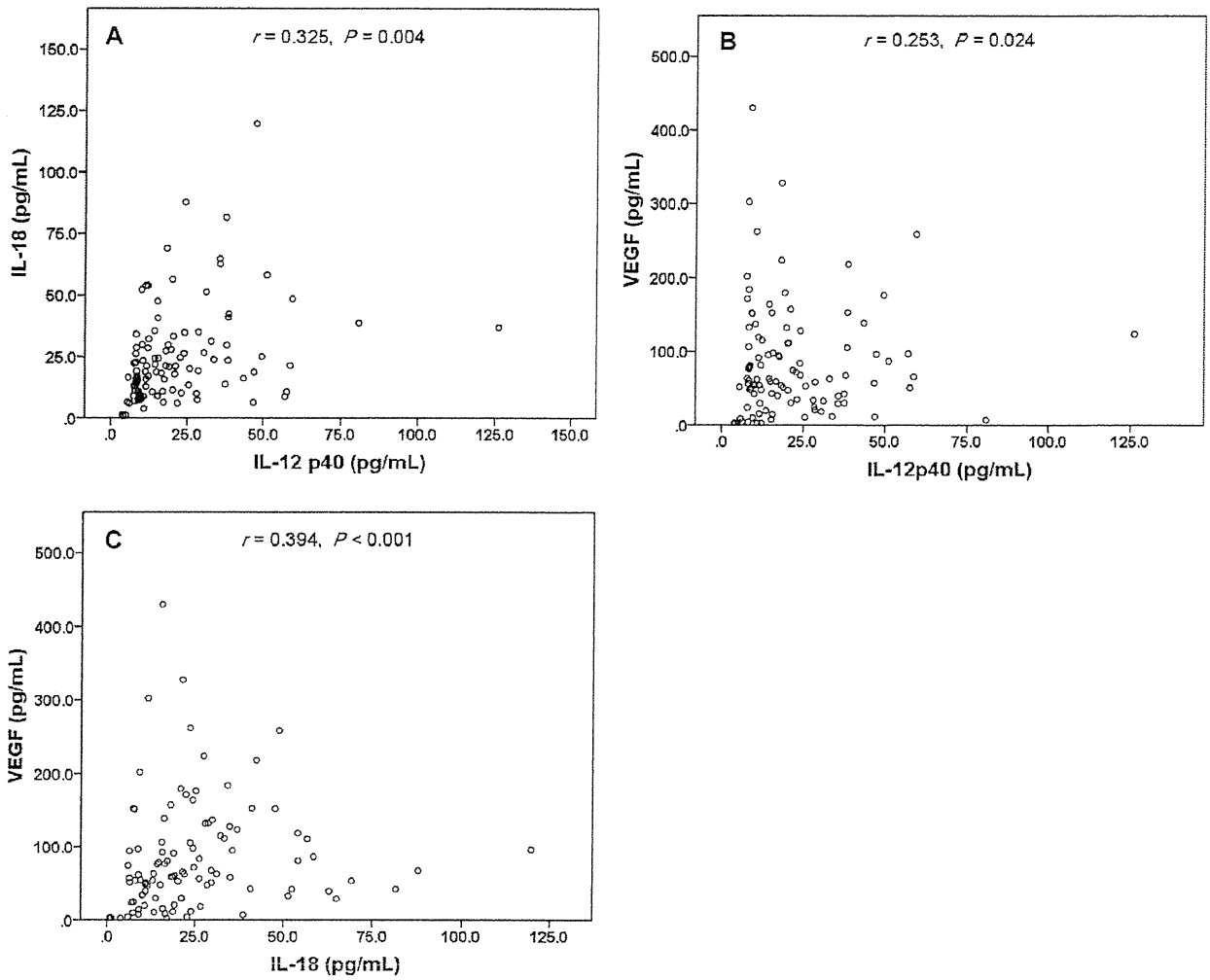

Figure 3. Correlation Between Serum Cytokines in 79 Patients with HCV Infection. (A–B) Serum IL-12p40 was significantly correlated with the level of (A) IL-18 ($r = .325$; $P = .004$) and (B) VEGF ($r = .253$; $P = .024$). (C) Serum IL-18 was correlated with the level of VEGF ($r = .394$; $P < .001$).
NOTE. HCV, hepatitis C virus; IL, interleukin; VEGF, vascular endothelial growth factor.

Table 2. Optimal Cutoff Value, Sensitivity, Specificity, Area Under The Curve, and Predictive Values of Serum IL-10, IL-12p40, IL-18, and VEGF at Baseline and After 4 Weeks of Treatment in 79 Patients with Chronic Hepatitis C

Cytokine	Collection Time	Cutoff Value	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	AUC (95% CI)	PPV (%)	NPV (%)
IL-10	baseline	5.0	100 (86–100)	80 (67–89)	.86 (.84–.98)	69	100
	4 wk	6.8	82 (69–91)	100 (86–100)	.86 (.78–.95)	100	71
IL-12p40	baseline	17.4	81 (63–93)	52 (37–67)	.70 (.59–.82)	52	81
	4 wk	21.3	81 (63–93)	60 (45–74)	.69 (.57–.81)	57	83
IL-18	baseline	15.4	97 (83–100)	46 (31–61)	.72 (.61–.83)	54	96
	4 wk	24.6	87 (70–96)	42 (28–57)	.62 (.50–.75)	49	83
VEGF	baseline	57.6	77 (59–90)	69 (54–81)	.74 (.63–.86)	62	83
	4 wk	62.6	74 (55–88)	67 (52–80)	.70 (.58–.82)	59	80

NOTE. CI, confidence interval; AUC, area under the curve; PPV, positive predictive value; NPV, negative predictive value; IL, interleukin

All AUC values were significantly higher than a 0.50 nonpredictive value ($P < .01$ for all comparisons). Cutoff values were determined by constructing receiver operating characteristic curves and are expressed as pg/mL. IL-10 is predictive of a nonresponse. IL-12p40, IL-18, and VEGF are predictive of a sustained virological response.

Table 3. Multivariate Analysis of Factors Independently Associated with a Sustained Virological Response to Pegylated Interferon and Ribavirin Therapy in Patients Infected with Hepatitis C Virus Genotype 1

Factors	OR	95% CI	*P*
Gender: male	10.932	2.178–54.780	.004
AST ⩾ 40 IU/L	.946	.906–.989	.013
IL-10 ⩾ 5.0 pg/mL	.823	.704–.962	.014
IL-12p40 ⩾ 17.4 pg/mL	1.071	1.009–1.137	.024
IL-18 ⩾ 15.4 pg/mL	1.085	1.024–1.150	.006

NOTE. OR, odds ratio; CI, confidence interval; AST, aspartate aminotransferase;IL, interleukin.

Only variables that achieved statistical significance ($P < .05$) in multivariate logistic regression analysis are shown.

patients (6%), respectively, which was statistically significant ($P < .001$).

Serum levels of IL-12p70 were significantly correlated with the number of substitutions in the ISDR (Kruskal–Wallis; $P = .027$). In addition, median baseline serum IL-12p70 levels were significantly higher in patients with mutant-type ISDR than in those with wild or intermediate types (15.6 vs 12.7 pg/mL; $P = .009$).

Factors Independently Associated with a Sustained Virological Response

We evaluated several factors found in association with an SVR from PEG-IFN and ribavirin therapy for their independence by multivariate analysis (Table 3). Male (odds ratio 10.93 [95% confidence interval 2.18–54.87], $P = .004$), AST ⩾40 IL/L (.95 [.91–.99], $P = .013$), IL-10 ⩾ 5.0 pg/mL (.82 [.70–.96], $P = .014$), IL-12p40 ⩾ 17.4 pg/mL (1.07 [1.01–1.14], $P = .024$), and IL-18 ⩾ 15.4 pg/mL (1.09 [1.02–1.15], $P = .006$) were independent risk factors related to an SVR. Conversely, core region or ISDR substitutions were not significant independent associations in this study.

Serum Cytokine Changes During and After Treatment

We next measured cytokine levels 4 weeks after the initiation of therapy and 6 months after its completion (Table 4). The levels of IL-10 ($P < .001$, Friedman test), IL-12p40 ($P = .008$), and IL-18 ($P < .001$) were significantly decreased in samples collected from patients who achieved an SVR. The reduction in serum cytokine levels from baseline to 4 weeks of treatment was determined and compared between SVR and non-SVR groups, and showed that the ratio of IL-10 had a significant negative association with both an EVR ($P = .024$) and an SVR ($P = .001$).

DISCUSSION

In this study, we measured the levels of 8 cytokines in patients with genotype 1 chronic hepatitis C and analyzed their association with the outcome of PEG-IFN and ribavirin therapy using a newly developed bead-array multiplex system. Serum IL-10, IL-12p40, IL-12p70, and IL-18 were higher in patients with HCV infection than in healthy participants. In addition, cytokines IL-10, IL-12p40, and IL-18 all decreased during treatment and remained low in patients with an SVR. These findings suggest that cytokines may in fact compromise host immune responses to the virus.

A strong association between high baseline serum IL-10 and a nonresponse to PEG - IFN and ribavirin therapy was found in our cohort, which is consistent with previous studies [7, 14, 15]. We found achievement of an EVR or SVR to be diminished in patients who had a lower IL-10 ratio between baseline and 4 weeks of treatment. In addition, using ROC curve analysis, we found sensitivity, specificity, and AUC were all high for IL-10, suggesting that serum IL-10 values at baseline and 4 weeks of treatment are predictive markers for treatment nonresponse (Table 2). Although humoral immunity is said to play a minor role in recovery from HCV infection and B-cell immunity is strongest in those with persistent infection [8, 16], a strong natural killer cell–mediated and Th1 cell–mediated immune response seems to be a key factor in protection from HCV infection. IL-10 was originally described as a cytokine synthesis inhibitory factor [17, 18], but recent studies have demonstrated that IL-10 produced by Th17 cells restrains the pathologic effects of Th17 [19, 20]. Furthermore, there is strong evidence of a substantial genetic component to IL-10 production [21, 22]; the –1082 G/G genotype is known to be related to increased IL-

Table 4. Serum Cytokine Levels Changes During and After Treatment of Pegylated Interferon Plus Ribavirin

Cytokines	Treatment Outcome	Baseline	Week 4	Week 72	*P*
IL-10	SVR	4.1 (3.3–25.4)	3.7 (3.1–19.9)	3.5 (2.9–9.0)	< .001
	Non-SVR	7.3 (3.7–10.8)	7.5 (3.9–8.8)	7.4 (3.9–10.9)	0.962
IL-12p40	SVR	24.1 (11.3–99.0)	22.1 (11.6–75.2)	18.4 (7.8–76.5)	0.008
	Non-SVR	17.2 (4.6–57.9)	19.2 (8.1–50.1)	21.6 (5.8–77.0)	0.281
IL-18	SVR	27.9 (13.8–100.6)	25.1 (13.2–95.2)	23.3 (6.6–48.5)	< .001
	Non-SVR	17.7 (1.1–59.9)	31.3 (10.3–90.6)	17.4 (5.4–52.0)	< .001

NOTE. Data are median (5th–95th percentile) values. IL, interleukin; SVR, sustained virological response.

10 production and is associated with a high risk of inefficient HCV clearance [23, 24] and resistance to IFN treatment [25–28].

In agreement with our findings, recent studies have indicated that Gln70 substitutions in the HCV core region are associated with treatment failure [11, 29–32]. Additionally, patients with Gln70 had higher IL-10 levels compared with those with Arg70. Among the 28 HCV patients who had Gln70, all 14 nonresponders had higher IL-10 (≥5.0 pg/mL), whereas 11 of 14 responders had lower IL-10 levels ($P < .001$). This association between Gln70 and elevated IL-10 levels is intriguing. Dolganiuc et al reported that HCV core and NS3 proteins in monocytes and dendritic cells induce IL-10 [33], so further studies are needed to clarify the relationship between IL-10 and core region amino acid substitutions.

This report demonstrates the beneficial role of IL-12 in achieving an SVR during PEG - IFN and ribavirin therapy. IL-12 is a proinflammatory cytokine that promotes the differentiation of Th1 cells, suppresses Th2 function, and amplifies the cytotoxicity of cytotoxic T lymphocytes and natural killer cells [34]. Thus, production of IL-12 is directed toward the elimination of intracellular pathogens and viruses. Elevated serum IL-12 has been noted in patients with chronic HBV or HCV infection, and is even more prominent among responders to IFN-α treatment [35, 36]. In our study, we noted significantly higher serum IL-12p70 in participants carrying mutant-type ISDR than in those with intermediate- or wild-type ISDR. This correlation between IL-12 and ISDR substitutions is striking and requires further study to verify its favorable effect during PEG-IFN and ribavirin therapy.

It is believed that the dynamics of the Th1/Th2 response determine the outcome of antiviral therapy to chronic hepatitis C [10] and that IL-18 is an important mediator of the Th1/Th2 balance. IL-18 plays a critical role in host defense against infection by intracellular microbes but also induces autoimmune diseases and propagates inflammation [37]. IL-18 is significantly upregulated in patients with chronic HCV infection and is correlated with hepatic injury [38, 39], indicating a key role in disease pathogenesis. However, the effect of IL-18 on antiviral therapy for chronic hepatitis C is still unclear. We found that IL-18 levels were significantly higher in patients with chronic HCV infection compared with healthy controls, but they were also higher at baseline in patients who achieved an SVR than in those who did not. In addition, there was a significant correlation between IL-18 and IL-12; in the presence of IL-12, IL-18 stimulates *IFNG* expression, thus promoting the Th1-mediated immune response. Without IL-12, IL-18 stimulates Th2 responses [37]. In this study, because serum IFN-γ levels were below detection thresholds, we could not assess the association of such cytokines.

Lastly, we observed that pretreatment serum VEGF levels were associated with an SVR. A previous study showed no association between baseline VEGF and treatment outcome, but only 36 patients, including 19 with genotype 1, were studied [40]. Hence, it is still unclear if this angiogenesis marker plays a critical role in response to antiviral therapy in chronic HCV infection. Furthermore, we correlated VEGF with IL-12 and IL-18 in our study. In particular, IL-18 enhances the production of VEGF in rheumatoid arthritis synovial fibroblasts, suggesting that IL-18 could be an angiogenic mediator with triggering effects on VEGF production [41]. Although the preoperative serum VEGF level was found to be a significant predictor of tumor recurrence and overall survival in patients with HCC [42], there have been no reports regarding treatment response in patients with chronic hepatitis C during antiviral therapy.

In multivariate analysis of our cohort, low IL-10, high IL-12p40, and high IL-18 were independent factors related to an SVR in patients treated with PEG-IFN and ribavirin. Our results indicate that such 3-cytokine profiling may offer clinicians another tool in predicting treatment outcome of HCV infection. Further investigation must be done in vitro and using many samples to validate the significance of our findings.

In conclusion, several cytokines were seen to be elevated in patients with chronic hepatitis C using the multiplex bead assay. Serum IL-10 levels and amino acid substitutions at the 70 aa core region of HCV are useful for predicting a nonresponse to PEG-IFN and ribavirin therapy in patients with chronic hepatitis C genotype 1. A higher level of serum IL-12 is considered to be favorable for response to antiviral therapy, and is correlated with substitutions in the ISDR. Lastly, IL-18 is notably high in patients with chronic HCV infection, and is correlated with IL-12.

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Association of IL28B Variants With Response to Pegylated-Interferon Alpha Plus Ribavirin Combination Therapy Reveals Intersubgenotypic Differences Between Genotypes 2a and 2b

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Genetic polymorphisms of the interleukin 28B (IL28B) locus are associated closely with outcomes of pegylated-interferon (PEG-IFN) plus ribavirin (RBV) combination therapy. The aim of this study was to investigate the relationship between IL28B polymorphism and responses to therapy in patients infected with genotype 2. One hundred twenty-nine chronic hepatitis C patients infected with genotype 2, 77 patients with genotype 2a and 52 patients with genotype 2b, were analyzed. Clinical and laboratory parameters, including genetic variation near the IL28B gene (rs8099917), were assessed. Drug adherence was monitored in each patient. Univariate and multivariate statistical analyses of these parameters and clinical responses were carried out. Univariate analyses showed that a sustained virological response was correlated significantly with IL28B polymorphism, as well as age, white blood cell and neutrophil counts, adherence to RBV, and rapid virological response. Subgroup analysis revealed that patients infected with genotype 2b achieved significantly lower rapid virological response rates than those with genotype 2a. Patients with the IL28B-major allele showed higher virus clearance rates at each time point than those with the IL28B-minor allele, and the differences were more profound in patients infected with genotype 2b than those with genotype 2a. Furthermore, both rapid and sustained virological responses were associated significantly with IL28B alleles in patients with genotype

Abbreviations: HCV, hepatitis C virus; HCC, hepatocellular carcinoma; IFN, interferon; PEG-IFN, pegylated-interferon; RBV, ribavirin; IL28B, interleukin 28B; SNPs, single nucleotide polymorphisms; BMI, body mass index; ALT, alanine transaminase; ISDR, the interferon sensitivity determining region; ITPA, inosine triphosphatase

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2b. IL28B polymorphism was predictive of PEG-IFN plus RBV combination treatment outcomes in patients infected with genotype 2 and, especially, with genotype 2b. In conclusion, IL-28B polymorphism affects responses to PEG-IFN-based treatment in difficult-to-treat HCV patients. ***J. Med. Virol. 83:871–878, 2011.*** © 2011 Wiley-Liss, Inc.

KEY WORDS: hepatitis C virus (HCV); chronic hepatitis C; genotype 2; PEG-IFN plus RBV therapy; combination therapy; IL28B; interferon-λ3

INTRODUCTION

Hepatitis C virus (HCV) infects around 170 million people worldwide and is characterized by a high probability of developing chronic inflammation and fibrosis of the liver, leading to end-stage liver failure and hepatocellular carcinoma (HCC) [Alter, 1997; Sakamoto and Watanabe, 2009]. Since the first report in 1986, type I interferons have been the mainstay of HCV therapy [Hoofnagle, 1994]. Current standards of care consist of a combination of ribavirin (RBV) plus pegylated interferon (PEG-IFN)-alpha for 48 weeks for infection with genotypes 1 and 4, and for 24 weeks for the other genotypes [Zeuzem et al., 2000; Fried et al., 2002]. Although this treatment improved substantially sustained virological response rates, it may result also in serious adverse effects and a considerable proportion of patients require early discontinuation of treatment. Patients of African origin have even poorer treatment outcomes [Rosen and Gretch, 1999]. Given this situation, a precise assessment of the likely treatment outcomes before the initiation of treatment may improve substantially the quality of antiviral treatment.

Recently, several studies have reported that genetic polymorphisms of the IL28B locus, which encodes interferon-λ3 (interleukin 28B), are associated with response to interferon-based treatment of chronic HCV infections with genotype 1 [Ge et al., 2009; Suppiah et al., 2009; Tanaka et al., 2009] and also spontaneous clearance of HCV [Thomas et al., 2009].

While chronic HCV infections with genotype 2 are associated with good treatment outcome, there are some refractory cases among patients infected with genotype 2, similar to genotype 1. The aims of this study were to analyze retrospectively clinical and virological factors associated with treatment response in patients with chronic HCV infection with genotype 2 who were treated with PEG-IFN plus RBV combination therapy and to clarify the relationship between IL28B polymorphism and the response to combination therapy.

PATIENTS AND METHODS

The authors analyzed retrospectively 129 patients with chronic HCV infection with genotype 2 who received combination therapy with PEG-IFN plus RBV between December 2004 and December 2009 at 10 multicenter hospitals (liver units with hepatologists) throughout Japan. All patients had chronic active hepatitis confirmed histologically or clinically and were positive for anti-HCV antibodies and serum HCV RNA by quantitative or qualitative assays. Patients with a positive test for serum hepatitis B surface antigen, coinfection with other HCV genotypes, coinfection with human immunodeficiency virus, other causes of hepatocellular injury (such as alcoholism, autoimmune hepatitis, primary biliary cirrhosis, or a history of treatment with hepatotoxic drugs), and a need for hemodialysis were excluded.

Study Design

Each patient was treated with combination therapy with PEG-IFN-α2b (Peg-Intron, Schering-Plough Nordic Biotech, Stockholm, Sweden, at a dose of 1.2–1.5 μg/kg subcutaneously once a week) or PEG-IFN-α2a (Pegasys; Roche, Basel, Switzerland, at a dose of 180 μg subcutaneously once a week) plus RBV (Rebetol, Schering-Plough Nordic Biotech or Copegus; Roche) 600–1,000 mg daily depending on the body weight (b.w.) (b.w. <60 kg: 600 mg po daily; b.w: 60–80 kg: 800 mg po daily; b.w. >80 kg: 1,000 mg po daily; in two divided doses). The duration of the combination therapy was set at a standard 24 weeks, but treatment reduction or discontinuation was permitted by doctor's decision. The rates of PEG-IFN and RBV administration achieved were calculated as percentages of actual total dose administered of a standard total dose of 24 weeks, according to body weight before therapy. During treatment, patients were assessed as outpatients at weeks 2, 4, 6, 8, and then every 4 weeks for the duration of treatment and at every 4 weeks after the end of treatment. Biochemical and hematological testing was carried out in a central laboratory. Serum HCV RNA was measured before treatment, during treatment at 4 weekly intervals, and after therapy at 4 weekly intervals for 24 weeks, by quantitative or qualitative assays.

Patient Evaluation

The following factors were analyzed to determine whether they were related to the efficacy of combination therapy: age, gender, body mass index (BMI), previous IFN therapy, grade of inflammation and stage of fibrosis on liver biopsy, pretreatment biochemical parameters, such as white blood cells, neutrophils, hemoglobin, platelet count, alanine transaminase (ALT) level, serum HCV RNA level (log IU/ml), and single nucleotide polymorphism (SNPs) in the *IL28B* locus (rs8099917). Liver biopsy specimens were evaluated blindly, to determine the grade of inflammation and stage of fibrosis, by an independent interpreter who was not aware of the clinical data. Activity of inflammation was graded on a scale of 0–3: A0 shows no activity, A1 shows mild activity, A2 shows moderate activity and A3 shows severe activity. Fibrosis was staged on a scale of 0–4:

F0 shows no fibrosis, F1 shows moderate fibrosis, F2 shows moderate fibrosis with few septa, F3 shows severe fibrosis with numerous septa without cirrhosis and F4 shows cirrhosis.

Informed written consent was obtained from each patient who participated in the study. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki and to the relevant ethical guidelines as reflected in a priori approval by the ethics committees of all the participating universities and hospitals.

SNP Genotyping

Human genomic DNA was extracted from whole blood of each patient. Genetic polymorphism of IL28B was determined by DigiTag2 assay by typing one tag SNP located within the IL28B locus, rs8099917 (22). Heterozygotes (T/G) or homozygotes (G/G) of the minor allele (G) were defined as having the IL28B minor allele, whereas homozygotes for the major allele (T/T) were defined as having the IL28B major allele.

TABLE I. Baseline Characteristics of Participating Patients Infected With HCV Genotype 2

Total number	129
Genotype (2a/2b)	77/52
IL28B SNPs (rs8099917)	
TT/TG/GG	100/28/1
Age (years)[a]	64 (20–73)
Gender (male/female)	64/65
Body mass index (kg/m^2)[a] (N = 80)	23.7 (16.9–33.5)
Previous interferon therapy (no/yes)	102/21 (unknown 6)
Histology at biopsy (N = 96)	
Grade of inflammation	
A0/1/2/3	10/53/29/4
Stage of fibrosis	
F0/1/2/3	7/59/19/11
White blood cells (/μl)[b] (N = 94)	5,115 ± 1,630
Neutrophils (/μl)[b] (N = 94)	2,765 ± 1,131
Hemoglobin (g/dl)[b] (N = 95)	14.2 ± 1.3
Platelet count ($\times 10^{-3}$/μl)[b] (N = 98)	187 ± 95
ALT (IU/L)[b] (N = 95)	82 ± 78
Serum HCV-RNA level (log(IU/ml))[a,c]	6.2 (3.6–7.4)
Treatment duration (>16, ≦24)	19/110

SNPs, single nucleotide polymorphisms; ALT, alanine transaminase.
[a]Data are shown as median (range) values.
[b]Data are expressed as mean ± SD.
[c]Data are shown as log(IU/ml)).

Outcomes

The primary end point was a sustained biochemical and virological response. A sustained virological response was defined as serum HCV RNA undetectable at 24 weeks after the end of treatment. Secondary end points were a rapid virological response (HCV RNA undetectable in serum at week 4) and end-of-treatment virological response. In addition, tolerability (adverse events) and drug adherence were recorded and factors potentially associated with virological response explored.

Statistical Analysis

SPSS software package (SPSS 18J, SPSS, Chicago, IL) was used for statistical analysis. Discrete variables were evaluated by Fisher's exact probability test and distributions of continuous variables were analyzed by the Mann–Whitney *U*-test. Independent factors possibly affecting response to combination therapy were examined by stepwise multiple logistic-regression analysis. All *P*-values were calculated by two-tailed tests, and those of less than 0.05 were considered statistically significant.

RESULTS

Clinical Characteristics and Response to Therapy

The clinical characteristics and response rates to therapy of 129 patients are summarized in Tables I and II. Sixty-eight patients achieved a rapid virological response, whereas 44 patients remained HCV-RNA positive at week 4. Treatment reduction or cessation was permitted also to avoid side effects, and one patient stopped treatment at week 12 because he was anticipated to be a non-responder. On an intention-to-treat analysis, serum HCV-RNA levels were negative at the end of treatment in 125 of the 129 patients (97%) treated and, among them, 98 (76%) achieved a sustained virological response. The rapid virological response rate of patients infected with genotype 2b was lower significantly than that of patients infected with genotype 2a ($P = 0.036$) (Table II). The sustained virological response rate decreased with RBV drug discontinuation and dose reduction (84% and 66% with ≧80% and <80% of RBV dose, $P = 0.021$, Table III). Adherences to PEG-IFN did not influence a sustained virological response or end of treatment response significantly, while RBV adherence was associated significantly with a sustained virological response (Table III).

Factors Associated With a Sustained Virological Response

Next the host clinical and viral factors associated with a sustained virological response were analyzed. Univariate statistical analysis showed that six parameters were associated significantly with the sustained virological response rates, including age, white blood cells, neutrophils, adherence to RBV, rapid virological response and an IL28B SNP (rs8099917) (Table IV). There was no significant association of sustained virological response with gender, previous interferon therapy, stage of fibrosis, pretreatment HCV titer or adherence to PEG-IFN. Further multivariate analyses were conducted using significant factors identified by the univariate analysis (Table V). The multiple logistic-regression analysis showed that only a rapid virological response was associated with a sustained virological response (OR = 0.170, $P = 0.019$).

TABLE II. Response Rates to Therapy

Character			Number/total number (%)
Overall			
RVR			68/112 (61)
ETR			125/129 (97)
SVR			98/129 (76)
Genotype	2a	2b	*P*-value
RVR	46/67 (69)	22/45 (49)	**0.036**
ETR	74/77 (96)	51/52 (98)	NS
SVR	56/77 (73)	42/52 (81)	NS

RVR, rapid virological response; ETR, end of treatment response; SVR, sustained virological response.
Bold indicated *P*-value of less than 0.05.

TABLE III. Response Rates to Treatment According to Drug Adherence

	≧80%	<80%	*P*-value
PEG-IFN adherence			
ETR	94/96 (98)	31/33 (94)	NS
SVR	75/96 (78)	23/33 (70)	NS
RBV adherence			
ETR	72/73 (99)	53/56 (95)	NS
SVR	61/73 (84)	37/56 (66)	**0.021**

ETR, end of treatment response; SVR, sustained virological response; PEG-IFN, pegylated interferon; RBV, ribavirin.
The rates of PEG-IFN and RBV administration achieved were calculated as percentages of actual total dose administered of a standard total dose of 24 weeks, according to body weight before therapy.
Bold indicated *P*-value of less than 0.05.

Comparison of Sustained Virological Response Rates According to IL28B SNPs

The PEG-IFN plus RBV treatment efficacy was compared after dividing the study subjects into two groups based on IL28B alleles (Table VI). Patients homozygous for the IL28B major allele (TT allele) achieved significantly higher rapid and sustained virological response rates than those heterozygous or homozygous for the IL28B minor allele (TG/GG alleles) ($P < 0.05$). In addition, responses to PEG-IFN plus RBV treatment were analyzed after dividing the study subjects into those with genotype 2a and with genotype 2b. The rapid and sustained virological response rates tended to be higher in patients homozygous for the IL28B major allele than those heterozygous or homozygous for the

TABLE IV. Clinical and Virological Characteristics of Patients Based on Therapeutic Response

	SVR (n = 98)	Non-SVR (n = 31)	*P*-value
Genotype (2a/2b)		56/42	21/10
IL28B SNPs (rs8099917)			
TT/TG + GG	81/17	19/12	**0.024**
Age (years)[a]	56 (20–73)	61 (40–72)	**0.002**
Gender (male/female)	51/47	13/18	NS
Body mass index (kg/m^2)[a]	22.8 (16.9–33.5)	24.1 (20.3–27.6)	NS
Previous Interferon therapy (no/yes)	80/14	22/7	NS
Grade of inflammation (A0-1/2-3)	46/28	15/7	NS
Stage of fibrosis (F0-2/3-4)	64/10	21/1	NS
White blood cells (/μl)[b]	5,318 ± 1,617	4,489 ± 1,540	**0.032**
Neutrophils (/μl)[b]	2,913 ± 1,139	2,278 ± 983	**0.021**
Hemoglobin (g/dl)[b]	14.2 ± 1.4	14.1 ± 1.1	NS
Platelet count (×10^{-3}/μl)[b]	193 ± 105	171 ± 54	NS
ALT (IU/ml)[b]	79 ± 73	94 ± 92	NS
Pretreatment Serum HCV-RNA level (log(IU/ml))[a,c]	6.1 (3.6–7.4)	6.3 (4.0–6.7)	NS
PEG-IFN adherence (≧80%/<80%)	75/23	21/10	NS
RBV adherence (≧80%/<80%)	61/37	12/19	**0.024**
RVR/non-RVR	57/24	11/20	**0.001**

SNPs, single nucleotide polymorphisms; ALT, alanine transaminase; RVR, rapid virological response.
[a]Data are show as median (range) values.
[b]Data are expressed as mean ± SD.
[c]Data are shown as log (IU/ml)).
Bold indicated *P*-value of less than 0.05.

TABLE V. Multivariate Analysis for the Clinical and Virological Factors Related to Sustained Response With Peg-IFN Plus RBV Therapy in 63 Patients

Factor	Category	Odds ratio (95% CI)	*P*-value
Regression analysis			
RVR	RVR	1	**0.019**
	Non-RVR	0.170 (0.039–0.744)	
RBV adherence	≧80%	1	0.061
	<80%	0.250 (0.059–1.064)	
IL28B SNPs (rs8099917)	TT	1	0.104
	TG + GG	0.252 (0.048–1.330)	
Age		1.087 (0.976–1.211)	0.128
Neutrophils		0.999 (0.997–1.001)	0.209
White blood cells		1.000 (0.999–1.002)	0.504

CI, confidence interval; SNPs, single nucleotide polymorphisms; RVR, rapid virological response, RBV, ribavirin.
Bold indicated *P*-value of less than 0.05.

IL28B minor allele infected with both genotype 2a and 2b, and these differences were more profound in patients infected with genotype 2b than with genotype 2a. The rapid and sustained virological response rates of patients with the major IL28B allele were higher significantly than those of patients with the minor IL28B allele infected only with genotype 2b (rapid virological response: 58% and 0% with IL28B major and hetero/minor, $P = 0.002$, sustained virological response: 88% and 44% with IL28B major and hetero/minor, $P = 0.009$).

Although the rapid virological response rate of patients infected with genotype 2b was lower significantly than that of patients infected with genotype 2a, the sustained virological response rate was higher in patients infected with genotype 2b than with genotype 2a (Table II), In order to investigate that discrepancy, sustained virological response rates in patients with or without rapid virological response were analyzed according to IL28B SNPs. In patients infected with genotype 2b and a non-rapid virological response, the sustained virological response rates differed significantly between IL28B major and hetero/minor groups (sustained virological response with non-rapid virological response: 75% and 29% with IL28B major and hetero/minor, $P = 0.044$), and no one achieved a rapid virological response among the patients infected with genotype 2b and with the IL28B hetero/minor allele. In patients infected with genotype 2a, on the contrary, there was no significant correlation of rapid and sustained virological response rates between IL28B SNPs (sustained virological response with rapid virological response: 78% and 70% with IL28B major and hetero/minor, $P = 0.630$, sustained virological response with non-rapid virological response: 57% and 43% with IL28B major and hetero/minor, $P = 0.552$).

Next, changes in virological response rates over time were investigated in patients treated with PEG-IFN plus RBV and the time course was analyzed after separating the patients infected with genotype 2a and 2b (Fig. 1). Patients with IL28B-TG and -GG showed significantly lower rates of rapid and sustained virological response, compared to patients with IL28B-TT, and greater differences were observed according to IL28B SNPs among patients infected with genotype 2b than with 2a.

Side Effects

Side effects leading to Peg-IFN plus RBV discontinuation occurred in eight patients (6.2%) and discontinuation of RBV alone occurred in four patients (3.1%).

TABLE VI. Rapid and Sustained Virological Response Rates to Treatment According to IL28B SNPs

Character	IL28B major	IL28B hetero/minor	*P*-value
Number/total number (%)			
Overall			
RVR	58/88 (66)	10/24 (42)	0.031
SVR	81/100 (81)	17/29 (59)	0.013
Genotype 2a			
RVR	36/50 (72)	10/17 (59)	NS
SVR	43/57 (75)	13/20 (65)	NS
Genotype 2b			
RVR	22/38 (58)	0/7 (0)	0.002
SVR	38/43 (88)	4/9 (44)	0.009

RVR, rapid virological response; ETR, end of treatment response; SVR, sustained virological response.

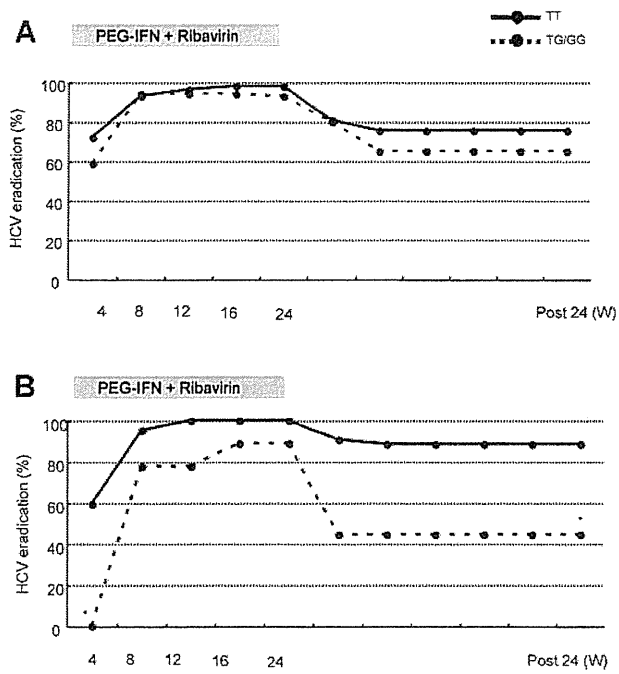

Fig. 1. Changes over time in virological response rates were confirmed in patients treated with PEG-IFN plus RBV, and the time courses were analyzed after separating the patients infected with genotypes 2a and 2b. Patients with the IL28B major (TT allele) are indicated in the figure by a continuous line and those with IL28B hetero or minor (TG or GG), by a dotted line. IL28B-TG and -GG patients showed significantly lower rates of rapid and sustained virological response, compared to IL28B-TT patients. *P*-values were two-tailed and those of less than 0.05 were considered to be statistically significant. $^{*}P < 0.01$.

Among the eight patients who withdrew from both drugs, four, including one who stopped at week 7, had achieved a sustained virological response. Among four patients who withdrew from RBV alone, three had achieved a sustained virological response. The events leading to drug withdrawal were HCC treatment (n = 2), general fatigue (n = 2), retinopathy, neuro-psychiatric event, severe dermatological symptoms suggestive of the drug-induced hypersensitivity syndrome, and arrhythmia.

DISCUSSION

Recent studies suggest that genetic variations in IL28B are strongly associated with response to therapy of chronic HCV infection with genotype 1 [Ge et al., 2009; Suppiah et al., 2009; Tanaka et al., 2009] and with spontaneous HCV clearance [Thomas et al., 2009]. In this study, univariate analyses showed that the sustained virological response was correlated significantly with IL28B polymorphism (rs8099917) as well as age, adherence to RBV and rapid virological response, and multiple logistic-regression analysis showed that only a rapid virological response was associated with a sustained virological response in all patients infected with genotype 2 (Table V). Although the IL28B polymorphisms are not so useful for predicting the clinical outcomes of PEG-IFN plus RBV combination therapy among patients with genotype 2, compared to genotype 1, IL28B polymorphism was predictive of PEG-IFN plus RBV treatment outcomes among patients with genotype 2 and, more remarkably, among patients with genotype 2b in this study. Indeed, both rapid and sustained virological response rates according to the rs8099917 genotypes were different significantly in patients with genotype 2b but not in patients with genotype 2a. Furthermore, in the plot of virological response (Fig. 1), a stronger effect of the IL28B allele was observed in patients with genotype 2b than with genotype 2a.

It has been reported that there was no significant association between genetic variation in IL28B and response to therapy of HCV patients infected with genotype 2 or 3, indicating that the prognostic value of the risk allele for treatment response might be limited to individuals with difficult-to-treat HCV genotypes [Rauch et al., 2010]. This report lacks details of the distribution of the various genotypes. The present study agrees with a more recent report that the IL28B polymorphism was associated with a sustained virological response in patients with chronic HCV infection with genotype 2 or 3 who did not achieve a rapid virological response [Mangia et al., 2010]. In Japan, the percentage of HCV infection with genotype 1b is 70%, genotype 2a is 20% and genotype 2b is 10%, whilst other genotypes are observed only rarely. In this study, the association of IL28B polymorphism with response to therapy was analyzed in more detail, considering the subtypes 2a and 2b, and IL28B polymorphism (rs8099917) found to be linked more closely to the virological response of patients infected with genotype 2b than those with genotype 2a. A recent in vitro study, which constructed several chimeric virus clones between HCV-2b and HCV-JFH1 (2a), also supported subgenotypic differences between genotype 2a and 2b [Suda et al., 2010]. The authors speculated that the prognostic value of the risk allele for treatment response might be more pronounced in individuals with difficult-to-treat HCV subgenotypes, such as patients infected with genotype 2b, compared with 2a. In addition, the prevalence of the IL28B minor allele is much higher in Caucasians and African Americans than in eastern Asian populations [Thomas et al., 2009], which suggest that the effects of IL28B polymorphism could be more pronounced in non-Asian populations. In the present results, however, the sustained virological response rate of patients infected with genotype 2b was higher than that of patients with genotype 2a overall. We speculate that, among patients infected with genotype 2b, only those with the IL28B minor variant might be treatment-refractory. That possibility might be validated further by a larger cohort study with genotype 2b.

The sustained virological response rates decreased significantly with failure of adherence to RBV (Table III), which was extracted as a factor associated with sustained virological response by univariate

analysis (Table IV). Regardless of the drug adherence, end of treatment response rates of patients infected with genotype 2 were around 94–99%, but the sustained virological response rates of the patients who received a total cumulative treatment dose of RBV of <80% was reduced significantly. As reported previously, increased RBV exposure during the treatment phase was associated with an increased likelihood of a sustained virological response [McHutchison et al., 2009] and these results confirm the importance of RBV in order to prevent relapse. Furthermore, host genetic variation leading to inosine triphosphatase (ITPA) deficiency protects against hemolytic anemia in chronic hepatitis C patients receiving RBV as revealed recently [Fellay et al., 2010]. We have reported also that the *ITPA* SNP, rs1127354, is confirmed to be a useful predictor of RBV-induced anemia in Japanese patients and that the incidence of early dose reduction was significantly higher in patients with ITPA-major (CC) variant as expected and, more importantly, that a significant higher sustained virological response rate was achieved in patients with the *ITPA-hetero*/minor (CA/AA) variant with non-genotype 1 or low viral loads [Sakamoto et al., 2010].

A rapid virological response was extracted in this study as a factor associated with sustained virological response only by multivariate analysis. It has been reported recently that a rapid virological response is an important treatment predictor and that drug adherence, which is reported to affect the therapeutic efficacy in patients infected with genotype 1, had no impact on the both sustained and rapid virological responses in combination therapy for patients infected with genotype 2 [Inoue et al., 2010]. The reasons why several host factors useful for predicting the response to therapy in patients with genotype 1, such as gender, age, progression of liver fibrosis and IL28B polymorphism had no influence on the efficacy in patients with genotype 2, can be attributed to IFN-sensitive genotypes. Similarly, the other viral factors useful for predicting the response to therapy, such as viral load and amino acid substitutions in the Core and NS5A regions had no influence on treatment outcomes. In this study, patients who achieved a rapid virological response had a high sustained virological response rate, regardless of IL28B polymorphism in patients with genotype 2a but, interestingly, none of the IL28B-TG and -GG patients with genotype 2b achieved a sustained virological response (although there were nine IL28B-TG and -GG patients with genotype 2b, two could not be determined as rapid virological response because the times at which they became HCV-negative were not recorded clearly, being described as 4–8 weeks.) These results also suggest that patients with both genotype 2b and IL28B minor allele are refractory cases.

IL28B encodes a protein also known as IFN-λ3 [O'Brien, 2009]. *IL28A* (IFN-λ2) and *IL29* (IFN-λ1) are found adjacent to *IL28B* on chromosome 19. These three IFN-λ cytokines, discovered in 2003 by two independent groups [Kotenko et al., 2003; Sheppard et al., 2003] have been suggested to be involved in the suppression of replication of a number of viruses, including HCV [Robek et al., 2005; Marcello et al., 2006; Tanaka et al., 2010]. Humans have these three genes for IFN-λ, and this group of cytokines is now collectively referred to as type III IFN [Zhou et al., 2007]. IFN-λ functionally resembles type I IFN, inducing antiviral protection in vitro [Kotenko et al., 2003; Sheppard et al., 2003] as well as in vivo [Ank et al., 2006]. Type III IFN utilizes a receptor complex different from that of type I IFN, but both types of IFN induce STAT1, STAT2, and STAT3 activation by activation of a highly overlapping set of transcription factors, and the two types of IFN seem to have similar biological effects at a cellular level. Some in vitro studies have suggested that IFN-α induces expression of IFN-λ genes [Siren et al., 2005]. Other in vitro studies also suggest that IFN-λ inhibits hepatitis C virus replication through a pattern of signal transduction and regulation of interferon-stimulated genes that is distinct from IFN-α and that the anti-HCV activity of either IFN-α or IFN-λ is enhanced by a low dose of the other [Marcello et al., 2006]. A novel mechanism of the interaction between IFN-α and IFN-λ may play a key role in the suppression of HCV [O'Brien, 2009].

In conclusion, IL28B polymorphism is predictive of PEG-IFN plus RBV treatment outcomes in patients infected with genotype 2, and more remarkably with genotype 2b. These results suggest that IL-28B polymorphism affects responses to IFN-based treatment in more difficult-to-treat subpopulations of HCV patients, and that intersubgenotypic differences between genotype 2a and 2b are revealed by responses to PEG-IFN plus RBV treatment according to IL28B variants.

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医療安全対策

透析患者におけるB型肝炎ウイルスマーカー測定の意義

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要　旨

透析患者は血液を介して感染するウイルスのハイリスクグループであり，B型肝炎ウイルス（HBV）もその1つである．透析施設におけるHBV感染の制御や，すでに透析を受けているHBVキャリアの診療にはウイルスマーカーの測定が欠かせない．近年，治療法の変化やマーカー測定法の進歩に伴い，ウイルスマーカーに対する考え方も変化している．このため，HBVマーカーの新しい理解は透析医にとっても重要な課題である．

はじめに

透析治療では，常に観血的処置を伴う医療行為を集団で行っており，透析患者は血液を介して感染する肝炎ウイルスのハイリスクグループである．さらに，透析患者では免疫能が低下しているため，感染の病態も健常者とは異なる可能性があり，診療にさいしては注意が必要である．

血液を介して感染するB型（HBV）およびC型肝炎ウイルスに対する感染対策は進歩し，最近では透析患者における感染の危険性は大きく低下した．しかし，透析患者がハイリスクグループであることには変わりなく，新規感染に対する対策は常に行う必要がある．また，透析患者では過去に感染したキャリアも多く，肝炎に対する対策も重要な課題である．

以前より透析施設では，患者および医療従事者に肝炎の集団発生がみられていたが，その大部分がHBV感染に伴うものである．また，透析患者でのHBV感染率は一般健常者に比較し高いことが知られている．さらに，透析患者の生命予後が大きく改善されると，ウイルス肝炎に伴う肝発癌の危険性も大きな問題となってきた．

肝炎ウイルス感染の監視やウイルス肝炎の診療には，関連したウイルスマーカーの測定が必須である．近年，治療法の変化やマーカー測定法の進歩に伴い，ウイルスマーカーに対する考え方も新しくなってきた．さらに，B型肝炎は複雑な病態を示すため多くのウイルスマーカーが使用されている[1]．これらのことから，HBVマーカーの理解は，非専門医には容易ではない領域と言える．

本稿では，透析医のために，透析患者の診療に必要なHBVマーカーの臨床的意義をできるだけわかりやすく解説する．

1　B型肝炎の病期と自然経過

HBVキャリアはその自然経過の中で，無症候性キャリア，慢性肝炎，肝発癌，急性増悪，再活性化などの多彩な病態を示す．これらの病態の変化はウイルス増殖と免疫応答の関係の変化に起因するものであり，B型肝炎の臨床ではこれらを十分理解する必要がある[2~4]．HBVキャリアの自然経過は，ALT値，HBe抗原，HBV DNA量，予測される免疫状態などから病期が分類されており，代表的なものを表1に示した[3]．

Clinical significance of hepatitis B viral markers in patients on hemodialysis
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表1　HBVキャリアの病期とウイルスマーカー

病期	肝炎	血中			肝臓	免疫状態
		DNA量	HBe抗原	HBs抗原	cccDNA	
免疫寛容期	−	8〜11	+	+	+	免疫寛容
慢性肝炎　eAg（+）	持続	6〜10	+	+	+	免疫排除
eAg（−）	変動	3〜8	−	+	+	
非活動性キャリア	−	<4	−	+	+	免疫監視
回復期	−	−	−	−	+	免疫監視

HBV DNA量：log copies/ml

1-1　免疫寛容期

免疫寛容期ではHBV増殖は活発であるがALT値は正常で，組織学的にも正常か軽度の炎症にとどまる．周産期にHBVに感染した場合，免疫寛容期は思春期〜若年成人まで続くことが多い．宿主の免疫はHBVを非自己とは認識せず，これを排除しようとしていないと考えられるので，この時期は免疫寛容期と呼ばれる．

1-2　免疫排除期

HBe抗原陽性の慢性肝炎では，HBV排除に働く宿主の免疫反応が起こり肝炎が惹起される．HBe抗原陽性の慢性肝炎が長期に続くと肝硬変へ進行するが，多くの患者ではHBe抗体へセロコンバージョンし非活動性キャリアとなる．

HBe抗原陰性になると総じて予後が良いと考えられていた．しかし，最近，逆に予後が悪い病態が報告され，重要な病期の一つとして分類されている．このHBe抗原陰性慢性肝炎は，HBe抗原が抗体へセロコンバージョンしてもHBV DNA量が十分低下せず慢性肝炎が持続する場合や，一旦非活動性キャリアとなった後に肝炎の再活性化が起こる場合がある[5]．特徴としては，HBV DNA量は中等度の範囲で変動し，間欠的に激しい肝炎を起こす傾向がある．また，この肝炎はHBe抗原非産生変異株により惹起され，肝硬変や肝癌へ進行しやすいことが報告されている．

1-3　免疫監視期

非活動性キャリア期ではHBVに対する宿主の免疫が優位になり，HBVの増殖は持続的に低下する．この結果，肝炎は沈静化し肝発癌率も低いので予後は良いと考えられている．しかし，自然経過または宿主の免疫抑制により，B型肝炎の再活性化がみられることがあるので経過観察は必要である．

非活動性キャリアを経過した後，一部ではHBs抗原が陰性化し，回復期となる．この時期は肝炎はなく肝発癌率も低いとされている．しかし，高齢者や肝硬変のHBs抗原消失例では肝発癌に対する注意が必要である．また，HBs抗原は陰性化しても肝細胞の核内にcccDNAの形でHBVが残存するので，HBVが完全に排除されたことにはならない[6,7]．

2　HBV感染の診断，HBs抗原とIgM-HBc抗体

HBVキャリアの診断にはHBs抗原の測定が最も優れている（表2）．HBs抗原はHBVの表面抗原であり，ビリオンの表面に存在する．しかし，これ以外にもHBs抗原粒子として血中に大量に分泌されるため，診断に用いられている．HBs抗原が陽性であるということは現在HBVに感染していることを示す．通常，HBVキャリアではHBc抗体も陽性である．

近年，HBs抗原の測定は非常に高感度となり，感度不足による偽陰性の危険性は大きく低下した．このため，キャリアと非キャリアの鑑別はHBs抗原の測定のみで十分とされている．ただし，HBs抗原検査試薬には一般測定用と精密測定用があり，前者は経済的であるが感度の点でやや劣るので，厳密にHBV感染を確認する場合には後者を用いる必要がある．

急性肝炎の診断にはHBs抗原に加えIgM-HBc抗体を同時に測定する必要がある．この理由の一つは，急性肝炎ではHBs抗原が早期に陰性化することがあり，HBs抗原だけの測定では診断ができない場合があるためである．このような場合でもIgM-HBc抗体は陽性（高力価）となるので，急性肝炎の診断が可能となる．第二の理由は，キャリアからの急性増悪との鑑別である．過去の感染状況が不明の場合，この急性増悪と急性肝炎を臨床的に鑑別することは困難であり，

表2　B型肝炎ウイルスマーカーの臨床的意義

マーカー	臨床的意義
HBs抗原	HBVに感染している（通常HBc抗体も陽性）
HBs抗体	HBVの感染既往（多くはHBc抗体も陽性） HBVワクチン接種後
HBc抗体	HBVの感染既往（多くはHBs抗体も陽性） HBVに感染している（HBs抗原も陽性）
IgM-HBc抗体	B型急性肝炎（高力価） B型慢性肝炎の急性増悪（低力価）
HBe抗原	HBVの増殖力が強い
HBe抗体	HBVの増殖力が弱い
HBV DNA	HBV量を反映
HBコア関連抗原	
核酸アナログ非使用時	HBV量を反映
核酸アナログ使用時	肝細胞中HBV cccDNA量を反映
HBV遺伝子型	感染経路や予後を推定
HBV遺伝子変異	病態や予後を推定

IgM-HBc抗体の測定が役立つ．すなわち，急性肝炎では抗体が高力価陽性（10.0 COI以上）となるのに対し，キャリアの急性増悪では陽性となっても低力価である．

3　HBVの活動性を示すマーカー

B型肝炎の診療では，HBVの活動性を測定するマーカーは欠くことができない．これは，キャリアの病期はHBVの活動性と肝炎の有無から判断されることや，抗ウイルス療法の効果判定にはこの測定が欠かせないからである．HBV活動性の指標としては，HBe抗原・抗体系が古くから用いられてきたが，最近はHBV DNA量がより重要視されるようになった．

3-1　HBe抗原・抗体

HBe抗原はHBV感染肝細胞から血中へ分泌されるたんぱく質で，ウイルス粒子とは別に存在する．HBVにとってのHBe抗原の役割は必ずしも明らかではないが，HBVの持続感染化と関連していると考えられている．

HBe抗原は臨床的にHBV増殖を反映するマーカーとして用いられており，陽性者ではHBVの増殖は盛んである（表2）．免疫寛容期では，基本的にHBe抗原陽性でウイルス量は多い．免疫排除期において，HBe抗原からHBe抗体にセロコンバージョンするとウイルス量は低下し肝炎が沈静化する．このため，HBe抗原のセロコンバージョンはB型肝炎の経過の中で大きな意味を持つ現象であり，重要な治療目標の一つである．しかし，HBe抗体陽性となってもウイルス量が十分低下しない症例や重症肝炎が惹起される症例もあり，セロコンバージョンだけでは不十分な場合もある．これがHBe抗原陰性の慢性肝炎であるが，HBe抗原陽性の慢性肝炎に比較し病変の進行が早いことがあり注意が必要な病態である．

3-2　血中HBV DNA量

血中HBV DNA量の測定は病態の把握や予後の予測に有用である．さらに，抗ウイルス療法の適応を決定したり治療効果を判定するのにも用いられる，最も重要なマーカーである．以前のHBV DNA量測定法は感度が低く，その有用性は限られていた．しかし，高感度で定量域の広い測定法の開発により，核酸アナログ治療にも対応する測定法となった．現在はTaqMan PCR法（2.1～9.0 log copy/ml）が，測定レンジがさらに広く感度も良いことから，臨床で広く使用されている．

HBV DNA量とその後の臨床経過には強い関連がある．すなわち，ウイルス量が多いほどその予後は悪く，肝硬変進展率や肝発癌率が高くなる[8,9]．逆に，HBV DNA量が4.0 log copy/ml未満になると肝炎は沈静化し肝発癌率も低下する．非専門医には定量値の意義を記憶することは苦痛と思われるので，図1にHBV

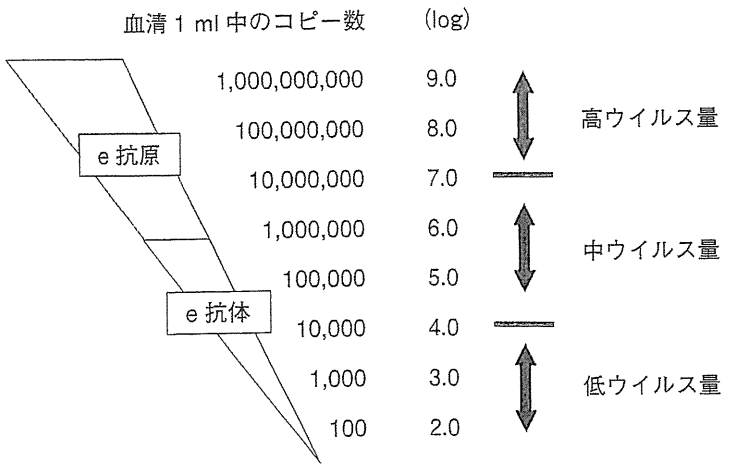

図 1　HBV DNA 量と HBe 抗原・抗体

DNA 量のイメージ図を示した．7.0 log copy/ml 以上は高ウイルス量で，抗ウイルス療法にも抵抗することが多い．4.0～7.0 log copy/ml は中ウイルス量であり，4.0 log copy/ml 未満が低ウイルス量となる．治療目標は低ウイルス量で安定させることである．市民講座などでは「イー，スー，チー」で覚えるように話している．

4　核酸アナログ薬治療と HBV マーカー

2000 年に核酸アナログ薬が導入され，B 型肝炎の治療は大きく進歩した．核酸アナログ薬は HBV に対して強い抗ウイルス効果を有し，免疫能が低下した透析患者でも HBV の増殖を強力に抑制する．核酸アナログ薬の治療効果は血中 HBV DNA 量を測定して判定する．すなわち，核酸アナログ薬投与開始後速やかに HBV DNA 量は低下し，多くの症例では陰性化する．また，耐性株出現時には最初に HBV DNA 量の増加がみられる．

核酸アナログ薬治療の問題点は，抗ウイルス効果が強力であっても最終的に HBV を完全に駆除することは困難であり，この結果として耐性株の出現や治療中止後の肝炎の再燃が起こることである．この問題を理解するために，HBV の複製過程と HBV cccDNA について，さらにこの cccDNA 量を反映するコア関連抗原量について説明する．

4-1　HBV の複製と肝細胞中 cccDNA

HBV の複製過程を図 2 に示した．HBV は肝細胞に感染後，不完全 2 重鎖の DNA 遺伝子が閉環し cccDNA (covalently closed circular DNA) となる．この cccDNA

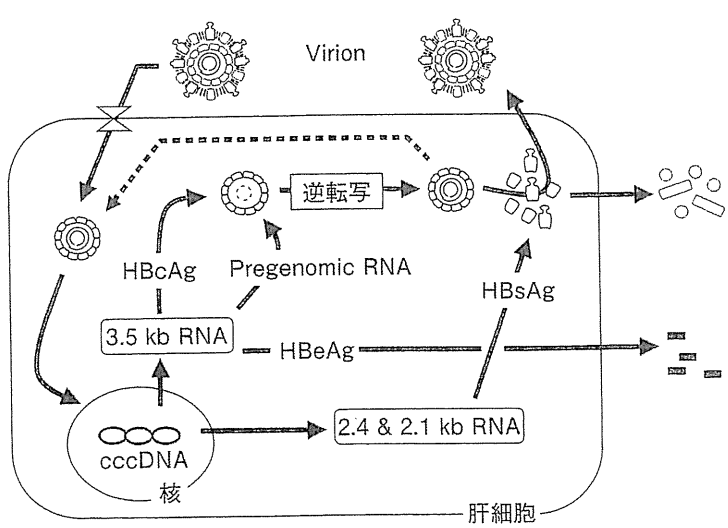

図 2　HBV 複製過程と逆転写

が核内に蓄積され，ここからpregenome RNAやmRNAが作られウイルスが複製される[10~12]．

HBV cccDNAはHBVの複製の起点であり，この存在が耐性株出現や治療中止後の肝炎の再燃と深くかかわっている．また，ミニ染色体とも言える安定した構造であり，長期に肝細胞中に残存することから，この存在がHBVの駆除を困難にしている．肝細胞中のcccDNA量を直接測定することは技術的に可能であるが，肝生検を要するため実際的ではない．このため，cccDNA量を反映する血清マーカーとしてHBコア関連抗原測定系が開発された．

4-2 血中HBコア関連抗原量

HBコア関連抗原測定系は，HBVのプレコア・コア遺伝子から転写・翻訳されるすべての抗原（c抗原，e抗原，等）をリニアエピトープとして同時に測定する方法である[13,14]．自然経過では，血中のコア関連抗原量とHBV DNA量は直線的に相関するので，ウイルス量の判定に使用可能である．これに対し，核酸アナログ投与下ではHBV DNA量と異なった動きを示す．すなわち，核酸アナログ投与開始後HBV DNA量は速やかに低下するのに対し，HBコア関連抗原量は緩徐に低下する．このHBコア関連抗原量の低下は肝細胞中のHBV cccDNA量の低下と相関し，核酸アナログ薬投与下において，HBコア関連抗原量はHBV cccDNA量と有意に相関する[15,16]．

HBVコア関連抗原量の特性を，HBV複製過程との関連で説明すると以下のようになる．HBVの複製で逆転写の過程が阻害されると血中へウイルス粒子が分泌されず，血中HBV DNA量は低下する．これに対し，HBコア関連抗原は，cccDNAから転写されるmRNAから直接翻訳されるので核酸アナログ薬の影響は受けにくい．この差が核酸アナログ薬投与下でのHBV DNA量とコア関連抗原量の推移の差に出ると考えられる．HBコア関連抗原量測定の有用性に関してはこれまで以下のものが報告されている[16~20]．HBコア関連抗原が低い症例では高い症例に比較して，①耐性株出現率が低い，②中止後の肝炎の再燃が弱い，③組織学的に進行しにくい，④肝発癌率が低いなどである．

5 その他のマーカー

5-1 HBs抗体

HBs抗原に対する抗体であり，中和抗体としてHBVに対する感染防御機能をもつ．HBs抗体が陽性であることは過去にHBV感染を受けたこと，またはHBワクチン接種を受けたことを示す（表2）．時にはHBIG投与後，輸血・血液製剤使用後などにもこの抗体が陽性となる．HBs抗体は定量的に測定することが可能であり，WHOの勧告ではHBs抗体価が10 mIU/ml未満になった時，追加のワクチン接種が推奨されている．

5-2 HBc抗体

これはHBc抗原に対する抗体であり，感染の比較的早期から血中に出現し，長年月持続する．HBV感染者を既往者も含めて最も広く拾い出す検査である（表2）．

既往感染者は低抗体価で，通常HBs抗体も同時に陽性である．HBVキャリアでは通常高抗体価であるが，肝炎を経験していない症例では低抗体価陽性または陰性である．従来より，HBc抗体を低抗体価と高抗体価に分けることによりHBV感染状態の把握を行ってきた．しかし，その後の研究や測定系の進歩により，この分類の意義は失われている．具体的には，HBV感染の判定にはHBs抗原の精密測定が，また急性肝炎かキャリアの急性増悪かの鑑別には後述のIgM-HBc抗体の測定が優れている．臨床的には，HBc抗体は定性レベルで陽性か陰性かの判定が重要である．すなわち，HBs抗原陰性でHBc抗体陽性の場合は，HBs抗体の有無にかかわらずHBVの既往感染であることを示す．

5-3 HBV遺伝子型

HBVの遺伝子型はA～Hの8型に分類されている．日本では遺伝子型BとCがほとんどで，前者は後者に比較し自然経過での予後は良く，抗ウイルス治療に対する反応性も良い．また，近年海外から持ち込まれた遺伝子型Aでは，成人の初感染でもキャリア化しやすいことが知られている．

5-4 プレコア変異，コアプロモーター変異

HBe抗原の合成が停止または減少するプレコアと，コアプロモーターの変異が測定可能である．これらの変異は，HBe抗原のセロコンバージョン予測や，急性増悪時の重症化予測などに有用である．

6 B型肝炎の再活性化

B型肝炎の再活性化は，HBVの増殖が十分抑制された状態から，なんらかの原因でHBVの増殖が再び活発になり肝炎が再燃することである[21~23]．原因の多くは強力な化学療法や免疫抑制療法によるものであるが，自然経過でも起こる．当然，腎移植でも再活性化は大きな問題であり，移植予定の患者はHBs抗原，HBs抗体，HBc抗体の測定を行いHBVの感染状況を確認する必要がある．さらに，HBs抗原陽性のキャリアが腎移植を受ける場合はHBVの再活性化は必須であり，核酸アナログ薬による予防を行う．

以前，HBV既往感染者（HBs抗原陰性でHBs抗体and/or HBc抗体陽性）ではHBVは完全に排除されたと考えられていたが，その後の研究で，ウイルス遺伝子がcccDNAの形で肝細胞核内に残存していることが明らかになった．通常，免疫監視によりHBVの増殖は起こらないが，免疫抑制下ではこのcccDNAを起点としてHBVの増殖が起こり，肝炎が再活性化する．この肝炎をde novo B型肝炎と呼んでいる．

腎移植では，ドナーがHBV既往感染の場合はde novo B型肝炎の危険性は低い．これに対し，レシピエントが既往感染の場合は再活性化に対する注意が必要である．この場合，核酸アナログ薬の予防投与は保険適用ではないので，現状では定期的な検査（HBV DNA，HBs抗原）でHBV再活性化の早期発見につとめることが推奨される．

医療の進歩に伴い化学療法や免疫抑制療法を行う機会が増え，さらに使用される薬物もより強力なものとなった．この結果，再活性化によるB型肝炎は増える傾向にある．再活性化による肝炎は劇症化率，死亡率は共に高く，重篤な病態を呈する．さらに，透析を含む多くの診療科が関係するため，近年注目されている病態である[24]．

おわりに

以上，B型肝炎ウイルスマーカーの意義を透析患者の診療を中心に述べた．ある意味で専門的な領域であり，透析医がすべてを理解する必要はない．しかし，透析患者でのHBV感染の危険性を考えると，基礎的な知識として知っておく意義は大きいと考える．

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Amino Acid Substitution in HCV Core/NS5A Region and Genetic Variation Near *IL28B* Gene Affect Treatment Efficacy to Interferon plus Ribavirin Combination Therapy

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Key Words
Hepatitis C virus · Interferon · Ribavirin · Core region · NS5A region · ISDR · IRRDR · *IL28B*

Abstract
Objective: To evaluate predictive factors of treatment efficacy to interferon (IFN)/ribavirin in patients infected with HCV genotype 1b (HCV-1b). ***Methods:*** This study investigated pretreatment predictors, including viral- (aa substitutions in core aa 70/91 and NS5A-ISDR/IRRDR) and host-related factors (genetic variation near *IL28B* gene), to 48-week IFN/ribavirin in 490 Japanese adults infected with HCV-1b. ***Results:*** The proportion of patients who showed end-of-treatment response (ETR), sustained virological response (SVR), and SVR after ETR was 76, 54, and 76%, respectively. There was a significant positive correlation between the number of aa substitutions in ISDR and those in IRRDR. Concerning the substitution of core aa 91, the number of aa substitutions in ISDR/IRRDR of patients with Leu91 was significantly higher than that of patients with Met91. Furthermore, levels of viremia were influenced by aa substitutions in core aa 91 and ISDR/IRRDR. By multivariate analysis, rs8099917 genotype was an important predictor of ETR and SVR. With regard to viral factors, core aa 70/91 was an important predictor of ETR, and SVR after ETR. ISDR was an important predictor of SVR, and SVR after ETR. ***Conclusion:*** aa substitution in core/NS5A region and genetic variation near *IL28B* were important predictors of treatment efficacy to IFN/ribavirin.

Introduction

Treatment of chronic hepatitis C virus (HCV) infection with interferon (IFN) combined with ribavirin carries potential serious side effects and is costly, especially when used long enough to achieve a high sustained virological response (SVR) in patients infected with HCV genotype 1b (HCV-1b) and high viral loads. For these rea-

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