larger than that in the monotherapy group ($P = 0.035$), when samples below the detection limit of the assay were assigned the viral load of the detection limit and one patient without detectable HCV RNA at day 14 was excluded from analysis.

In patients given both drugs, correlation was not significant between the first phase and early second phase decreases ($r = -0.084$, $P = 0.73$), between the early second phase and late second phase decreases ($r = -0.12$, $P = 0.63$), nor between the late second phase and first phase decreases ($r = 0.29$, $P = 0.23$). In patients given IFN-α2b only, correlation was not significant in any of these comparisons ($r = -0.27$, $P = 0.54$; $r = 0.36$, $P = 0.39$; $r = -0.29$, $P = 0.51$, respectively).

DISCUSSION

Earlier [25], we compared the analytical sensitivity and validity of TaqMan PCR for measurement of HCV RNA with those of other widely used quantitative methods. The sensitivity of TaqMan PCR was the highest, and the method gave accurate results throughout the wide detection range. In this study, the baseline HCV level of all patients was within the detection range of TaqMan PCR, but was more than the upper detection limit (500 kIU/mL) of the Amplicor Monitor test in 18 of the 30 patients. The proportion of patients without detectable HCV RNA during therapy was smaller with TaqMan PCR than with the Amplicor Monitor test. TaqMan PCR was more suitable for the monitoring of changes in HCV RNA levels during antiviral therapy, because of its wide detection range.

We found that the second phase decrease in HCV monitored by TaqMan PCR is correlated with the sustained virological response to IFN-α monotherapy [12]. Neumann *et al.* [9] suggested that the rapid viral decrease in the first phase reflects the dose-dependent effects of IFN on HCV production, and that the slower decrease in the second phase arises from the death of hepatocytes infected with HCV. The rate of HCV-infected cell death may depend on cellular immunity involving cytotoxic T-lymphocytes. Therefore, the results of our previous study may mean that a strong cellular immune response is needed for sustained loss of HCV by treatment with IFN alone.

For two reasons, we divided the second phase into the early second phase (first 2 weeks) and the late second phase (after the first 2 weeks) and evaluated changes in HCV RNA. First, our protocol of IFN administration, which is common in this country, consisted of daily induction therapy for the first 2 weeks and then thrice weekly maintenance therapy. As expected, in both groups, viral decrease in the early second phase (still induction therapy) was larger than that in the late second phase (maintenance therapy). Secondly, it takes several weeks for serum ribavirin concentrations to reach a plateau [19,20]. The exact mechanism of action of ribavirin when combined with IFN is not known [26]. Ribavirin may inhibit HCV RNA-dependent RNA polymerase, the capping structure of viral messenger RNA, and inosine monophosphate dehydrogenase. Other immunomodulatory actions also may contribute to the effects of this drug [27,28]. No matter which action is most important, these pharmacokinetic results suggested that the synergistic effects of ribavirin given with IFN might appear slowly after the earliest phase of treatment. The most striking finding in our study was that in the late second phase, the rate of decrease in HCV in the combination therapy group was larger than that in the monotherapy group. Because the observation period in this study was 12 weeks, we do not know which phase(s) of viral decrease is associated with sustained virological response to combination therapy. However, faster viral decline in the late second phase would contribute to the long-term outcome of treatment.

Changes in serum HCV RNA during the first few weeks of IFN monotherapy are useful in prediction of the outcome [29,30]. Reliable prediction of the response to therapy early would be useful, because IFN is expensive and sometimes has serious side-effects. In this study, however, the viral decrease in the first 2 weeks was similar in the two groups, and was not correlated with the viral decrease afterwards. Perhaps viral decline in the first few days of treatment cannot be used for prediction of the long-term response to combination therapy with IFN-α and ribavirin.

The attachment of a polyethyleneglycol (PEG) moiety to IFN-α produces a biologically active molecule, PEG-IFN-α, with a long half-life and favourable pharmacokinetics. Randomized controlled trials have shown that weekly treatment with PEG-IFN-α plus ribavirin is well tolerated, and gives higher rates of sustained virological response than treatment with unmodified IFN-α plus ribavirin [31,32]. Changes in HCV levels during combination therapy with PEG-IFN-α and ribavirin should be further evaluated in large clinical studies.

In summary, in a group of patients with HCV genotype 1 and high viral loads treated with IFN-α2b plus ribavirin and such a group treated with IFN-α2b alone, differences in the rate of decrease in viral RNA in the first phase, during the first 24 h of therapy, and in the early second phase, between days 1 and 14, were not significant. Later in the second phase, between days 14 and 84, the rate of decrease in the combination therapy group was greater than that in the monotherapy group. Our results suggest that a long-term favourable response to therapy with IFN-α2b and ribavirin for chronic hepatitis C is associated with rapid viral disappearance in this later phase.

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Adefovir Dipivoxil to Prevent Exacerbation of Lamivudine-resistant Hepatitis B Infection during Chemotherapy for Non-Hodgkin's Lymphoma

TO THE EDITOR: Lamivudine is a potent inhibitor of hepatitis B virus (HBV) replication and is widely used for the treatment of chronic hepatitis B (1). In HBV carriers receiving cytotoxic or immunosuppressive chemotherapy for hematological malignancies, acute exacerbation of hepatitis following viral reactivation is well documented; therefore, prophylactic or therapeutic use of lamivudine is highly recommended (2, 3). However, prolonged treatment may induce the emergence of lamivudine-resistant variants with mutations in the reverse transcriptase (rt) domain of the HBV polymerase gene. There is currently no consensus on the management of exacerbations of chronic HBV infection in patients with lamivudine-resistant variants during chemotherapy. Adefovir dipivoxil, another nucleotide analogue, has recently demonstrated potency against HBV, including lamivudine-resistant strains (4–6). We hereby report a patient with lamivudine-resistant HBV who was treated with adefovir dipivoxil during chemotherapy for non-Hodgkin's lymphoma.

A 44-year-old woman with malaise and generalized lymphadenopathy was admitted to our hospital in December 2002. About 5 years previously, chronic hepatitis B had been diagnosed. Her mother and all 4 siblings were also carriers of HBV. Treatment with lamivudine was started at a daily dose of 100 mg in June 2001. The subsequent clinical course is shown in Figure 1. Shortly after the start of therapy, the serum HBV DNA level declined, and transaminase activity normalized by week 20. At week 40 of therapy, viral DNA became undetectable by transcription-mediated amplification assay ($<3.7 \log_{10}$ copies/ml). At week 64, however, lamivudine-resistant HBV variants emerged, and viral DNA became detectable again in serum. On admission (at week 70), alanine aminotransferase (ALT) activity was 21 IU/L, aspartate aminotransferase (AST) activity 45 IU/L, lactate dehydrogenase activity 872 IU/L, and γ-glutamyltransferase activity 29 IU/L. The hepatitis B e (HBe) antigen was positive

and the anti-HBe negative, with an HBV DNA level of 6.7 $\log_{10}$ copies/ml. The genotype of the HBV was type C. The stop codon mutation at nucleotide (nt) 1896 in the precore region of HBV DNA was not found, but mutations were found at nt 1762 and nt 1764 in the basal core promoter. Mutations related to lamivudine resistance were detected from leucine to methionine at amino acid rt180 and from methionine to valine at rt204.

On the basis of the findings of lymph node biopsy, ultrasonography, computed tomography, and gallium scintigraphy, diffuse large-B-cell non-Hodgkin's lymphoma (stage III) was diagnosed. Five courses of chemotherapy with adriamycin, cyclophosphamide, and vincristine were administered between December 2002 and April 2003. After three courses of chemotherapy, lymph nodes regressed considerably in size. However, lymphoma recurred after the fifth course; involvement of the liver and spleen were found on gallium scintigraphy. The patient did not respond to subsequent intensive chemotherapy with a combination of cytarabine and carboplatin in May 2003, two courses of rituximab plus etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin between June and July 2003, or two courses of fludarabine, mitoxantrone, and dexamethasone in August 2003. On September 25, the patient died suddenly of rupture of splenic lesions.

At the start of antineoplastic chemotherapy, the ALT activity was 58 IU/L, and the AST activity 71 IU/L with an HBV DNA level of 8.3 $\log_{10}$ copies/ml. To prevent chemotherapy-induced exacerbation of lamivudine-resistant HBV infection, treatment with adefovir dipivoxil at 10 mg daily was added to the lamivudine. The serum HBV DNA level fell immediately after the commencement of adefovir dipivoxil and became undetectable at week 20 of therapy. HBe antigen did not seroconvert to anti-HBe. ALT activity was 170 IU/L at week 8 of therapy, decreased thereafter, and normalized at week 16 of therapy. During 36 weeks of therapy with adefovir dipivoxil, suppression of HBV replication was sustained, allowing chemotherapy to proceed without delay or modification, albeit the response to chemotherapy was not favorable. There were no

clinically significant adverse events related to adefovir dipivoxil, such as nephrotoxicity, which has been reported with higher doses (≥30 mg daily).

Ohmoto *et al.* described a case of lamivudine-resistant HBV reactivation that was successfully treated with a combination of lamivudine and interferon-α during chemotherapy for non-Hodgkin's lymphoma (7). However, randomized controlled trials have not shown long-term beneficial effects of this combination (8, 9). Interferon-α sometimes causes serious adverse effects, including hemopoietic toxicity. Adefovir dipivoxil is generally well tolerated. Resistance to adefovir dipivoxil was not found in large, placebo-controlled 48-week studies (5, 6), although a novel mutation associated with resistance has more recently been identified in the HBV polymerase gene (10).

There remain two unsolved issues related to the treatment schedule. First, should adefovir dipivoxil be initiated as prophylaxis in patients with lamivudine-resistant HBV at the start of chemotherapy? Second, how long should lamivudine be continued after the initiation of adefovir dipivoxil? Delayed treatment due to acute exacerbation of HBV may preclude the subsequent completion of chemotherapy protocols. Withdrawal of lamivudine can cause breakthrough of wild-type HBV strains. We believe that prophylactic use of adefovir dipivoxil with continued use of lamivudine (at least for the first few weeks) is indicated in the management of hematological malignancies by chemotherapy.

In conclusion, adefovir dipivoxil should be considered to prevent viral reactivation in patients who carry lamivudine-resistant HBV variants during intensive chemotherapy. Further studies are needed to establish optimal treatment regimens.

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LEGEND

Figure 1. ALT (●) and HBV DNA (▲) levels during the clinical course of the patient from the time of initiation of lamivudine treatment. CHO, adriamycin, cyclophosphamide, and vincristine; AraC, cytarabine; CBDCA, carboplatin; EPOCH-R, rituximab plus etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin; FND, fludarabine, mitoxantrone, and dexamethasone. Broken line shows the detection limit of the transcription-mediated amplification assay (3.7 $\log_{10}$ copies/ml of HBV DNA).

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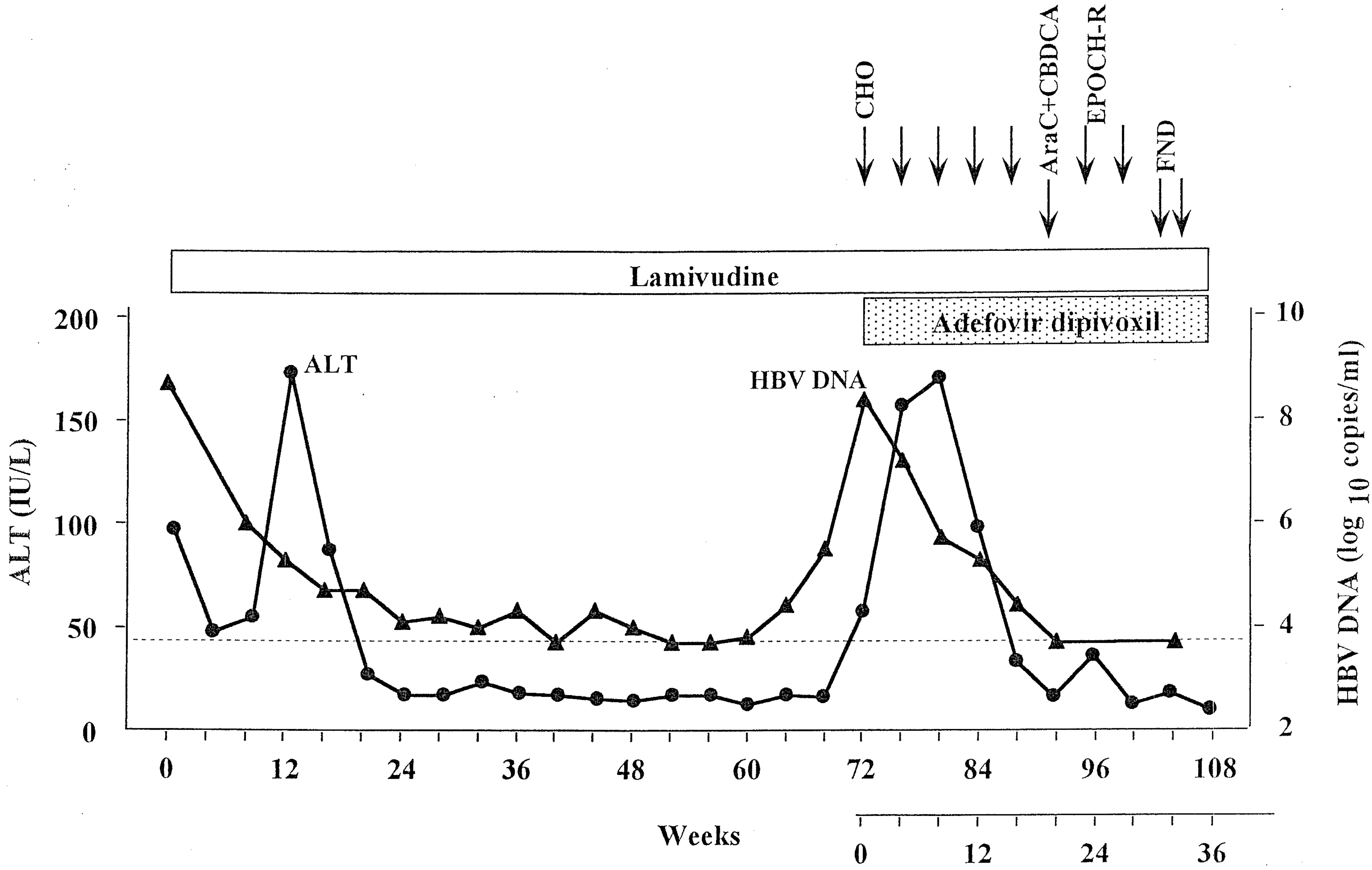

CHO
AraC+CBDCA
EPOCH-R
FND
Lamivudine
Adefovir dipivoxil
ALT
HBV DNA
ALT (IU/L)
200
150
100
50
0
HBV DNA (log 10 copies/ml)
10
8
6
4
2
0
12
24
36
48
60
72
84
96
108
Weeks
0
12
24
36

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A randomized controlled trial of consensus interferon with or without lactoferrin for chronic hepatitis C patients with genotype 1b and high viral load

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Abstract

Recently, lactoferrin has been reported to have anti-HCV effects. The aim of this study was to investigate the effect of combination therapy using consensus interferon (CIFN) and lactoferrin in patients with chronic hepatitis C. Twenty-one patients with chronic HCV infection, who were positive for HCV-RNA genotype 1b with serum viral loads from 100 to 700 KIU/ml, were randomly assigned to two groups; the CIFN + Lac group received CIFN with lactoferrin and the CIFN group received CIFN alone. Nine patients in each group completed this trial; the other patients dropped out because of side effects. Three, two and four patients were categorized as complete responders, relapsers and non-responders, respectively, in the CIFN + Lac group, and four, one and four in the CIFN group, respectively. There was no statistically significant difference in virologic response between the two groups. During the follow up after CIFN therapy with continued lactoferrin, there were two relapsers in the CIFN + Lac group and their HCV-RNA titers before treatment were over 400 KIU/ml. In conclusion, the combination therapy of CIFN and lactoferrin did not increase the response rate or prevent relapse after discontinuation of IFN.
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Keywords: Chronic hepatitis C; Consensus interferon; Lactoferrin

1. Introduction

Interferon-α (IFN) treatment has been shown to be effective in a proportion of patients with chronic hepatitis C virus (HCV) infection. However, the overall rate of HCV eradication is at most 30–40% of patients treated with IFN [1–3], and patients without HCV eradication are at risk for liver cirrhosis and hepatocellular carcinoma [4,5]. Consensus interferon (CIFN), a recombinant type-1 interferon, has been shown to be more effective in the treatment of HCV patients than other IFNs [6,7].

Bovine lactoferrin, a milk protein in the iron transporter family, has been reported to effectively prevent HCV infection in cultured human hepatocytes [8–10]. Lactoferrin is a potential candidate as an anti-HCV reagent that may be effective for the treatment of patients with chronic hepatitis. Therefore, the aims of this study were to evaluate the efficacy of CIFN combination therapy with lactoferrin in patients with chronic hepatitis C who had never undergone IFN therapy and to investigate whether this combination therapy prevents relapse after the discontinuation of treatment.

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2. Patients and methods

2.1. Patients

Patients with chronic HCV infection who had never received IFN therapy were eligible for this study. The patients were continuously positive for HCV-RNA genotype 1b with serum titers from 100 to 700 KIU/ml using the amplicor HCV monitor assay and were diagnosed with chronic active hepatitis from a liver biopsy. Pathological diagnosis was made using the Inuyama criteria renewed in 1996 [11]. Patients with decompensated liver cirrhosis were not enrolled in this trial.

2.2. Study design

This study was a randomized controlled trial. Twenty-one patients gave written informed consent and were then randomly assigned to two groups: the CIFN + Lac group received CIFN with lactoferrin, and the CIFN group received CIFN alone. All patients were given CIFN subcutaneously every day for 14 days and then three times a week for 24 weeks at a dose of 18 mega units (MU). The 10 patients in the CIFN + Lac group were given 3 g lactoferrin orally three times a day with CIFN for 26 weeks, and lactoferrin administration was continued for 24 weeks after the discontinuation of CIFN, for a total of 50 weeks. The 11 patients in the CIFN group were given CIFN according to the protocol for 26 weeks. Serum HCV-RNA was measured before treatment and every month for one year during and after CIFN therapy. Based on the virologic response, patients were classified into three groups: complete responders, who had undetectable serum HCV-RNA at the end of treatment and sixth months after IFN therapy; relapsers, who had undetectable HCV-RNA at the end of treatment but HCV-RNA relapse during follow up; and non-responders, who had detectable HCV-RNA during and after treatment.

2.3. Statistical analyses

The group means of age, ALT level, HCV RNA titer, leucocyte count and platelet count were evaluated by Fisher's exact test. Proportions were also compared between the groups by Fisher's exact test. Differences with a P value <0.05 were considered significant.

3. Results

Table 1 shows the biochemical, virologic and histological features of the patients who were randomly assigned to the CIFN + Lac group or the CIFN group. One patient in each group needed a CIFN dose reduction from 18 to 12 MU because of malaise. One patient in the CIFN + Lac group and two in the CIFN group discontinued the therapy because of severe general malaise. Leucocyte, neutrophil and platelet counts decreased in both groups during CIFN administration but no patients discontinued treatment. No differences were seen in any features between the two groups.

There were three, two and four complete responders, relapsers and non-responders, respectively, in the CIFN + Lac group and four, one and four in the CIFN group, respectively. There was no significant difference in virologic response between the two groups (Table 1). The ALT levels during CIFN administration increased even in the complete responders, but decreased after completion of the therapy. The HCV-RNA titer before treatment in the complete responders was below 400 KIU/ml in both groups. Even in the CIFN + Lac group, virologic relapse could not be prevented after discontinuation of CIFN (Fig. 1).

Table 1
Characteristics of the patients

Characteristics	CIFN + Lac	CIFN	P
Number of entry	10	11	
Sex (male:female)	7:3	8:3	NS
Age[a] (years)	56.1 ± 11.3	56.0 ± 12.8	NS
Histological findings (staging)			
F1	2	2	
F2	2	3	NS
F3	5	5	
F4	1	1	
Pretreatment ALT[a] (IU/l)	78 ± 47	71 ± 36	NS
Pretreatment HCV RNA titer[a] (KIU/ml)	357 ± 190	326 ± 160	NS
Pretreatment leucocyte counts[a] ($\times 10^3$ mm^{-3})	4.5 ± 0.8	4.7 ± 0.9	NS
Pretreatment platelet counts[a] ($\times 10^3$ mm^{-3})	14.0 ± 5.9	15.6 ± 6.4	NS
Discontinuation for malaise	1	2	
CIFN dose reduction (18 MU → 12 MU)	1	1	
Patients who completed this trial	9	9	
Virologic response			
100 < pretreatment HCV-RNA < 400	CR = 3, Rel = 1, NR = 0	CR = 4, Rel = 0, NR = 1	NS
400 ≤ pretreatment HCV-RNA < 700	CR = 0, Rel = 1, NR = 4	CR = 0, Rel = 1, NR = 3	

[a] Mean ± S.D.; NS: not significant; CR: complete response, Rel: relapse, NR: no response.

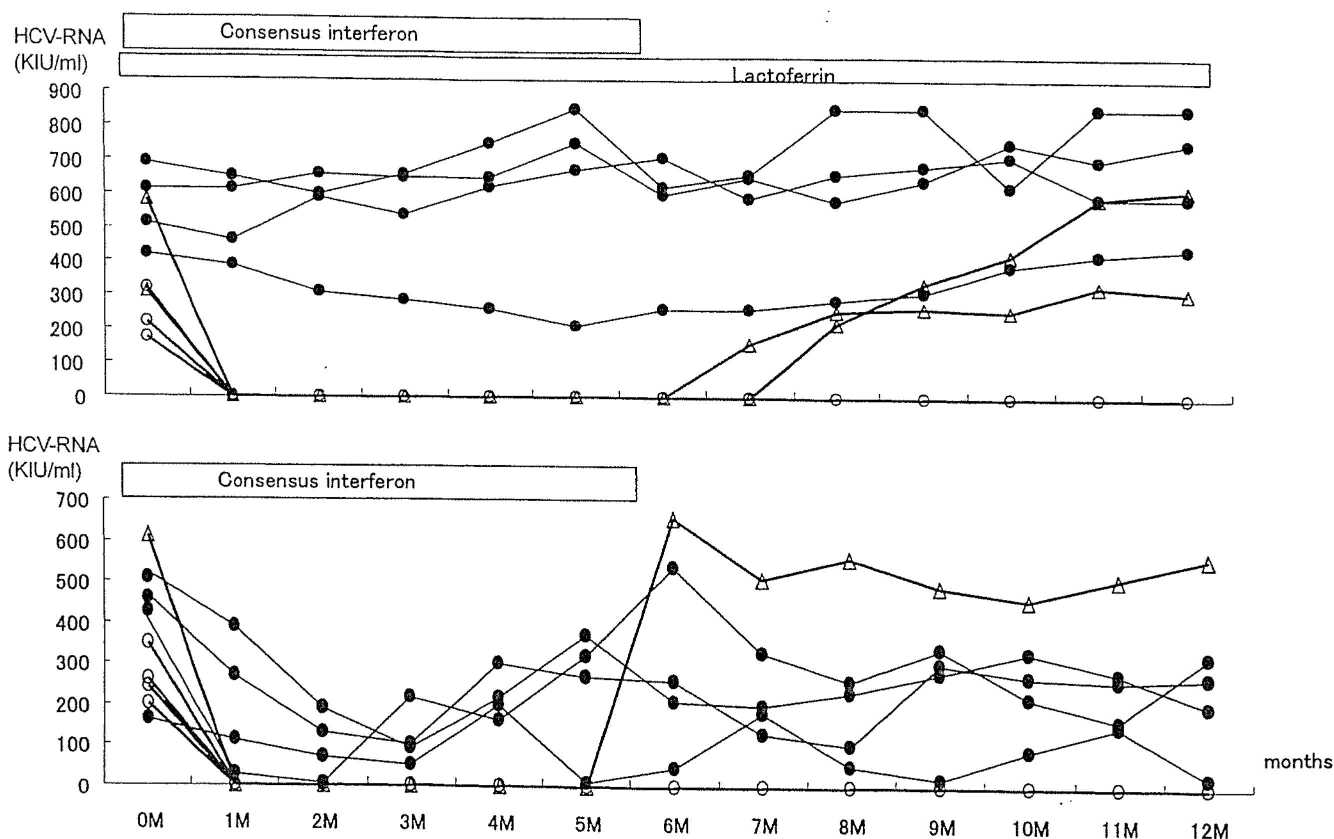

Fig. 1. Serum HCV-RNA levels during and after therapy in the CIFN + Lac group and the CIFN group. There was no significant difference in HCV-RNA levels during and after therapy in either group. In addition, relapsers were also observed in both groups. The open circle, open triangle and closed circle indicate complete virologic responders, relapsers and non-responders, respectively.

4. Discussion

The sustained complete virologic response rate to IFN therapy for 6 months in patients with chronic hepatitis C genotype 1b and high HCV-RNA titers is reported to be very poor, less than 10%. By extending the duration of IFN treatment up to one year, a significant increase of the response rate to about 25% was observed [12,13]. The suppression of HCV replication with longer IFN administration is considered to lead to better virologic response. One of the ways to prevent relapse after the discontinuation of IFN and increase the response rate would, to some extent, depend on the duration or combination of IFN treatment.

CIFN is a genetically engineered molecule derived from commonly observed amino acids of several natural IFN subtypes to develop a novel type I IFN [6,7,14]. CIFN has been shown to be effective in naive patients, in patients who have either not responded to previous IFN therapy or relapsed after discontinuation of IFN therapy [6,7,15]. It was reported that the virologic response rate was higher than IFN-α 2b in the treatment of naive patients infected with HCV genotype 1b (24% versus 15%). However, the patients infected with HCV genotype 1b in these reports had low viral loads. The real response rate to CIFN in patients infected with HCV genotype 1b in high titer is therefore not known.

Lactoferrin, which is an 80 kDa, iron-binding glycoprotein, has several biological activities, including anti-viral activity. Lactoferrin has been reported to inhibit HCV infection in cultured human hepatocytes [9]and to inhibit HCV viremia in low pretreatment HCV-RNA titers of patients with chronic hepatitis C [8]. We hypothesized that CIFN and lactoferrin in combination may yield higher response rates during the first 26 weeks of use and that 24 weeks of continued lactoferrin use after the discontinuation of CIFN may prevent virologic relapse. In this study, however, no significant difference in virologic response was observed between the two groups. The HCV-RNA titers before the treatment of responders in both groups were below 400 KIU/ml. Two relapsers were found in the CIFN + Lac group and their titers of HCV-RNA before treatment was over 400 KIU/ml. Discontinuation of therapy for adverse events did not occur in both groups with and without lactoferrin.

In conclusion, CIFN and lactoferrin in combination did not improve the response rate or prevent relapse after discontinuation of CIFN. High viral loads of over 400 KIU/ml may indicate a low probability of complete virologic response even with the combined treatment of CIFN and lactoferrin.

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HEPATOLOGY

Lack of association between occult hepatitis B virus DNA viral load and aminotransferase levels in patients with hepatitis C virus-related chronic liver disease

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Abstract

Background and Aim: Occult hepatitis B virus (HBV) infection in hepatitis C virus (HCV)-infected patients might enhance the severity of chronic liver disease (CLD). To elucidate the correlation between occult HBV infection and the clinical course of HCV-related CLD, we evaluated whether the fluctuation of occult HBV-DNA directly affects the serum alanine aminotransferase (ALT) level.

Methods: Forty-one patients with HCV-related CLD who received regular outpatient treatment and 42 age-, sex-, and antibody to hepatitis B core antigen positivity-matched healthy volunteers were enrolled. Serum HBV-DNA was quantitatively detected using real-time detection polymerase chain reaction (RTD–PCR). Serial serum samples in three patients were measured for HBV-DNA, ALT and HCV core antigen.

Results: Hepatitis B virus DNA was amplified in eight of the HCV-related CLD patients (19.5%), which was significantly higher than that of healthy volunteers (2.4%). No significant difference between the genotype 1 HCV-related CLD group and the genotype 2 group was found. Based on the analyses using serial serum samples, the elevation of HBV-DNA did not occur before the ALT flares, but occurred at the same time or after the ALT flares.

Conclusions: The prevalence of occult HBV infection of HCV-related CLD is significantly higher than that of control. Occult HBV infection has no influence on ALT flares among patients with HCV-related CLD.

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Key words: aminotransferase, hepatitis C virus, occult hepatitis B virus infection, real-time detection polymerase chain reaction.

INTRODUCTION

Hepatitis B virus (HBV) infection is usually diagnosed when circulating hepatitis B surface antigen (HBsAg) is identified, and the disappearance of this antigen indicates the clearance of HBV. However, HBV-DNA has been identified in the sera and liver tissues of HBsAg-negative patients with chronic liver disease (CLD)[1] who recovered from self-limited acute hepatitis B,[2] resolved chronic HBV infection[3] or chronic hepatitis C.[4–6] HBV-DNA has also been reported in HBsAg-negative volunteer blood donors[7] and healthy individuals.[8] In particular, the prevalence of HBV-DNA detection in patients with chronic HCV infection is high.[4–6,9–12] The HBV-DNA persistence in HBsAg-negative subjects is defined as occult HBV infection.[12]

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The clinical impacts of occult HBV infection have been reported, namely, the transmission of HBV from HBsAg-negative donors to recipients by liver graft occurs.[13,14] HBV reactivation in patients without HBsAg occurred by immunosuppressive treatment.[15] Although there has been a report that occult HBV infection in anti-HCV-positive chronic hepatitis enhances the severity of the liver disease,[4] the significance of occult HBV infection has still not been clearly elucidated.

In chronic hepatitis C, HCV RNA loads are not correlated to alanine aminotransferase (ALT) fluctuation.[16] In HCV-related CLD patients with occult HBV infection, fluctuation of HBV-DNA might directly affect the ALT level, and such an accumulation might increase the severity of liver disease in occult HBV-infected patients.

To elucidate the correlation between occult HBV infection and the clinical course of HCV-related CLD, we evaluated whether the fluctuation of occult HBV-DNA directly affects the serum ALT levels. First we analyzed the prevalence of occult HBV infection in patients with HCV-related CLD compared with healthy volunteers in a matched cross-sectional study, and then evaluated whether fluctuation of occult HBV-DNA directly affects the clinical course of HCV-related CLD by using serial serum samples.

METHODS

Patients

Forty-one patients with HCV-related CLD who visited Nagoya City University Hospital from August 2001 to September 2001 were consecutively selected. All were positive for antibody to HCV (anti-HCV), but negative for HBsAg. All patients had undergone diagnostic liver biopsy, apart from six patients with clinically evident cirrhosis. For HCV genotypes, 28 (68.3%) were genotype 1b, 11 (26.8%) were genotype 2, and two were not detectable. The background of the 41 patients is listed in Table 1.

For controls, 230 healthy volunteers were enrolled from a local medical examination center in Nagoya, Japan. HBsAg was found in one. Anti-HCV was found in one. Antibody to hepatitis B core antigen (anti-HBc) was found in 52 (22.6%). Forty-two subjects matched by age, sex, and anti-HBc-positivity to the 41 CLD patients were randomly selected for the control study. All patients and volunteers provided written informed consent.

Serological testing

Anti-HCV was determined using a commercially available second-generation enzyme immunoassay (EIA; EIA II, Ortho, Raritan, NJ, USA). Reverse transcription–polymerase chain reaction (RT–PCR) for the detection of the HCV-genome was carried out using a qualitative commercial RT–PCR assay (AMPLICOR HCV test; Nippon Roche, Tokyo, Japan). HCV core antigen was measured in serum using an EIA method (Ortho Diagnostics, Tokyo, Japan). Serological markers of HBV infection such as HBsAg, antibody to hepatitis B core antigen (anti-HBc), and antibody to HBsAg (anti-HBs) were determined by radioimmunoassay (RIA) using commercially available kits (Dinabot, Tokyo, Japan). The serum was considered anti-HBc positive when the percentage inhibition in the assay was

Table 1 Background of 41 patients with hepatitis C virus (HCV)-related chronic liver disease

Characteristics	
Sex (male : female)	19:22
Age (years)	60.0 ± 8.5†
Disease (CH:LC)	34:7
HCV genotype (1:2)‡	28:11
HCV-RNA level (high : low)§	33:8
ALT (IU/L)	61.4 ± 43.5†
Positive for anti-HBc	18 (43.9%)

†Values are means ± SD. ‡HCV genotype was not detected in two. §HCV RNA level: high indicates an HCV RNA level of ≥100 KIU/mL; low indicates an HCV RNA level of <100 KIU/mL. ALT, alanine aminotransferase; anti-HBc, antibody to hepatitis B core antigen; CH, chronic hepatitis; LC, liver cirrhosis.

Table 2 Prevalence of occult hepatitis B virus (HBV)-DNA in hepatitis C virus (HCV)-related chronic liver disease (CLD) patients and sex-, age-, and antibody to hepatitis B core antigen (anti-HBc)-positivity-matched healthy volunteers

Characteristics	HCV-related CLD patients (n = 41)	Healthy volunteers (n = 42)	P-value
Sex (male : female)	21:20	20:22	NS
Age (years)†	60.0 ± 8.5	60.0 ± 15.1	NS
Positive for HBsAg	0	0	NS
Positive for anti-HBc (%)	18 (43.9)	20 (47.6)	NS
ALT (IU/L)†	61.4 ± 43.5	19.3 ± 10.9	<0.0001
Positive for HBV-DNA (%)	8 (19.5)	1 (2.4)	<0.05

†Values are means ± SD. ALT, alanine aminotransferase; HBsAg, hepatitis B surface antigen; NS, not significant.

>30%. Anti-HBc of the volunteers was measured by a PHA kit (recombinant HBcAg, Institute of Immunology, Tokyo, Japan). The HCV genotypes were determined using RTD–PCR using type-specific primers for the core region of the viral genome.[17]

Hepatitis B virus DNA in serum

Serum HBV-DNA was quantitatively detected using real-time detection polymerase chain reaction (RTD–PCR) based on Taqman chemistry as reported previously.[18] In brief, total DNA was extracted from 100 μL of serum using microspin columns (QIAamp Blood kit, Qiagen KK Tokyo, Japan). Purified DNA was resuspended in 50 μL of distilled water and a 25 μL aliquot of DNA solution (50 μL serum equivalent) was used for RTD–PCR. Amplification was carried out using primers corresponding to conserved sequences of the surface region. A portion of the HBV surface region was amplified using primers: forward primer HBSF2 (5′CTTCATCCTGCTGCTATGCCT3′, nucleotide position (nt) 406–426) and reverse primer HBSR2 (5′AAAGCCCAGGATGGGAT3′, nt 646–627), and HBSP2 (5′ATGTTGCCCGTTTGTCCTCTAAT TCCA3′, nt 461–488). In preliminary experiments, the efficacy of RTD–PCR was evaluated by quantitatively measuring the sequential levels of synthetic standard HBV-DNA. The detection limit of this system was as low as five DNA copies/assay, and a linear standard curve was obtained between five and 10^6 DNA copies/assay.

Statistical analysis

Statistical analysis was carried out using Fisher's exact test, χ^2 test, and Student's *t*-test as appropriate. A *P*-value <0.05 was considered significant.

RESULTS

With the RTD–PCR method, HBV-DNA was amplified in eight of the HCV-related CLD patients (19.5%) and one of the 42 healthy volunteers (2.4%). The prevalence of occult HBV infection of HCV-related CLD was significantly higher than that of age-, sex-, and anti-HBc-positivity-matched healthy volunteers. This difference was significant ($P < 0.05$; Table 2). Interestingly, all subjects with HBV-DNA were positive for anti-HBc. The comparison of the genotype 1 HCV-related CLD group and the genotype 2 HCV-related CLD group indicated that no significant difference between the two groups in terms of occult HBV-DNA positivity was found (Table 3).

To investigate whether the fluctuation of occult HBV-DNA directly affected the clinical course of HCV-related CLD, the correlation between ALT, HCV RNA levels, and HBV-DNA levels was examined. Of eight HBV-DNA-positive CLD patients, three patients who had elevated serum ALT levels of more than three-fold the upper normal limit during the clinical course were studied. The characteristics are shown in Table 4.

For patient 1, there were four spikes of ALT elevation; two were post-interferon (post-IFN) relapses and

Table 3 Prevalence of occult hepatitis B virus (HBV)-DNA in genotype 1 and genotype 2 hepatitis C virus (HCV)-related chronic liver disease patients

Characteristics	Genotype 1 (*n* = 28)	Genotype 2 (*n* = 11)	*P*-value
Sex (male : female)	13:15	6:5	NS
Age (years)†	56.9 ± 16.7	62.1 ± 9.46	NS
Disease	22:6	10:1	NS
HCV-RNA level (high : low)‡	25:3	5:6	<0.05
Positive for anti-HBc (%)	11 (39.3)	5 (45.5)	NS
ALT (IU/L)†	66.6 ± 48.2	52.7 ± 32.4	NS
Positive for HBV-DNA (%)	5 (17.9)	2 (18.2)	NS

†Mean ± SD. ‡HCV-RNA level (high : low): high indicates an HCV RNA level of ≥100 KIU/mL; low indicates an HCV RNA level of <100 KIU/mL. ALT, alanine aminotransferase; anti-HBc, antibody to hepatitis B core antigen; NS, not significant.

Table 4 Clinical characteristics of three patients

	Patient no.		
	1	2	3
Sex	M	M	F
Age (years)	23	26	24
Liver disease	CH	CH	CH
Hepatitis C virus genotype	1b	1b	1b
Follow-up period (years)	7.5	7.5	6.5
Blood transfusion	–	–	–
Maximum ALT (IU/L) (means ± SD)	227.0 (87.7 ± 44.1)	270.0 (66.5 ± 50.9)	487.0 (92.0 ± 93.2)

ALT, alanine aminotransferase; CH, chronic hepatitis; F, female; M, male.

the other two were natural elevations. In the post-IFN relapse, the elevation of HCV core antigen was observed before the elevation of ALT. HBV-DNA became positive between the HCV core antigen elevation and ALT elevation. In natural elevations, neither the HCV core antigen elevation nor the HBV-DNA elevation was observed before the ALT elevation. HBV-DNA was positive after the ALT elevation (Fig. 1a). For patient 2, a post-IFN relapse and a natural elevation were observed. In the post-IFN relapse, the elevation of HCV core antigen was observed before the elevation of ALT, and HBV-DNA was positive when the ALT reached the peak. In the natural ALT elevation, HBV-DNA was positive after the ALT elevation (Fig. 1b). For patient 3, post-IFN relapse and a natural elevation were observed. In the natural elevation HBV-DNA was positive after the ALT elevation. The elevation of HBV-DNA did not occur before ALT flares, but occurred at the same time or after the ALT flares. (Fig. 1c).

DISCUSSION

Our RTD–PCR method indicated that HBV-DNA was amplified in eight (19.5%) HCV-related CLD patients and one (2.4%) healthy volunteer in a matched cross-sectional study. This difference was significant ($P < 0.05$). The prevalence of occult HBV infection in HCV-related CLD has been reported to be 21–87%.[4–6,9,11] For the detection of occult HBV infection, most studies applied nested PCR. In this study, an RTD–PCR method was used and the detection limit was 100 copies/mL. The detection limit was minimal, stable and reproducible for the quantitation of HBV-DNA. Because of this detection limit, the HBV-DNA detection rate in this study was slightly lower than the rates in the previous reports.[11]

All the occult HBV-positive patients in our study were positive for anti-HBc, as also found in some previous reports.[8,19] Our data showed that anti-HBc was found in 18/41 (43.9%) HCV-related CLD patients and 52/230 (22.6%) healthy volunteers. Our result was slightly higher than the results of others, who reported 32–38% anti-HBc positivity in HCV-related CLD patients.[4,5,10] There may be regional differences, however, another reason may be the difference in the sensitivity of the assays. For example, using the RIA/EIA method, the percentage inhibition is divided into three categories: true positive, true negative, and in between. In our data, true positive and in between were defined to be positive, as reported by others.[8,20] Lower prevalence data might have defined positive as true positive. The prevalence of anti-HBc in healthy volunteers who attended a periodical medical check up has not been reported, although that of anti-HBc in blood donors has been reported to be 1–4%.[21,22] The different prevalence could depend on the background of the blood donors in other studies and the healthy volunteers in our study; in particular, the mean age of the blood donors was 36 years compared with 60 years for the healthy volunteers. Thus, a comparison of these two groups is not adequate.

No correlation between occult HBV-DNA fluctuation and serum ALT fluctuation in a serial sample study

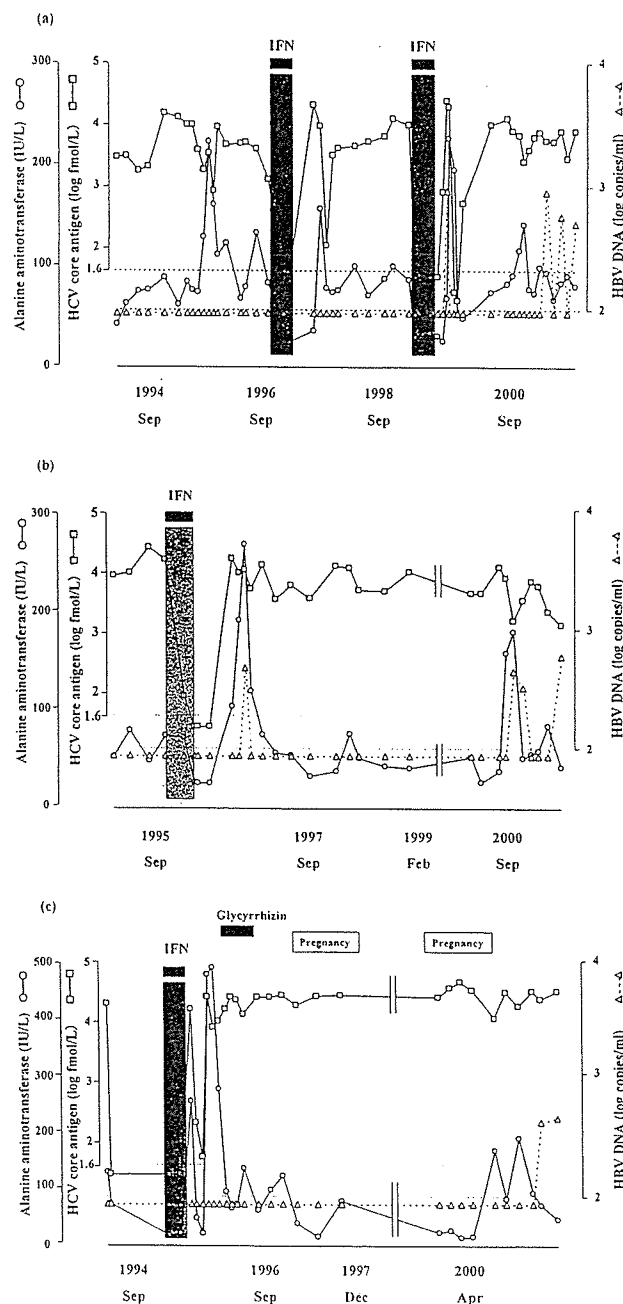

Figure 1 Clinical course of chronic liver disease patients based on hepatitis B virus (HBV)-DNA levels, hepatitis C virus (HCV) core antigen levels, and alanine aminotransferase (ALT) levels. (△) HBV-DNA levels, (□) HCV core antigen levels, (○) ALT levels. The upper and lower dashed lines indicate the lower detection limit of HCV core antigen and HBV-DNA, respectively. (a) Patient 1, (b) patient 2, (c) patient 3. IFN, interferon.

has been reported. Our serial data were similar to the data in other studies,[6,23] in that occult HBV-DNA was not detected continuously. As HBV-DNA appearance after ALT elevation was found in some of these patients, this might reflect the release from the liver. More sensitive quantitative PCR assays might reveal precisely the

manner in which the viral loads change. It has been reported that HCV core protein inhibits HBV-DNA replication in HCV and HBV dual infection.[24,25] The HCV core protein is thought to suppress the activity of HBV enhancer 1 and 2.[25] The HCV core antigen might have some effect on the fluctuation of HBV-DNA. However, no correlation was shown between the occult HBV-DNA fluctuation and serum HCV core antigen levels in this study.

In conclusion, the prevalence of occult HBV infection was significantly higher in HCV-related CLD patients than in age-, sex-, and anti-HBc positivity-matched-healthy volunteers, although the fluctuation of occult HBV-DNA did not directly affect the serum ALT flares. The clinical significance of occult HBV infection in HCV-related CLD is not still clear as yet, and further studies are needed.

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B型及びC型肝炎ウイルスの感染者に対する治療の標準化に関する臨床的研究

平成16年度～平成18年度　総括･分担研究報告書

主任研究者　　熊田　博光

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Ⅲ．平成17年度 研究成果の刊行に関する一覧表

Ⅲ. 研究成果の刊行に関する一覧表

書　籍

著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	ページ
熊田博光	ウイルス性肝炎（E型肝炎及びA型肝炎を除く）	日本医師会感染症危機管理対策室	感染症の診断・治療ガイドライン2004	医学書院	東京	2005	186-189
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