even at 6–12 months after the vaccination. Such an observation suggests that NK cells primed with DC vaccine may contribute to long-term tumor rejection. Further exploration is needed to disclose how NK cells work in DC vaccine therapies.

In the patient displaying an SD response (Case 1), CEA(24)-pentamer-positive cells increased gradually with the injection of OPA-DCs. Interestingly, despite expansion of CTLs, we detected no IFN-γ-producing or antigen-specific-lytic ability of CTLs against antigen-loaded T2-A24 cells. One of the reasons for such a discrepancy may be the impaired differentiation of CTLs, exhibited by the predominance of Tcm phenotype as antigen-specific CTLs in this study. Sallusto et al. [15] identified different populations of memory CD8+ T cells in the peripheral blood of humans. The Tcm cells are reported to attain potent proliferative or IL-2-producing ability critical for maintenance of memory-T-cell pools, whereas Tem or Tem/td cells possess potent effector function such as IFN-γ/perforin production or cytotoxicity [15]. It is still uncertain which cells, Tcm or Tem, are more advantageous in providing protective immunity against cancer [38]. Mornarini et al. [39] reported that although most melanoma patients displayed antigen-specific CTLs belonging to Tcm subsets in tumor-invaded lymph nodes, few perforin-producing Tem or Tem/td cells infiltrated in the neoplastic tissues, and they found no evidence for tumor regression. Although the factors that regulate CTL differentiation are still unclear, further understanding of such regulators could provide clues to developing effective vaccines.

In summary, quickly inducible OPA-DC vaccine is tolerated and could induce preferable immunological responses in patients with advanced-stage CRC. OPA-DC vaccine exerted significant NK-activating ability, and such a response was partially linked to favorable clinical outcomes in the patients. Most antigen-specific CTLs induced with the OPA-DC vaccine belonged to a Tcm subset. In order to develop more practically effective DC vaccine against CRC, further investigation is necessary to explore the modality to induce coordinated and durable activation of both NK cells and CTLs.

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Amino Acid Substitution in the Core Protein has no Impact on Relapse in Hepatitis C Genotype 1 Patients Treated With Peginterferon and Ribavirin

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Previous reports demonstrated that amino acid (aa) substitutions in the hepatitis C virus (HCV) core protein are predictors of non-virological responses to pegylated interferon (Peg-IFN) and ribavirin combination therapy. The aim of this study was to investigate the impact of core aa substitutions on viral kinetics during the treatment and relapse after the treatment. The 187 patients with HCV genotype 1 enrolled in this study were categorized into four groups according to core aa substitution patterns: double-wild group (n = 92), Arg70/Leu91; 70-mutant group (n = 42), Gln70/Leu91; 91-mutant group (n = 31), Arg70/Met91; and double-mutant group (n = 22), Gln70/Met91. The relationship between the core aa substitutions and the virological response was examined. Multivariate logistic regression analyses showed that substitution at aa 70 was significantly associated with a poor virological response during the first 12 weeks (decline of <1 log from baseline at week 4, <2 log at week 12), and substitution at aa 91 was significantly associated with detectable HCV RNA at week 24. With respect to relapse, only the ribavirin exposure (odds ratio (OR), 0.77; 95% confidence interval (CI), 0.60–0.98) and HCV RNA disappearance between weeks 13 and 24 (OR, 23.69; 95% CI, 5.44–103.08) were associated independently with relapse, with no correlation being found with the core aa substitutions and relapse. In conclusion, the results showed that core aa substitutions can be strong predictive factors at pretreatment of the non-response, but not for relapse, for virological responders with HCV RNA disappearance during treatment. *J.*

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KEY WORDS: amino acid substitution; core protein; hepatitis C virus; peginterferon and ribavirin combination therapy; relapse

INTRODUCTION

The current standard of care for chronic hepatitis C patients is combination therapy using pegylated interferon (Peg-IFN) and ribavirin [Anonymous, 2002; Strader et al., 2004; Dienstag and McHutchison, 2006]. However, the treatment outcome in response to this combination therapy among patients infected with hepatitis C virus (HCV) genotype 1 is still unsatisfactory and the chance of sustained virological response ranges from 42% to 52% [Manns et al., 2001; Fried et al., 2002; Hadziyannis et al., 2004]. Therefore, tailoring treatment regimens for individual patients has become an important issue.

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Outcome of therapy is influenced by various factors. Some host factors, such as age, sex, body weight, insulin resistance, and liver fibrosis have been reported as pretreatment factors affecting virological response to this combination therapy [Manns et al., 2001; Fried et al., 2002; Hadziyannis et al., 2004; Romero-Gomez et al., 2005]. Recently, several genome-wide association studies identified single nucleotide polymorphisms (SNPs) near the interleukin (IL)-28B gene, which encodes interferon (IFN) lambda-3, as associated with response to Peg-IFN plus ribavirin treatment among patients infected with HCV of European [Suppiah et al., 2009], African [Ge et al., 2009], and Asian ancestry [Tanaka et al., 2009]. These studies suggest that host genetic variants may be associated strongly with response to IFN-alpha-based therapy. However, the ethical problem to perform host genetic search for all patients remains, and the sustained virological response rate is only 48–69% in patients having favorable IL-28B genotype to this combination therapy [Thompson et al., 2010].

Response-guided therapy is a dynamic approach to management of chronic hepatitis C patients based on the virological response at weeks 4 and 12 of treatment. At present, it is regarded as an excellent strategy for optimizing the treatment duration for individual patients. Earlier HCV RNA disappearance has been shown to lead to a higher sustained virological response rate [Ferenci et al., 2005; Berg et al., 2006; McHutchison et al., 2009], while patients without an early virological response, defined as showing an at least 2 log decrease from the baseline of HCV RNA levels at week 12 is recommended for discontinuing the treatment under the current guidelines [Anonymous, 2002; Strader et al., 2004; Dienstag and McHutchison, 2006].

In addition to viral kinetics during treatment, other viral factors have also been reported to be associated with this combination therapy outcome [Manns et al., 2001; Fried et al., 2002; Hadziyannis et al., 2004; Shirakawa et al., 2008]. Previous studies indicated that amino acid (aa) 70 and/or 91 substitutions in the HCV core protein were independent pretreatment predictors of null or weak response to this combination therapy in genotype 1 patients [Akuta et al., 2007b,c]. The HCV core protein has been reported to inhibit signal transducer and activator of transcription (STAT)-1 phosphorylation, and disrupt the normal IFN-stimulated transcriptional response to viral infection [Lin et al., 2006]. It is supposed that the HCV core region might be associated with resistance to IFN therapy involving the Janus activated kinase (Jak)-STAT signaling cascade [Blindenbacher et al., 2003; Bode et al., 2003; Melen et al., 2004; de Lucas et al., 2005]. Recently, Okanoue et al. [2009] have demonstrated that wild type of core aa 70 and 91 are important for positive prediction of the virological response. However, the impact of core aa substitutions on the extent of HCV RNA decline during the treatment or virological relapse after completion of treatment has not yet been investigated in detail. Approximately 30% of genotype 1 patients who become HCV RNA negative at the end of the treatment will experience relapse [Hadziyannis et al., 2004]. Being able to distinguish between end-of-treatment responders with a high probability of relapse and those with a low probability of relapse will be useful in reducing relapse rates and improving treatment outcome.

The aim of this study was to evaluate the impact of aa substitutions in the HCV core protein on viral kinetics and virological relapse in patients with HCV genotype 1 treated by Peg-IFN alpha-2b and ribavirin combination therapy.

PATIENTS AND METHODS

Patient Selection and Study Design

Patients considered to be eligible for this study were those who were infected with HCV genotype 1, had a viral load more than 10^5 IU/ml, had started Peg-IFN alpha-2b (Schering-Plough K.K. Tokyo, Japan) and ribavirin (Schering-Plough K.K.) combination therapy from December 2005 to June 2008 at Osaka University Hospital and three other medical institutions taking part in the Osaka Liver Forum, and had been examined with respect to the aa sequences at positions 70 and 91 in the HCV core protein with pretreatment serum samples. Patients with the following criteria were excluded: hepatitis B virus or human immunodeficiency virus co-infection; decompensated liver disease; severe cardiac, renal, hematological, or chronic pulmonary disease; poorly controlled psychiatric disease; poorly controlled diabetes; and immunologically mediated disease. As a result of screening at the institutions concerned, 187 patients with HCV genotype 1 were enrolled in this study. Liver biopsy had been performed within 12 months prior to the treatment, and histological results were classified according to the METAVIR scoring system [Bedossa and Poynard, 1996].

Written informed consent was obtained from each patient, and the study protocol was reviewed and approved according to the ethical guidelines of the 1975 Declaration of Helsinki by Institutional Review Boards at the respective sites.

Peg-IFN alpha-2b and ribavirin dosages were based on body weight according to the manufacturer's instructions: Peg-IFN alpha-2b was given subcutaneously weekly (45 kg or less, 60 μg/dose; 46–60 kg, 80 μg/dose; 61–75 kg, 100 μg/dose; 76–90 kg, 120 μg/dose; and 91 kg or more, 150 μg/dose), and ribavirin was given orally daily (60 kg or less, 600 mg/day; 61–80 kg, 800 mg/day; and 81 kg or more, 1,000 mg/day). The drug doses were also modified based on the manufacturer's instructions according to the severity of the adverse hematologic effects.

Detection of Amino Acid Substitutions in Core Region

The nucleotide sequence encoding aa 1–191 (the core protein of HCV) was analyzed by direct sequencing as described by Akuta et al. [2005, 2007b]. In brief, HCV

RNA was extracted from the serum samples and converted to cDNA and two nested rounds of polymerase chain reaction (PCR) were performed. Primers used in the PCR were as follows: the first PCR was performed using cc11 (sense, 5′-GCC ATA GTG GTC TGC GGA AC-3′) and e14 (antisense, 5′-GGA GCA GTC CTT CGT GAC ATG-3′) primers. The second PCR was performed using cc9 (sense, 5′-GCT AGC CGA GTA GTG TT-3′) and e14 (antisense) primers. All samples were denatured initially at 95°C for 15 min. The 35 cycles of amplification were set as follows: denaturation for 1 min at 94°C, annealing of primers for 2 min at 55°C, and extension for 3 min at 72°C with an additional 7 min for extension. Then 1 μl of the first PCR product was transferred to the second PCR reaction. The conditions for the second PCR were the same as the first PCR, except that the second PCR primers were used instead of the first PCR primers. The amplified PCR products were purified by the QIA quick PCR Purification Kit (Qiagen, Tokyo, Japan) after agarose gel electrophoresis and then used for direct sequencing. Dideoxynucleotide termination sequencing was performed with the Big Dye Deoxy Terminator Cycle Sequencing Kit (Perkin–Elmer, Tokyo, Japan). The obtained nucleotide and amino acid sequences were compared with the prototype sequence of genotype 1b HCV-J (GenBank Accession No. D90208) [Kato et al., 1990]. Wild types virus encoded arginine (Arg) and leucine (Leu) at aa 70 and 91, respectively, and the aa substitutions were glutamine (Gln) or histidine (His) at aa 70 and methionine (Met) at aa 91. If the intensities of the band were similar, the case was regarded as competitive. Two patterns of mutant and competitive were labeled as mutant. In this study, patients were categorized into four groups according to aa substitution patterns: double-wild group, Arg70/Leu91; 70-mutant group, Gln or His70/Leu91; 91-mutant group, Arg70/Met91; and double-mutant group, Gln or His70/Met91.

Virological Tests

Serum HCV RNA level was quantified by PCR assay (COBAS Amplicor HCV Monitor Test v2.0, Chugai-Roche Diagnostics, Tokyo, Japan), with a sensitivity limit of 5,000 IU/ml and a dynamic range from 5,000 to 5,000,000 IU/ml.

Serum HCV RNA was assessed by qualitative PCR assay (COBAS Amplicor HCV Test v2.0, Chugai-Roche Diagnostics), with a detection limit of 50 IU/ml.

Efficacy Assessments

Patients who achieved negative HCV RNA at week 12 were defined as having a complete early virological response. Patients who became HCV RNA negative between weeks 13 and 24 were defined as having a late virological response. According to the established guidelines, the treatment was considered to have failed if the patients showed an insufficient virological response at week 12 (a detectable HCV RNA and a decrease of <2 log from the baseline level) or at week 24 (a detectable HCV RNA), and therapy was discontinued. The end-of-treatment response was defined as undetectable HCV RNA at week 48. Patients with end-of-treatment response and undetectable HCV RNA 24 weeks after completion of therapy were defined as having sustained virological response. Relapse was defined as a case in which HCV RNA had been undetectable at the end-of-treatment, but detectable during the 24-week follow-up after the treatment.

Drug Exposure

The amounts of Peg-IFN alpha-2b and ribavirin actually taken by each patient during the treatment period were evaluated by reviewing the medical records. The mean doses of both drugs were calculated individually as averages on the basis of body weight at baseline; Peg-IFN alpha-2b expressed as μg/kg/week and ribavirin as mg/kg/day.

Data Collection

The medical records were retrospectively reviewed and the factors necessary for this examination were extracted: age, sex, body weight, body mass index (BMI), basic laboratory assessments, liver histology, quantitative and qualitative HCV RNA, dose of Peg-IFN alpha-2b and ribavirin received at each administration, and the response to treatment.

Statistical Analysis

Continuous variables are reported as the mean with standard deviation (SD) or median level, while categorical variables are shown as the count and proportion. In univariate analysis, the Mann–Whitney *U*-test (between two groups) or Kruskal–Wallis test (among more than three groups) was used to analyze continuous variables, while chi-squared and Fisher's exact tests were used for analysis of categorical data. For all tests, two-sided *P* values were calculated, and the results were considered statistically significant if $P < 0.05$. Variables that achieved statistical significance ($P < 0.05$) or marginal significance ($P < 0.10$) on univariate analysis were subjected to multivariate logistic regression analysis. Stepwise and multivariate logistic regression models were used to explore the independent factors that could be used to predict a virological response. Statistical analysis was performed using the SPSS program for Windows, version 15.0J (SPSS, Chicago, IL).

RESULTS

Baseline Characteristics of Study Groups

The total study population was predominately male (55.6%), with a mean age of 56.2 years. The baseline characteristics of all patients and the four study groups according to core aa substitution patterns are shown in Table I. Mean age of patients in the double-mutant group was higher than the other three groups ($P = 0.003$). More patients in the double-wild group had

TABLE I. Baseline Demographic and Viral Characteristics of Patients

Characteristic	Total (n = 187)	Double-wild (n = 92)	70-Mutant (n = 42)	91-Mutant (n = 31)	Double-mutant (n = 22)	*P* value[a]
Age (years)	56.2 ± 9.3	55.7 ± 9.2	57.0 ± 9.8	52.4 ± 9.9	61.8 ± 4.7	0.003
Sex (male/female)	104/83	51/41	26/16	18/13	9/13	0.444
Body weight (kg)	60.9 ± 11.6	60.9 ± 11.7	62.2 ± 11.7	62.5 ± 13.2	56.0 ± 7.5	0.193
Body mass index (kg/m^2)	22.8 ± 3.1	22.8 ± 3.0	22.8 ± 3.1	23.1 ± 3.6	22.1 ± 2.4	0.627
Past IFN therapy (naïve/experienced)	118/69	45/47	34/8	20/11	19/3	<0.001
HCV RNA (×10^3 IU/ml)[b]	1,700	2,100	1,400	1,500	1,230	0.122
Fibrosis (0–2/3–4)[c]	105/29	56/11	22/6	14/7	13/5	0.366
Activity (0–1/2–3)[d]	83/50	42/24	18/10	11/10	12/6	0.771
White blood cell (×10^6/l)	4,980 ± 1,520	4,990 ± 1,420	5,180 ± 1,760	4,890 ± 1,430	4,660 ± 1,560	0.795
Red blood cell (×10^{12}/l)	4.34 ± 0.46	4.33 ± 0.46	4.41 ± 0.52	4.39 ± 0.42	4.18 ± 0.32	0.145
Hemoglobin (g/dl)	13.9 ± 1.4	13.9 ± 1.4	14.0 ± 1.7	14.2 ± 1.4	13.5 ± 1.1	0.253
Platelet (×10^9/l)	161 ± 54	167 ± 49	165 ± 65	154 ± 60	138 ± 30	0.067
ALT (IU/l)	74 ± 61	73 ± 67	79 ± 56	81 ± 64	57 ± 37	0.263
γ-GTP (IU/l)	62 ± 74	47 ± 54	81 ± 89	70 ± 93	78 ± 78	0.032

IFN, interferon; HCV, hepatitis C virus; ALT, alanine aminotransferase; γ-GTP, gamma-glutamyl transpeptidase.
[a]*P* value for comparison among double-wild, 70-mutant, 91-mutant, and double-mutant.
[b]Values expressed as median.
[c]Data for 53 patients are missing.
[d]Data for 54 patients are missing.

been treated previously for HCV infection ($P < 0.001$). Patients in the double-wild group had significantly lower gamma-glutamyl transpeptidase (γ-GTP) levels ($P = 0.032$).

Progress of Patients

The progress of patients in this study is shown in Figure 1. Of the 187 patients, 183 completed 4 weeks of treatment. Among them, 133 were assessed based on HCV RNA dynamics between baseline and week 4. Those completing 12 weeks of treatment totaled 181, of which 154 were assessed for HCV RNA dynamics between baseline and week 12. Those completing 24 weeks of treatment totaled 153, and all were assessed for HCV RNA quantitatively or qualitatively at week 24. Those completing 48 weeks of treatment totaled 114. These 114 patients and the 55 patients who had discontinued treatment because of treatment failure entered a follow-up period. Among these 169 patients, 164 completed 24 weeks follow-up and the sustained virological response (SVR) rate

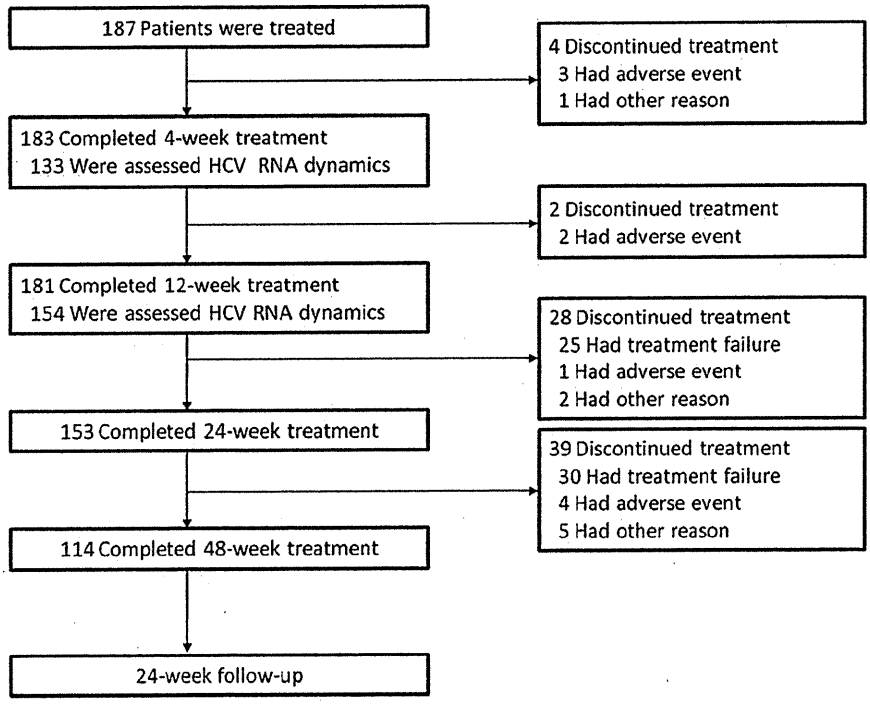

Fig. 1. Treatment and follow-up of the study patients. Treatment was discontinued for patients with <2 log decrease from the baseline HCV RNA level at week 12 or detectable HCV RNA at week 24.

TABLE II. Multivariate Analysis for Factors Associated With <1 log Decrease in HCV RNA Level at Week 4, <2 log Decrease at Week 12, Detectable HCV RNA at Week 24, and Relapse After Treatment

Factor	Category	Odds Ratio	95% CI	*P* value
HCV RNA <1 log decrease at week 4				
White blood cells ($\times 10^6$/l)	<5,000/5,000≤	—	—	NS
γ-GTP (IU/l)	<40/40≤	—	—	NS
Peg-IFN dose (μg/kg/week)	By 0.1 μg/kg/week	0.80	0.67–0.97	0.020
Core aa 70	Wild/mutant	1/2.80	1.16–6.75	0.022
HCV RNA <2 log decrease at week 12				
γ-GTP (IU/l)	<40/40≤	—	—	NS
Peg-IFN dose (μg/kg/week)	By 0.1 μg/kg/week	—	—	NS
Core aa 70	Wild/mutant	1/2.72	1.09–6.78	0.032
Detectable HCV RNA at week 24				
Platelet ($\times 10^9$/l)	<150/150≤	—	—	NS
γ-GTP (IU/l)	<40/40≤	1/2.46	1.02–5.95	0.045
Core aa 91	Wild/mutant	1/4.11	1.73–9.78	0.001
Relapse after treatment				
Ribavirin dose (mg/kg/day)	By 1 mg/kg/day	0.77	0.60–0.98	0.036
Virological response	Complete early virological response/late virological response	1/23.69	5.44–103.08	<0.001

CI, confidence interval; NS, not significant difference; γ-GTP, gamma-glutamyl transpeptidase; Peg-IFN, pegylated interferon; aa, amino acid.

was 48.2% (79/164), based on per-protocol set. Among the 106 patients who had an end-of-treatment response and completed follow-up, 27 showed relapse during the follow-up period; the relapse rate was 25.5% (27/106).

IMPACT OF CORE-RELAPSE AFTER TREATMENT (TABLE II)

Impact of core aa substitutions on <1 log viral decrease rate at week 4, <2 log at week 12, detectable HCV RNA at week 24, and virological relapse after treatment (Table II).

The impact of core aa substitutions on <1 log viral decrease rate at week 4, <2 log at week 12, detectable HCV RNA at week 24, and virological relapse after treatment (Table II).

The impact of the core aa substitutions on <1 log viral decrease at week 4, which is a predictor of non-sustained virological response; fewer than 5% of patients without 1 log decrease at week 4 had an sustained virological response [McHutchison et al., 2009] was examined. Among the 133 patients who completed 4 weeks of treatment, 31 failed to show a ≥1 log decrease of HCV RNA level at week 4. Univariate analysis for factors associated with <1 log decrease of HCV RNA level at week 4 was performed on the following variables: age, sex, body weight, BMI, history of past IFN therapy, baseline HCV RNA level, histological fibrosis and activity, white blood cell count, red blood cell count, hemoglobin level, platelet count, alanine aminotransferase (ALT) level, γ-GTP level, dose exposure of Peg-IFN and ribavirin, and aa substitutions in the HCV core protein. The results indicated that pretreatment white blood cell count, γ-GTP level, the mean dose of Peg-IFN during the first 4 weeks of treatment and single-spot substitution in the HCV RNA core position at aa 70 contributed to a <1 log decrease of HCV RNA level at week 4. Analysis of these factors by multivariate logistic regression analysis showed that substitution of aa 70 (odds ratio (OR) 2.80, 95% confidence interval (CI) 1.16–6.75, $P = 0.022$) as well as the mean dose of Peg-IFN (OR 0.80, 95% CI 0.67–0.97, $P = 0.020$) was independently associated with viral decline (<1 log) at week 4.

Next, the impact of the core aa substitutions on <2 log viral decrease rate at week 12, which is presently considered to be the most reliable predictor of non-sustained virological response [Fried et al., 2002; Davis et al., 2003] was examined. Among the 154 patients who completed 12 weeks of treatment, 25 failed to show a ≥2 log decrease of HCV RNA level at week 12. Univariate analysis was performed on the same factors in the preceding examination. As a result, pretreatment γ-GTP level, the mean dose of Peg-IFN during the first 12 weeks of treatment and single-spot substitution in the HCV RNA core position at aa 70 contributed to a <2 log decrease of the HCV RNA level. These factors were then analyzed by multivariate logistic regression analysis; only substitution of aa 70 (OR 2.72, 95% CI 1.09–6.78, $P = 0.032$) was found to be independently associated with an insufficient virological response (<2 log HCV RNA decrease from baseline level) at week 12.

The impact of the core aa substitutions on detectable HCV RNA at week 24, which is another non-sustained virological response predictor [Davis et al., 2003] was also examined. Among 153 patients who completed 24 weeks of treatment, 30 still had detectable HCV RNA at week 24. Univariate analysis revealed that pretreatment platelet count, γ-GTP level, and single-spot substitution in the HCV RNA core position at aa 91 contributed to the HCV RNA remaining positive. Multivariate logistic regression analysis, using these factors, indicated that substitution of aa 91 (OR 4.11, 95% CI 1.73–9.78, $P = 0.001$) as well as γ-GTP level (>40 IU/l) (OR 2.46, 95% CI 1.02–5.95, $P = 0.045$) was

independently associated with detectable HCV RNA at week 24.

Next, the factors associated with virological relapse after the treatment was examined. Univariate analysis was performed on the virological response (complete early virologival response or late virological response) in addition to the factors in the preceding examination, revealing the mean dose of ribavirin during the full treatment period and a late virological response, but not aa substitutions (single-spot substitution in the HCV RNA core position at aa 70, $P = 0.467$; aa 91, $P = 0.776$).

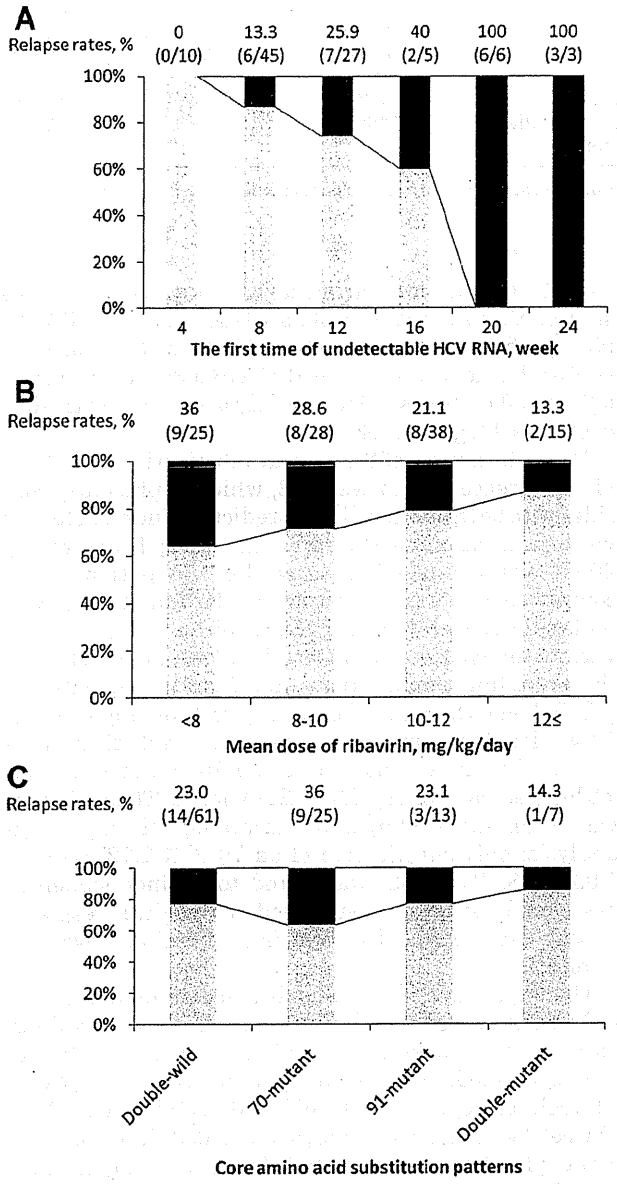

Fig. 2. Relapse rates according to the timing of HCV RNA disappearance (**A**), mean ribavirin dose (**B**), and core amino acid substitution patterns (**C**) in patients who had end-of-treatment response and completed 24-week follow-up. Relapse rates are shown as percentages and the number of patients with relapse in relation to the total number of patients examined is shown at the top of each column. Gray bar, sustained virological response; black bar, relapse.

These factors were analyzed by multivariate logistic regression analysis. This analysis revealed that the mean ribavirin dose (OR 0.77, 95% CI 0.60–0.98, $P = 0.036$) and a late virological response (OR 23.69, 95% CI 5.44–103.08, $P < 0.001$) were independently associated with relapse.

Relapse Rates According to the Timing of HCV RNA Disappearance, Ribavirin Dose, and Core aa Substitution Patterns

The relapse rates were indicated according to the time to the first non-detection of HCV RNA, mean ribavirin dose and core aa substitution patterns (Fig. 2). The relapse rate was 0% (0/10) in patients with undetectable HCV RNA during 1–4 weeks, and increased 13.3% (6/45) during 5–8 weeks, 25.9% (7/27) during 9–12 weeks, 40% (2/5) during 13–16 weeks, 100% (6/6) during 17–20 weeks, and 100% (3/3) during 21–24 weeks (Fig. 2A). Similarly, the relapse rates increased as the mean ribavirin dose decreased; 13.3% (2/15) in patients receiving ≥12 mg/kg/day of ribavirin, 21.1% (8/38) at 10–12 mg/kg/day, 28.6% (8/28) at 8–12 mg/kg/day, and 36% (9/25) at <8 mg/kg/day (Fig. 2B). On the other hand, the relapse rates were similar among the four core aa substitution patterns; 23.0% (14/61) in patients in the double-wild group, 36% (9/25) in 70-mutant group, 23.1% (3/13) in 91-mutant group, and 14.3% (1/7) in double-mutant group (Fig. 2C). In the subgroup of patients receiving <10 mg/kg/day of ribavirin, no significant difference of the relapse rates was observed between double-wild group and 70-mutant and/or 91-mutant group (31.3% (10/32) in double-wild group vs. 33.3% (7/21) in 70-mutant and/or 91-mutant group), and also in the patients receiving ≥10 mg/kg/day of ribavirin (13.8% (4/29) in double-wild group vs. 25% (6/24) in 70-mutant and/or 91-mutant group) (Fig. 3). Among patients with complete early virological response, the relapse rates were also similar between double-wild group and 70-mutant and/or 91-mutant group (13.7% (7/51) in double-wild vs. 18.4% (7/38) in 70-mutant and/or 91-mutant group). The impact of core aa substitutions on relapse rates in patients with late virological response could not be assessed because of the small number of patients.

DISCUSSION

Kobayashi et al. [2010] investigated the clinical and virological factors influencing these core aa substitutions in patients infected with HCV genotype 1 who had not received antiviral therapy, and found that HCV variants with wild type of core aa 70 and 91 significantly decreased with age, while those with the mutant type of core aa 70 and/or 91 significantly increased with age. Furthermore, they demonstrated that the proportion of patients with the mutant type of core aa 70 HCV variant significantly increased with an elevated γ-GTP level and a decrease in platelet counts. In this study, the significant differences of baseline demographics between patient groups according to core aa substitution pat-

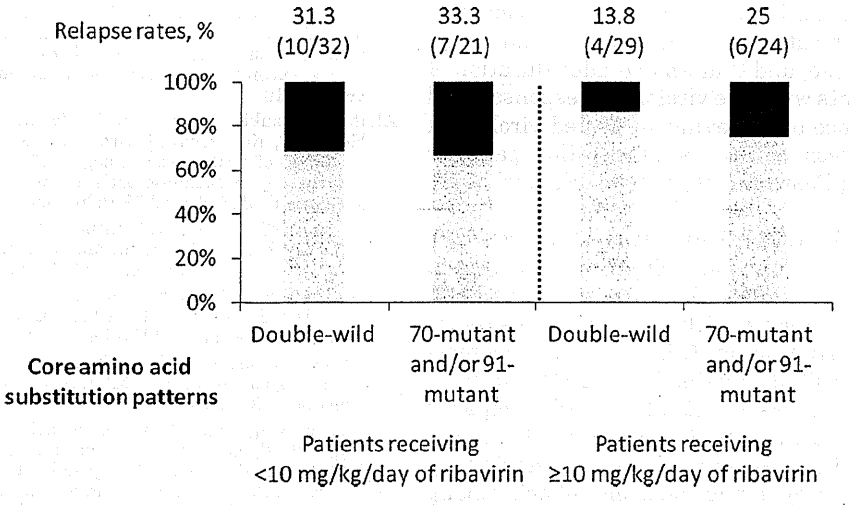

Fig. 3. Relapse rates according to core amino acid substitution patterns in patients receiving <10 mg/kg/day and receiving ≥10 mg/kg/day of ribavirin. Relapse rates are shown as percentages and the number of patients with relapse in relation to the total number of patients examined is shown at the top of each column. Gray bar, sustained virological response; black bar, relapse.

terns were similarly found in age, platelet count, and γ-GTP level. Accordingly, this study cohort had no specific bias and seems to reflect the natural background of the patients according to the HCV variance. In this study, the impact of HCV core aa substitutions on the virological response were evaluated by multivariate analysis, in order to resolve the bias of patient background factors among the groups classified according to the core aa substitution patterns. Recently, Abe et al. [2010] reported that the human genotype of the rs8099917 SNP at the IL28B locus was associated with lower γ-GTP level and viral wild type of core aa 70 and 91. Possibly these differences of IL28B genotype may influence the difference of patient background factors. Further studies are needed to clarify the relationship between human genetic variation and HCV core amino acid substitutions.

The HCV core protein has been reported to have an effect on a variety of cellular functions [Lai and Ware, 2000; Joo et al., 2005; Ariumi et al., 2007; Waris et al., 2007; Osna et al., 2008]. Currently, aa substitutions in the HCV core region has been thought to be related with outcome of antiviral therapy [Akuta et al., 2005; Donlin et al., 2007] and also the development of hepatocellular carcinoma [Akuta et al., 2007a; Hu et al., 2009]. Importance of core aa substitutions, especially at aa 70 and 91, comes to be recognized, and the new method to detect these substitutions easily has been proposed [Nakamoto et al., 2009]. As for the mechanism of antiviral activity on core aa substitutions, Ikeda et al. [2010] showed that core aa substitutions were not associated with intracellular antiviral response to IFN-alpha by in vitro analysis. The mechanism of antiviral activity and hepatocarcinogenesis on core aa substitutions has not been elucidated enough, so far. Further in vitro studies will be needed to clarify this.

Previous studies showed that patients with substitution of core aa 70 often had slow or no decrease in HCV RNA levels during the early phase of IFN-alpha treatment [Akuta et al., 2005, 2007b,c; Donlin et al., 2007]. Consistent with these reports, multivariate analysis in this study revealed that substitution of core aa 70 could be independently associated with insufficient viral decline during the first 12 weeks after the treatment (decline of <1 log from baseline at week 4, <2 log at week 12). This suggests that patients with substitution of core aa 70 are likely to fail to have a sustained virological response. On the other hand, dose exposure of Peg-IFN during the first 4 weeks of treatment was also independently linked to a minimal decline in HCV RNA (<1 log) at week 4 in this study. This suggests that maintaining the dose of Peg-IFN as high as possible until the disappearance of HCV RNA can help avoid treatment failure [McHutchison et al., 2002; Oze et al., 2009], especially in patients with substitution of core aa 70. On the other hand, substitution of core aa 91 was independently associated with detectable HCV RNA at week 24. This suggests that patients with substitution of core aa 91 are likely to achieve non-sustained virological response even if they had a ≥2 log decline in the HCV RNA level at week 12. The reason for the difference of the impact on virological response is not yet clear.

Multivariate logistic regression analysis also showed that the dose exposure of ribavirin during the full treatment period and having late virological response were independently associated with relapse. As for ribavirin exposure, it has been previously demonstrated that the relapse rate among patients responding to the treatment showed a decline in relation to the increase in the dose of ribavirin [Hiramatsu et al., 2009]. In this study, relapse rates were also decreased from 36% to 13.3% with increasing dose exposure of ribavirin among patients with end-of-treatment response. These results

confirm that maintaining a sufficient dose of ribavirin during the full treatment period could reduce the possibility of relapse, and that an extended duration of therapy for patients with late virological response could increase the chance of achieving sustained virological response, regardless of core aa substitution patterns [Berg et al., 2006; Pearlman et al., 2007; Ferenci et al., 2010].

In this study, the COBAS Amplicor HCV Test v2.0, with a lower limit of detection of 50 IU/ml, was used to assess the serum HCV RNA. Recently, real-time PCR-based HCV RNA assays with a higher sensitivity, COBAS TaqMan HCV assay (Chugai-Roche Diagnostics), with a lower limit of detection of 15 IU/ml, have been introduced. Sarrazin et al. [2010] compared virological response rates that were originally tested by COBAS Amplicor assay with those retested by COBAS TaqMan assay, using the same cohort. Among genotype 1 patients, complete early virological response and sustained virological response rates were similar when virological responses were defined as <50 IU/ml by Amplicor assay (77% and 87%) and <15 IU/ml by TaqMan assay (76% and 88%). Therefore, measuring HCV RNA by the Amplicor assay in this study would have little affects on the results.

In conclusion, the results have demonstrated that substitution of core aa 70 could be independently associated with an insufficient decline in HCV RNA level during first 12 weeks, and substitution of core aa 91 was independently associated with detectable HCV RNA at week 24, all of which were considered to be important negative predictors of attaining sustained virological response in patients with HCV genotype 1 treated with Peg-IFN plus ribavirin. On the other hand, only dose exposure of ribavirin and no complete early virological response was independent predictors of virological relapse among patients with end-of-treatment response, not substitution of core aa 70 or 91. The aa substitution patterns of the HCV core protein can be an important pretreatment predictor for non-response in patients with HCV genotype 1 treated with Peg-IFN plus ribavirin, but not for relapse after the completion of therapy.

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診断と治療のTopics

C型肝炎

Hepatitis C among HIV-infected patients

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Summary

いまや，HIV感染者の死亡原因の第２位が肝疾患関連死である。HIV感染はC型慢性肝疾患の進展を加速させ，逆にHCV感染は抗HIV療法(ART)での肝障害の頻度・程度を悪化させる可能性があるなどHIV感染の病態を修飾する。HCVに対するワクチンがない現状では，まず感染防止，感染した場合は慢性化の阻止，慢性肝炎の場合は早期の治療介入などに重点を置いた対応が求められる。

Key words

- HCV
- インターフェロン治療
- リバビリン
- telaprevir

疫学

C型肝炎ウイルス(hepatitis C virus；HCV)は全世界で約１億3,000万人が感染し，400～500万人がHIVと重複感染している[1]。また，アメリカではHIV感染者の約30％がHCV陽性と報告されている[2)3)]。一方，日本では2004年の厚生労働省の研究班による全国調査でHIV感染者の19.2％がHCV抗体陽性で，このうち約80％がHCV-RNA陽性と報告されている[4]。日本では，HIV・HCV重複感染者の多くが血液製剤による感染が原因である。その他では，男性同性愛者間，異性間性交渉，注射による薬物乱用[5]などが感染経路である。

アメリカの大規模研究(n＝23,441)におけるHIV感染患者の死因調査で，AIDS(31.1％)に次いで肝疾患関連死(14.5％)が多いことが報告されている[6]。そして，HCVが重複感染している場合には死亡に至る相対危険度が6.7倍高値であり，C型肝炎をコントロールすることが重要である。

HCVの急性感染

HIV感染が確認されたとき，必ずHCVおよびB型肝炎ウイルス(hepatitis B virus；HBV)感染の有無は確認されているはずである。HIV感染が診断された時点でHCV感染を認めなくても，経過中にAST/ALT上昇を認めた場合はHCVマーカーを再検索するが，HCV抗体だけではなく，必ずHCV-RNAを測定すべきである。AST/ALTが異常値をとった時点でもHCV抗体が陽性化しないケースがあるため，必ずHCV-RNAまで

調べなければならない。これは，HIV感染の有無にかかわらず実施すべき手順である[7]。また，AST/ALTが正常範囲でも感染のリスク行動が継続されている場合，毎年1回HCV抗体を測定することもHCV感染を早期に把握するうえで有用といえる。

HCVは，いったん感染すると70～90%と高率にキャリア化することが知られており，HIV感染者でも同様のことがいえる。そのため，C型急性肝炎と診断された場合，治療介入を検討することになる。日本ではペグインターフェロン(peginterferon；Peg-IFN)単独治療を選択することが多く(保険適応外)，HIV非感染者ではgenotypeにかかわらず90%近いHCV排除率が得られる。しかし，HIV感染者に対し急性期にインターフェロン(interferon；IFN)導入を図った治療成績の報告は少ない。一般的には，HCV初感染が確認されて6ヵ月経過してもHCV血症が持続する場合に，慢性化と判定してIFN治療を行うことが多い。

一方，HIV・HCV感染が同時にみつかった場合，HCV感染時期を特定する情報は問診以外になく，診断に難渋する。ただ，若年者であった場合，C型肝炎の罹病期間は短いと考えられる。HCVの初感染からの期間が短いほどIFN治療効果は高いため，若年者では積極的にIFN治療を行うことを勧める。

C型慢性肝疾患の疫学

Benhamouら[8]は，HIV感染C型慢性肝炎症例と患者背景をマッチさせたHIV非感染C型慢性肝炎症例の肝線維化の進展速度を比較し，HCV単独感染例に比べHIV重複感染例は進展が速いことを報告している。肝線維化進展速度を年率で表現すると，HIV重複感染例では0.153/年であり，計算上HCV初感染から26年で肝硬変になるのに対して，HCV単独感染例では0.106/年であり，計算上HCV初感染から38年で肝硬変になるという(図1)。

またPineda[9]らは，C型肝硬変症例が非代償期に入ってからの生存期間をHIV感染の有無で検討している。HIV感染群がHIV非感染群に比し，若い(中央値 38歳 vs. 66歳)，男性が多い(86% vs. 58%)，HBs抗原陽性者が多い(24% vs. 4%)など背景因子に違いを認めるものの，平均生存期間がHIV感染群で16ヵ月，HIV非感染群で48ヵ月と，HIV感染が肝硬変の終末期においても病状悪化の一因になっていることが示されている。

C型慢性肝炎に対する抗ウイルス療法

以上のことから，HIV感染C型慢性肝炎に対しては，早期にHCV排除を目指した治療介入が望まれる。治療は，HIV感染の有無にかかわらずガイドラインに沿ったものとなる。日本人のHCVキャリアにおけるgenotypeやウイルス量の分布は，genotype 1型(ほとんどが1b型)が約70%で，高ウイルス量(≧5.0 $\log_{10}$IU/mL)が約50%，低ウイルス量(<5.0 $\log_{10}$IU/mL)が約20%という割合である。一方，残り約30%がgenotype 2型で，高ウイルス量と低ウイルス量は半数ずつという内訳である。つまり，genotype 1型・高ウイルス量症例が日本人のHCVキャリアの約半

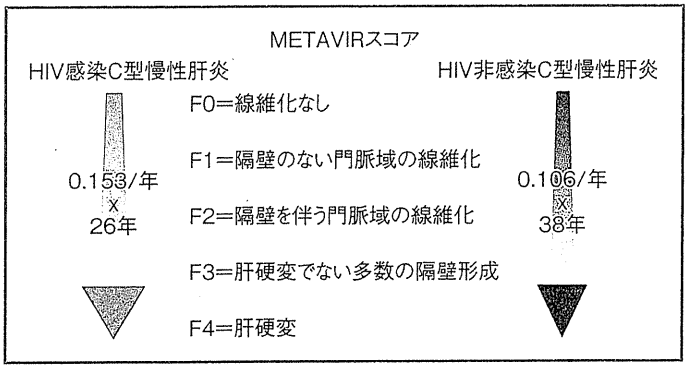

図1．HIV感染の有無とC型慢性肝炎の肝線維化進展速度

数となるが，この集団は現在の標準治療であるPeg-IFN・リバビリン併用療法をもってしても，ウイルス排除率は45～50％に留まる。他方，genotype 1型・低ウイルス量症例およびgenotype 2型症例では，ガイドラインで推奨される治療(表1)を行うことで90％近いウイルス排除率が期待できる。HIVを重複感染しているとウイルス排除率が若干抑えられるが，やはり「genotype 1型かつ高ウイルス量」症例以外であれば，病状の許すかぎり(合併症の有無，本人のモチベーションなどを考慮して)積極的にIFN治療を行うべきと考える。しかし，genotype 1型・高ウイルス量症例に対するPeg-IFN・リバビリン併用療法のウイルス排除率は低値であり[10)-13)](表2)，今後プロテアーゼ阻害薬との3剤併用療法が認可された場合は第一選択となるだろう(表1)。

次に，診療ガイドラインの要点を記載する。まず，初回治療で低ウイルス量症例なら，genotypeにかかわらずIFN単独治療もしくはPeg-IFN単独治療を行う。一方，高ウイルス量症例ならPeg-IFN・リバビリン併用療法を行い，genotype 1型なら48週治療，genotype 2型なら24週治療を行う。Genotype 1型の場合，HCV-RNAの陰性化が12週目以降36週目までなら治療期間を72週に延長する。ただ，現実的には24週目までに陰性化しないとウイルス排除率は低く，24週目の時点で継続治療の必要性を検討するべきである。HIV感染を合併する場合はウイルス排除率が低いため，海外から治療期間の工夫が提言されている[14)](図2)。すなわち，4週目までにHCV-RNAの陰性化が得られなければ，genotype 1型なら72週，genotype 2型なら48週まで治療期間を延長するというものであ

表1．C型慢性肝炎に対する初回治療ガイドライン(2011年)

	genotype 1型	genotype 2型
高ウイルス量 (5.0 log_{10}IU/mL 300fmol/L 1 Meq/mL以上)	・Peg-IFNα-2b＋リバビリン(48～72週間) ・Peg-IFNα-2a＋リバビリン(48～72週間) ※ 精神症状が課題なら ・IFNβ＋リバビリン(48～72週間) ★telaprevir認可後は ・Peg-IFNα-2b＋リバビリン ＋telaprevir(24週間)	・Peg-IFNα-2b＋リバビリン(24週間) ※精神症状が課題なら ・IFNβ＋リバビリン(24週間)
低ウイルス量 (5.0 log_{10}IU/mL 300fmol/L 1 Meq/mL未満)	・IFN(24週間) ・Peg-IFNα-2a(24～48週間)	・IFN(8～24週間) ・Peg-IFNα-2a(24～48週間)

表2．HIV感染C型慢性肝炎に対するPeg-IFN・リバビリン併用療法の治療成績

	APRICOT[10)]	ACTG A5071[11)]	RIBAVIC[12)]	Barcelona[13)]
症例数	868	133	412	95
Peg-IFN	2a	2a	2b	2b
リバビリン	800mg	600～1,000mg	800mg	800～1,200mg
CD4値およびHIV-RNA	「≧200/mm^3」or「100～199/mm^3 でHIV-RNA＜5,000copies/mL」	＞100/mm^3 かつHIV-RNA＜10,000copies/mL	＞200/mm^3	＞250/mm^3 かつHIV-RNA＜10,000copies/mL
ALT	2度は上昇	不問	不問	正常上限の1.5倍以上
genotype 1型の割合	60％	77％	48％	55％
bridging fibrosisを認める慢性肝炎＋肝硬変の割合	12％	11％(肝硬変)	39％	29％
genotype 1型のウイルス排除率	29％	14％	17％	38％

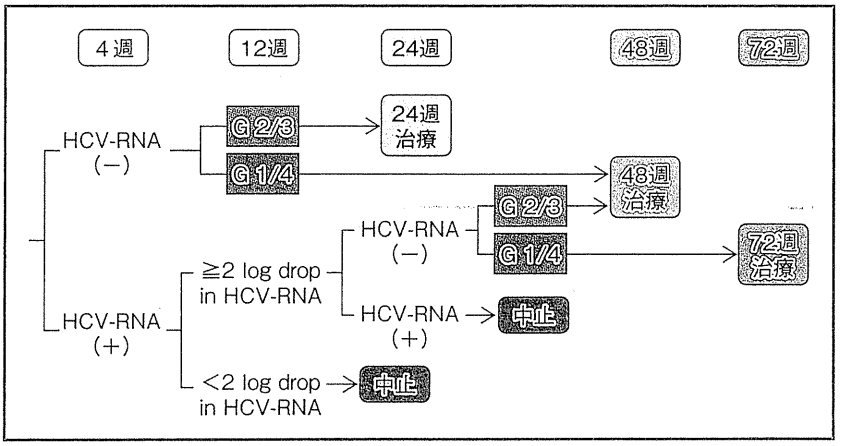

図2．HIV感染C型慢性肝炎に対するIFN治療期間の提言
G：genotype

る。また，血液製剤によってHCV感染を起こした症例も多くgenotype 3型やgenotype 4型症例を経験するが，この提言ではgenotype 3型はgenotype 2型の，genotype 4型はgenotype 1型の治療に準じている。

最近，HCV genotype 1型・高ウイルス量症例に対するPeg-IFN・リバビリン併用療法の反応を規定する因子としてインターロイキン(interleukin；IL)-28Bの一塩基多型(single nucleotide polymorphism；SNP)が報告された[15)16)](表3)。すなわち，IL-28BのSNP(保険適応外)がメジャーホモ接合体なら治療の反応性が良好で(厳密にいうと，経過中にリバビリンを減量することによって治療終了後に再燃するケースはあるのだが)，ヘテロ接合体もしくはマイナーホモ接合体なら治療反応性が不良である(図3)。治療反応性が不良と予測される場合は，プロテアーゼ阻害薬との3剤併用療法を行うことを検討すべきであろう。

また，抗HIV療法(antiretroviral therapy；ART)で使う薬物のなかには，リバビリンとの薬物相互作用で注意を要するものがある[17)]。リバビリンはジダノシン(ddI)の細胞内濃度を増大し，膵炎や乳酸アシドーシスを起こすが，同様のことが他の非核酸系逆転写酵素阻害薬(non-nucleoside reverse transcriptase inhibitor；NNRTI)でも観察される。また，ジドブジン(AZT)はリバビリンと併用すると高度の貧血を起こすことがあり，できれば併用を避ける。一方IFNでは，エファビレンツ(EFV)との併用で精神神経症状の増悪をきたすことがあり，できれば併用を避ける。

表3．Genotype 1型に対するPeg-IFN・リバビリン併用療法の治療効果を規定するIL-28BのSNP

SNP	rs8099917[15)]	rs12979860[16)]
メジャーホモ接合体	TT	CC
ヘテロ接合体	TG	TC
マイナーホモ接合体	GG	TT

日本人の頻度としては，メジャーホモ接合体が約4分の3，残りが4分の1の割合である。
rs8099917は真ん中に9が3つ並ぶため，トリプルナインと報告者は名付けている。一方，rs12979860はDuke大学からの報告なので，デュークスニップと学会などで呼ばれることがある。塩基配列TTは，rs8099917ではメジャーホモ接合体，rs12979860ではマイナーホモ接合体と，正反対の意味になるので注意したい。両者は連鎖不均衡を示し，同じことを示していると考えて差し支えない。

しかし，ART薬剤の進化は著しく，現在これらの薬剤は決して必須ではないため対応は可能である。今後もHCVに対する抗ウイルス薬の開発ラッシュが予定されているが，必ずART薬剤との薬理相互作用は検討されているので，情報は提供されると思われる。

C型肝硬変・肝癌に対する治療

C型肝硬変に関しては，包括的治療ガイドラインが示されている。HIV感染者に対して特別なものはなく，このガイドラインを意識した診療に努める。①治癒目的のIFN治療を行うか，②発癌予防および肝癌再発予防でIFN治療を行うか，③IFN治療を行わず(もしく

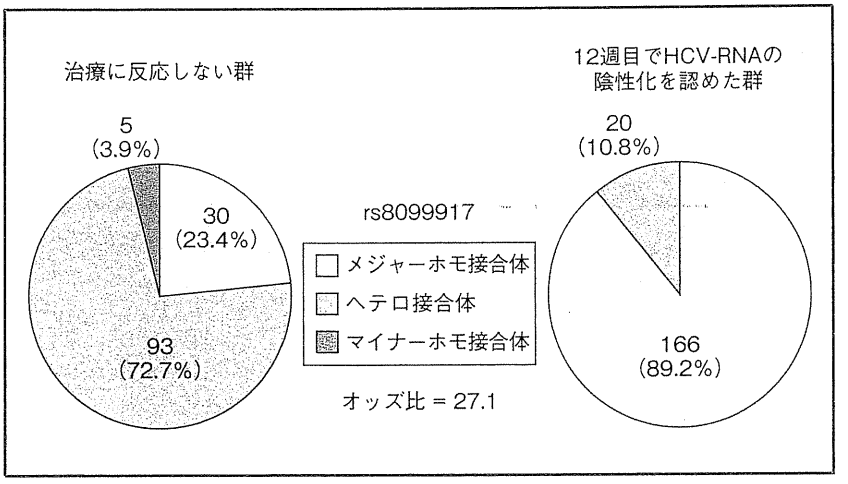

図3．IL-28BのSNP別にみたgenotype 1型に対するPeg-IFN・リバビリン併用療法の治療反応性[15]

は行えず），肝庇護療法を行うかを総合的に判断する。従来，C型肝硬変に対するIFN治療ではリバビリンを併用できなかったが，最近，治癒目的でリバビリンとの併用が保険認可された。C型肝炎のガイドラインは毎年更新されるので，日本肝臓学会のホームページなどを注視していただきたい。IFN治療ができない場合でも根気よく肝庇護療法を行い，肝病変の進展を遅らせることを目指したい。

肝癌も治療のガイドラインが示されている。早期発見には，綿密なサーベイランスが重要で，その必要性を患者に理解してもらうことが基本である。

最後に

HCV・HIV重複感染者の多くが血液製剤を介した感染で，感染期間が長くなるに伴い肝病変が進行している。適切な治療を早急に実施する必要性を感じる。ただ，現在のIFN治療は身体にかかる負担が大きい。経口の抗ウイルス薬でHCV感染を克服できる時代が早く到来することを期待したい。

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