chronic HCV infection are susceptible to bacterial infections [9], it remains uncertain whether the induction of hyporesponsiveness to TLR ligands is dependent on viral and/or host factors.

Patients in the advanced stages of chronic liver disease owing to viral infection or alcohol abuse exhibit increased susceptibility to microbial infections and are considered to be immunocompromised hosts [10]. Impaired activation of TLR signalling may also be involved in the susceptibility to infections in patients with liver cirrhosis. Riordan *et al.* [11] showed reduced production of TNF in response to microbial antigens by peripheral blood mononuclear cells (PBMCs) from liver cirrhosis patients. Thus, it is likely that suppression of TLR signalling pathways underlies the susceptibility to bacterial infection in patients with liver cirrhosis. However, it remains unknown whether blunted TLR activation is a common feature of APCs isolated from liver cirrhosis patients with chronic hepatitis B virus (HBV) or HCV infection. More importantly, the clinical parameters associated with suppression of TLR activation have not been identified. In this study, we examined the activation of TLR signalling using PBMCs from patients with chronic HBV or HCV infection. We found that PBMCs from patients with HCV, but not HBV, infection have defective proinflammatory cytokine responses to TLR ligands and that impaired TLR signalling in PBMCs from HCV-infected patients is strongly correlated with the severity of liver dysfunction.

MATERIALS AND METHODS

Patients and cells

Ethical permission for this study was granted by the review board of Kinki University. Healthy controls (n = 17), treatment-naïve patients with chronic HCV infection (n = 17) and treatment-naïve patients with chronic HBV infection (n = 10) were enrolled in the study after informed consent was obtained. Chronic HBV and HCV infection was confirmed by a positive result for serum HBsAg and serum anti-HCV antibodies, respectively. PBMCs (2×10^6/mL) isolated from each patient were stimulated with core antigen (5 μg/mL; Biodesign International, Saco, ME, USA), peptidoglycan (PGN, 10 μg/mL; Sigma-Aldrich, Saint Louis, MO), Pam_3CSK4 (PAM, 10 μg/mL; InvivoGen, San Diego, CA, USA) or lipopolysaccharide (LPS, 1 μg/mL; Sigma-Aldrich) as previously described [12,13]. Culture supernatants were collected 24 h after stimulation.

Virological assays

Serum levels of HCV core antigen were measured using an enzyme immunoassay as previously described [14]. Briefly, 100 μL of serum was mixed with 50 μL of a pretreatment solution containing 0.3% Triton X-100, 1.5% 3-[(3-cholamidopropyl) dimethylammonio]propanesulfonic acid and 15% sodium dodecyl sulphate. After incubation at 56 °C for 30 min, 100 μL of the pretreatment solution was added to wells coated with monoclonal antibodies against HCV core antigen (c11-3 and c11-7) and filled with 100 μL of reaction buffer (1% bovine serum albumin, 5 mM ethylenediaminetetraacetic acid, 0.1 M NaCl, 3% mouse serum, 0.3% Triton X-100, 0.1 M phosphate buffer, pH 7.2). The mixture was incubated for 90 min at room temperature and the wells washed with buffer. Alkaline phosphatase-conjugated monoclonal antibodies against the HCV core antigen (c11-10 and c11-14) were then added to the wells and incubated for 30 min at room temperature. After washing, CDP star (Tropix Inc., Bedford, MA, USA) was added and incubated for 15 min at room temperature. The relative chemiluminescence was measured and the concentration of HCV core antigen was read according to a standard curve generated using recombinant HCV core antigen. The concentration was expressed as femtomol per L (fmol/L), and the cut-off value was set at 20 fmol/L. Serum levels of HBV-DNA were measured using a transcription-mediated amplification assay (Chugai Diagnostics Science Co., Ltd., Tokyo, Japan), which has a quantitative range between 3.7 and 8.7 log genome equivalents/mL.

Measurement of clinical parameters

Platelet counts and haemoglobin levels were determined using an automated hematology analyzer (CELL-DYN Sapphire; Abbott Japan Co, Tokyo, Japan). Serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase, total bilirubin and albumin were measured using an automated analyzer (Hitachi 7700; Hitachi Instruments Service Co, Tokyo, Japan). Prothrombin time (PT) was measured using an automated coagulometer (Coagrex 800; Sysmex Co., Kobe, Japan).

Enzyme-linked immunosorbent assays

Measurement of IL-6 and IL-8 was performed using enzyme-linked immunosorbent assay (ELISA) kits (BD Pharmingen, San Diego, CA, USA) as previously described [12,13,15].

Statistical analysis

Student's t-test was used to evaluate the significance of any differences between groups. Statistical analysis was performed using StatView v.4.5 (Abacus Concepts). A P value < 0.05 was regarded as statistically significant. Correlations between cytokine production and clinical parameters were analysed using the Spearmen rank correlation. Platelet counts and serum levels of PT, ALT, and core antigen were also analysed.

RESULTS

Impaired production of IL-6 and IL-8 in response to Toll-like receptor ligands by peripheral blood mononuclear cells isolated from patients with chronic hepatitis C virus infection

In our previous report [8], we showed that peripheral blood monocytes isolated from patients with chronic HCV infection exhibited defective production of IL-6 and IL-8 upon stimulation with TLR ligands, presumably because of chronic exposure to the TLR2 agonist, HCV core antigen. Thus, chronic activation of TLR2 by the HCV core antigen is associated with hyporesponsiveness to TLR ligands during chronic HCV infection. However, it remains unknown whether peripheral blood monocytes from patients with chronic HBV infection also show defective proinflammatory cytokine responses to TLR ligands. In this study, to address this issue, we stimulated PBMCs isolated from patients with HCV or HBV infection with TLR2 and TLR4 ligands. Table 1 lists the characteristics of the patients enrolled in the study. As shown in Fig. 1, PBMCs from patients with HCV infection showed reduced production of both IL-6 and IL-8 upon stimulation with TLR2 (PGN, PAM, core antigen) and TLR4 (LPS) ligands compared with healthy controls. In contrast, stimulation with TLR ligands induced comparable levels of IL-6 and IL-8 production by PBMCs from patients with chronic HBV infection and healthy controls. This suggests that hyporesponsiveness to TLR ligands is a specific feature of PBMCs in patients with chronic HCV infection.

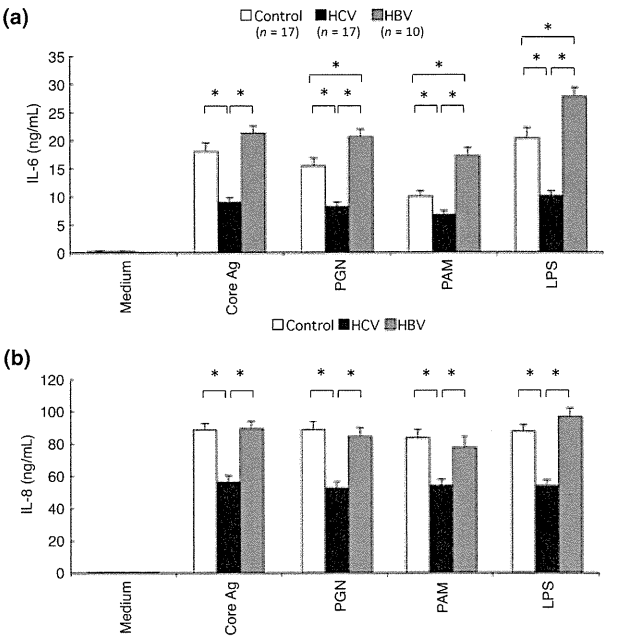

Fig. 1 Production of IL-6 and IL-8 by peripheral blood mononuclear cells isolated from patients with chronic viral hepatitis. PBMCs (2×10^6/mL) isolated from patients with hepatitis B virus or hepatitis C virus infection or healthy controls were stimulated with core antigen (Core-Ag, 5 μg/mL), PGN (10 μg/mL), Pam_3CSK4 (10 μg/mL) or lipopolysaccharide (1 μg/mL). Culture supernatants were collected 24 h after stimulation, and the levels of IL-6 (a) and IL-8 (b) were measured. Results are expressed as the means ± SE. *$P < 0.05$.

Table 1 Clinical characteristics of patients

	HBV (n = 10)	HCV (n = 17)	P value
Age (years)	56 ± 11 (34–65)	64 ± 12 (36–79)	0.021
Gender (male/female)	7/3	11/6	0.887
Child-pugh grade (A/B/C)	8/1/1	13/4/0	0.907
Laboratory data			
AST (IU/L)	60 ± 26 (26–115)	88 ± 40 (26–175)	0.053
ALT (IU/L)	75 ± 78 (18–250)	89 ± 54 (30–216)	0.132
γ-GTP (IU/L)	70 ± 75 (11–252)	111 ± 135 (13–556)	0.295
Total bilirubin (mg/dL)	1.3 ± 0.6 (0.6–2.5)	0.9 ± 0.4 (0.5–1.7)	0.066
Albumin (g/dL)	3.7 ± 0.6 (2.4–4.6)	3.6 ± 0.5 (2.3–4.2)	0.940
Prothrombin time (%)	76 ± 24 (30–108)	77 ± 9.5 (65–98)	0.692
Haemoglobin (g/dL)	13.5 ± 1.7 (10.2–16.6)	13.2 ± 1.7 (9.9–15.5)	0.808
Platelet count ($\times 10^4/\mu L$)	11.8 ± 6.7 (5.1–25)	12.8 ± 7.7 (4.5–29.3)	0.920
HCV Core-Ag (fmol/L)	N.D.	2766 ± 3118 (20–9500)	N.D.
HBV-DNA (LGE/mL)	5.9 ± 1.9 (3.7–8.5)	N.D.	N.D.

Data are expressed as the median ± standard deviation (range) or frequency. ND, not done; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase.

Impaired production of IL-6 in response to Toll-like receptor ligands by peripheral blood mononuclear cells isolated from patients with chronic hepatitis C virus infection does not correlate with serum levels of the core antigen

We next examined whether there was a correlation between the dose of core antigen and the production of proinflammatory cytokines, because stimulation of monocytes with core antigens leads to hyporesponsiveness to TLR ligands via induction of IRAK-M expression (one of the most potent negative regulators of TLR signalling) [8]. As shown in Fig. 2, there was no significant correlation between serum levels of the core antigen and TLR-induced IL-6 or IL-8 production, suggesting that the serum concentration of the core antigen is not primarily responsible for hyporesponsiveness to TLR ligands during chronic HCV infection. In addition, no correlation was found between patient age and the production of proinflammatory cytokines in patients with chronic HBV and HCV infection (data not shown).

Impaired production of IL-6 by peripheral blood mononuclear cells isolated from patients with chronic hepatitis C virus infection in response to Toll-like receptor ligands is associated with liver dysfunction

As TLR-induced proinflammatory cytokine responses are not associated with serum concentrations of the core antigen, we next determined the host factors that showed a strong correlation with hyporesponsiveness to TLR ligands. As shown in Fig. 3, no correlation was found between serum levels of ALT and TLR-induced IL-6 production. In contrast, reduced production of IL-6 by PBMCs from chronic HCV patients was associated with decreased platelet counts and prolonged PT (Figs 4 & 5). Because these parameters are very sensitive markers of liver function [10], the data suggest that liver dysfunction is associated with hyporesponsiveness to TLR ligands in patients with chronic HCV infection. No correlation was seen between platelet counts and TLR-induced IL-6 production in patients with chronic HBV infection (data not shown).

DISCUSSION

In this study, we examined proinflammatory cytokine responses to TLR ligands by PBMCs isolated from patients with chronic HBV or HCV infection. Surprisingly, reduced production of IL-6 and IL-8 in response to TLR ligands was observed in PBMCs from HCV-infected patients, but not those from HBV-infected patients. This finding suggests that HCV-associated immunomodulatory proteins play an important role in the generation of hyporesponsiveness to TLR ligands. In this regard, we previously reported that TLR2 activation of the HCV core antigen results in reduced pro-inflammatory cytokine responses to subsequent stimulation with TLR ligands in human APCs [8]. Given the fact that peripheral blood APCs from patients with chronic HCV infection are always exposed to circulating HCV core

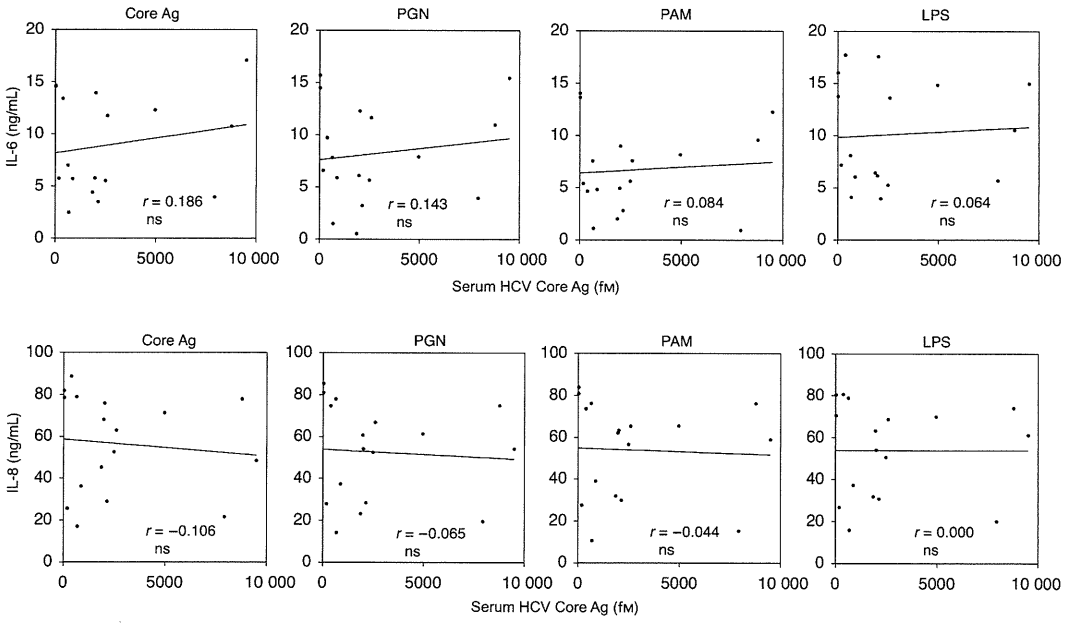

Fig. 2 Production of IL-6 and IL-8 by peripheral blood mononuclear cells (PBMCs) isolated from patients with chronic HCV infection does not correlate with serum concentrations of hepatitis C virus (HCV) core antigen. Production of IL-6 and IL-8 by PBMCs in response to HCV core antigen and Toll-like receptor ligands was compared with the serum levels of HCV core antigen in each patient with chronic HCV infection. No correlation was seen between IL-6 and IL-8 production and serum concentrations of HCV core antigen.

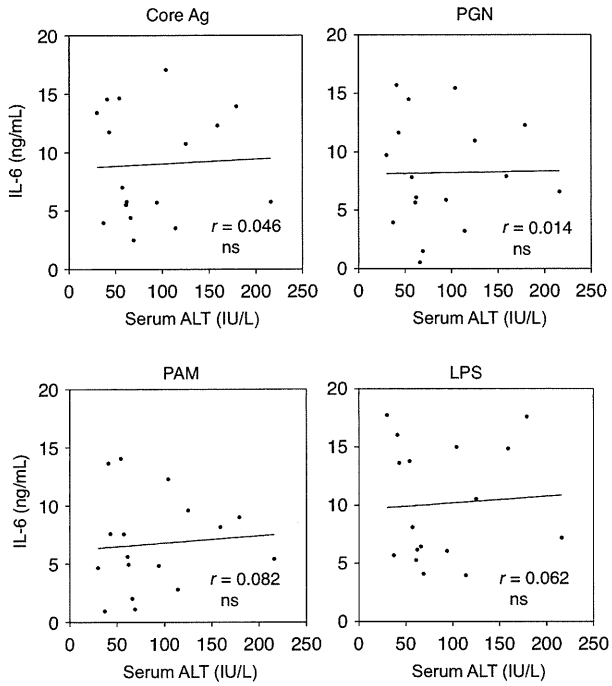

Fig. 3 Production of IL-6 by peripheral blood mononuclear cells (PBMCs) isolated from patients with chronic HCV infection does not correlate with serum levels of ALT. Production of IL-6 by PBMCs in response to HCV core antigen and TLR ligands was compared with serum ALT levels in each patient with chronic HCV. No correlation was seen between IL-6 production and serum ALT levels.

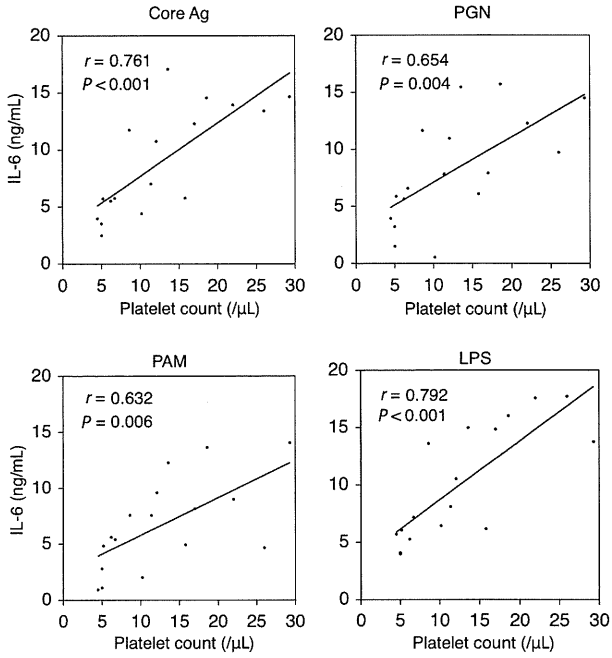

Fig. 4 Production of IL-6 by peripheral blood mononuclear cells isolated from patients with chronic hepatitis C virus (HCV) infection is correlated with thrombocytopenia. Production of IL-6 by PBMCs in response to HCV core antigen and Toll-like receptor ligands was compared with the platelet count in each patient with chronic HCV infection. IL-6 production correlated with platelet counts.

antigen, we speculate that chronic activation of TLR2 by the HCV core antigen induces hyporesponsiveness to TLR ligands. This idea partially explains the mechanisms underlying impaired TLR signalling specific to patients with HCV infection. However, there was no correlation between serum doses of HCV core antigen and TLR-induced cytokine production, suggesting that impaired production of IL-6 induced by TLRs cannot be explained by the dose of circulating HCV core antigen alone and that other viral proteins such as NS3/4A and host factors may be involved in the generation of hyporesponsiveness to TLR ligands. It should be noted that the role played by HCV core antigen-induced TLR2 activation in the generation of hyporesponsiveness to TLR ligands cannot be excluded by the negative correlation between core antigen concentrations and IL-6 production. Given the fact that peripheral blood APCs in patients with chronic HCV infection are constantly exposed to HCV core antigen for many years after the initial acute infection, the duration of the chronic infection may also be an important factor. Thus, both viral factors and host factors are associated with impaired TLR responses in patients with chronic HCV infection.

PBMCs from patients with chronic HBV infection showed comparable levels of IL-6 and IL-8 production with those from healthy controls. Thus, our data suggest that impaired production of TLR-induced cytokines is a specific immunological feature of chronic HCV infection. Although the mechanisms responsible for the different TLR responses observed during HCV and HBV infection are currently unknown, TLR stimulation by viral proteins may be involved. HCV-associated proteins, such as the core and NS3 antigens, are potent activators of TLRs [6–8], whereas HBV-associated proteins, such as the HBc and HBe antigens, fail to activate TLRs [16]. Therefore, it is possible that APCs from patients with chronic HCV infection are tolerant to TLR ligands as a result of constant exposure to the core and NS3 antigens. Taken together, these findings suggest that viral factors appear to play a role in the generation of hyporesponsiveness to TLR ligands in patients with chronic HCV infection.

Several lines of evidence suggest that chronic HCV infection causes impaired innate and adaptive immune responses. For example, adaptive T-helper type 1 and 17 responses are suppressed in patients with chronic HCV infection [17,18]. In addition, APCs isolated from patients with HCV infection exhibit defective proinflammatory cytokine responses upon stimulation with TLR ligands [19,20]. However, these previous studies did not address or determine the clinical parameters that are associated with impaired cytokine responses in

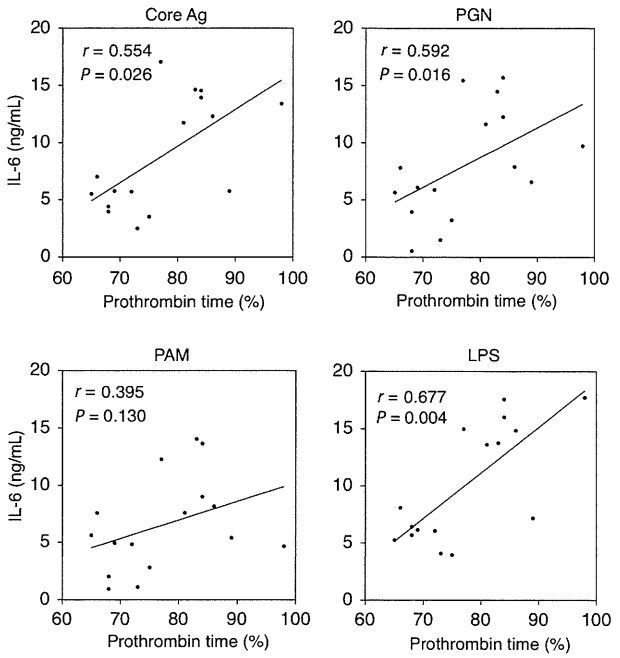

Fig. 5 Production of IL-6 by peripheral blood mononuclear cells (PBMCs) isolated from patients with chronic hepatitis C virus (HCV) infection is correlated with prothrombin time (PT). Production of IL-6 by PBMCs in response to HCV core antigen and Toll-like receptor ligands was compared with the PT in each patient with chronic HCV infection. Production of IL-6 correlated with PT.

HCV infection. In this study, we clearly show that the reduction in TLR-induced IL-6 production correlates with the severity of liver dysfunction as assessed by platelet counts and PT in patients with chronic HCV infection. Because these parameters are sensitive markers for the evaluation of liver function, our data suggest that liver dysfunction may play a role in impaired TLR-induced cytokine responses in patients with chronic HCV infection. The mechanisms by which liver dysfunction predispose the host to defective TLR signalling are currently unknown. Kakazu *et al.* [21] showed that decreased levels of plasma branched-chain amino acids (BCAAs), which are the characteristic feature of severe liver dysfunction, impair APC function in patients with HCV. Furthermore, they also demonstrated that addition of BCAAs to the culture medium normalized IL-12 production by APCs isolated from cirrhotic patients. Therefore, reduced levels of BCAAs because of severe liver dysfunction, together with viral factors, contribute to an abnormal immune environment during chronic HCV infection in which APCs fail to produce TLR-induced host defence factors.

No correlation was found between serum levels of ALT and hyporesponsiveness to TLR ligands in patients with chronic HCV infection. In contrast, reduced production of IL-6 by PBMCs from chronic HCV patients was associated with decreased platelet counts and prolonged PT. As serum ALT is the most useful marker for assessing liver damage [22], our data suggest that liver dysfunction as assessed by PT rather than liver damage as assessed by ALT levels is associated with hyporesponsiveness to TLR ligands in chronic HCV infection.

In contrast to HCV infection, production of IL-6 did not correlate with thrombocytopenia in patients with chronic HBV infection. Thus, liver function does not seem to affect host innate immunity through TLRs in patients with chronic HBV infection. However, we need to be cautious in interpreting the data regarding HBV infection. Tejima *et al.* [23] reported that chronic HCV patients present with more severe levels of thrombocytopenia than chronic HBV patients, even in those with the same grade of splenomegaly and liver stiffness. They also found reduced levels of prothrombin activity in chronic HCV infection patients compared with chronic HBV infection patients [23]. Thus, virological aetiology may contribute to thrombocytopenia and reduced levels of serum PT in a specific manner, and it may not be accurate to directly compare the function of HBV- or HCV-infected livers using the clinical parameters chosen in the present study.

In conclusion, our results clearly show that PBMCs isolated from patients with chronic HCV infection have impaired proinflammatory cytokine responses to TLR ligands. In addition, there is a strong correlation between reduced proinflammatory responses to TLR ligands and the severity of liver dysfunction in these patients. Both viral and host factors contribute to the generation of impaired responses to TLR ligands during chronic HCV infection. Moreover, our results also suggest that platelet counts and PT can be used as markers to determine TLR responses in patients with chronic HCV infection. Future immunological studies using a large number of patients with chronic HBV or HCV infection at the same stage of liver fibrosis are required to identify the host defence responses mediated by TLRs in these patients.

ACKNOWLEDGEMENTS

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Correlation between Insulin Resistance and Outcome of Pegylated Interferon and Ribavirin Therapy, Hepatic Steatosis, Hepatic Fibrosis in Chronic Hepatitis C-1b and High Viral Load

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Key Words

Insulin resistance · Hepatic fibrosis · Hepatic steatosis · Genotype 1b · High viral load · Pegylated interferon · Ribavirin · Sustained virological response

Abstract

Background/Aims: Insulin resistance (IR) has been reported to be an independent predictor of treatment outcome in chronic hepatitis C patients. ***Methods:*** We analyzed the relationship between IR and the outcome of pegylated interferon and ribavirin (PEG-IFN/RBV) therapy, taking into account host factors of body mass index and histological index, such as rate of fatty change and fibrosis. Japanese patients (n = 30; 19 men and 11 women; median age 60.0 ± 8.7 years) with chronic hepatitis C-1b with a high viral load were treated with PEG-IFN-α2b/RBV for 48 weeks. ***Results:*** Sustained virological response (SVR) was seen in 60% (18/30) and non-SVR in 40% (12/30). HOMA-IR (homeostasis model of assessment-insulin resistance index) at the start and at 24 weeks of treatment showed no statistical difference between SVR and non-SVR. Correlation was observed between HOMA-IR and body mass index (r = 0.45, p = 0.013). Among 20 patients, steatosis and fibrosis were assessed by biopsy. Correlation was observed between HOMA-IR and steatosis (r = 0.57, p = 0.0093), whereas no correlation was observed between HOMA-IR and fibrosis. ***Conclusion:*** A larger prospective study is needed to clarify the role of IR in the outcome of PEG-IFN/RBV combination therapy and hepatic fibrosis in Japanese patients.

Introduction

Chronic hepatitis C genotype 1b and a high viral load are known to be highly refractory to interferon (IFN) therapy. In Japan, such patients account for approximate-

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Table 1. Patient baseline characteristics

Age, years	60.0 ± 8.7
Males/females	19/11
Body weight, kg	60.2 ± 10.9
BMI	22.9 ± 3.8
HCV-RNA, log IU/ml	4.5 ± 1.7
AST, IU/l	63.4 ± 44.8
ALT, IU/l	71.3 ± 63.3
FBS, mg/dl	92.8 ± 16.4
γ-GTP, IU/l	84.1 ± 99.1
T-Cho, mg/dl	170.0 ± 39.6
TG, mg/dl	85.7 ± 39.4
HOMA-IR	2.1 ± 1.1

Data are shown as mean ± SD.

ly 70% of chronic hepatitis C cases, and various strategies have been investigated to improve treatment outcome. Currently, the first choice for refractory patients is a 48-week combined administration of pegylated IFN and ribavirin (PEG-IFN/RBV). Nonetheless, sustained virological response (SVR) is achieved in at most 50% of such patients [1, 2].

Host factors including HLA class I [3] and II [4], ethnicity [5] and body mass index (BMI) [6] also influence SVR [7]. Indeed, overweight [6] patients have shown characteristic resistance to combination therapy, and increased insulin resistance (IR) has been identified as an independent variable associated with a poor response [8]. Experimental and clinical studies have shown the role of hepatitis C virus (HCV) infection in the development of IR [9]. Patients with mild chronic hepatitis demonstrate a higher homeostasis model of assessment-insulin resistance index (HOMA-IR) than do healthy controls matched for age and BMI [8]. IR has also been implicated in the progression of fibrosis [10] and the development of steatosis [11, 12]. The latter finding is, however, observed mainly in European and American, including Caucasian and African, patients. The aim of this study was to analyze the relationship between IR and the outcome of PEG-IFN/RBV therapy, taking into account host factors of BMI and histological index, such as rate of fatty change and fibrosis, in Japanese patients with chronic hepatitis C-1b and high viral loads.

Patients and Methods

Patients

A total of 30 patients (19 men, 11 women; age 60.0 ± 8.7 years) seen at Kobe Asahi Hospital and diagnosed with chronic HCV-1b infection on the basis of the presence of anti-HCV antibodies and HCV-RNA, were enrolled in the study. The patients were treated with PEG-IFN-α2b (1.5 μg per kilogram body weight, once a week subcutaneously) and RBV (600–1,000 mg daily, per os) for 48 weeks, according to the standard treatment protocol for Japanese patients established by a hepatitis study group of the Ministry of Health, Labor and Welfare, Japan. The HCV genotype was determined according to the method of Okamoto et al. [13]. Informed consent in writing was obtained from each patient, and the study protocol conformed to the ethical guidelines approved by the Ethics Committee in Kobe Asahi Hospital. The baseline characteristics of 30 patients are listed in table 1.

Laboratory and Histological Tests

Serum samples were collected from the patients at intervals of 4 weeks before, during and after the treatment, and tested for HCV-RNA based on the COBAS TaqMan HCV test (Roche Diagnostics Corp., Basel, Switzerland). Fasting glucose and insulin were obtained at the start of and at 24 weeks of the treatment with PEG-IFN/RBV; IR was assessed by HOMA: [fasting insulin [μU/ml] × (fasting glucose [mg/dl]/18)]/22.5 [14, 15]; BMI was calculated as weight divided by height (kg/m^2).

Twenty biopsies were assessed for staging fibrosis and grading steatosis: fibrosis was staged on a scale from 0 to 4 according to the new classification by Desmet et al. [16]: with 0, no fibrosis; 1, mild fibrosis; 2, moderate fibrosis; 3, severe fibrosis, and 4, cirrhosis. Steatosis was graded on a scale from 0 to 4 according to the percentage of cells with fat: with 0, <5%; 1, 5–32%; 2, 33–65%; 3, ≥66%.

Statistical Analysis

Statistical differences in treatment responses according to patient baseline parameters of age, sex, body weight, BMI, HCV-RNA load, aspartate aminotransferase (AST), alanine aminotransferase (ALT), fasting blood sugar (FBS), γ-glutamyl transpeptidase (γ-GTP), total cholesterol (T-Cho) and triglyceride (TG) were determined by Fisher's exact test or the Mann-Whitney U test. Differences between the start of and at 24 weeks of therapy were assessed by the Wilcoxon signed rank test. Correlation between IR and the staging of fibrosis, the grading of steatosis and BMI was assessed by single regression analysis. Variables with a p value of <0.05 were considered statistically significant.

Results

Among the 30 patients, SVR was seen in 60% (18/30) and non-SVR in 40% (12/30). The baseline characteristics and the clinical responses are shown in table 2. Sex, body weight, BMI, HCV-RNA load, AST, ALT, FBS, γ-GTP, T-Cho and TG showed no significant difference between SVR and non-SVR, but age did ($p = 0.03$).

HOMA-IR at the start and at 24 weeks of treatment was 2.2 ± 1.0 and 2.5 ± 3.9 in SVR, and 2.0 ± 1.2 and 1.4 ± 0.5 in non-SVR, respectively, with no statistical difference between the two groups. BMI was ≥25 in 20% (6/30) and <25 in 80% (24/30) of patients, and correlation

Table 2. Baseline characteristics and the clinical response in SVR and non-SVR

	SVR (n = 18)	Non-SVR (n = 12)	p value
Age, years	57.1 ± 8.8	64.3 ± 6.6	0.03
Males/females	12/6	7/5	0.80
Body weight, kg	62.3 ± 12.0	57.0 ± 8.4	0.21
BMI	23.8 ± 4.0	21.8 ± 3.2	0.28
HCV-RNA, log IU/ml	4.7 ± 1.9	4.2 ± 1.4	0.85
AST, IU/l	65.2 ± 51.3	60.0 ± 34.7	0.82
ALT, IU/l	82.3 ± 76.0	54.8 ± 33.7	0.46
FBS, mg/dl	95.4 ± 20.0	88.8 ± 8.1	0.63
γ-GTP, IU/l	108.1 ± 121.9	48.3 ± 24.4	0.66
T-Cho, mg/dl	176.6 ± 44.0	160.3 ± 31.2	0.23
TG, mg/dl	88.1 ± 47.8	82.3 ± 23.2	0.85
HOMA-IR	2.2 ± 1.0	2.0 ± 1.2	0.53

Data are shown as mean ± SD.

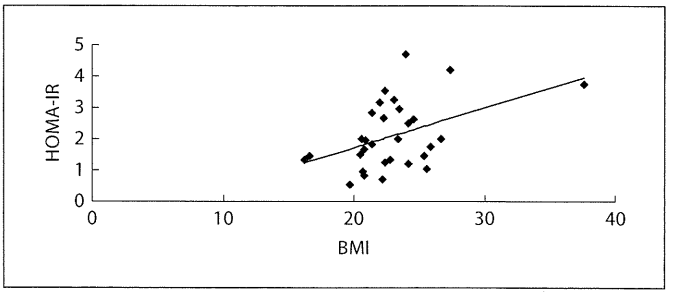

Fig. 1. Correlation observed between HOMA-IR and BMI (r = 0.45, p = 0.013).

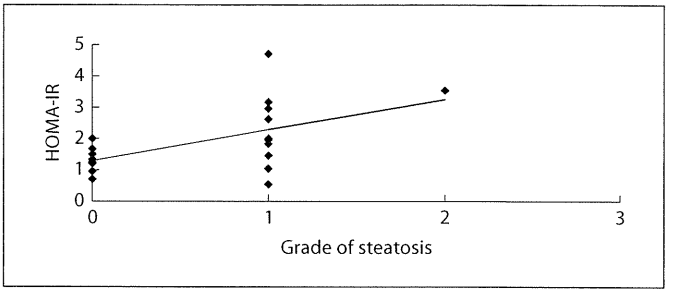

Fig. 2. Correlation observed between HOMA-IR and grade of steatosis (r = 0.57, p = 0.0093).

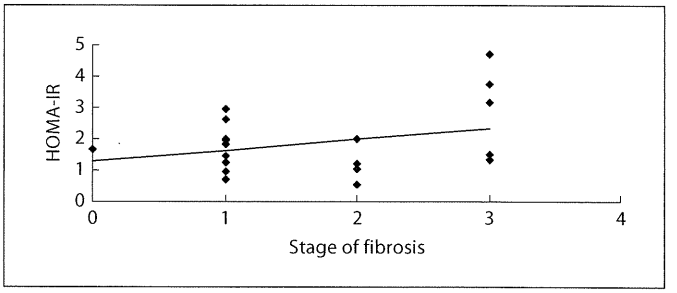

Fig. 3. No correlation was observed between HOMA-IR and stage of fibrosis (r = 0.32, p = 0.17).

was observed between HOMA-IR and BMI (r = 0.45, p = 0.013; fig. 1). Among 20 patients, steatosis assessed by histology revealed grade 0 in 45% (9/20), grade 1 in 50% (10/20), grade 2 in 5% (1/20), and grade 3 in 0% (0/20; fig. 2). Fibrosis was observed at stage F0 in 5% (1/20), F1 in 45% (9/20), F2 in 20% (4/20), F3 in 30% (6/20; fig. 3). Correlation was observed between HOMA-IR and steatosis (r = 0.57, p = 0.0093), whereas no correlation was observed between HOMA-IR and fibrosis (r = 0.32, p = 0.17).

Discussion

HCV genotype and HCV viral load remain the most important predictors of response to PEG-IFN/RBV combination therapy [17]. In contrast to HCV genotypes 2 and 3, which are significantly more susceptible to combination therapy with good outcome after standard or short-term treatment [18, 19], genotype 1 infection calls for developing more effective therapy and elucidating predictors of response conducive for optimizing individualized regimens. Therefore, the effect of host factors on the rate of SVR in anti-HCV therapy becomes a compelling concern for patients with unfavorable virological predictors.

IR, the stage of fibrosis and HCV genotype are independent predictors of response to anti-HCV therapy among Spanish patients [n = 159 (including genotype 1, n = 113); age, 41.7 years; BMI, 26.8]. In patients with genotype 1, a significantly lower SVR rate is observed in those with HOMA IR >2 than in those with HOMA-IR ≤2 (32.8%, 23/70 vs. 60.5%, 26/43, p = 0.007). The authors suggest that HOMA-IR might assist in further refining the prediction of antiviral response in genotype 1 patients [11]. In American, including African and Caucasian, patients with genotype 1 (n = 399; age, 47.7 years; BMI, 29.5), SVR rates of 49% for patients with HOMA-IR ≤2, and 36% for patients with HOMA-IR >2 have been

observed, the authors concluding that IR is independently associated with a low SVR rate [12]. HCV genotype 1b-infected Taiwanese patients (n = 150; age, 51.1 years; BMI, 23.5) with high IR demonstrated a lower SVR rate than those with low IR, suggesting the possible value of evaluating IR to predict response in HCV genotype 1b infection and a high pretreatment serum HCV-RNA level [20]. Noteworthy in that study is that the effect of HOMA-IR on response is observed in genotype 1b patients and particularly, for the first time, in those classified as 'difficult to treat' (genotype 1b infection and a high HCV-RNA level). Japanese patients with genotype 1b and a high viral load (n = 51; age, 57 years; BMI, 23.2) achieving SVR have lower HOMA-IR compared with non-SVR patients [21].

In the current study (BMI, 22.9), IR showed no difference between SVR and non-SVR patients, implying that the association of IR with response might be explained, in part, by ethnicity (Romero-Gomez, Spanish; Conjeevaram, American; the current study, Japanese), sample size (113, Spanish; 399, American; 150, Taiwanese, Dai's group; 51 Japanese, Mizuta, and 30 in the current study) and age (41.7, Spanish; 47.7, American; 51.1, Taiwanese, and 60.0 in the current study).

Since IR is a potentially modifiable factor, the response to the therapy might be improved by the modulation of insulin signaling and by improvements in IR and glucose control. The considerable potential for evaluating novel therapies and targets including insulin-sensitizing drugs for chronic hepatitis C patients deserves prospective investigation. Prospective studies for effective approaches resolving the IR issue before the initiation of combination therapy for chronic hepatitis C can significantly raise the SVR rate. HCV might induce IR irrespective of the severity of liver disease [8], and IR could be associated with severe hepatic fibrosis and might contribute to the progression of fibrosis in chronic HCV infection [8, 22, 23].

Around one to two thirds of liver biopsies from chronic hepatitis C patients show histological evidence of steatosis, which has been associated with being overweight, with hepatic fibrosis and TG levels [12, 24, 25]. Associations among IR, steatosis and liver fibrosis have been observed in chronic hepatitis C patients [25–29]. IR has been suggested as the cause, more than the consequence, of hepatic steatosis and fibrosis in patients with HCV, particularly in those with genotype 1 infection [30]. The mechanisms of the more obvious and crucial influence of IR, more than that of steatosis and fibrosis, need further study. In the current study, IR was found to be associated with BMI and steatosis, but not with hepatic fibrosis. The differences in the results regarding IR-associated hepatic fibrosis might also be explained by ethnic difference, sample size and age. Further large-scale study on Japanese patients is needed to clarify the role of IR in hepatic fibrosis.

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Disclosure Statement

None of the authors has any conflict of interest.

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Double-Filtration Plasmapheresis plus Interferon-β for HCV-1b Patients with Non-Sustained Virological Response to Previous Combination Therapy

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Key Words

Double-filtration plasmapheresis · Interferon-β · Ribavirin · Rapid virological response · Complete early virological response · Viral dynamics · Relapse · Null virological response

Abstract

Background and Aims: Double-filtration plasmapheresis (DFPP) together with interferon (IFN) administration produces a substantial reduction in the viral load during the early stages of treatment. ***Methods:*** Based on their responses to previous pegylated IFN and ribavirin (PEG-IFN/RBV) therapy, 20 patients were divided into null virological response (NVR; n = 12) and relapse (n = 8) groups. DFPP was used in combination with IFN-β/RBV with subsequent administration of PEG-IFN-α2a/RBV therapy (DFPP + IFN-β/RBV then PEG-IFN/RBV). Early viral dynamics was assessed, focusing especially on complete early virological response (cEVR) associated with sustained virological response. Additionally, the interleukin 28B gene, the IFN/RBV resistance-determining region, the IFN sensitivity-determining region and the core regions were analyzed. ***Results:*** Rapid virological response was achieved in 0% (0/12) of NVR and in 75% (6/8) of relapse patients, with a significant difference between the two groups (p = 0.001). Similarly, cEVR was achieved in 8% (1/12) of NVR and in 88% (7/8) of relapse patients, with a significant difference between the two groups

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(p = 0.037). By multivariate logistic regression analysis, interleukin-28B major was a significant determiner of cEVR (odds ratio = 24.19, p = 0.037). ***Conclusion:*** DFPP + IFN-β/RBV then PEG-IFN/RBV therapy is indicated more for relapse than for NVR patients.

Introduction

Currently, the therapy of first choice for patients refractory to chronic hepatitis C (CHC) treatment is the combined administration of pegylated interferon and ribavirin (PEG-IFN/RBV) for 48 weeks; however, the subsequent sustained virological response (SVR) is achieved in no more than 50% of the patients with genotype 1b and high viral loads [1]. To enhance this SVR rate, several trials have been undertaken, two of which are (1) re-treatment with combination therapy and (2) double-filtration plasmapheresis (DFPP). By the protocol-defined primary analysis of the former, the SVR rate has been 16% at the most, even for a 72-week induction group [2]. The use of DFPP (approved in Japan in April 2008 for the re-treatment of chronic CHC patients with genotype 1b and high viral loads) together with IFN administration has produced a substantial reduction in the viral load during the early stages of treatment, and has effected a high SVR [3, 4], suggesting that this treatment is a new modality for difficult-to-treat CHC patients.

Recent reports have revealed factors associated with response to PEG-IFN/RBV therapy: single nucleotide polymorphisms, as host genetic factors, located in interleukin (IL) 28B (rs8105790, rs11881222, rs8103142, rs28416813, rs4803219, rs8099917, rs7248668, and rs12979860) on chromosome 19 [5–8]; amino acid (aa) substitutions in nonstructural protein 5a (NS5A), especially those in the IFN/RBV resistance-determining region (IRRDR) [9] and the IFN sensitivity-determining region (ISDR) [10], and the core regions of HCV [11], as viral genetic polymorphisms.

In this study, DFPP was used in combination with IFN-β/RBV with subsequent administration of PEG-IFN/RBV (DFPP + IFN-β/RBV then PEG-IFN/RBV) therapy to enhance the efficacy of the treatment of CHC patients whose hepatitis C virus (HCV) had not been eradicated by earlier PEG-IFN/RBV combination therapy; we also assessed early viral dynamics, focusing especially on complete early virological response (cEVR) associated with SVR. Additionally, IL28B, the core regions, ISDR and IRRDR were analyzed before treatment.

Patients and Methods

Patients

Twenty patients whose HCV had not been eradicated by previous PEG-IFN/RBV combination therapy carried out in several institutions between 2008 and 2010 were enrolled in this study. Based on the response to the previous treatment (PEG-IFN/RBV), the patients were divided into 2 groups: continuous viremia throughout the observation period (10 patients) or unknown outcome regarding viral dynamics (2 patients), referred to as the null virological response (NVR) group (n = 12), and transient disappearance of serum HCV RNA at a certain point in time with a subsequent rebound in viremia either before or after the end of the treatment, referred to as relapse group (n = 8). All the patients received DFPP + IFN-β/RBV then PEG-IFN/RBV therapy for a planned 48 weeks. Since none of the patients has completed the 48-week treatment, the results at 12 weeks are presented here, and the early viral dynamics are analyzed. Each patient gave written informed consent and agreed to receive the treatment; the study was approved by the review board of Kobe Asahi Hospital.

DFPP and Blood Collection

Blood collected from the peripheral vein for DFPP by a Plasmaflo™ OP-18W filter (Asahi Kasei Medical, Tokyo, Japan) was separated into plasma and cell components. The virus was then removed from the plasma by a second filter (Cascadeflo™ EC-50W; Asahi Kasei Medical) of an average pore of 30 nm. For each session, the final volume of treated plasma was 50 ml/kg, the number of sessions was 5 over 2 weeks, and the intervals between administrations of DFPP, based on the reduced plasma fibrinogen levels during DFPP, were decided by the physicians and as required by the patients.

IFN in Combination with DFPP

During and after DFPP, the patients received IFN-β (3 MU twice daily) and RBV for 4 weeks with subsequent administration of PEG-IFN-α2a (180 μg/per week) and RBV for a planned 48 weeks. The RBV dose in combination with IFN-β and PEG-IFN-α2a was 600–1,000 mg/day, per os and according to body weight.

Measurement of HCV RNA and Viral Dynamics

In the previous PEG-IFN/RBV therapy, HCV RNA levels had been measured before the start and at 4 weeks of treatment by real-time PCR (COBAS TaqMan HCV test; Roche, detection limit 1.2 log IU/ml), by HCV core antigen (IRM assay; Ortho Clinical Diagnostics), or by RT-PCR (Amplicor; Roche). Before the start of DFPP + IFN-β/RBV therapy, all the patients were confirmed to be HCV RNA positive with high transaminase levels, and with HCV RNA genotype 1b at levels exceeding 10^5 log IU/ml. Also, the patients were negative for hepatitis B surface antigen. In DFPP + IFN-β/RBV then PEG-IFN/RBV therapy, HCV RNA levels were measured by real-time PCR before the start, at 24 and 48 h, and 1, 2, 4, 8 and 12 weeks of treatment. The quantity of HCV RNA was converted to a log value at the start of the treatment (A) and at each virus measurement point (B); Δlog was then calculated as follows: Δlog = logA – logB = log (A/B). HCV RNA negative at 4 and 12 weeks of treatment was regarded as rapid virological response (RVR) and cEVR, respectively.

Table 1. Patient baseline characteristics

Group by previous treatment response	Case No.	Age years	Sex	HCV-RNA log IU/ml	AST IU/l	ALT IU/l	γ-GTP IU/l	Hemoglobin g/dl	Platelets $\times 10^4/\mu l$
NVR (n = 12)	1	43	F	6.2	38	25	50	8.2	14.3
	2	43	M	7.2	30	57	63	17.3	21.3
	3	67	F	7.6	82	60	69	13.0	9.7
	4	49	M	6.0	45	31	82	15.8	9.6
	5	52	F	5.8	14	10	11	10.2	19.4
	6	66	F	5.3	28	20	15	10.1	6.5
	7	47	F	6.8	51	73	161	14.2	16.7
	8	52	F	5.5	24	16	11	12.1	21.0
	9	64	M	6.7	35	41	24	15.8	15.0
	10	66	M	5.5	31	34	26	9.7	13.3
	11	55	M	6.3	62	60	151	13.1	7.9
	12	62	F	6.6	24	7	19	12.9	13.7
	Average	55.5		6.3	38.7	36.2	56.8	12.7	14.0
Relapse (n = 8)	13	61	F	6.5	25	20	26	10.7	6.7
	14	61	F	5.5	28	21	11	10.6	19.1
	15	65	F	6.4	48	71	23	11.0	15.0
	16	68	F	6.3	30	21	42	11.9	9.2
	17	67	M	3.2	17	10	16	9.6	20.7
	18	62	M	7.1	30	35	18	14.0	18.7
	19	43	M	5.6	26	37	20	15.1	30.5
	20	67	F	3.7	37	36	22	9.9	21.5
	Average	61.8		5.5	30.1	31.4	22.3	11.6	17.7
NVR vs. relapse	p value	0.15	0.85	0.33	0.33	0.82	0.18	0.35	0.26

Genetic Variation near the IL28B Gene

Genetic polymorphism, rs8099917 around the IL28B gene was determined by real-time PCR with the TaqMan assay [5]. Homozygosity for the major sequence (MA) was defined as having the IL28B major allele, whereas heterozygosity (HE) or homozygosity for the minor sequence (MI) was defined as having the IL28B minor allele.

Sequence Analysis of HCV NS5A and Core

Before the start of treatment, measurements were made of the HCV aa substitutions in nonstructural protein 5a (NS5A), including those in IRRDR and ISDR. The optimal cutoff number of mutations for predicting SVR has been estimated at 6 in IRRDR [9], but at 2 in ISDR [10]. Correlations between core regions (arginine at position 70, leucine at position 91) and treatment response have been estimated [11].

Statistical Analysis

Statistical differences in treatment responses according to patient baseline parameters of age, sex, HCV-RNA load, aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GTP) and hemoglobin were assessed by Fisher's exact test or the Mann-Whitney U test. Differences between viral dynamics were assessed by the Wilcoxon signed rank test or the Mann-Whitney U test. The treatment responses according to NS5A, core mutations and the IL28B gene were evaluated using univariate and multivariate logistic regression analyses. Variables with a p value of <0.05 were considered statistically significant. The odds ratios and 95% confidence intervals were also calculated. All statistical analyses were carried out with EXCEL multivariate statistical analysis software version 6.0 (ESUMI Inc., Tokyo, Japan).

Results

Treatment Reponses and Viral Dynamics

The baseline characteristics of the patients and the laboratory data of age, sex, HCV-RNA, AST, ALT, γ-GTP, hemoglobin and platelets showed no significant difference between NVR and relapse patients (table 1).

In DFPP + IFN-β/RBV then PEG-IFN/RBV therapy, RVR was achieved in 0% (0/12) of NVR and in 75% (6/8) of relapse patients, with a significant difference between the two groups (p = 0.001). Similarly, cEVR was achieved in 8% (1/12) of NVR and in 88% (7/8) of relapse patients, with a significant difference between the two groups (p = 0.037).

DFPP + IFN-β/RBV then PEG-IFN/RBV therapy induced an average viral reduction of 3.24 log in the 20 pa-

Table 2. Viral dynamics of DFPP + IFN-β/RBV then PEG-IFN/RBV and previous PEG-IFN/RBV treatment

Group by previous treatment response	Case No.	DFPP + IFN-β/RBV then PEG-IFN/RBV therapy									Previous PEG-IFN/RBV therapy		
		HCV RNA								re-sponse	HCV RNA		re-sponse
		before treatment log IU/ml	log drop, log IU/ml								before treatment	log drop log IU/ml 4 weeks	
			24 h	48 h	1 week	2 weeks	4 weeks	8 weeks	12 weeks				
NVR (n = 12)	1	6.2	0.5	0.6	0.2	1.3	2.2	4.1	5.0		745 fmol/l	0.1	NVR
	2	7.2	0.6	1.0	1.4	2.5	2.9	4.1	6.0		426 kIU/ml	+0.1	NVR
	3	7.6	1.1	1.1	1.5	3.3	5.4	–	7.6	cEVR	–	–	–
	4	6.0	–0.1	0.6	1.5	2.0	3.4	–	4.8		6.9 log IU/ml	0.2	NVR
	5	5.8	0.4	0.7	1.0	1.6	–0.2	0.1	–0.3		6.3 log IU/ml	0.2	NVR
	6	5.3	0	0.5	0.8	1.2	1.3	0.8	0.1		11,500 fmol/l	0.8	NVR
	7	6.8	0.6	0.3	0.3	0.4	0.4	0.3	0.3		2,900 kIU/ml	0.3	NVR
	8	5.5	1.4	0.9	1.5	1.2	1.9	1.5	1.9		782 fmol/l	0.6	NVR
	9	6.7	1.1	0.8	0.4	0.8	0.8	–	0.9		–	–	–
	10	5.5	1.0	1.5	1.1	1.0	1.4	1.9	–		6.2 log IU/ml	0.7	NVR
	11	6.3	0.5	0.8	0.3	1.1	1.3	1.0	0.7		5.8 log IU/ml	+0.2	NVR
	12	6.6	1.5	1.8	1.3	2.6	2.7	–	3.2		–	–	NVR
	Average	6.3	0.7	0.9	0.9	1.6	2.0[a]	1.7	2.7		–	0.3[a]	
Relapse (n = 8)	13	6.5	1.2	2.1	3.4	5.0	4.8	5.3	5.3		8,450 fmol/l	2.6	PR
	14	5.5	1.3	2.6	2.6	4.3	5.5	5.5	–	RVR	9,700 fmol/l	2.7	PR
	15	6.4	1.5	2.4	3.8	5.2	6.4	6.4	6.4	RVR	6.5 fmol/l	2.8	PR
	16	6.3	0.9	1.5	2.4	3.7	5.0	6.3	6.3	cEVR	540 kIU/ml	2.7	PR
	17	3.2	2.0	2.0	2.0	2.0	3.2	3.2	3.2	RVR	6.4 log IU/ml	1.2	PR
	18	7.1	2.8	3.8	4.7	5.7	7.1	7.1	7.1	RVR	–	–	PR
	19	5.6	1.7	2.7	4.4	5.6	5.6	–	5.6	RVR	6.8 log IU/ml	2.5	PR
	20	3.7	2.5	3.7	3.7	3.7	3.7	–	3.7	RVR	629 fmol/l	1.2	PR
	Average	5.5	1.7	2.6	3.4	4.4	5.2[b]	5.6	5.4		–	2.2[b]	
Total (n = 20)	Average						3.24					1.14	
NVR vs. relapse	p value	0.334	0.003	0.0003	0.0002	0.001	0.001	0.005	0.037				

[a] p = 0.01; [b] p = 0.03.

tients at week 4, with a significant difference as compared with the previous PEG-IFN/RBV therapy (1.14 log): NVR 2.0 vs. 0.3 log, p = 0.01, and relapse 5.2 vs. 2.2 log, p = 0.03; also, the early viral dynamics between NVR and relapse patients showed an average viral reduction of 0.7 vs. 1.7 log at 24 h (p = 0.003); 0.9 vs. 2.6 log at 48 h (p = 0.0003); 0.9 vs. 3.4 log at 1 week (p = 0.0002); 1.6 vs. 4.4 log at 2 weeks (p = 0.001); 2.0 vs. 5.2 log at 4 weeks (p = 0.001); 1.7 vs. 5.6 log at 8 weeks (p = 0.005); 2.7 vs. 5.4 log at 12 weeks (p = 0.037), with a significant difference at each observation point (table 2).

Correlation between cEVR and IL28B, Core Regions, ISDR, IRRDR

In the NVR group, IL28B major (MA) was demonstrated by 17% (2/12) of patients, HCV core aa 70 wild by 67% (8/12), HCV core aa 91 wild by 50% (6/12), HCV isolates involving 2 or more mutations (ISDR ≥2) by 0% (0/12) and HCV isolates involving 6 or more mutations (IRRDR ≥6) by 30% (3/10). In the relapse group, IL28B major was demonstrated by 75% (6/8) of patients, HCV core aa 70 wild by 63% (5/8), HCV core aa 91 wild by 38% (3/8), ISDR ≥2 by 0% (0/8) and IRRDR ≥6 by 50% (4/8). Among 8 of the 20 cEVR patients, IL28B major was demonstrated by 75% (6/8), HCV core aa 70 wild by 62% (5/8), HCV core aa 91 wild by 38% (3/8), ISDR ≥2 by 0% (0/8) and IRRDR ≥6 by 38% (3/8; table 3). Only IL28B major showed a statistically significant difference between cEVR and non-cEVR by univariate analysis (p = 0.018; data not shown). Multivariate logistic regression analysis also identified IL28B major as a significant determiner of cEVR (odds ratio = 24.19, p = 0.037; table 4).

Table 3. Correlation between treatment responses and IL28B, core, ISDR, IRRDR

Case No.	Response to DFPP + IFN-β/ RBV then PEG-IFN/RBV	IL28B geno-type	HCV core aa 70	HCV core aa 91	ISDR mutations	IRRDR mutations
NVR (n = 12)						
1		MI	wild	mutant	1	4
2		HE	wild	wild	0–1	–
3	cEVR	MA	wild	wild	0	3
4		MA	wild	wild	0	5
5		HE	wild	wild	1	–
6		HE	wild	wild	1	2
7		HE	mutant	mutant	1	7
8		HE	wild	mutant	1	4
9		HE	mutant	mutant	0	7
10		HE	wild	wild	0	3
11		HE	mutant	mutant	1	3
12		HE	mutant	mutant	1	7
Relapse (n = 8)						
13		MA	wild	wild	0	6
14	RVR, cEVR	MA	wild	mutant	1	6
15	RVR, cEVR	MA	mutant	mutant	1	5
16	cEVR	MA	wild	wild	0	8
17	RVR, cEVR	HE	mutant	wild	1	8
18	RVR, cEVR	MA	mutant	mutant	0	4
19	RVR, cEVR	MA	wild	mutant	1	3
20	RVR, cEVR	HE	wild	mutant	0	4

MA = Homozygosity for the major sequence; HE = heterozygosity; MI = homozygosity for the minor sequence.

Discussion

Granulocyte apheresis, plasma exchange and hemofiltration are modalities that have shown a reduction in HCV RNA in blood during the treatment of HCV-infected patients for cryoglobulinemia and vasculitis [3, 12–16]. The mechanisms of plasmapheresis have been described as related to the enhancement of the effects of IFN therapy by synergistically removing HCV from the blood [17]. Hemodialysis, hemofiltration and peritoneal dialysis given to chronic dialysis patients infected with HCV significantly lower HCV RNA levels in the blood [18]. Thus, the potential for effective IFN therapy combined with early physical removal of the virus is of particular interest.

The SVR rate is closely related to viral dynamics in the early stages of IFN therapy [19], and even to the combination therapy of IFN and DFPP [3]. In PEG-IFN/RBV therapy, reduction in the HCV RNA viral load by 4 weeks is considered essential, and a 2 log reduction is a prerequisite to achieving SVR [20]. Since a daily dose of IFN-β 6 MU is effective in reducing the HCV RNA load [21], all the patients in our study receiving a daily dose of IFN-β 6 MU (especially 3 MU twice daily) for 4 weeks demonstrated a reduction of ≥2 log at 4 weeks in the viral load of 65% (13/20) of patients.

In DFPP + PEG-IFN therapy, viral reduction after 4 weeks of treatment has been 2.43 ± 1.07 log IU/ml with overall SVR achieved in 70.8% (17/24) of patients [3], and 2.79 ± 1.85 log IU/ml with cEVR in 57.5% (104/181) [4]. The current study showed a viral reduction of 3.24 ± 1.00 log IU/ml with a cEVR rate of 45% (8/20 patients). Consequently, we expect to achieve high SVR rates at the end of the 48 weeks, as in the report [3].

The prerequisite for cEVR has been emphasized in predicting SVR and non-SVR in CHC patients undergoing IFN treatment; those who do not reach EVR fail to respond to further therapy. Treatment discontinued in patients not reaching EVR would reduce drug costs by more than 20%; consequently, early confirmation of viral reduction after initiating antiviral therapy for CHC is highly desirable [19]. Re-treatment with PEG-IFN-α2a/RBV of 107 relapsers to PEG-IFN/RBV therapy has achieved cEVR in 43% [22]; re-treatment with PEG-IFN-α2a/RBV of 469 relapse and NVR patients to PEG-IFN-α2b/RBV has achieved cEVR in only 13% [23]. Re-treatment with DFPP + PEG-IFN/RBV of 73 relapse and NVR patients to previous PEG-IFN/RBV therapy has achieved cEVR in 63.0% of relapsers and in 18.9% of NVR patients [4]. Therefore, it is expected that the addition of DFPP to PEG-IFN/RBV therapy will be effective in the re-treatment of relapse and NVR patients to previous PEG-IFN/RBV therapy.

In the current study, we clarified that early viral dynamics with DFPP + IFN-β/RBV then PEG-IFN/RBV therapy is superior to the previous PEG-IFN/RBV combination therapy. There was a significant difference in viral reduction at 24 and 48 h, and 1, 2, 4, 8 and 12 weeks between NVR and relapse patients. The rate of RVR and cEVR showed a significant difference between NVR and relapse patients: among the 20 patients, RVR was obtained in 75% (6/8) of relapse patients but in 0% (0/12) of NVR patients, and cEVR in 88% (7/8) of relapse patients but in only 8% (1/12) of NVR patients. On the basis of the above results, DFPP + IFN-β/RBV then PEG-IFN/RBV therapy is indicated more for relapse than for NVR patients. We could conclude that relapse patients would be better candidates than NVR patients.

Table 4. Multivariate analysis of factors associated with cEVR

Factor	Category	Patients	Odds ratio	95% CI	p value
IL28B genotype	major (MA)	75% (6/8)	24.19	1.22–479.58	0.037
	minor (HE+MI)	25% (2/8)			
HCV core aa 70	wild	62% (5/8)	0.82	0.03–19.43	0.903
	mutant	38% (3/8)			
HCV core aa 91	wild	38% (3/8)	3.18	0.12–87.4	0.494
	mutant	62% (5/8)			
ISDR mutations	≥2	0% (0/8)	0.83	0.05–14.2	0.898
	≤1	100% (8/8)			
IRRDR mutations	≥6	38% (3/8)	0.2	0.01–3.76	0.285
	≤5	62% (5/8)			

Recently, a new triple combination therapy comprising PEG-IFN-α, RBV, and a protease inhibitor such as telaprevir or boceprevir has been approved in the US for CHC patients with genotype 1 and high viral loads. Results have shown SVR was attained in 70% of naïve, 80% of relapse and 30% of NVR patients. The discontinuation rate due to adverse events such as anemia and skin eruption has not been low [24].

In this study, 80% of cEVR patients are expected to attain SVR, and despite the small number of subjects, the estimated SVR rate in our relapse patients is comparable to that of the new triple combination therapy. In view of the adverse events attending the triple combination therapy, however, DFPP could become an alternative for CHC patients with genotype 1b and high viral loads.

Among viral genetic polymorphisms such as HCV core, ISDR, IRRDR, and host genetic factor such as IL28B, only IL28B was defined as a determinant of cEVR by univariate and multivariate analyses.

Because of the small number of patients in the present study, further prospective study is needed to identify eligible candidates for DFPP + IFN-β/RBV then PEG-IFN/RBV therapy and independent predictive factors for cEVR and SVR.

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Disclosure Statement

None of the authors has any conflict of interest.

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Association of Interleukin-28B and Hepatitis C Genotype 1 with a High Viral Load and Response to Pegylated Interferon plus Ribavirin Therapy

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Key Words
Chronic hepatitis C · Interleukin-28B · Pegylated interferon plus ribavirin therapy · Drug dose · Core aa70

Abstract
Background: Pegylated interferon (PEG-IFN) plus ribavirin therapy is the current standard treatment for chronic hepatitis C (CHC) genotype 1 with high viral load. A common genetic variation near the IL28B gene has been found to affect the response to PEG-IFN plus ribavirin therapy for CHC. The aims of this study were to analyze the association between the rs8099917 genotype and treatment response in a cohort study of CHC. ***Methods:*** This study evaluated clinical and laboratory parameters retrospectively in a cohort of 122 patients with chronic hepatitis C with genotype 1 and a high viral load who received PEG-IFN plus ribavirin therapy. We carried out univariate and multivariate statistical analyses of parameters and clinical responses. ***Results:*** Sixty-three of 122 patients (51.6%) had sustained virological responses (SVRs). Patients with the rs8099917 genotype TT achieved significantly higher SVR rates ($p < 0.01$). Univariate analysis revealed that SVRs were associated with BMI, fibrosis, albumin, total cholesterol, PEG-IFN dose, ribavirin dose and the rs8099917 genotype. Multivariate analysis revealed that the rs8099917 genotype (odds ratio 7.434, 95% CI 2.278–24.257, $p = 0.001$) and total PEG-IFN dose (odds ratio 7.162, 95% CI 1.565–18.15, $p = 0.007$) were significant factors. ***Conclusions:*** The rs8099917 genotype and total PEG-IFN dose were associated with SVR in patients with hepatitis C virus genotype 1.

Introduction

Infection with hepatitis C virus (HCV) is a global health problem, with an estimated 120–130 million carriers worldwide [1]. HCV is a causative agent of acute and chronic hepatitis, as well as liver cirrhosis and hepatocellular carcinoma [2–4]. Antiviral therapy with pegylated interferon (PEG-IFN) and ribavirin can be efficacious for patients with chronic hepatitis C and the prognosis of patients from whom HCV is successfully eradicated improves markedly [5]. However, this treatment is effective only in 50% of patients and has severe side effects, often requiring discontinuation or dose modification [6]. Variations in treatment response have been investigated and several contributory factors identified, including age, liver fibrosis, HCV genotype, HCV RNA levels and race.

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Among the viral factors, amino acid (aa) substitutions at positions 70 and 91 of the HCV core protein and accumulation of substitutions in the IFN sensitivity-determining region (ISDR) of the NS5A protein have been shown to be associated with treatment outcome [7, 8].

Host factors also have been shown to be associated with the outcome of therapy, including age, sex, race, liver fibrosis and obesity [9, 10]. Recently, several highly correlated common single nucleotide polymorphisms on a linkage disequilibrium block in the vicinity of three IFN-γ genes on chromosome 19, encoding IFN-γ_1 *(IL29)*, -γ_2 *(IL28A)*, and -γ_3 *(IL28B)*, have been implicated in three genome-wide association studies in affecting the response to PEG-IFN/ribavirin among patients infected with HCV genotype 1 [11–13].

In this study, we investigated whether the rs8099917 polymorphisms are associated with susceptibility to HCV infection and with response to therapy with PEG-IFN and ribavirin in patients with chronic HCV infection.

Patients and Methods

Patients

This was a retrospective trial at Kinki University Hospital. A total of 122 patients with chronic hepatitis C with genotype 1 and a high viral load and who completed treatment with a combination of PEG-IFN-α2b and ribavirin between January 2005 and December 2008 were enrolled. The serum HCV RNA was measured before commencement of treatment and every month during the treatment by quantitative Amplicor HCV monitor assay (version 2.0, Roche Diagnostics; detection limit 500 IU/ml). When the quantitative assay showed undetectable levels of HCV RNA, a qualitative Amplicor HCV assay (version 2.0, Roche Diagnostics; detection limit 50 IU/ml) was used. Viral aa substitutions were determined for the ISDR in NS5A and substitutions at aa positions 70 and 91 in the core region, as described previously [7, 8, 14]. We genotyped each patient for the IL28B single nucleotide polymorphisms reported previously to be associated with treatment outcome, rs8099917 [12]. All subjects gave written informed consent to participate in the study according to the process approved by the ethical committee of Kinki University Hospital and conforming to the ethical guidelines of the 1975 Declaration of Helsinki.

Treatment

All patients were treated with PEG-IFN-α2b (Peg-Intron; Schering-Plough, Kenilworth, N.J., USA) plus ribavirin (Rebetol; Schering-Plough) for 48 weeks. PEG-IFN-α2b was administered at 1.5 μg/kg subcutaneously each week. Ribavirin was administered orally at a dose of 600 mg/day to patients weighing <60 kg, 800 mg/day to those weighing 60–80 kg, 1,000 mg/day to those weighing 80–100 kg, and 1,200 mg/day to those weighing >100 kg. However, the patients who discontinued treatment because of side effects of previous IFN plus ribavirin therapy were administered 400 mg/day. The dose reduction and discontinuation of the combination treatment was determined according to standard protocols.

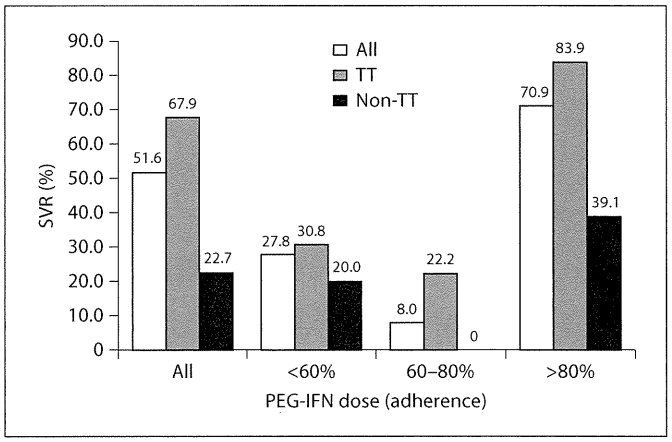

Fig. 1. Effect of IFN plus ribavirin therapy on patients with genotype 1 infection. SVR rates were analyzed according to the IL28B genotype, PEG-IFN dose and IL28 genotype.

Statistical Analysis

Data are expressed as median (range) values. Differences between groups were examined for significance using the Mann-Whitney U test and Fisher's exact test where appropriate. Multivariate analysis was performed using the logistic regression model. All the analyses described above were performed using the SPSS program (version 11.5; SPSS, Chicago, Ill., USA).

Results

Patient Characteristics

Baseline patient characteristics are shown in table 1. The median age on therapy was 61 (range 20–78) years. Sustained virological response (SVR) was achieved by 51.6% (63 of 122 patients). According to the genetic variation of rs8099917, SVR was achieved by 67.9% (53 of 78 patients) and 22.7% (10 of 44 patients) of patients with TT and non-TT, respectively (fig. 1). According to the substitution of core aa70, SVR was achieved by 46.1% (12 of 26 patients) and 21.0% (4 of 19 patients) of patients with wild type and mutant type, respectively.

Predictive Factors for SVR

As shown in table 2, univariate analysis revealed that SVR was associated with BMI, fibrosis, albumin, total cholesterol, total PEG-IFN dose, ribavirin dose and the rs8099917 genotype. Using BMI, fibrosis, albumin, total cholesterol, PEG-IFN dose, ribavirin dose and the rs8099917 genotype, multivariate analysis was performed using the logistic regression model. This analysis revealed that the rs8099917 genotype (odds ratio 7.434, 95% CI