	0.943
	Progressive	20 (50%)	4 (50%)	

IL: Interleukin, CH: chronic hepatitis

Table 7. The 5-year Changes of Forns Index between IL-10 -1087/-824 Haplotypes in CH Patients

IL-10 haplotypes	Low (AT/AT, AT/AC, AC/AC) (n=41)	Intermediate / High (AT/GC, AC/GC, GC/GC) (n=8)	p value
Forns index difference (Mean ±SD)	0.312 ± 0.91	0.661 ± 1.61	0.394

IL-10 haplotypes		Low	Intermediate / High	p value
Category of Liver fibrosis progression	Non-progressive	11 (28%)	3 (38%)	0.716
	Progressive	29 (72%)	5 (62%)	

recent IFN treatment strategies including IFN modifications (recombinant and PEGlyated), the duration of treatment, and combination with ribavirin administration might affect the results of these studies. For example, ribavirin administration promotes Th1 lymphocytes and suppresses IL-10 production (38). However, discrepancies still remain between the studies that have involved identical IFN treatment programs (33, 36). The combinations of gene polymorphisms of other cytokines suggest that this is the key factor in association studies. IL-10 belongs to the cytokine superfamily (IL-10, -19, -20, -22, -24, -26, -28 and -29) and they all share their receptors with each other. For example, IL-28A and B (alternatively named interferon lambda 2 and 3, respectively) bind to IFNLR1 (IL-28R) and IL-10R2, which is shared with IL-10, -22 and -26 (39). Recently, the genome-wide association study has revealed a significant relationship between gene polymorphisms of IL-28B and sustained HCV clearance by IFN alpha therapy (40). For example, the minor G allele in the non-coding region rs8099917 (8kb upstream of IL-28B gene) strongly associates with unsuccessful IFN alpha therapy. The functions of these polymorphisms have not been elucidated clearly, but some reports have suggested the possible relationship between the risk allele G and the lower expression level of IL-28B (41, 42). These functional polymorphisms of the IL-10 superfamily, which relate to their expression profiles may affect their competitive binding to the common IL-10R cell surface receptor because IL-10R surface expression is relatively low on immune-related cells such as hepatospecific lymphocytes and kupffer cells (43, 44) The interrelationships between the members of the IL-10 superfamily gene polymorphisms are expected to be studied in the future.

In conclusion, we have identified the IL-10 lower transcriptional -824 T allele, -1087 A allele and -1087/-824 haplotypes AT and AC as the risk factors for the progression of liver fibrosis in HCV-related chronic liver disease. Further studies using a larger sample size of Japanese patients with HCV-related chronic liver disease are required to confirm the association of IL-10 polymorphisms with the progression of liver fibrosis.

The authors state that they have no Conflict of Interest (COI).

References

1. Baldo V, Baldovin T, Trivello R, Floreani A. Epidemiology of HCV infection. Curr Pharm Des **14**: 1646-1654, 2008.
2. Marcellin P, Asselah T, Boyer N. Fibrosis and disease progression in hepatitis C. Hepatology **36**: S47-S57, 2002.
3. McCaughan GW, George J. Fibrosis progression in chronic hepatitis C virus infection. Gut **53**: 318-321, 2004.
4. Knapp S, Hennig WBJ, Frodsham AJ, et al. Interleukin-10 promoter polymorphisms and the outcome of hepatitis C virus infection. Immunogenetics **55**: 362-369, 2003.
5. Tilg H, Kaser A, Moschen AR. How to modulate inflammatory cytokines in liver diseases. Liver International **26**: 1029-1039, 2006.
6. Nelson DR, Lauwers GY, Lau JY, Davis GL. Interleukin 10 treatment reduces fibrosis in patients with chronic hepatitis C: a pilot trial of interferon nonresponders. Gastroenterology **118**: 655-660, 2000.
7. Yee LJ, Tang J, Gibson AW, Kimberly R, Van Leeuwen DJ, Kaslow RA. Interleukin 10 polymorphisms as predictors of sustained response in antiviral therapy for chronic hepatitis C infection. Hepatology **33**: 708-712, 2001.
8. Edwards-Smith CJ, Jonsson JR, Purdie DM, Bansal A, Shorthouse C, Powell EE. Interleukin-10 promoter polymorphism predicts initial response of chronic hepatitis C to interferon alfa. Hepatology **30**: 526-530, 1999.
9. Mok CC, Lanchbury JS, Chan DW, Lau CS. Interleukin-10 promoter polymorphisms in southern Chinese patients with systemic lupus erythematosus. Arthritis Rheum **41**: 1090-1095, 1998.
10. Reichert S, Machulla HKG, Klapproth J, et al. The interleukin-10 promoter haplotype ATA is a putative risk factor for aggressive periodontitis. J Periodont Res **43**: 40-47, 2008.
11. Sugimoto M, Furuta T, Shirai N, et al. Effects of interleukin-10 gene polymorphism on the development of gastric cancer and peptic ulcer in Japanese subjects. J Gastroenterol Hepatol **22**: 1443-1449, 2007.
12. Abe H, Aizawa Y, Takahashi H, Amano K, Zeniya M. Single nucleotide polymorphisms in the interleukin-10 gene promoter and clinical outcome of chronic hepatitis C virus infection. Jikeikai Med J **50**: 159-166, 2003.
13. Koda M, Matunaga Y, Kawakami M, Kishimoto Y, Suou T, Murawaki Y. FibroIndex, a practical index for predicting significant fibrosis in patients with chronic hepatitis C. Hepatology **45**: 297-306, 2007.
14. Forns X, Ampurdanès S, Llovet JM, et al. Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. Hepatology **36**: 986-992, 2002.
15. Chan HLY, Tse AML, Chim AML, et al. Association of cytokine gene polymorphisms and liver fibrosis in chronic hepatitis B. J Gastroenterol Hepatol **23**: 783-789, 2008.
16. Bouzgarrou N, Hassen E, Farhat K, et al. Combined analysis of interferon-gamma and interleukin-10 gene polymorphisms and chronic hepatitis C severity. Hum Immunol **70**: 230-236, 2009.
17. Abbott WGH, Rigopoulou E, Haigh P, et al. Single nucleotide polymorphisms in the interferon-gamma and interleukin-10 genes do not influence chronic hepatitis C severity or T-cell reactivity to hepatitis C virus. Liver Int **24**: 90-97, 2004.
18. Kusaka K, Harihara Y, Torzilli G, et al. Objective evaluation of liver consistency to estimate hepatic fibrosis and functional reserve for hepatectomy. J Am Coll Surg **191**: 47-53, 2000.
19. Zhang LJ, Wang XZ. Interleukin-10 and chronic liver disease. World J Gastroenterol **12**: 1681-1685, 2006.
20. Nelson DR, Tu Z, Soldevila-Pico C, et al. Long-term interleukin 10 therapy in chronic hepatitis C patients has a proviral and anti-inflammatory effect. Hepatology **38**: 859-868, 2003.
21. Kakumu S, Okumura A, Ishikawa T, et al. Serum levels of IL-10, IL-15 and soluble tumor necrosis factor-alpha (TNF-a) receptors in type C chronic liver disease. Clin Exp Immunol **109**: 458-463, 1997.
22. Gramenzi A, Andreone P, Loggi E, et al. Cytokine profile of peripheral blood mononuclear cells from patients with different outcomes of hepatitis C virus infection. J Viral Hepat **12**: 525-530, 2005.
23. Chen TY, Hsieh YS, Wu TT, et al. Impact of serum levels and gene polymorphism of cytokines on chronic hepatitis C infection. Translational Res **150**: 116-121, 2007.
24. Wan S, LeClerc JL, Schmartz D, et al. Hepatic release of interleukin-10 during cardiopulmonary bypass in steroid-pretreated patients. Am Heart J **133**: 335-339, 1997.
25. Thompson KC, Trowern A, Fowell A, et al. Primary rat and mouse hepatic stellate cells express the macrophage inhibitor cytokine interleukin-10 during the course of activation in vitro. Hepatology **28**: 1518-1524, 1998.
26. Saito K, Ait-Goughoulte M, Truscott SM, et al. Hepatitis C virus inhibits cell surface expression of HLA-DR, prevents dendritic cell maturation, and induces interleukin-10 production. J Virol **82**: 3320-3328, 2008.
27. Miyazoe S, Hamasaki K, Nakata K, et al. Influence of interleukin-10 gene promoter polymorphisms on disease progression in patients chronically infected with hepatitis B virus. Am J Gastroenterol **97**: 2086-2092, 2002.
28. Feld JJ, Ayers M, El-Ashry D, Mazzulli T, Tellier R, Heathcote EJ. Hepatitis B virus DNA prediction rules for hepatitis B e antigen-negative chronic hepatitis B. Hepatology **46**: 1057-1070, 2007.
29. Chen W, Shi M, Shi F, et al. HBcAg-pulsed dendritic cell vaccine induces Th1 polarization and production of hepatitis B virus-specific cytotoxic T lymphocytes. Hepatol Res **39**: 355-365, 2009.
30. Chen CJ, Yang HI, Iloeje UH. Hepatitis B virus DNA levels and outcomes in chronic hepatitis B. Hepatology **49**: S72-S84, 2009.
31. Mangia A, Santoro R, Piattelli M, et al. IL-10 haplotypes as possible predictors of spontaneous clearance of HCV infection. Cytokine **25**: 103-109, 2004.
32. Yee LJ, Tang J, Gibson AW, Kimberly R, Van Leeuwen DJ, Kaslow RA. Interleukin 10 polymorphisms as predictors of susutained response in antiviral therapy for chronic hepatitis C infection. Hepatology **33**: 708-712, 2001.
33. Morgan TR, Lambrecht RW, Bonkovsky HL, et al. DNA polymorphisms and response to treatment in patients with chronic hepatitis C: results from the HALT-C trial. J Hepatol **49**: 548-556, 2008.
34. Constantini PK, Wawrzynowicz-Syczewska M, Clare M, et al. 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, 2002.
35. Vidigal PG, Germer JJ, Zein NN. 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**: 526-530, 2002.
36. Chuang JY, Yang SS, Lu YT, et al. IL-10 promoter gene polymorphisms and sustained response to combination therapy in Taiwanese chronic hepatitis C patients. Dig Liver Dis **41**: 424-430, 2009.
37. Abbas OM, Abdel-Rahman MH, Omar NA, Badran HM, Amir EM. Interleukin-10 promoter polymorphisms in hepatitis C patients with and without Schistosoma mansoni co-infection. Liver Int **29**: 1422-1430, 2009.
38. Cramp ME, Rossol S, Chokshi S, Carucci P, Williams R, Naoumov NV. Hepatitis C virus-specific T-cell reactivity during interferon and ribavirin treatment in chronic hepatitis C. Gastroenterology **118**: 346-355, 2000.
39. Commins S, Steinke JW, Borish L. The extended IL-10 superfamily: IL-10, IL-19, IL-20, IL-22, IL-24, IL-26, IL-28, and IL-

29. J Allergy Clin Immunol **121**: 1108-1111, 2008.

40. Rauch A, Kutalik Z, Descombes P, et al. Genetic variation in IL28 B is associated with chronic hepatitis C and treatment failure: a genome-wide association study. Gastroenterology **138**: 1338-1345, 2010.

41. Suppiah V, Moldovan M, Ahlenstiel G, et al. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. Nat Genet **41**: 1100-1104, 2009.

42. Tanaka Y, Nishida N, Sugiyama M, Tokunaga K, Mizokami M. lambda-Interferons and the single nucleotide polymorphisms: A milestone to tailor-made therapy for chronic hepatitis C. Hepatol Res **40**: 449-460, 2010.

43. Carson WE, Lindemann MJ, Baiocchi R, et al. The functional characterization of interleukin-10 receptor expression on human natural killer cells. Blood **85**: 3577-3585, 1995.

44. Knolle PA, Uhrig A, Protzer U, et al. Interleukin-10 expression is autoregulated at the transcriptional level in human and murine Kupffer cells. Hepatology **27**: 93-99, 1998.