(Roche Diagnostics, Tokyo, Japan). TaqMan has a lower limit of quantitation of 15 IU/mL and an outer limit of quantitation of 6.9×10^7 IU/mL (1.2 to 7.8 logIU/mL referred to $\log_{10}$ units/mL).[24,25] Therefore, TaqMan assay is able to do both qualitative and quantitative analysis for HCV RNA. The HCV genotype was determined by type-specific primers of the core region of the HCV genome. The protocol for genotyping was carried out as previously described.[3]

Efficacy of treatment

Sustained virological response (SVR) was defined as serum HCV RNA undetectable at 24 weeks follow-up after the end of treatment. SVR was defined as non-detectable HCV-RNA as measured by TaqMan assay, with the results labeled as positive or negative. The analysis of SVR rate was done on an intention-to-treat basis.

Minimum acceptable dosage

We previously reported that the minimum acceptable dosage necessary for Japanese genotype 1 patients to obtain an SVR is at least 80% or more of the target dosage of PEG-IFN α-2b and a minimum acceptable dosage of 60% or more of the target RBV.[23,26] Therefore, we compared the SVR rates of patients with NALT and elevated ALT who received at least 80% or more of the target dosage of PEG-IFN α-2b and 60% or more of the target RBV (minimum acceptable dosage).

Statistical analysis

Continuous data are expressed as mean values, the values ± standard deviation (SD), or the values ± standard error (SE) of the mean. The statistics were done using a commercially available software package (BMDP Statistical Software Inc., Los Angeles, CA, USA) for the IBM 3090 system computer. The χ^2 test, Student's *t*-test and Fisher's exact test were used to determine the differences in baseline clinical characteristics, safety, efficacy of the combination therapy, adherence to the total dose, and the association between the adherence and SVR. Univariate analysis was carried out on 13 background factors that had previously been evaluated in the literature for their possible association with SVR. Logistic regression models were used to evaluate possible predictors of SVR, and results were reported as odds ratios (OR) and their 95% confidence intervals (CI). A *P*-value of less than 0.05 was considered significant.

RESULTS

SVR rate by intention-to-treat analysis

ANALYSIS OF VIRAL response and ALT change was done at 24 weeks after the end of treatment. Of the 989 patients, 374 (37.8%) achieved SVR in the intention-to-treat analysis. The SVR rate was not significantly different between group A (48 of 114, 42.1%) and group B (326 of 875, 37.3%) ($P = 0.749$). The SVR rate was significantly higher for the women of group A (37 of 77, 48.1%) than for those of group B (120 of 373, 32.2%) ($P = 0.009$), but no significant difference was found for the men (group A: 11 of 37, 29.7% vs. group B: 206 of 502, 41.0%).

The SVR rates of patients with at least the minimum acceptable dosage during treatment were 43.0%, 49 of 114 patients in group A and 33.4%, 292 of 875 in group B. When the men received at least the minimum acceptable dosage, the SVR rate was not significantly different between groups A and B (group A: 9 of 16, 56.3% vs. group B: 122 of 185, 65.9%), and no significant difference was found between groups A and B for the women (group A: 22 of 33, 66.7% vs. group B: 58 of 107, 54.2%). The rate of SVR for patients under 65 years was significantly higher than for patients 65 years or older in groups A and B (41 of 80, 51.3% vs. 7 of 34, 20.6%:$P = 0.003$, 274 of 627, 43.7% vs. 52 of 248,21.0%: $P < 0.001$). Among the group B patients who received at least the minimum acceptable dosage of treatment, the SVR rate was significantly higher for patients under 65 years than for patients 65 years or older (158 of 239, 66.1% vs. 22 of 53, 41.5%: $P = 0.002$). However, there was no significant difference of SVR rate between patients under 65 years and patients 65 years or older in group A (25 of 39, 64.1% vs. 6 of 10, 60.0%, $P = 0.810$).

In our analysis of whether or not the SVR rate differed according to the age and sex of patients who received at least the minimum acceptable dosage, the rate of SVR of group A patients was not significantly different by sex or age (men: 8 of 15, 53.3% vs. 1 of 1, 100%, women: 17 of 24, 70.8% vs. 5 of 9, 55.6%). On the other hand, among the men of group B, the SVR rate was significantly higher for patients under 65 years than for patients 65 years or over (108 of 154, 70.1% vs. 8 of 22, 36.4%, $P = 0.003$). There was no significant difference of the rate between patients under 65 years and patients 65 years or older among the women of group B (50 of 85, 58.8% vs. 14 of 31, 45.2%).

We compared the SVR rates by platelet count status, over 150×10^9/L or not, and by whether or not the

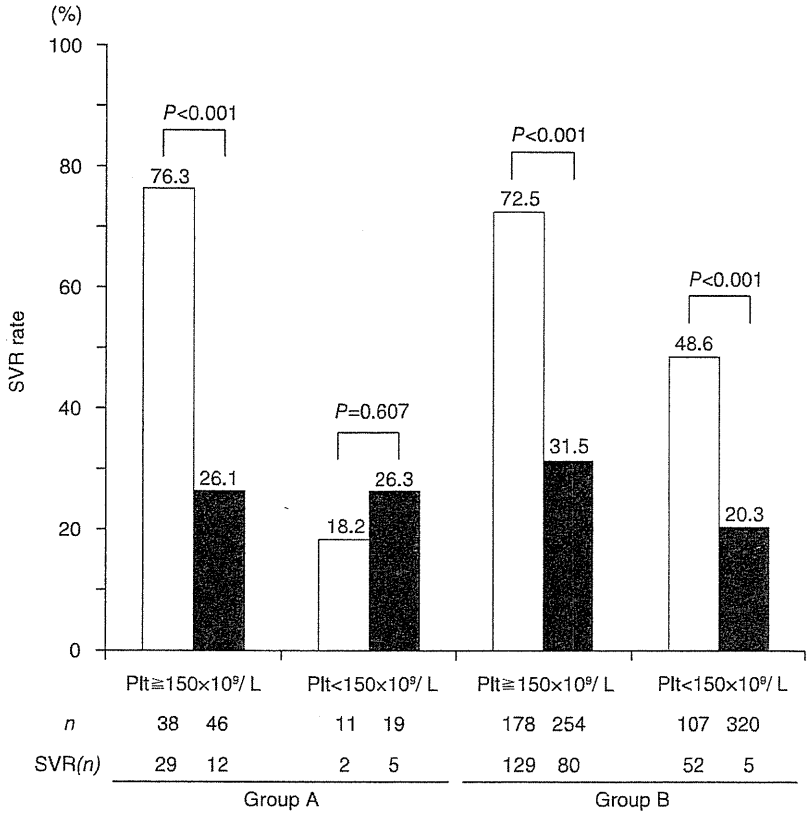

Figure 1 Comparison of the sustained virological response (SVR) rate and platelet count of patients who received the minimum acceptable dosage of pegylated interferon α-2b and ribavirin. In group A (alanine aminotransferase [ALT] <30 IU/L) patients whose platelet count was over 150×10^9/L, the SVR rate was significantly higher for those who received the minimum acceptable dosage than for those who did not (29 of 38, 76.3% vs. 12 of 46, 26.1%, $P < 0.001$). In group B (ALT ≧ 30 IU/L), the SVR rate was significantly higher for those who received the minimum acceptable dosage, with no relation to platelet count. The white column means an SVR rate of patients who received the minimum acceptable dosage. The black column means an SVR rate of patients who did not receive the minimum acceptable dosage.

patient received at least the minimum acceptable treatment dosage. In group A patients whose platelet count was over 150×10^9/L, the SVR rate was significantly higher for those who received at least the minimum acceptable dosage than for those who did not (29 of 38, 76.3% vs. 12 of 46, 26.1%, $P < 0.001$). In group B, the SVR rate was significantly higher for those who received the minimum acceptable dosage with no relation to platelet count (over 150×10^9/L: 129 of 178, 72.5% vs. 80 of 254, 31.5%, $P < 0.001$, under 150×10^9/L: 52 of 107, 48.6% vs. 65 of 320, 20.3%, $P < 0.001$) (Fig. 1). Further, in group A patients whose platelet count was over 150×10^9/L and who received at least the minimum acceptable dosage, the SVR rate was not significantly different by sex or age (under 65 men: 8/11, 72.7%, under 65 women: 15/20,75.0%, over 65 men: 1/1, 100%, over 65 women, 5/6, 83.3%). Furthermore, we compared the SVR rates of patients whose liver fibrosis was F2-4, and found no significant difference between groups A and B.

In a comparison of the SVR rate of patients with or without one or more previous courses of IFN plus RBV, there was no significant difference between groups A and B.

Background factors associated with SVR

To determine the relative weight of the background factors influencing SVR, both univariate and multivariate analyses were performed. Univariate analysis showed that age (<65 years old), homeostasis model assessment-insulin resistance (HOMA-IR) (<2) and total cholesterol (≧220 mg/dL) were significantly associated with SVR in the NALT group, butγGTP, HCVRNA level and LDL-C were not (Table 2). In the multivariate analysis, age (odds ratio [OR] 0.236, $P = 0.017$) and total cholesterol (OR 4.098, $P = 0.039$) were independent factors associated with an SVR in the NALT group (Table 3).

Change of ALT levels after the combination therapy of PEG-IFN α-2b plus RBV

After 6 months of the combination therapy, the mean ALT level of the group A patients who achieved an SVR

Table 2 Univariate analysis of background factors influencing a sustained virological response (SVR)

Factors	Group A (ALT < 30 IU/L) (n = 114)			Group B (ALT ≧ 30 IU/L) (n = 875)		
	Odds ratio	95% CI	*P*-value	Odds ratio	95% CI	*P*-value
Sex	1			1		
Men						
Women	2.186	0.949–5.038	0.066	0.682	0.515–0.902	0.007
Age (years)						
<65	1			1		
≧65	0.247	0.096–0.631	0.004	0.341	0.242–0.481	<0.001
Histological Staging						
F 0–1	1			1		
F 2–3	0.349	0.128–1.207	0.103	0.382	0.264–0.553	<0.001
Serum HCV RNA level (logIU/mL)						
<6	1			1		
≧6	0.486	0.198–1.192	0.115	0.449	0.317–0.636	<0.001
γGTP (IU/)						
<44	1			1		
≧44	0.523	0.196–1.394	0.195	0.407	0.306–0.541	<0.001
Albumin (mg/dL)						
≧3.5	1			1		
<3.5				0.169	0.072–0.398	<0.001
Platelet count (×10^9/L)						
≧150	1			1		
<150	0.312	0.121–0.805	0.886	0.422	0.317–0.561	<0.001
Hemoglobin (g/dL)						
≧14	1			1		
<14	1.304	0.564–3.016	0.534	0.703	0.533–0.928	0.013
Fasting plasma glucose (mg/dL)						
<95	1		1			
≧95	0.471	0.210–1.057	0.068	0.553	0.411–0.744	0.001
HbA1c (%)						
<6.4	1			1		
≧6.4				0.235	0.103–0.535	0.001
HOMA-IR						
<2	1			1		
≧2	0.156	0.052–0.466	<0.001	0.188	0.121–0.290	<0.001
Total cholesterol (mg/dL)						
<220	1			1		
≧220	3.462	1.051–11.396	0.041	1.394	0.732–2.653	0.312
Tryglyceride (mg/dL)						
<150	1			1		
≧150	1.00	0.267–4.533	0.895	0.747	0.453–1.234	0.255
HDL-C (mg/dL)						
<40	1			1		
≧40	3.182	0.605–16.725	0.172	1.065	0.623–1.822	0.817
LDL-C (mg/dL)						
<140	1			1		
≧140	1.067	0.090–12.706	0.959	0.985	0.402–2.410	0.973

ALT, alanine aminotransferase; CI, confidence interval; γ-GTP, γ-glutamyltranspeptidase; HDL-C, High density lipoprotein-cholesterol; HOMA-IR, homeostasis model assessment-insulin resistance; LDL-C, Low density lipoprotein-cholesterol.

Table 3 Multivariate analysis of background factors influencing an sustained virological response (SVR) in normal alanine aminotransferase (NALT) patients

Factors	Odds ratio	95% CI	*P*-value
Age (years)			
<65	1		
≧65	0.236	0.072–0.771	0.017
HCV RNA (logIU/mL)			
<6	1		
≧6	0.391	0.131–1.167	0.092
Total cholesterol (mg/dL)			
<220	1		
≧220	4.098	1.077–15.591	0.039

CI, confidence interval.

decreased from 24.4 ± 3.4 IU/L to 16.3 ± 10.1 IU/L for the men and from 23.6 ± 3.5 IU/L to 14.1 ± 5.9 IU/L for the women. ALT-flare ups were observed for 34.0% (18 of 53) of the non-responsive group A patients. The mean ALT level was 63.6 ± 35.1 IU/L, and only three of these patients (16.7%) had serum ALT activity >100 IU/L (max 163 IU/L).

DISCUSSION

THIS IS THE first report of a large multicenter trial of the efficacy and safety of PEG-IFN α-2b plus RBV treatment of Japanese chronically infected HCV patients with NALT. A large randomized controlled trial of PEG-IFN α-2a 180 μg/week plus RBV at a fixed dose of 800 mg/day for American HCV patients with NALT reported an SVR rate of 40% for patients with genotype 1 treated for 48 weeks,[16] comparable to that achieved by patients with elevated ALT activity.[19,20] Our results were similar (37.8%), which indicates that Japanese NALT patients are suitable candidates for PEG-IFN α and RBV combination treatment.

Puoti *et al.*[17] reported that, for patients treated with PEG-IFN α-2a 180 μg/week plus an optimal RBV dosage (1000–1200 mg/day), the SVR rate was improved to 62% for HCV-1 NALT patients. In Japan, RBV taken orally at a daily dose of 600–1000 mg based on body weight is the recommended treatment of the Japanese Ministry of Health, Labor and Welfare. Thus, we are not able to use the same dose of RBV as used in the United States and European countries. On the other hand, Hiramatsu *et al.* have reported that maintaining a high dose (≧12 mg/kg/day) of RBV during the full treatment period could strongly suppress the relapse rate with chronic hepatitis C genotype 1 responding to α-2b plus RBV.[27] However, in their study, 165 (16.8%) of 984 patients who were enrolled discontinued the treatment because of adverse events or voluntary withdrawal, and 331 patients (33.6%) discontinued the treatment because of non-response. SVR in the intention-to-treat analysis was only 347 of 984 (35.3%), and the rate was similar to ours. Maintaining a higher dose of RBV results in higher rates of discontinuation due to adverse events, which leads to a decrease in SVR. Thus we feel it is best to reduce the dose of RBV. Therefore, we analyzed the SVR rates of our patients who were given less than the minimum acceptable dosage.

Our results indicate that taking at least the minimum acceptable dosage during treatment increased the SVR rate of NALT patients with genotype 1 by two to three times more than patients who did not take the minimum acceptable dosage. The current results confirm our previous study,[23,28] as well as indicate that receiving at least the minimum acceptable dosage is also very important for NALT patients to achieve SVR. The SVR rate was almost the same for patients taking a higher total dosage of RBV and those receiving the minimum acceptable dosage, and prescribing the minimum acceptable dosage would be safe and more cost effective than prescribing a higher dosage of RBV for NALT patients.

For HCV patients with NALT, Puoti *et al.*[17] stated that young patients without contraindications should take a combination therapy of PEG-IFN α plus RBV rather than to take a watchful-waiting strategy, we feel that older patients with NALT also may be acceptable candidates for PEG-IFN α plus RBV treatment. Moreover, results that the men over 65 years-of-age with elevated ALT had a lower SVR rate (36.4%) than those under 65 years (70.1%) indicate that it is necessary to treat the men with interferon at a younger age and before the exacerbation of ALT.

In this study, patients with NALT had milder histological disease than those with elevated ALT, which may be related to the higher rate of SVR in the NALT group.

Okanoue *et al.* reported that HCV carriers with ALT<30 IU/L and PLT counts >150 × 10^9/L were recommended to have follow up without antiviral treatment, because over 90% show normal or minimal liver damage with good prognosis from the point of view of the prevention of HCC.[29] Our data showed a higher SVR rate if NALT patients received at least the minimum acceptable dosage when liver fibrosis was not advanced. Therefore, from the point of view of eliminating HCV,

we feel that NALT patients also should receive PEG-IFN α plus RBV treatment if liver fibrosis is not advanced.

Further, our data demonstrated that total cholesterol could be useful for predicting which NALT patients will achieve SVR. These results showed that the total cholesterol level is inversely associated with liver fiborosis.[30,31] Therefore, serum total cholesterol might be helpful for a determination to treat NALT patients with PEG-IFN α-2b plus RBV, whether or not liver fibrosis is advanced, even when we cannot do liver biopsy. We feel that whether or not to initiate therapy should be decided not only by age and serum ALT level, but also by serum total cholesterol and the guidelines of AASLD as above mentioned.[12]

Although IFN α treatment for patients with NALT has been reported to cause ALT-flare ups after treatment,[32,33] we previously reported that the number of patients with elevated ALT levels in a 2-year follow up was not significantly different between patients treated with IFN α and untreated patients.[34] There has been only one report that PEG-IFN α-2a plus RBV combination treatment did not cause ALT flare-ups after treatment,[16] but the precise relationship remains to be elucidated. Our data indicated that the ALT flare up rate after treatment was 15.8%, and watching non-SVR patients carefully after treatment is important to check for ALT flare ups. Along with a report that over 60% of patients with NALT have an elevated ALT level at 3 years,[35] we considered that the PEG-IFN α plus RBV combination treatment is also safe for patients with NALT, although we must note that we did not follow up a full 2 years to observe the change of ALT levels.

This study has a limitation that liver biopsy was done only for about half of the enrolled patients and that we could not measure biomarkers of liver fibrosis such as hyaluronic acid, so we could not precisely estimate the liver fibrosis. However, because the present study was a large multicenter design, the findings are of great interest for clarifying the efficacy and safety of PEG-IFN α-2b plus RBV combination treatment for patients with NALT.

CONCLUSIONS

THE EFFICACY AND safety of PEG-IFN α-2b plus RBV combination therapy for patients with chronic HCV infection who have NALT is similar to that of patients with elevated ALT levels. These results indicate that patients with NALT are suitable candidates for treatment with PEG-IFN α-2b plus RBV.

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Original Article

Occurrence of clinical depression during combination therapy with pegylated interferon alpha or natural human interferon beta plus ribavirin

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Aim: The onset of depression symptoms during pegylated interferon α plus ribavirin (PEG-IFN/RBV) combination therapy has led to treatment discontinuation in some cases. In the present study, we conducted a questionnaire survey during treatment to determine whether natural human interferon β plus ribavirin (IFNβ/RBV) therapy is associated with a lower incidence of depression symptom onset compared with PEG-IFN/RBV therapy.

Methods: Seventy-seven patients with chronic hepatitis C received PEG-IFN/RBV (PR) or IFNβ/RBV (FR) therapy. A questionnaire survey was administered at the start of treatment, and at 4 and 12 weeks, using the Beck Depression Inventory II (BDI-II) and the Pittsburgh Sleep Quality Index (PSQI).

Results: BDI-II scores in the PR group increased at 4 and 12 weeks, but remained unchanged in the FR group. At 12 weeks, the mean BDI-II score and incidence of abnormalities with a BDI-II score of ≥14 were significantly lower in the FR group than in the PR group. BDI-II scores during IFNβ/RBV therapy in 11 patients currently using antidepressants remained unchanged up to 12 weeks. None of these 11 patients required addition or dose increases of antidepressants, and there was no evidence of worsened depression symptoms. Nine PR patients had BDI-II scores of ≥14 and PSQI scores of ≥11 at 12 weeks.

Conclusions: IFNβ/RBV therapy was associated with a lower incidence of depression symptom onset during treatment. In patients already diagnosed with depression, there was no evidence that IFNβ/RBV therapy caused any worsening of symptoms, indicating that IFNβ/RBV therapy is safe for patients with depression.

Key words: Beck Depression Inventory II, chronic hepatitis C, depression, natural interferon β, pegylated interferon α, Pittsburgh Sleep Quality Index.

INTRODUCTION

INTRODUCTION OF PEGYLATED interferon α plus ribavirin (PEG-IFN/RBV) combination therapy has led to an improved sustained virological response (SVR) in patients with chronic hepatitis C who are receiving interferon therapy.[1–6] An additional new treatment regimen has been introduced by adding Telaprevir to this PEG-IFN/RBV therapy.[7,8] However, adverse effects of PEG-IFN/RBV include the onset of symptoms of depression.[9–11] Thus, there are some difficulties in treating patients with depression or sleep disorders with PEG-IFN/RBV therapy.

In Japan, natural human interferon β (IFNβ), which has a low association with the onset of symptoms of depression, has been used in interferon therapy for chronic hepatitis C.[12,13] IFNβ plus ribavirin (IFNβ/RBV) combination therapy is now used.[14] However, there are no existing reports on the relationship between PEG-IFN/RBV or IFNβ/RBV therapy and the onset of depression symptoms. Therefore, in the present study, in order to determine if IFNβ/RBV therapy is associated with a lower incidence of the onset of symptoms of depression compared to PEG-IFN/RBV therapy, and to evaluate the safety of the IFNβ/RBV therapy in patients with depression, we conducted a questionnaire survey during PEG-IFN/RBV or IFNβ/RBV therapy to investigate the frequency, timing, and intensity of depression symptoms.

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METHODS

Study population

A TOTAL OF 77 Shinkokura Hospital patients with chronic hepatitis C who received IFN therapy for at least 12 weeks between January 2010 and April 2011 were included in the study. The study protocol was in compliance with both the Good Clinical Practice Guidelines and the 1975 Declaration of Helsinki, and was approved by the Institutional Review Board. Each patient gave informed consent before participating in this trial. Patients were assigned to one of the following three groups: (1) the PEG-IFN/RBV (PR) group, consisting of 41 patients who received PR therapy for a period of 24 to 48 weeks; (2) the IFNβ/RBV (FR) group, consisting of 25 patients who received the FR therapy for a period of 24 to 48 weeks; and (3) the FR-d group, consisting of 11 patients with depression who were on antidepressants and who received the FR therapy for a period of 24 to 48 weeks. Patients in the FR-d group received regular psychiatric consultation and experienced dose reduction, dose increase, or addition of antidepressants during treatment. Patients with depression, those with a previous history of depression, those who were on antidepressants, or those who were on sleep-inducing drugs were excluded from the PR and FR groups. Patients reporting some type of sleep disorder during treatment were given sleep-inducing drugs at the discretion of their primary physician. Treatment regimens of PR or FR therapy were determined by the physician. None of the patients required dose reduction of IFN due to neutropenia or thrombocytopenia prior to 12 weeks. This study is a prospective, non-randomized open trial.

Criteria for exclusion from the study were as follows: (i) clinical or biochemical evidence of hepatic decomposition and advanced cirrhosis identified by ascites, encephalopathy, or hepatocellular carcinoma; (ii) IFNβ/RBV: white blood cell count of less than 3000/mm^3 and platelet count of less than 50 000/mm^3, PEG-IFN/RBV: white blood cell count of less than 4000/mm^3 and platelet count of less than 80 000/mm^3; (iii) concomitant liver disease other than hepatitis C (hepatitis B surface antigen- or human immunodeficiency virus-positive); (iv) excessive active alcohol consumption exceeding 60 g/day or drug abuse; (v) severe psychiatric disease; and (vi) antiviral or corticosteroid therapy within the 12 months prior to enrollment.

Interferon treatment

Patients in the PR group received the following treatment regimen. In brief, PEG-IFNα-2b (PEG-Intron; MSD Co., Tokyo, Japan) was injected subcutaneously at a median dose of 1.5 lg/kg (range: 1.3–2.0 lg/kg) once a week. Ribavirin (Rebetol; MSD Co., Tokyo, Japan) was administered at a dose of 200–600 mg twice a day after breakfast and dinner (daily dose: 600–1000 mg). Patients in the FR and FR-d groups received the following treatment regimen. Briefly, IFNβ (Feron; Toray Industries Inc., Tokyo, Japan) was given intravenously at a dose of 6 million units (MU) daily for 4 weeks, followed by three times a week for 20–44 weeks. Ribavirin (Rebetol; MSD Co., Tokyo, Japan) was administered at a dose of 200–600 mg twice a day after breakfast and dinner (daily dose: 600–1000 mg). Hepatitis C virus (HCV) RNA concentrations were determined using the COBAS TaqMan HCV test (Roche Diagnostics). The linear dynamic range of the assay was 1.2–7.8 log IU/mL. Patients were considered to have an SVR if HCV RNA remained undetectable at 24 weeks after the completion of treatment. Urinalysis and measurement of serum albumin levels were performed once every 4 weeks, from the start of treatment to Week 24.

Questionnaire

A questionnaire survey was conducted immediately before the start of treatment and at 4 weeks and 12 weeks using the Beck Depression Inventory II (BDI-II) and the Pittsburgh Sleep Quality Index (PSQI).[15,16] The questionnaire survey was administered by one expert investigator, who remained blinded to the treatment regimens prescribed to patients, the timing of treatment, and other information. Patients with a BDI-II score of 14 or more were considered to have the onset of depression symptoms. Patients with a PSQI score of 11 or more were identified as having sleep disorder. All patients were given a questionnaire at 12 weeks, while a questionnaire was administered to 58 subjects at the baseline and at 4 weeks, including 28, 19, and 11 patients in the PR, FR, and FR-d groups, respectively.

Statistical analysis

Nonparametric tests (χ^2 test and Fisher's exact probability test) were used to compare the characteristics of the groups, as well as the BDI-II score and the PSQI score at 12 weeks. Univariate and multivariate logistic regression analyses were used to determine the factors that significantly contributed to the onset of symptoms of depression. The odds ratios (OR) and 95% confidence intervals (95% CI) were also calculated. All *P*-values less than 0.05, as determined by the two-tailed test, were considered significant. Variables were entered into

multiple logistic regression analysis to identify significant independent predictive factors. The potential pretreatment factors associated with patients having the onset of depression included the following variables: age, sex, HCV genotype, type of IFN, hemoglobin, platelet count, alanine aminotransferase (ALT), albumin, gamma-glutamyl transpeptidase (γ-GTP), total cholesterol, fasting blood sugar, and HCV RNA level.

RESULTS

Baseline background and IFN treatment

TABLE 1 SHOWS THE background of patients in the PR and FR groups. The mean age was significantly higher in the FR group (64.1 years) than in the PR group (52.5 years; $P < 0.001$). The PR group had more men than the FR group, although statistical significance was not reached. Baseline laboratory data showed a significantly lower platelet count in the FR group ($P < 0.05$). Significantly lower γ-GTP values were observed in the FR group ($P < 0.05$). The other laboratory parameters were comparable between the two groups. More patients with genotype 1 were in the PR group than the FR group, although no statistical significance was found. A total of 59 of 66 patients were evaluable for SVR. The proportion of patients with genotype 1 achieving an SVR was 33% (3/9) in the FR group and 48% (12/25) in the PR group. The PR group had a higher SVR rate, although statistical significance was not reached. The SVR rate among patients with genotype 2 was similar in the FR (85%, 11/13) and PR (83%, 10/12) groups. Over 24 weeks of treatment, 8% of patients (3/36) experienced at least one proteinuria event. None of the patients had a serum albumin level of ≤3.3 g/dL.

Change in the BDI-II score and the PSQI score during IFN treatment

Changes in the BDI-II score over time are shown in Figure 1. BDI-II scores in the PR group were increased relative to baseline at 4 and 12 weeks. Corresponding scores in the FR group remained unchanged. At 12 weeks, BDI-II scores were significantly lower in the FR group (5.8) than in the PR group (12.6; $P < 0.05$). The FR-d group had already high BDI-II scores of 23.0 at baseline, but BDI-II scores remained unchanged during treatment. No patients required dose increase or addition of antidepressants during treatment. There was no evidence of worsened depression symptoms during FR therapy.

In the PR group, the incidence of the onset of symptoms of depression, defined as a BDI-II score of 14 or more, increased from 0% at baseline to 21% at 4 weeks

Table 1 Clinical background before combination therapy of interferon β plus ribavirin (IFNβ/RBV) or pegylated interferon α plus ribavirin (PEG-IFN/RBV) in chronic hepatitis C patients

		IFNβ /RBV $n = 25$		PEG-IFN/RBV $n = 41$		IFNβ /RBV with depression $n = 11$	
Study variables		Mean	(SD)	Mean	(SD)	Mean	(SD)
Age	years	64.1	(12.7)**	52.5	(10.2)**	49.2	(9.7)
Gender							
Male		13	(52%)	30	(73%)	5	(45%)
Female		12	(48%)	11	(27%)	6	(55%)
Baseline hemoglobin	g/dL	14.0	(1.4)	14.7	(1.4)	14.0	(2.0)
Baseline platelet	10^9/L	165	(57)*	192	(59)*	202	(78)
Baseline ALT	IU/L	81.2	(81.1)	73.4	(64.0)	65	(43.1)
Baseline γ-GTP	IU/L	47.9	(36.5)*	92.0	(58.5)*	92.1	(96.3)
Baseline total cholesterol	mg/dL	177.1	(23.3)	177.5	(43)	201.5	(38.3)
Baseline fasting blood sugar	mg/dL	118.7	(58.4)	117.5	(33)	10.5.0	(30.8)
Baseline HCV	log IU/mL	5.8	(1.1)	6.1	(0.9)	5.9	(1.1)
HCV genotype							
1		12	(48%)	28	(68%)	5	(45%)
2		13	(52%)	13	(32%)	6	(55%)

*$P < 0.05$ (IFNβ/RBV vs. PEG-IFN/RBV).
**$P < 0.001$ (IFNβ/RBV vs. PEG-IFN/RBV).
ALT, alanine aminotransferase; HCV, hepatitis C virus; γ-GTP, albumin, gamma-glutamyl transpeptidase.

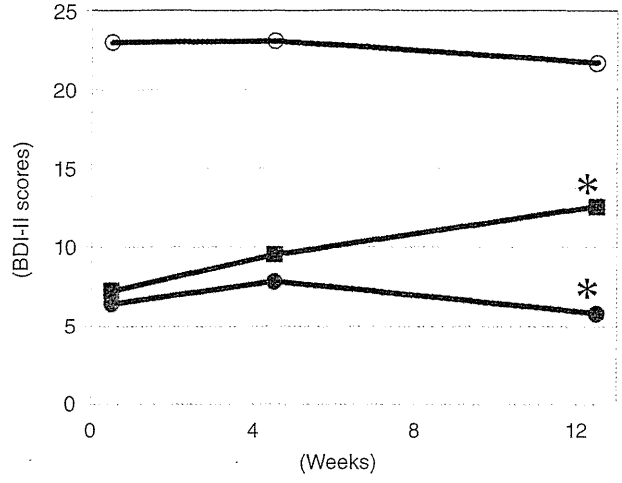

Figure 1 Changes in Beck Depression Inventory II (BDI-II) score for pegylated interferon α plus ribavirin (PEG-IFN/RBV) or interferon β plus ribavirin (IFNβ/RBV) therapy (●: IFNβ/RBV [FR] group, ○: FR-d group [FR patients with depression], ■: PEG-IFN/RBV [PR] group. *$P < 0.05$, FR vs. PR at week 12).

($n = 6$) and 34% at 12 weeks ($n = 14$). In the FR group, the incidence of the onset of symptoms of depression was 10% at 4 weeks ($n = 2$) and 8% at 12 weeks ($n = 2$), compared with 0% at baseline, indicating that the incidence did not change between 4 and 12 weeks. The incidence of the onset of depressive symptoms at 4 weeks was lower, but not significantly, in the FR group than in the PR group. Figure 2 shows the BDI-II score with a treatment regimen of IFN therapy at 12 weeks. The incidence of the onset of depressive symptoms (BDI-II score of 14 or more) was significantly lower in

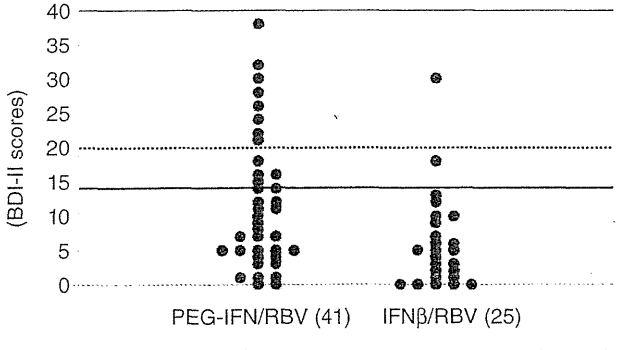

Figure 2 Distribution of Beck Depression Inventory II (BDI-II) scores for treatment regimens of interferon (IFN) therapy at 12 weeks (solid line: BDI-II score of 14, dotted line: BDI-II score of 20).

the FR group (8%, $n = 2$) than in the PR group (34%, $n = 14$; $P < 0.05$). The incidence of the onset of moderate depression symptoms (a BDI-II score of 20 or more) was higher in the PR group (20%, $n = 8$) than in the FR group (4%, $n = 1$). Mean PSQI scores at baseline, 4 weeks, and 12 weeks were 5.44, 6.62, and 7.37 in the PR group and 5.69, 6.01, and 6.88 in the FR group, respectively, indicating higher scores in the PR group than in the FR group from Week 4 onward. The incidence of sleep disorder, defined as a PSQI score of 11 or more, was higher in the PR group at both 4 and 12 weeks (18% and 27%, respectively) than in the FR group (0% and 8%, respectively).

BDI-II score and PSQI score at 12 weeks

Figure 3 shows the correlation between the BDI-II score and the PSQI score at 12 weeks. Some correlation was found between these scores with an overall coefficient of correlation (r) of 0.6755 ($P < 0.0001$). A strong correlation was noted between the BDI-II score and the PSQI score in the PR group, with an r-value of 0.7586 ($P < 0.0001$). In contrast, no correlation was observed in the FR group, with an r-value of 0.3589 ($P = 0.0786$). The incidence of sleep disorder (a PSQI score of 11 or more) at 12 weeks was lower in the FR group (8%, $n = 2$) than in the PR group (27%, $n = 11$). Only nine patients in the PR group had a BDI-II score of 14 or more and a PSQI score of 11 or more, whereas there were no such patients in the FR group, with the difference reaching statistical significance ($P < 0.05$). Three of the nine patients with a BDI-II score of 14 or more

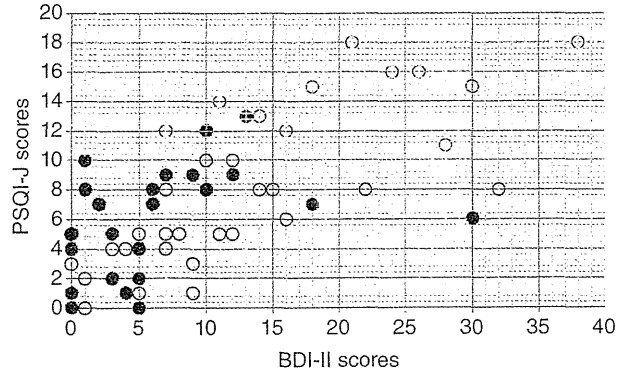

Figure 3 Graph showing correlation between Beck Depression Inventory II (BDI-II) and the Pittsburgh Sleep Quality Index (PSQI) scores at 12 weeks (correlation coefficient, Total: r = 0.6755, $P < 0.0001$; pegylated interferon α plus ribavirin [PEG-IFN/RBV]: r = 0.7586, $P < 0.0001$; interferon β plus ribavirin [IFNβ/RBV]: r = 0.3589, $P = 0.0786$).

and a PSQI score of 11 or more at 12 weeks discontinued treatment prior to 24 weeks due to depression symptoms.

Predictive factors contributing to the onset of depression symptoms during IFN therapy

Results from univariate and multivariate logistic regression analyses of the factors contributing to the onset of depression symptoms during IFN therapy are shown in Table 2. The univariate regression analysis showed that the type of IFN (PEG-IFNα) was the only factor that contributed to the onset of depressive symptoms ($P < 0.027$). The multivariate logistic regression analysis confirmed that the type of IFN (PEG-IFNα/RBV) was the only contributing significant independent predictive factor.

DISCUSSION

PR THERAPY FOR chronic hepatitis C involves long-term treatment, ranging from 24 to 48 weeks. The duration of treatment in patients with HCV genotype 1 and a high viral load may range from 48 and 72 weeks.[17] Currently available PR therapy yields only a low SVR rate in patients who discontinue treatment early. Thus, it is important to complete treatment as prescribed. The onset of depression symptoms associated with PEG-IFNα treatment is one of the reasons for early discontinuation of treatment due to adverse effects. In Japan, IFNβ, which is associated with a low incidence of the onset of depression symptoms, has been used in patients with depression.[12–14] In addition, due to the milder side effects of IFNβ, we have used it in IFN therapy for hemodialyzed patients with chronic hepatitis C.[18] The SVR rate among patients with HCV genotype 1 who were treated with IFNβ/RBV was lower (approximately 40%) than that among those treated with PEG-IFN/RBV[11], while patients with HCV genotype 2 who were treated with IFNβ/RBV had an SVR rate of approximately 87%, which was similar to that observed in those treated with PEG-IFN/RBV [19].

There have been no reported studies on the relationship between FR therapy and the onset of depression symptoms. In the present study, we demonstrated that FR therapy produced a significantly lower frequency of depression symptoms than PR therapy. We also found no evidence of worsened depression symptoms during the FR therapy in patients with depression.

In the present study, a questionnaire was conducted using BDI-II and PSQI scores to assess depression symptoms and sleep disorder. The BDI-II is way to measure the severity of depression symptoms and consists of 21 questions. Symptoms with a total score of ≥14, ≥20, and ≥29 are considered mild, moderate, and severe, respectively.[15] The PSQI is a questionnaire that is used to measure the quality of sleep. Original versions of both questionnaires have been translated into Japanese, and the translated versions were used in our study.

In the present study, we found that the percentage of patients with a BDI-II score of 14 or more in the PR group was approximately 20% as early as 4 weeks after

Table 2 Results from univariate and multivariate logistic regression analyses of the factors contributing to the onset of depressive symptoms

			Simple regression		Multiple logistic regression	
Factor	Range		Odds ratio	*P*-value	Odds ratio	*P*-value
Age	≧60 / <60	(years)	0.308	0.066	–	–
Sex	Male / Female		0.808	0.728	–	–
Genotype	1 / 2		0.900	0.859	–	–
Type of IFN	PEG-IFN/IFNβ		0.168	0.027	0.168	0.027
Hemoglobin	<14 / ≧14	(g/dL)	1.310	0.647	–	–
Platelet	<15 / ≧15	(10^4/μL)	3.294	0.143	–	–
ALT	≧50 / <50	(IU/L)	1.269	0.682	–	–
γ-GTP	≧45 / <45	(IU/L)	0.990	0.986	–	–
Total cholesterol	≧220 / <220	(mg/dL)	1.667	0.652	–	–
FBS	<110 / ≧110	(mg/dL)	0.682	0.531	–	–
Viral load	≧6.0 / <6.0	(LogIU/mL)	0.829	0.750	–	–

ALT, alanine aminotransferase; FBs, fasting blood sugar; IFN, interferon; γ-GTP, gamma-glutamyl transpeptidase; PEG-IFN/RBV, pegylated interferon α plus ribavirin.

the start of treatment and increased to 34% within the first 12 weeks. However, in the FR group, 10% or less of patients only experienced the onset of mild depressive symptoms and the percentage was comparable at 4 and 12 weeks, after which no patients discontinued treatment due to depression symptoms. At 12 weeks particularly, both the mean BDI-II score and the incidence of abnormalities (a BDI-II score of 14 or more) were significantly lower in the FR group than in the PR group, indicating that FR therapy was less likely to induce the onset of depression symptoms than PR therapy. It appears that assessing the onset of depressive symptoms is useful at 12 weeks of IFN treatment. However, assessment at 4 weeks of treatment also appears to be necessary, when possible, because the onset of depression symptoms may be observed as early as 4 weeks.

The onset of depression symptoms during PR therapy has been associated with sleep disorder. In the present study, there was a strong association between the BDI-II scores and PSQI scores. Careful management is required in patients reporting sleep disorder, which is one of the early symptoms of depression.

Some of the patients receiving PR therapy, who had a BDI-II score of 14 or more and a PSQI score of 11 or more at 12 weeks, discontinued treatment due to the subsequent onset of depressive symptoms; more careful management is required in these patients.

Patients with depression were also included in the present study (FR-d group). There was no increase over time in the BDI-II score of patients with depression and none of the patients with depression required additional or an increased dose of antidepressants; there was no evidence that the depression symptoms worsened. This suggests that FR therapy is safe in both patients with depression and patients at risk for symptoms of depression.

The BDI-II and the PSQI, which were used in the present study, are simple questionnaires, which take several minutes to complete and appear to be useful instruments in assessing the onset of depressive symptoms during IFN therapy. IFNβ/RBV therapy should be used in patients with depression or sleep disorder. Patients showing the onset of depression or sleep disorder during PEG-IFN/RBV therapy should be switched to IFNβ/RBV therapy to continue IFN therapy, having given due consideration to the discontinuation of therapy.

IFNB/RBV THERAPY WAS associated with a low incidence of the onset of depression symptoms during treatment, and was also safe in patients with depression, who showed no evidence of worsening of symptoms during treatment. Depression symptoms during PEG-IFN/RBV therapy were strongly associated with sleep disorders and commonly occurred within the first 12 weeks of treatment. Patients with the onset of both symptoms of depression and sleep disorders should be closely monitored, as they are more likely to discontinue treatment after these conditions develop.

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HEPATOLOGY

Increase in platelet count based on inosine triphosphatase genotype during interferon beta plus ribavirin combination therapy

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Key words
chronic hepatitis C, inosine triphosphatase, natural interferon β, platelet count, ribavirin.

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Abstract

Background and Aim: The inosine triphosphatase (*ITPA*) genotype is associated with ribavirin-induced anemia and pegylated interferon α (PEG IFN-α)-induced platelet reduction during PEG IFN-α plus ribavirin combination therapy. Natural IFN-β plus ribavirin therapy is associated with increases in platelet counts during treatment. We investigated decreases in platelet counts according to *ITPA* genotype during natural IFN-β/ribavirin therapy to determine if patients with low platelet counts were eligible for this combination therapy.

Methods: A total of 187 patients with chronic hepatitis C received PEG IFN-α/ribavirin or natural IFN-β/ribavirin therapy. Decreases in platelet counts based on *ITPA* genotype were investigated during treatment through 24 weeks.

Results: Platelet counts decreased during week 1 of PEG IFN-α/ribavirin therapy, but increased during week 2, after which platelet counts decreased gradually. Platelet counts decreased until week 4 of natural IFN-β/ribavirin therapy, after which platelet counts increased. Platelet counts after week 8 were higher relative to pretreatment platelet counts. Patients with the *ITPA*-CC genotype showed a smaller decrease in platelet counts during natural IFN-β/ribavirin therapy than those with the *ITPA*-CA/AA genotype; platelet counts after week 8 of this therapy were higher than pretreatment platelet counts, regardless of pretreatment platelet counts. Multivariate logistic regression analyses showed that natural INF-β/ribavirin therapy was the only significant independent predictor for an increase in platelets through week 8.

Conclusion: Natural IFN-β/ribavirin therapy is safe for patients with the *ITPA*-CC genotype, even if their pretreatment platelet counts are low.

Introduction

The introduction of pegylated interferon-α (PEG IFN-α) plus ribavirin (PEG-IFN/RBV) combination therapy has led to an improved sustained virological response (SVR) rate in patients with chronic hepatitis C receiving IFN therapy.[1–6] However, cytopenia has been observed during PEG-IFN/RBV therapy. Specifically, cases of RBV-induced anemia and PEG-IFN-induced thrombocytopenia or neutropenia have been reported, and we have previously described cases of RBV-induced anemia.[7] A genome-wide association study (GWAS) identified the inosine triphosphatase gene (*ITPA*) single nucleotide polymorphism (SNP) as being strongly associated with RBV-induced anemia.[8–10] This *ITPA* SNP was also reported to play a role in the decreases in platelet counts that occur during PEG-IFN/RBV therapy.[11,12] In Japan, natural INF-β plus ribavirin (IFN-β/RBV) therapy has been indicated for the treatment of chronic hepatitis C. This therapy is associated with greater increases in platelet counts than seen with PEG-IFN/RBV therapy.[13] Therefore, we investigated the association between the *ITPA* genotype and decreases in platelet count during IFN-β/RBV therapy to determine if patients with a low platelet count were eligible for IFN-β/RBV therapy.

Methods

Patients. A total of 187 patients with chronic hepatitis C who received IFN therapy for at least 24 weeks at the Shinkokura Hospital between January 2009 and April 2011 were included in the study. The study protocol was in compliance with the Good Clinical Practice Guidelines and the 1975 Declaration of Helsinki, and was approved by the Institutional Review Board. Each

patient provided informed consent before participating in this trial.

Criteria for exclusion were as follows: (i) clinical or biochemical evidence of hepatic decomposition or advanced cirrhosis identified by ascites, encephalopathy, or hepatocellular carcinoma; (ii) IFN-β/RBV: a white blood cell count of less than 3×10^9/L and a platelet count of less than 50×10^9/L, PEG-IFN/RBV: a white blood cell count of less than 4×10^9/L and a platelet count of less than 80×10^9/L; (iii) concomitant liver disease other than hepatitis C (hepatitis B surface antigen- or human immunodeficiency virus-positive); (iv) excessive active alcohol consumption exceeding 60 g/day or drug abuse; (v) severe psychiatric disease; and (vi) antiviral or corticosteroid therapy in the 12 months prior to enrollment.

IFN-β/RBV combination therapy. Interferon-β (Feron; Toray Industries, Tokyo, Japan) was given intravenously at a dose of 6 million units (MU) daily for 4 weeks, followed by three times a week for 20–44 weeks. The ribavirin (Rebetol; MSD, Tokyo, Japan) dose was adjusted according to body weight (600 mg for ≤ 60 kg; 800 mg for > 60 to ≤ 80 kg; and 1000 mg for > 80 kg), based on the guidelines of the Ministry of Health, Labor and Welfare of Japan.[5] The drug was administered orally after breakfast and dinner.

PEG-IFN/RBV combination therapy. Pegylated interferon-α-2B (PEG-Intron; MSD) was injected subcutaneously at a median dose of 1.5 μg/kg (range: 1.3–1.5 μg/kg) once a week. Ribavirin was administered twice a day according to body weight, as described for IFN-β/RBV combination therapy.

This study was a prospective, nonrandomized open trial. Platelet counts and hemoglobin levels were measured at baseline and at weeks 1, 2, 4, 8, 12, and 24.

We genotyped each patient for two SNPs: rs8099917, an *IL28B* SNP previously reported to be associated with therapy outcome, and rs1127354 (14), an *ITPA* SNP reported to be associated with ribavirin-induced anemia[14] and decreases in platelet counts.[11] Samples were genotyped using the Illumina HumanHap610-Quad Genotyping BeadChip or with the Invader or TaqMan assay, as described elsewhere.[15–17]

Statistical analysis. Statistical analysis was performed using PASW Statistics, version 18 (SPSS, Chicago, IL, USA) and R, version 2.11. Categorical data were analyzed using the χ^2 test and Fisher's exact tests, and continuous data were analyzed using the nonparametric Mann–Whitney *U*-test. Univariate and multivariate logistic regression analyses were used to determine the factors that significantly contributed to the increase in platelets $> 0 \times 10^9$/L from week 0 through week 8. The odds ratios (OR) and 95% confidence intervals (95% CI) were also calculated. All *P*-values found to be less than 0.05 by the two-tailed test were considered significant. Variables that achieved statistical significance ($P < 0.1$) on univariate analysis were entered into a multiple logistic regression analysis to identify significant independent predictive factors. The potential pretreatment factors associated with increases in platelets $> 0 \times 10^9$/L from week 0 to week 8 included the following variables: age, sex, method of IFN treatment, hepatitis C virus (HCV) genotype, *ITPA* genotype, *IL28B* genotype, hemoglobin, platelet count, alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GTP), and HCV RNA level.

Results

The clinical backgrounds of chronic hepatitis C patients before combination therapy with IFN-β/RBV or PEG-IFN/RBV are shown in Table 1. The mean age of patients receiving IFN-β/RBV therapy was 59.3 years and that of patients receiving PEG-IFN/RBV therapy was 57.9 years, with no difference between the two patient groups. The PEG-IFN/RBV group had more men, although the number was not significantly higher. All baseline laboratory parameters, including hemoglobin levels, platelet counts, ALT levels, γ-GTP levels, and HCV loads, showed no differences

Table 1 Clinical background before combination therapy with interferon β plus ribavirin (IFN-β/RBV) or pegylated interferon plus ribavirin (PEG-IFN/RBV) in chronic hepatitis C patients

		IFN-β/RBV	PEG-IFN/RBV	*P*-value
		n = 45	*n* = 137	
Age	Year (SD)	59.3 (14.3)	57.9 (10.4)	ns
Sex	M/F	22/23	73/64	ns
Hb	g/dL (SD)	14 (1.5)	14.2 (1.4)	ns
Platelet	10^9/L (SD)	178 (59)	183 (59)	ns
ALT	IU/L (SD)	84.1 (63.3)	76.5 (64)	ns
γ-GTP	IU/L (SD)	79.1 (56.29)	69.5 (58.5)	ns
HCV	logIU/mL (SD)	6.7 (1.1)	6.4 (0.9)	ns
HCV genotype	1/2	21/24	102/35	< 0.001
ITPA (rs1127354)	CC/CA or AA	36/9	99/38	ns
IL28B (rs8099917)	TT/TG or GG	35/10	96/41	ns
Decrease in platelet count at week 1	10^9/L (SD)	−47 (32)	−47 (43)	ns
Decrease in platelet count at week 4	10^9/L (SD)	−42 (33)	−28 (33)	< 0.05
Decrease in platelet count at week 8	10^9/L (SD)	19 (36)	−35 (43)	< 0.0001

ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase; HCV, hepatitis C virus; ITPA, inosine triphosphate pyrophosphatase; ns, not significant; SD, standard deviation.

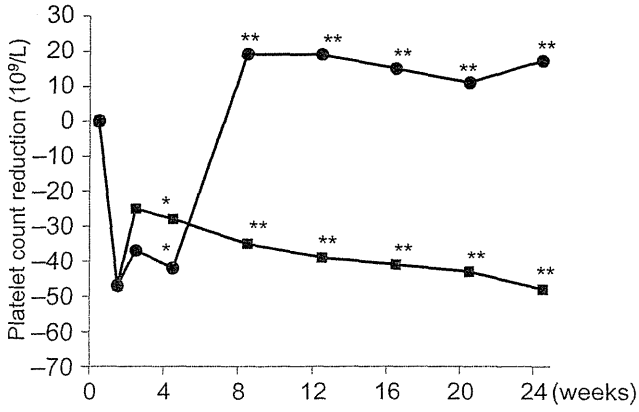

Figure 1 Decreases in platelet count during combination therapy with IFN-β/RBV or PEG-IFN/RBV (closed circle, IFN-β/RBV; closed square, PEG-IFN/RBV; $*P < 0.05$, IFN-β/RBV *versus* PEG-IFN/RBV at week 2; $**P < 0.0001$, IFN-β/RBV *versus* PEG-IFN/RBV at weeks 8, 12, 16, 20, and 24). IFN-β, interferon β; RBV, ribavirin; PEG-IFN, pegylated interferon.

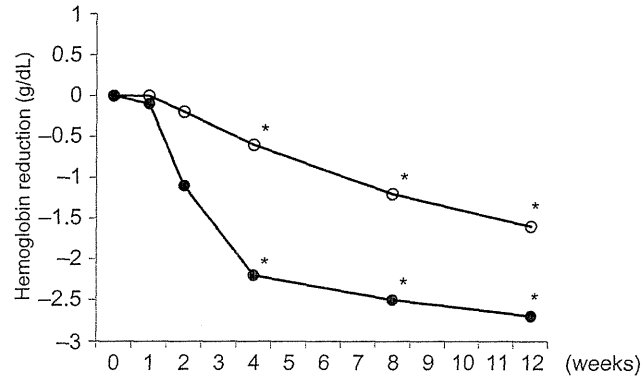

Figure 2 Decreases in hemoglobin levels according to inosine triphosphate pyrophosphatase *(ITPA)* genotype during combination therapy with IFN-β/RBV (closed circle, *ITPA*-CC; open circle, *ITPA*-CA/AA; $*P < 0.01$, *ITPA*-CC *versus ITPA*-CA/AA (rs1127354) at weeks 4, 8, and 12). IFN-β, interferon β; RBV, ribavirin.

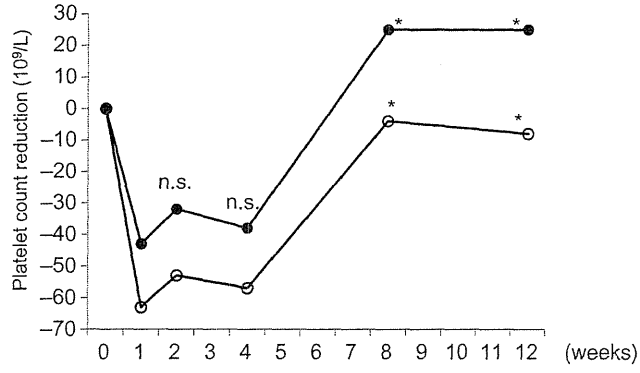

Figure 3 Decreases in platelet count according to inosine triphosphate pyrophosphatase *(ITPA)* genotype during combination therapy with IFN-β/RBV (closed circle, *ITPA*-CC; open circle, *ITPA*-CA/AA; $*P < 0.05$, *ITPA*-CC *versus ITPA*-CA/AA (rs1127354) at weeks 8 and 12). IFN-β, interferon β; RBV, ribavirin.

between the two patient groups. Significantly more patients with HCV genotype 1 were in the PEG-IFN/RBV group ($P < 0.001$). A total of 74% (135/182) patients had the *ITPA*-CC genotype, while 72% of patients had the *IL28B* TT genotype. The frequencies of the *ITPA*-CC genotype and the *IL28B* TT genotype were comparable between the two patient groups. There was no difference in the decreases in platelet counts at week 1; however, at weeks 4 and 8, decreases in platelet counts differed significantly between the two patient groups ($P < 0.05$, $P < 0.0001$).

Platelet count decreases that occurred during combination therapy with IFN-β/RBV or PEG-IFN/RBV are depicted in Figure 1. A decrease in platelet counts of 47×10^9/L was observed at week 1 during IFN-β/RBV therapy. Subsequently, platelet counts transiently increased at week 2, but reduced again at week 4. Platelet counts reduced for 4 weeks after the start of treatment, as IFN-β/RBV therapy involved continuous, daily dosing with IFN-β for 4 weeks after the start of treatment. As per the treatment protocol, IFN-β administration was subsequently reduced to thrice-weekly dosing. At week 8, platelet counts increased and were significantly higher than the pretreatment platelet counts ($P < 0.001$). Platelet counts remained unchanged after week 8. A reduction of 47×10^9/L was observed at week 1 during PEG-IFN/RBV therapy, similar to the reduction that was observed during IFN-β/RBV therapy. Subsequently, platelet counts increased at week 2, decreased at week 4, and gradually decreased further after week 8. The decrease in platelet counts at week 4 during IFN-β/RBV therapy was significantly larger than the decrease observed during PEG-IFN/RBV therapy ($P < 0.05$). However, platelet counts after week 8 of IFN-β/RBV treatment were significantly higher than those during PEG-IFN/RBV therapy ($P < 0.0001$), due to a rapid increase in platelet counts after week 4 of the IFN-β/RBV regimen.

Decreases in hemoglobin levels in relation to the *ITPA* genotype (rs1127354: CC, CA/AA) are shown in Figure 2. At week 2, a large decrease in hemoglobin levels was observed in patients with the *ITPA*-CC genotype. There was no difference in hemoglobin levels based on *ITPA* genotype up to week 2 in patients receiving IFN-β/RBV therapy. Patients with the *ITPA*-CC genotype showed a significantly larger decrease in hemoglobin levels at weeks 4, 8, and 12 than those with the *ITPA*-CA/AA genotype ($P < 0.01$).

Platelet counts during combination therapy with IFN-β/RBV according to the *ITPA* genotype is shown in Figure 3. Similar changes in platelet count decreases were observed in patients with the *ITPA*-CC and *ITPA*-CA/AA genotypes. Patients with the *ITPA*-CC genotype showed a smaller decrease in platelet counts at weeks 1, 2, 4, 8, 12, 24 during therapy compared to those with the *ITPA*-CA/AA genotype. Specifically, patients with the *ITPA*-CC genotype showed a statistically lower degree of platelet decrease at weeks 8, 12, and 24 than those with the *ITPA*-CA/AA genotype ($P < 0.05$). Patients with the *ITPA*-CC genotype had significantly increased platelet counts at week 8 compared with the pretreatment platelet counts ($P < 0.0001$).

Decreases in platelet counts during combination therapy with PEG-IFN/RBV in relation to the *ITPA* genotype are shown in

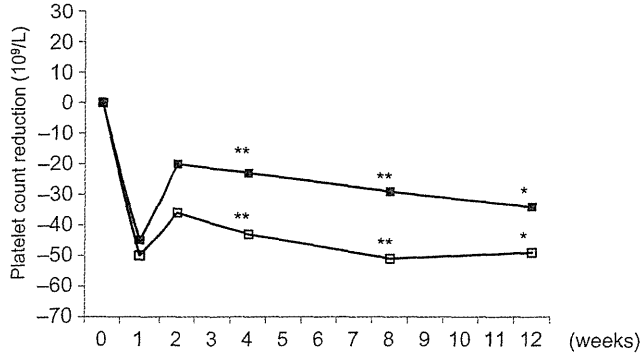

Figure 4 Decreases in platelet count according to inosine triphosphate pyrophosphatase *(ITPA)* genotype during combination therapy with PEG-IFN/RBV (closed square, *ITPA*-CC; open square, *ITPA*-CA/AA; $*P < 0.05$, *ITPA*-CC *versus* *ITPA*-CA/AA (rs1127354) at week 12; $**P < 0.01$, *ITPA*-CC *versus* *ITPA*-CA/AA (rs1127354) at weeks 4 and 8). PEG-IFN, pegylated interferon; RBV, ribavirin.

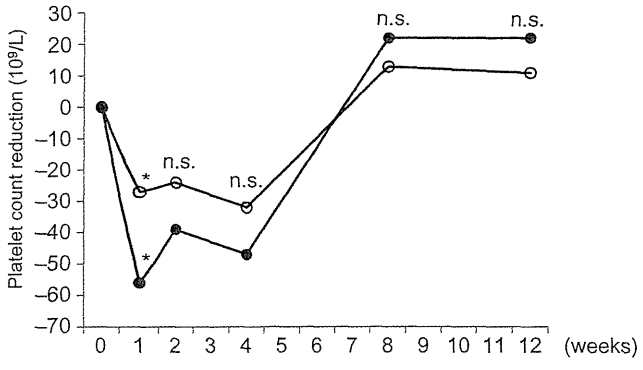

Figure 5 Decreases in platelet count relative to pretreatment platelet counts during combination therapy with IFN-β/RBV (closed circle, $\geq 150 \times 10^9$/L; open circle, $< 150 \times 10^9$/L; $*P < 0.05$, $\geq 150 \times 10^9$/L *versus* $< 150 \times 10^9$/L at week 1). IFN-β, interferon β; RBV, ribavirin.

Figure 4. Similar changes in platelet count decreases were observed in patients with the *ITPA*-CC and *ITPA*-CA/AA genotypes. Patients with the *ITPA*-CC genotype showed a lower degree of platelet reduction at weeks 1, 2, 4, 8, 12, 24 during therapy compared to those with the CA/AA genotype. Specifically, patients with the *ITPA*-CC genotype had a significantly smaller decrease in platelet counts at weeks 4, 8, and 12 than those with the *ITPA*-CA/AA genotype ($P < 0.01$, $P < 0.05$).

Platelet reduction during combination therapy with IFN-β/RBV compared with pretreatment platelet counts is shown in Figure 5. At week 1, patients with a low pretreatment platelet count ($< 150 \times 10^9$/L) showed a significantly smaller decrease in platelet counts than those with a high pretreatment platelet count ($\geq 150 \times 10^9$/L; $P < 0.01$). Five patients had pretreatment platelet counts of $\leq 100 \times 10^9$/L, and a decrease in platelet counts of $\leq 40 \times 10^9$/L was observed in these patients at week 1. Patients with low pretreatment platelet counts showed a small decrease in platelet counts at week 1, after which there was no difference in platelet counts between the groups of patients with high and low

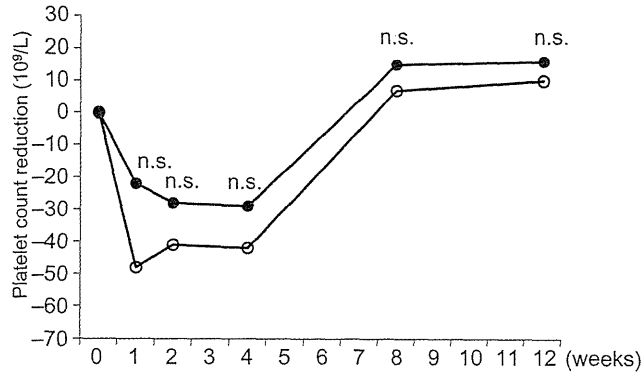

Figure 6 Decreases in platelet count according to inosine triphosphate pyrophosphatase *(ITPA)* genotype during combination therapy with IFN-β/RBV in patients with pretreatment platelet counts ($< 150 \times 10^9$/L) (closed circle, *ITPA*-CC; open circle, *ITPA*-CA/AA). IFN-β, interferon β; RBV, ribavirin.

pretreatment platelet counts. Among patients with both high and low pretreatment platelet counts, platelet counts at week 8 were significantly increased compared with pretreatment platelet counts ($P < 0.01$, $P < 0.05$).

Decreases in platelet counts according to *ITPA* genotype during combination therapy with IFN-β/RBV for patients with pretreatment platelet counts ($< 150 \times 10^9$/L) are shown in Figure 6. For patients with pretreatment platelet counts of $< 150 \times 10^9$/L, patients with the *ITPA*-CC genotype showed a smaller decrease in platelet counts than those with the *ITPA*-CA/AA genotype.

The results of univariate and multivariate logistic regression analyses of factors associated with the increase in platelets $> 0 \times 10^9$/L from week 0 to 8 are shown in Table 2. Univariate and multivariate logistic regression analyses revealed that IFN-β/RBV therapy was the only significant independent predictor for the increase in platelets $> 0 \times 10^9$/L from week 0 to week 8.

Only one patient in the IFN-β/RBV group was withdrawn from the study by week 24. The reason for discontinuation was proteinuria. The dose of IFN was reduced only in the one patient. The dose of ribavirin was reduced in four of 45 patients, all of whom had the *ITPA*-CC genotype.

Discussion

This study showed that the platelet counts of patients undergoing IFN-β/RBV combination therapy for chronic hepatitis C infection after week 8 are higher than those before treatment. Moreover, patients with the *ITPA*-CC genotype showed a smaller decrease in their platelet counts not only during IFN-β/RBV, but also with PEG-IFN/RBV therapy, compared to those with the *ITPA*-CA/AA genotype. In particular, the results demonstrated that platelet counts after week 8 during IFN-β/RBV therapy were higher than pretreatment platelet counts, regardless of pretreatment platelet counts. Compared with pretreatment platelet counts, patients with the *ITPA*-CC genotype had markedly increased platelet counts after week 8 of IFN-β/RBV therapy. Multivariate logistic regression analyses showed that IFN-β/RBV therapy was the factor that contributed to increased platelet counts at week 8 relative to pretreatment platelet counts.

Table 2 Results of univariate and multivariate logistic regression analyses of factors associated with the increase in platelets > 0 (10^9/L) from week 0 to week 8

			Simple regression		Multiple logistic regression	
Factor	Range		Odds ratio	*P*-value	Odds ratio	*P*-value
Age (years)	≥ 60/< 60		1.219	0.389	–	–
Sex	Male/Female		1.219	0.554	–	–
Genotype	1/2		1.303	0.451	–	–
Method of IFN therapy	IFN-β/RBV/PEG-IFN/RBV		20.797	< 0.0001	23.596	< 0.0001
ITPA	CC/CA or AA		0.468	0.073	–	–
IL28B	TT/TG or GG		0.569	0.153	–	–
Baseline hemoglobin	< 14/≥ 14	g/dL	0.569	0.153	–	–
Baseline platelet count	< 150/≥ 150	10^9/L	0.737	0.399	–	–
Baseline ALT	≥ 50/< 50	IU/L	1.646	0.140	–	–
Baseline γ-GTP	≥ 45/< 45	IU/L	1.603	0.166	–	–
Baseline viral load	≥ 6.0/< 6.0	LogIU/mL	1.833	0.091	–	–

ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase; IFN-β, interferon-β; ITPA, inosine triphosphate pyrophosphatase; RBV, ribavirin; PEG-IFN, pegylated interferon.

A GWAS identified several new host genetic variants that may be important for PEG-IFN/RBV therapy in chronic hepatitis C. One of these was the SNP in the *IL28B* gene that was strongly associated with therapy outcome,[18–21] and another was the *ITPA* gene that was associated with RBV-induced anemia during PEG-IFN/RBV therapy in chronic hepatitis C.[8–10]

Tanaka *et al.* reported that one SNP (rs11697186) located on the *DDRGK1* gene on chromosome 20 showed strong associations with a decrease in platelet counts in response to PEG-IFN/RBV therapy, and fine mapping with 22 SNPs around the *DDRGK1* and *ITPA* genes showed that rs11697186 had strong linkage disequilibrium with rs1127354, known as a functional variant of the *ITPA* gene.[11] We investigated the changes in platelet count decreases during IFN-β/RBV or PEG-IFN/RBV therapy relative to the *ITPA* genotype (CC, CA/AA). PEG-IFN/RBV therapy was associated with a larger decrease in hemoglobin levels among patients with the *ITPA*-CC genotype than those with the *ITPA*-CA/AA genotype.[8–10] A reactive increase in platelet counts was observed from week 1 through week 4 of treatment, with patients with the *ITPA*-CC genotype showing a higher degree of a reactive increase in platelet counts. This trend was similar to findings reported by Tanaka *et al.*, who reported that a reactive increase in platelet counts occurred secondary to RBV-induced anemia through week 4.[11]

In this investigation, decreases in hemoglobin levels were also observed from weeks 2 through 4 during IFN-β/RBV therapy. Secondarily, a temporary reactive increase in platelet counts occurred. IFN-β/RBV therapy involves continuous daily dosing of IFN-β for 4 weeks, and therefore, platelet counts typically decrease up until week 4, after which platelet counts rapidly increase following a reduction in the dosing frequency of IFN-β to thrice-weekly dosing. However, patients receiving IFN-β/RBV therapy had higher platelet counts at week 8 than pretreatment platelet counts. Arase *et al.* reported that platelet counts increased following a reduction in the dosing frequency of IFN-β from continuous daily dosing to thrice-weekly dosing.[13] We could demonstrate evidence of a relationship between the reduction of the dosing frequency of IFN-β and increases in platelet counts because we developed a treatment protocol using a 4-week continuous daily dosing of IFN-β and complied strictly with the protocol-defined duration of continuous daily dosing of 4 weeks. A higher degree of these recurrent increases in platelet counts was observed in patients with the *ITPA*-CC genotype than in those with the *ITPA*-CA/AA genotype. As with PEG-IFN/RBV therapy, patients with the *ITPA*-CC genotype showed a smaller decrease in platelet counts during IFN-β/RBV therapy. In the present study, our results demonstrated that the *ITPA* genotype was strongly involved in platelet reduction during IFN therapy, in both PEG-IFN RBV and IFN-β/RBV therapy.

The *ITPA* genotype is strongly associated with ribavirin-induced anemia and IFN-induced platelet reduction, although the reasons for these associations are not clear. Erythropoietin (EPO) is produced when hemoglobin reduction occurs as a result of ribavirin-induced anemia. The sequence homology of thrombopoietin (TPO) and EPO may explain the synergy of the physiological roles of TPO and EPO in platelet production. When EPO is elevated, as in iron deficiency anemia, an amino acid sequence similar to TPO may increase the platelet count.[22]

In Japan, the IFN-β/RBV regimen used in the present study has been indicated for chronic hepatitis C patients receiving IFN-based therapy. The SVR rate among patients with HCV genotype 1 who were treated with IFN-β/RBV was lower (approximately 40%) than that among those treated with PEG-IFN/RBV.[13] We reported that IFN-β/RBV therapy was associated with a lower incidence of depressive symptoms or sleep disorders than PEG-IFN/RBV therapy.[23] Therefore, we have also used IFN-β/RBV therapy in elderly patients or patients with concurrent depression. Patients with HCV genotype 2 who were treated with IFN-β/RBV had an SVR rate of approximately 87%, which was similar to that observed in those treated with PEG-IFN/RBV.[24] This study is a prospective, nonrandomized open trial. Thus, the SVR rate among patients with HCV genotype 1 who were treated with PEG-IFN/RBV was higher than the SVR rate of those treated with IFN-β/RBV. IFN-β/RBV therapy was performed only in patients with depression or sleep disorder, thus the number of enrolled patients with HCV genotype 1 who were treated with IFN-β/RBV was small. As for patients with HCV genotype 2, since there was no difference in the SVR rate between IFN-β/RBV and PEG-IFN/RBV therapies, the number of enrolled patients was not different.

Therefore, more patients with HCV genotype 1 were included in the PEG-IFN/RBV group.

In this investigation, there were few discontinuations, dose reductions of IFN, and dose reductions of ribavirin in the IFN-β/RBV group. This is likely due to the fact that few patients developed anorexia, no patients showed weight loss, and dietary intake was adequate during the IFN-β/RBV therapy.

In the present study, patients with the *ITPA*-CC genotype showed a higher increase in platelet counts after week 8 during IFN-β/RBV therapy than those with the *ITPA*-CA/AA genotype. Platelet counts after week 8 were increased compared with pretreatment platelet counts, regardless of pretreatment platelet counts. In patients with low pretreatment platelet counts, patients with the *ITPA*-CC genotype showed a smaller decrease in platelet count than those with the CA/AA genotype, and the platelet counts were increased after week 8. The IFN-β/RBV regimen appears to be a safe strategy for IFN therapy for patients with the *ITPA*-CC genotype, even if they have low pretreatment platelet counts.

The present study demonstrated that as with PEG-IFN/RBV therapy, patients with the *ITPA*-CC genotype showed a smaller decrease in platelet counts during IFN-β/RBV therapy. Platelet counts after week 8 of IFN-β/RBV therapy were increased compared with pretreatment platelet counts, regardless of pretreatment platelet counts. Therefore, we concluded that IFN-β/RBV therapy is safe for patients with the *ITPA*-CC genotype, even if their pretreatment platelet counts are low.

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Kaposi's sarcoma-associated herpesvirus genome replication, partitioning, and maintenance in latency

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Kaposi's sarcoma-associated herpesvirus (KSHV) is thought to be an oncogenic member of the γ-herpesvirus subfamily. The virus usually establishes latency upon infection as a default infection pattern. The viral genome replicates according to the host cell cycle by recruiting the host cellular replication machinery. Among the latently expressing viral factors, LANA plays pivotal roles in viral genome replication, partitioning, and maintenance. LANA binds with two LANA-binding sites (LBS1/2) within a terminal repeat (TR) sequence and is indispensable for viral genome replication in latency. The nuclear matrix region seems to be important as a replication site, since LANA as well as cellular replication factors accumulate there and recruit the viral replication origin in latency (ori-P) by its binding activity to LBS. KSHV ori-P consists of LBS followed by a 32-bp GC-rich segment (32GC). Although it has been reported that LANA recruits cellular pre-replication complexes (pre-RC) such as origin recognition complexes (ORCs) to the ori-P through its interaction with ORCs, this mechanism does not account completely for the requirement of the 32GC. On the other hand, there are few reports about the partitioning and maintenance of the viral genome. LANA interacts with many kinds of chromosomal proteins, including Brd2/RING3, core histones, such as H2A/H2B and histone H1, and so on. The detailed molecular mechanisms by which LANA enables KSHV genome partitioning and maintenance still remain obscure. By integrating the findings reported thus far on KSHV genome replication, partitioning, and maintenance in latency, we will summarize what we know now, discuss what questions remain to be answered, and determine what needs to be done next to understand the mechanisms underlying viral replication, partitioning, and maintenance strategy.

Keywords: Kaposi's sarcoma-associated herpesvirus, human herpesvirus 8, latency-associated nuclear antigen, ori-P, DNA replication, genome maintenance, pre-replication complex, nuclear matrix

INTRODUCTION

Kaposi's sarcoma (KS)-associated herpesvirus (KSHV) is a gamma-2 herpesvirus discovered from KS specimens in 1994 (Chang et al., 1994). KSHV is closely associated with KS and several non-Hodgkin lymphomas, including primary effusion lymphoma (PEL) and multicentric Castleman's disease (MCD; Cesarman et al., 1995, 1996; Soulier et al., 1995). While KS is the most common cancer in acquired immune deficiency syndrome patients (Potthoff et al., 2010), KSHV is detected in about 95% of all types of KS lesions by PCR analysis (Dupin et al., 1995; Huang et al., 1995; Moore and Chang, 1995). PEL is a rare B cell lymphoma originated from preterminal B cells, and PEL in AIDS patients is often associated with KSHV as well as EBV. Several KSHV-infected PEL cell lines have been established, and EBV is frequently lost in the course of establishment (Arvanitakis et al., 1996; Gaidano et al., 1996; Renne et al., 1996; Said et al., 1996; Carbone et al., 1997, 1998; Katano et al., 1999). MCD is a plasmacytic lymphadenopathy with polyclonal hyper-immunoglobulinemia and high levels of serum IL-6 (Frizzera et al., 1983; Yoshizaki et al., 1989).

Like all herpesviruses, KSHV has two life cycles: latent and lytic replication phases (for review, see Boshoff and Chang, 2001). Whereas KSHV is usually in latency when it infects KS and PEL cells, in MCD some cells express lytic genes (Katano et al., 2000; Parravicini et al., 2000). On the other hand, it has been reported that KSHV infection itself and/or viral lytic proteins promote cell proliferation and angiogenesis as well as lymphatic reprogramming (Ciufo et al., 2001; Gao et al., 2003; Carroll et al., 2004; Hong et al., 2004; Naranatt et al., 2004; Pan et al., 2004; Wang et al., 2004; Sharma-Walia et al., 2006; Qian et al., 2007, 2008; Sadagopan et al., 2007; Ye et al., 2007).

In latency, the KSHV genome is present as an episome, which is capable of autonomously replicating during S phase of the host cell cycle without integration into host chromosomes, and only limited genes are expressed during latency. Therefore, there is no generation of progeny virions. It is very important to elucidate and learn the virus's survival strategy in order to control infection and to formulate treatment for KSHV-related diseases.

In this review, we would like to focus on studies on the mechanisms underlying viral DNA replication, genome segregation and maintenance, and gene expression regulation in latency, and to discuss these topics in the light of studies on cellular mechanisms.

GENE EXPRESSION CONTROL IN KSHV LATENCY

The KSHV genome is a double-stranded linear DNA in the virion. It is circularized upon infection and is maintained as an episome in the infected nucleus. The complete genome is about 160–170 kbp,