with genotype C infection. Although the number of patients studied was not large enough for statistical evaluation, the transition to chronic infection may be more frequent in infection with genotype A than the other genotypes, insofar as higher viral loads can predict chronic infection [Fong et al., 1994]. Further studies on more patients are required to evaluate whether or not viral persistence occurs more often after HBV infection with genotype A than the other genotypes.

Patients with fulminant hepatic failure in the present study were infected with either genotypes B or C; no patient with genotype A developed hepatic failure. As mutations at nt 1896 in the precore and nt 1762/1764 in the BCP regions, which are found frequently in patients with fulminant hepatic failure [Carman et al., 1991; Kosaka et al., 1991; Liang et al., 1991; Omata et al., 1991; Hawkins et al., 1994; Sato et al., 1995; Baumert et al., 1996; Chu et al., 1996], were not detected in patients with genotype A, low frequency of fulminant hepatic failure associated with genotype A infection may be attributed to the lack of these mutations. The high frequency of HBeAg in genotype A infection may also be related to low frequency of fulminant hepatic failure. However, interpretation on this data should be made carefully, because the number of patients studied was small. Further research is necessary to determine if the genotype itself affects the clinical course of acute hepatitis B.

In summary, (1) infection with HBV genotype A is common in patients with acute hepatitis in Japan; (2) patients with genotype A are more frequent in metropolitan areas and may be associated with particular sexual behavior; (3) patients with genotype A have a milder but longer course of infection, which may lead to increased risk of progression to chronic disease.

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Progressive Disappearance of Anti-Hepatitis B Surface Antigen Antibody and Reverse Seroconversion after Allogeneic Hematopoietic Stem Cell Transplantation in Patients with Previous Hepatitis B Virus Infection

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Reactivation of resolved hepatitis B virus (HBV) infection, which is known as reverse seroconversion (RS), has been reported as a rare complication of allogeneic hematopoietic stem cell transplantation. We retrospectively studied HBV serologic markers in 14 recipients with pretransplant anti-hepatitis B surface antigen antibody (anti-HBs). Progressive decreases in anti-HBs titer were observed in all cases. In 12 cases, anti-HBs titer had decreased to under the protective value. RS occurred in seven cases after disappearance of anti-HBs. Although reseroconversion occurred in five cases, two cases remained in an HBV-carrier status after resolution of hepatitis. In the other five cases, RS did not occur even after disappearance of anti-HBs. The actual risks of anti-HBs disappearance and RS were estimated to be 75.0% and 39.8% at 2 years and 100.0% and 70.0% at 5 years, respectively. In conclusion, RS is a late-onset complication with high frequency that can be predicted by careful monitoring of progressive decrease in anti-HBs titer.

Keywords: Hepatitis B virus, Reverse seroconversion, Reactivation hepatitis.

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Appearance of anti-hepatitis B surface antigen antibody (anti-HBs) and clearance of hepatitis B virus (HBV) from serum usually indicate resolution of hepatitis in patients infected with HBV. However, most patients in whom HBV has been eliminated from the serum still have HBV DNA in the liver that is detectable by using polymerase chain reaction (PCR) (*1*). Reactivation of this dormant HBV in the liver has been observed in an immunocompromised status such as hematopoietic stem cell transplantation (HSCT), renal transplantation, intensive chemotherapy, or use of rituximab (*2–5*). Reactivation of hepatitis in anti–HBs-positive patients is known as reverse seroconversion (RS). There have been several case reports of RS occurring after allogeneic HSCT (allo-HSCT) as a rare complication (*6–12*). However, precise frequency of RS and results of long-term follow-up after RS have not been reported. In some cases, disappearance of anti-HBs was observed several months before RS (*4, 6, 7, 9*). In this study, we investigated the time course of immunologic status against HBV and the incidence of RS in patients with preHSCT anti-HBs.

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PATIENTS AND METHODS

Patients

Fifty-six patients who had undergone allo-HSCT and had been followed for at least 1 year after the transplantation in our institute during the period from February 1990 to March 2003 were enrolled as subjects of this study. Fourteen of the 56 patients were preHSCT anti-HBs positive. Thirteen of the 14 patients were also positive for anti–hepatitis-B core antigen antibody (anti-HBc), and one patient was negative for anti-HBc. Patients' characteristics are shown in Table 1. We retrospectively studied hepatitis B surface antigen (HBsAg) (chemiluminescent immunoassay [CLIA]), anti-HBs (CLIA), hepatitis B e antigen (HBeAg) (radioimmunoassay [RIA]), anti-hepatitis e antigen antibody (anti-HBe) (RIA), and HBV-DNA (PCR) in those 14 patients using cryopreserved serum samples stored at −20°. No patients had a prior history of vaccination or HBV-specific immunoglobulin (Ig) usage. All donors were negative for HBsAg, and seven donors were confirmed to be negative for anti-HBs. Anti-HBs in the other seven donors who donated bone marrow before 1998 were not investigated in our institute or in the Japan Marrow Donor Program because RS was not commonly recognized as a complication of allo-HSCT at that time. Therefore, there was no donor who was confirmed to be anti-HBs positive in this study. The follow-up period varied from 15 to 92 months (median 48 months). Ten grams of Ig was administered intravenously on day 0 and every other week until day 100 for prophylaxis of opportunistic infection. Chronic graft-versus-host disease (cGvHD) was observed in 10 cases, and prednisolone was administered for treatment of cGvHD in 2 of those 10 cases. Only one case (case 1 in Fig. 1A) had relapse of hematologic malignancy during

TABLE 1. Patients' characteristics

Sex	
Male	9
Female	5
Age (years)	22–52 (median: 35)
Follow-up (months)	15–92 (median 47.5)
Diagnosis	
CML	5
ALL	4
MDS	3
SAA	2
Donor	
HLA-identical sibling	7
1-locus-mismatched sibling	1
HLA-identical unrelated	4
1-locus-mismatched unrelated	2
Conditioning regimen	
CY+VP+TBI	4
MCNU+CY+TBI	3
BU+CY	2
ALG+CY+TLI	2
MCNU+CY+TBI+SI	1
CY+TBI	1
CY+TBI+SI	1
GvHD prophylaxis	
MTX+CSA	12
MTX+FK506	2
aGvHD	
Grade 0–I	11
Grade II	2
Grade III	1
cGvHD	Yes 10, No 4

Bu, busulfan; CY, cyclophosphamide; TBI, total body irradiation; TLI, total lymphoid irradiation; SI, splenic irradiation; ALG, antilymphocyte globulin; VP, etoposide; MTX, methotrexate; CsA, cyclosporine A; FK506, tacrolimus; HLA, human leukocyte antigen.

the follow-up period.

Statistical Analysis

Primary endpoint was disappearance of anti-HBs, and secondary endpoint was occurrence of RS. RS was defined as disappearance of anti-HBs and appearance of HBsAg and HBV-DNA in serum with or without clinical hepatitis. The actual risks of endpoints were estimated using the Kaplan-Meier method.

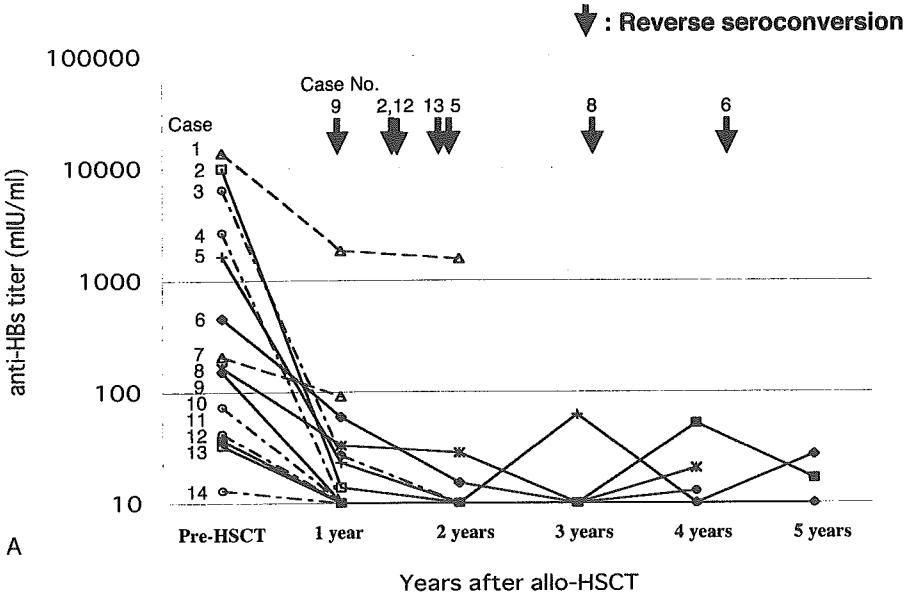
RESULTS

Progressive decreases in anti-HBs titer were observed in all 14 cases (Fig. 1A). In 12 of the 14 cases, anti-HBs titer had decreased to under the protective value (<10.0 mIU/mL) at 10 to 38 (median 13) months after HSCT. RS occurred in seven cases with clinical hepatitis. Four of those seven cases received transplantation from anti-HBs-negative donors, and the donors for the other three cases were not investigated for anti-HBs. Therefore, there was no patient with RS whose donor was confirmed to be anti-HBs positive. RS occurred 12 to 51 (median 20) months after HSCT. Peak value of aspartate aminotransferase during RS hepatitis varied from 196 to 1,460 (median 212) IU/L. One patient, reported previously (*12*), needed hospitalization for treatment of hepatitis. Duration of hepatitis varied from 1 to 9 (median 4) months. Because of transient hepatic injury, RS was not diagnosed at onset in four of the seven cases with clinical hepatitis. Reappearance of anti-HBs and disappearance of HBV-DNA occurred in five cases after adequate therapy (supportive therapy in 4 cases and lamivudine therapy in 1 case; reseroconversion). HBsAg and HBV-DNA were still detected in the remaining two cases after resolution of transient hepatitis (healthy carrier status). RS did not occur in the other five cases even after loss of anti-HBs (sustained seronegative status). In cases with RS, complete disappearance of anti-HBs occurred 0 to 18 (median 1) months before the occurrence of RS. Hepatitis was subsided, and HBsAg disappeared with acquisition of anti-HBe shortly after RS. On the other hand, reappearance of anti-HBs was delayed by 6 to 51 (median 32) months after onset of RS. cGvHD was observed in 10 cases, and 2 of those 10 cases were treated with prednisolone. Although all seven cases with RS had cGvHD, three of the five cases with sustained seronegative status were free from cGvHD. Only one patient (case 1), who had a relapse of hematologic malignancy during the follow-up period, had a higher anti-HBs titer than those in other patients.

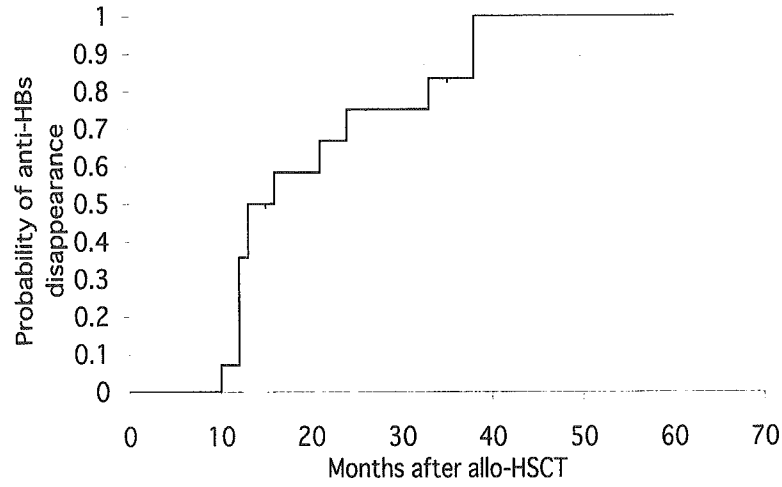
DISCUSSION

Although cases with HBsAg have clearly been shown to be a high-risk group for liver complications, little attention has been paid to cases with anti-HBs when performing HSCT (*13*). Frequency of RS after HSCT was found to be 14% to 50% in studies using a small series of patients or with short-term observation (*14–16*). Our data suggest a higher frequency than those previously reported. In our study, the actual risks of disappearance of anti-HBs and RS were estimated to be 75.0% and 39.8% at 2 years and 100.0% and 70.0% at 5 years, respectively (Fig. 1, B and C). Progressive disappearance of anti-HBs would be an inevitable phenomenon occurring with progressive loss of recipient-type immune cells regardless of pretransplantation anti-HBs titer. RS is not such a rare event in long-term follow-up. One reported risk factor for RS is cGvHD (*14*). It has also been reported that the presence of cGvHD might result in earlier disappearance of recipient-oriented IgG (*17*). In our study, disappearance of anti-HBs occurred regardless of the existence of cGvHD. However, all seven patients with RS had cGvHD. Although the presence of cGvHD may result in earlier onset of RS, no correlation was found between severity of cGvHD and onset of RS in our study.

The onset of hepatitis due to RS after HSCT has been reported to be relatively late (6–52 months; median 19 months) (*6–12, 14, 16*) compared with that in cases with HBsAg in pretransplantation. This is probably caused by prolonged existence of recipient-type memory B-cell immunity. Recipient-derived IgG decreases gradually after HSCT and still remains detectable for 1 to 2 years after allo-HSCT (*17*). RS hepatitis is thought to be a phenomenon caused by naive donor immunity after loss of recipient-oriented immunity against HBV. In many cases, RS hepatitis was transient and



A



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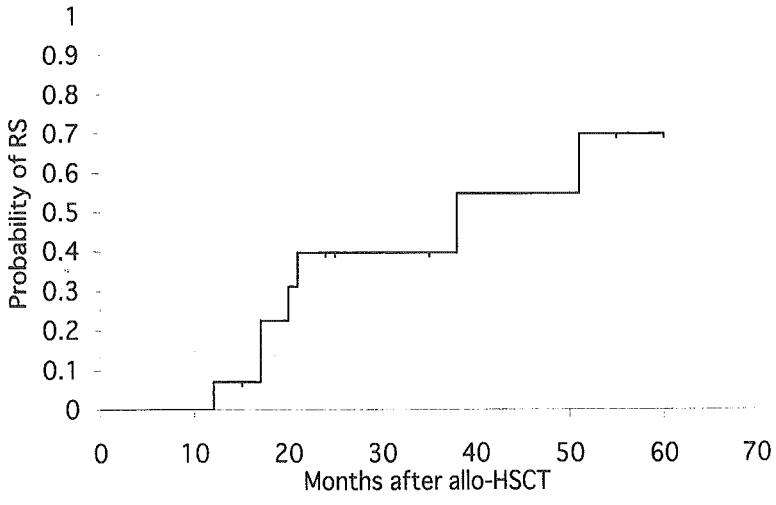

C

FIGURE 1. (A). Progressive disappearance of anti–hepatitis-B surface antigen antibody (anti-HBs) and occurrence of reverse seroconversion (RS). (B). Actual risk of anti-HBs disappearance in the 14 patients with pretransplant anti-HBs. (C). Actual risk of RS in the 14 patients with pretransplant anti-HBs. HSCT, hematopoietic stem cell transplantation.

self-limited. It is possible that some cases might be overlooked or misdiagnosed as hepatic injury caused by cGvHD. On the other hand, we had five cases that remained seronegative without HBV reactivation. This is thought to reflect minimal viral load or dormant status of HBV. These cases were followed for 0 to 47 (median 13) months after loss of anti-HBs without RS. It is uncertain whether these patients can continue to be free from RS. Timing of RS is thought to be determined by not only the patient's immunologic status but also viral activity. Recipients would be good candidates for vaccination when they have lost anti-HBs.

Several studies have recommended prophylactic vaccination of the donor, which is supported by the fact that no reactivation was seen in patients who received transplantation from anti–HBs-positive donors (*9–11*, *15*, *16*). On the other hand, attempts to overcome immunodeficiency by immunization of the donor have not always been successful (*18*). Long-term immunity, defined as persistence of antibody presence, is not achieved without reexposure to the specific antigen by either reimmunization or reinfection regardless of the immune status of the donor (*18*, *19*). Some other studies have shown transfer of HBV-specific immunity by allo-HSCT and good response to posttransplantation booster vaccination, which achieved and maintained protective levels of anti-HBs titer (*20*, *21*). Immunization immediately after the allo-HSCT period has not been successful, probably because of the absence of T–cell-dependent B-cell immune responses (*20*). However, because RS is a late-onset complication, reflecting the time required for reconstitution of donor-derived immunity, vaccination on demand for a recipient depending on anti-HBs titer would be theoretically effective even if the donor has anti-HBs. An appropriate vaccination schedule (timing and frequency) should be studied prospectively. If immunization is unsuccessful, long-term anti–HBs-specific Ig infusions might be considered.

In conclusion, RS is a late-onset complication with high frequency that can be predicted by careful monitoring of progressive disappearance of anti-HBs. Clinicians should consider the possibility of RS in all recipients with anti-HBs. Vaccination on demand for recipients depending on the decrease in anti-HBs titer would be prophylactic for reactivation of HBV.

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Long-term follow-up of chronic hepatitis B after the emergence of mutations in the hepatitis B virus polymerase region

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SUMMARY. Treatment of chronic hepatitis B has been greatly improved by the use of lamivudine, but mutations occur in the polymerase region of hepatitis B virus (HBV) and lamivudine-resistant mutants frequently develop. The emergence of lamivudine-resistant strains of HBV is a problem for treating chronic hepatitis B using lamivudine. We observed biochemical and virological changes in 15 patients with chronic hepatitis B for a median period of 29 months (range: 4–42 months) after the emergence of lamivudine-resistant mutants of HBV. Patterns of mutation of the polymerase gene were examined by sequencing the LLAQ motif in domain B and the YMDD motif in domain C. Exacerbation of liver dysfunction occurred in 14 (93.3%) of the 15 patients at a median of 4 months after the emergence of mutations. However, exacerbation of liver dysfunction was observed only in four patients (26.7%) at the time of appearance of the first mutations and in 80.0% of the patients at the time of appearance of the second mutations. Increase in serum alanine aminotransferase (ALT) levels was significantly greater at the time of appearance of second mutations ($P = 0.0096$). In most cases, wild-type HBV was mutated with the substitution of only rtM204I at first, and rtL180M/M204I mutations and then rtL180M/M204V mutations subsequently appeared. Further mutations of the polymerase region caused clinical deterioration. Thus as mutations emerge in the polymerase region, the clinical outcome deteriorates. Thus, monitoring the patterns of mutation of the polymerase gene is useful when using lamivudine for treating HBV.

Keywords: breakthrough, hepatitis B virus, lamivudine, LLAQ, mutation, YMDD.

INTRODUCTION

Lamivudine is a nucleoside analogue that suppresses the replication of hepatitis B virus (HBV) by inhibiting the viral RNA-dependent DNA polymerase. Treatment of chronic hepatitis B has been greatly improved by the use of lamivudine, and the rates of seroconversion (loss of HBe antigen and appearance of anti-HBe) in HBe antigen-positive patients have been reported to be 16–22% after 1 year and 35–40% after 3 years of lamivudine therapy [1–4]. Moreover, in HBe antigen-negative patients, normalization of alanine aminotransferase (ALT) and suppression of serum HBV-DNA to undetectable levels have been achieved [5]. However, it has been reported that lamivudine-resistant HBV mutations of the polymerase region develop in 30% of patients after 1 year and in 49–57% of patients after 3 years of lamivudine therapy [3,6]. Breakthrough hepatitis induced by lamivudine-resistant mutations is sometimes difficult to treat and can be fatal, and is one of the biggest problems in lamivudine treatment of chronic hepatitis B. Although new nucleoside analogues such as adefovir dipivoxil and entecavir used in the United States, Europe, Australia and some Asian countries have demonstrated clinical activity against lamivudine-resistant strains of HBV [7–9], lamivudine is still a key drug in the treatment of chronic hepatitis B. Elucidation of the clinical course of hepatitis B after the emergence of lamivudine-resistant mutations is important. In this paper, we report the long-term biochemical and virological changes in HBV after the emergence of mutations in chronic hepatitis B patients.

Abbreviations: ALT, alanine aminotransferase; HBV, hepatitis C virus; PCR, polymerase chain reaction; IFN, interferon.

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PATIENTS AND METHODS

Patients

During the period from March 1999 to February 2002, 40 patients with chronic hepatitis B were treated with lamivudine (100 mg/day) at the Hokkaido University Hospital, and HBV mutations emerged in 15 (37.5%) patients.

Table 1 Patients' characteristics at the start of lamivudine treatment

Total	$n = 15$
Age median (range)	36 (23–59)
Sex	
Male	11 (73.3%)
Female	4 (26.7%)
Background liver disease	
Chronic hepatitis	13 (86.7%)
Cirrhosis	2 (13.3%)
Knodell's Histologic Activity Index ($n = 10$)	
Necroinflammatory score	6.9 ± 3.1
Fibrosis score	1.9 ± 1.3
HBeAg positive	14 (93.3%)
ALT	407 ± 439 (IU/L)
HBV-DNA	7.7 ± 1.3 (LGE/mL)

Age is expressed as median (range) and values of Knodell's Histologic Activity Index and ALT and HBV-DNA are expressed as mean ± SD.

Laboratory testing

Serum ALT levels and HBe antigen, anti-HBe and serum HBV-DNA levels were checked biweekly or at least once a month. HBe antigen and anti-HBe levels were determined using radioimmunoassay kits (Abbot, North Chicago, IL, USA), and serum HBV-DNA levels were measured using transcription-mediated amplification (TMA) assay kits (Chugai Diagnostic Science Co., Ltd, Tokyo, Japan).

Sequencing of the polymerase region

DNA was extracted from 200 μL of serum of each patient by using a QIAamp DNA blood kit (Qiagen, Chatswoth, CA, USA). Five microlitres of DNA template was mixed with 12.5 μL of PCR Master Mix (Promega, Madison, WI, USA), 0.4 μM of sense and antisense primers, and 5.5 μM nuclease-free water for amplification by PCR. The sense primer for PCR was 5′-TGGCTATCGCTGGATGTGTCT-3′ and the antisense primer was 5′-TTGTTCAGTGGTTCG-TAGGGC-3′. The conditions of polymerase chain reaction (PCR) were as follows: 94 °C for 2 min for the initial incubation, 94 °C for 30 s for denaturing, 57 °C for 30 s for annealing, 72 °C for 1 min for extension for 35 cycles, and a final extension step of 72 °C for 5 min. The DNA product was purified by using a QIAquick PCR Purification Kit (Qiagen). The PCR products were reacted by using an ABI PRISM BigDye Terminator v3.1 Ready Reaction Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) with the sequence primer of 5′-CCCTCATGTTGCTGTA-CAAAACCT-3′. Then sequence reaction products were purified by using a DyeEx Spin Kit (Qiagen) and sequenced by using an ABI PRISM 310 Genetic Analyzer (Applied Biosystems). We checked two motifs of the HBV polymerase region, the leucine–leucine–alanine–glutamine (LLAQ) motif from codon rt179 to codon rt182 in domain B of the polymerase region, and the tyrosine–methionine–aspartate–aspartate (YMDD) motif from codon rt203 to codon rt206 in domain C. Emergence of lamivudine-resistant mutations was defined as detection of LLAQ and/or YMDD motif mutations. Sequencing of the polymerase region was performed at intervals of 2 weeks to 3 months partly retrospectively, and serial changes in mutation patterns of the HBV polymerase region were observed. In this study, the first mutation was defined as any mutation in the polymerase region detected for the first time and the second or further mutations were defined when new patterns of mutations emerged after previous mutations.

Statistical analysis

Mann–Whitney's *U*-test was used to compare the periods to the emergence of mutations, and Student's *t*-test test was used to compare serum ALT and HBV-DNA levels. All *P*-values were two-sided, and $P < 0.05$ was considered to be statistically significant. All serum HBV-DNA levels less than the limit of detection (<3.7 LGE/mL) were analysed as being 3.7 LGE/mL, and levels above the upper limit of detection (more than 8.8 LGE/mL) were analysed as being 8.8 LGE/mL.

RESULTS

Patients

We observed biochemical and virological changes, including patterns of mutations in the polymerase region, in those 15 lamivudine-resistant patients. Baseline characteristics of the patients at the start of lamivudine treatment are shown in Table 1. The 15 patients included 11 males and four females with a median age of 36 years (range: 23–59). Liver biopsies were performed in 10 patients. In those 10 patients, the necroinflammatory score of hepatitis was 6.9 ± 3.1 (mean ± SD) and the fibrosis score was 1.9 ± 1.3 in the Knodell's Histologic Activity Index [10]. HBe antigen was positive in 14 (93.3%) of the 15 patients. The mean serum ALT level and mean serum HBV-DNA level in the 15 patients were 407 ± 439 IU/L and 7.7 ± 1.3 log genome equivalent (LGE)/mL, respectively. The median follow-up periods were 35 months (range: 27–50 months) from the start of lamivudine treatment and 29 months (range: 4–42 months) after the emergence of lamivudine-resistant mutations.

Period to the emergence of mutations

The median period from the start of lamivudine therapy to emergence of mutations was 16 months (range: 5–34). The

median period was 22 months (range: 12–30 months) in patients with HBV-DNA levels <7.7 LGE/mL (mean serum HBV-DNA level of 15 patients at the start of lamivudine therapy), and it was 15 months (range: 7–18) in patients with higher levels. Patients with higher serum HBV-DNA levels developed mutations more rapidly ($P = 0.0056$).

Patterns of mutation in the HBV polymerase region

Mutation patterns of the polymerase region changed serially (Table 2). The median number of changes in mutation pattern during the follow-up period was 2 (range: 1–4). In nine (60.0%) of the 15 patients, the first mutation pattern was wild type in domain B and YIDD (rtM204I) in domain C. The first mutation patterns of the LLAQ motif in domain B were LMAQ (rtL180M) in five patients (83.3%) and LLTQ (rtA181T) in one patient (16.7%), and those of the YMDD motif in domain C were YIDD (rtM204I) in 10 patients (76.9%) and YVDD (rtM204V) in three patients (23.1%). Only four patients (26.7%) showed liver dysfunction at the time of the appearance of the first mutations. In 10 patients, further mutations appeared in the polymerase region 7 months (range: 1–27 months) after the appearance of the first mutations, and exacerbation of liver dysfunction occurred in eight (80.0%) of the 10 patients. Second or further mutations occurred in domain B and/or domain C, and each mutation caused exacerbation of liver dysfunction. In most cases, mutation with substitution of only rtM204I appeared at first, and mutation with rtL180M/M204I and then mutation with rtL180M/M204V subsequently emerged. Further mutations of the polymerase region caused worse clinical outcomes (Table 3).

Serum ALT and HBV-DNA levels

Exacerbation of liver dysfunction was observed frequently. Serum ALT levels remained within the normal range in only one patient (6.7%), and they were lower than 100 IU/L in five patients (33.3%), 100–500 IU/L in four patients (26.7%), and exceeded 500 IU/L in five patients (33.3%). The median period from the emergence of mutations to the start of exacerbation of liver dysfunction was 4 months (range: 2–10), and the peak serum ALT level was 367.8 ± 385.8 IU/L (mean ± SD). The peak serum ALT level after the appearance of the first mutations was 191.8 ± 281.6 IU/L and that after the appearance of the second mutations was 570.6 ± 390.3 IU/L, exacerbation of liver dysfunction being significantly more

Table 3 Patterns of mutations in the polymerase region and exacerbation of liver dysfunction

Domain C: wild Domain B: wild	→	YIDD wild	→	YIDD LMAQ	→	YVDD LMAQ
n		12		3		10
Extraction of liver dysfunction (ALT >200 IU/L)		4 (33.3%)		2 (66.7%)		7 (70.0%)

Table 2 Mutation patterns of the HBV polymerase region

case	First mutation	Second mutation	Third mutation	Fourth mutation	Follow-up period (months)
1	LLAQ YIDD*	LMAQ YVDD*			42
2	LLTQ YMDD	LMTQ YVDD	LMAQ YVDD		36
3	LLAQ YIDD				35
4	LLAQ YIDD	LMAQ YIDD*	LLAQ YIDD		33
5	LLAQ YIDD	LMAQ YVDD*	LLAQ YIDD		32
6	LLAQ YIDD*	LMAQ YIDD*	LMAQ YMDD†	LMAQ YVDD*	31
7	LMAQ YVDD				29
8	LLAQ YIDD†	LLAQ YMDD	VLAQ YMDD		26
9	LMAQ YIDD	LMAQ YVDD†			24
10	LLAQ YIDD				19
11	LLAQ YIDD				19
12	LLAQ YIDD	LMAQ YVDD*			17
13	LMAQ YVDD	LLAQ YIDD*			13
14	LMAQ YVDD†				11
15	LMAQ YMDD	LMAQ YVDD†			8

*Liver dysfunction: ALT >500 IU/L; †Exacerbation of: ALT >200 IU/L.
Values with '*' and '†' mean that the mutations were accompanied with exacerbation of liver dysfunction. Values with '†' mean ALT levels of 200–500 IU/L and '*' mean more than 500 IU/L.

severe after the appearance of second mutations ($P = 0.0096$).

The mean serum HBV-DNA level was 5.00 ± 0.39 LGE/mL (mean ± SE) at the first detection of mutations. The peak value of serum HBV-DNA levels was 6.27 ± 0.59 LGE/mL after the appearance of the first mutations, and it increased further after the appearance of the second mutations, peaking at 7.18 ± 0.54 LGE/mL (Fig. 1).

Clinical course

The clinical courses of 11 patients who did not use other antiviral drugs such as interferon (IFN) or new nucleoside analogues after the emergence of mutations were observed. The median follow-up period was 30 months (range: 8–42). Serum ALT levels remained within the normal range in only one patient and normalized in five patients after temporary worsening. In two patients, seroconversion of HBe antigen to anti-HBe occurred after temporary worsening of liver function, and serum HBV-DNA levels were decreased to undetectable levels. However, the exacerbation of liver dysfunction continued in five patients.

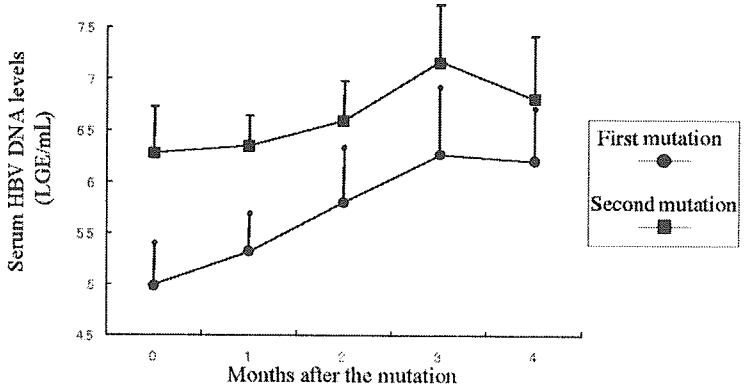
Four patients with severe breakthrough hepatitis were treated with IFN, and the median follow-up period for those patients was 6.5 months (range: 3–14). Six million units of IFN-beta was administered everyday for the first 4 weeks and thereafter three times a week for 20 weeks. Serum ALT levels were normalized in three patients, and serum HBV-DNA levels decreased to undetectable levels in two patients. One of the three patients who had HBe antigen before IFN treatment achieved seroconversion.

DISCUSSION

Lamivudine is a nucleoside analogue that suppresses replication of HBV by inhibiting the viral RNA-dependent DNA polymerase. It has been reported that resistance to lamivudine often develops after 6 months of treatment [11,12] Mutations occur in the polymerase region of HBV-DNA, and HBV becomes resistant to lamivudine. There have been many reports on mutations in the polymerase region and viral resistance, most of them focusing on the YMDD motif from codon rt203 of domain C and the LLAQ motif from codon rt179 of domain B of the HBV polymerase region [13–16]. In this study, we observed changes in serum ALT and HBV-DNA levels, HBe antigen, and anti-HBe in relation to serial changes in mutation patterns of the HBV polymerase region for a median period of 29 months after the emergence of mutations.

Serum HBV-DNA levels increased as soon as the first mutation occurred in the polymerase region, indicating that monitoring of serum HBV-DNA levels is important and that serum HBV-DNA level can be used as a predictive factor for the appearance of mutations as reported previously [17]. On the contrary, increases in serum ALT levels were delayed and ALT level peaked at a median of 4 months after the emergence of mutations. It has been reported that biochemial breakthrough phenomena are usually observed several months after the first detection of strains resistant to lamivudine [18,19]. This time lag is thought to be due to the duration until the occurrence of second or further mutations in the polymerase region.

In our series, rtL179V, rtL180M and rtA181T mutations were observed in domain B, and rtM204I and rtM204V mutations were observed in domain C. These mutations are almost the same as those reported previously [20,21]. In most cases, a mutation with only rtM204I appeared at first, and rtL180M/M204I mutations and then rtL180M/M204V mutations appeared subsequently in the polymerase region. Exacerbation of liver dysfunction at the time of appearance of the first HBV mutations occurred in only 26.7% of our patients. However, when further mutations appeared in 10 patients (66.7%) a median of 7 months after the appearance



No. evaluated

First mutation	15	15	14	12	11
Second mutation	10	10	9	8	7

Fig. 1 Changes of serum HBV-DNA levels after the emergence of mutations. The changes of serum HBV DNA levels after the first mutations to the second were shown by closed circles and after the second mutations to the third by closed squares. Serum HBV-DNA levels were measured by TMA assay. Vertical bars mean standard errors.

of first mutations, worsening of liver impairment occurred in 80.0% of the patients. Further mutations resulted in worsening of clinical courses. Although mutations of the YMDD motif in domain C have stronger effects on resistance to lamivudine than those of the LLAQ motif in domain B, it has been reported that single C-domain mutants have remarkedly decreased abilities of replication [22–24] and that B-domain mutation rtL180M rescues the defective replication competence of domain C mutants [25,26]. In another study, the effect of the addition of rtL180M mutation was examined by using a three-dimensional homology model of the catalytic core of HBV, and it was also shown that the rtM204V mutant is more resistant to lamivudine than the rtM204I mutant and that rtL180M substitution makes each mutant more resistant to lamivudine *in vitro* [27]. These results explain our finding that exacerbation of liver dysfunction occurred more frequently in cases with further mutations because rtM204V and/or rtL180M mutations were detected more frequently in our series when further mutations occurred in the polymerase region. Although small amounts of HBV mutants could not be detected because of the detection limit of direct sequencing, our results showed the significant correlation between accumulation of mutations and exacerbation of liver dysfunction. Direct sequencing was a useful method to detect mutations in the polymerase region clinically. But it is more effective to detect especially second or further mutations by more sensitive methods such as peptide nucleic acid mediated PCR clamping [28,29] because these mutations frequently caused severe liver dysfunction.

Exacerbation of liver dysfunction occurred without increase in serum HBV-DNA level after the emergence of mutations in some of our patients, indicating that some other factors may also lead to exacerbation of liver dysfunction. The polymerase gene of HBV overlaps with the surface antigen gene, and mutations in the polymerase region result in a change in the relevant amino acid of the surface antigen. It is possible that changes in the amino acid of the surface antigen may induce exacerbation of liver dysfunction.

In 11 patients, antiviral drugs other than lamivudine were not used. Fatal cases after discontinuation of lamivudine treatment have been reported [30], and it has been reported that lamivudine still suppresses the replication of wild-type HBV-DNA after the emergence of mutations [11,31]. We therefore continued lamivudine treatment after the appearance of lamivudine-resistant mutations. Serum ALT levels were normalized in six patients, and two of those six patients achieved seroconversion after temporary worsening. Thus, good clinical courses following temporary worsening were observed in some cases, indicating that observation without using other antiviral drugs is one of choices for patients with breakthrough hepatitis. However, exacerbation of liver dysfunction continued in five of our patients, and HBe antigen reappeared in two of them who had anti-HBe before the emergence of mutations. It has been reported that breakthrough hepatitis can be fatal in patients with advanced liver disease [32]. Patients with continuous liver dysfunction after emergence of mutations and patients with advanced liver disease should therefore be treated with new antiviral drugs such as adefovir dipivoxil.

Although the follow-up period was short, a good clinical response to IFN in patients with breakthrough hepatitis was obtained. Suzuki *et al.* [33] reported that IFN therapy was effective for lamivudine-resistant HBV mutants and that it may induce virological and clinical improvement accompanied by seroconversion. Although IFN has some side-effects and it is difficult to use for cirrhotic patients, it is one of the treatment options for breakthrough hepatitis. However, it has also been reported that serum HBV-DNA levels increased again and that hepatitis reccurred after reducing the dose of IFN [33]. Indeed, hepatitis recurred after cessation of IFN treatment in one of our patients. Care should therefore be taken in reducing the dose of IFN or terminating IFN treatment.

CONCLUSIONS

Exacerbation of liver dysfunction was observed frequently after the emergence of mutations in the HBV polymerase region. In most cases, the mutation pattern was a substitution of only rtM204I at first, and rtL180M/M204I mutations and then rtL180M/M204V mutations subsequently appeared. Exacerbation of liver dysfunction became more severe as more mutations occurred in the polymerase region. These results suggest that monitoring the patterns of mutation of the polymerase gene is useful when using lamivudine for treatment of HBV.

In some cases, serum ALT levels were normalized and seroconversion was achieved after temporary worsening of liver impairment, indicating that treatment is not necessarily required for all cases after the appearance of lamivudine-resistant mutations.

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症例報告

HIV・HCV重複感染の治療経過中，急速に致死的肝不全を来した血友病Aの1例

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目的：本邦では血液製剤由来のHIV患者のほとんどがHCVにも重複感染しているが，HAARTと抗HCV薬のリバビリン併用にて時に重篤な乳酸アシドーシス（LA）が生じることがある。今回，HAART施行中にリバビリンを併用した経過中にLAを契機に致死的肝不全を来した1例を経験したので，本症例の剖検所見に文献的考察を加えて報告する。

症例：症例は35歳男性。血友病Aに対し使用した血液製剤でHIV・HCVに感染した。1990年より抗HIV療法を開始し，薬剤変更を経て2003年1月よりd4T＋ddI＋PI（RTV＋LPV）で治療していた。同年9月よりHCVに対しIFNα＋リバビリンにて加療開始したが，10月にLAを発症した。投薬中止しメイロン投与等対症療法を施行したが，肝不全が進行し，11月に永眠された。

結論：本症例ではLA発症を契機に急速な致死的肝不全を来したわけであるが，その原因としては，①LAそれ自体，②IFN・リバビリン併用療法の副作用，③HCVによる代償性肝硬変から非代償性肝硬変への急速な進展，の3点が考えられた。

キーワード：HIV感染症，HCV感染症，リバビリン，乳酸アシドーシス，ヌクレオシド系逆転写酵素阻害薬（NRTI）

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緒　　言

HIV感染患者では，HCVに重複感染している例が多く，特に血友病患者においては，本邦で実に98.4%にも上る[1)]。Highly Active Anti-Retroviral Therapy（HAART）によってHIV感染者のAIDS発症が抑制され，生命予後が延長するのに伴い，HCV感染症が生命予後規定因子として重要になってきた。一方，HAARTのヌクレオシド系逆転写酵素阻害薬（NRTI）の副作用の一つとして乳酸アシドーシス（LA）があるが，抗HCV治療としてリバビリンを併用したのちにLAが生じたとの報告があり，特にNRTIの中でもジダノシン（ddI），サニルブジン（d4T）との併用で頻度が高いとされている[2,3)]。今回我々はHIV・HCVの重複感染に対し，HAART施行中にリバビリンを投与したのちにLAを契機に致死的肝不全を来した血友病Aの1例を経験したので，その臨床経過に剖検結果と文献的考察を加えて報告する。

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症　　例

症例：35歳，男性

主訴：全身倦怠感，嘔気，腹部不快感

現病歴：小児期より血友病Aに対し凝固因子補充療法施行され，中学生時に非A非B型肝炎との診断を受けたが，肝障害は軽度で，以後特別な治療は施行されなかった。その後18歳時にHIV感染が判明した。1990年にCD4陽性リンパ球数の50/μlまでの低下と同時に帯状疱疹を発症し，ジドブジン（AZT）＋ddIの投与を開始した。その後はCD4陽性リンパ球数，臨床症状ともに安定していたが，服薬コンプライアンスの問題から，2001年にAZT＋ラミブジン（3TC）＋ネルフィナビル（NFV）に変更した。この時点までは，GOT，GPTは20～40 IU/l程度で明らかな肝障害の進行は認められなかった。2002年にCD4陽性リンパ

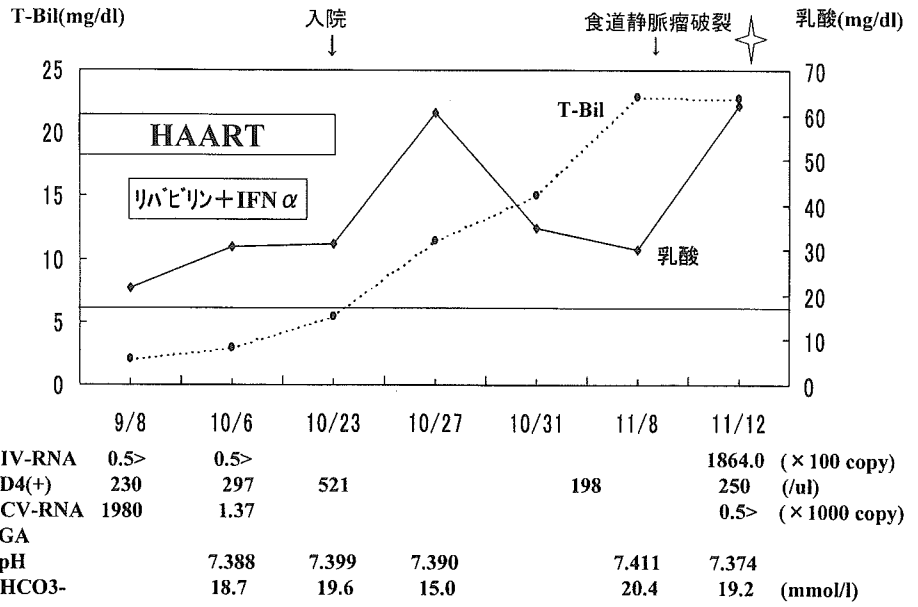

図 1 臨床経過

球数が 65/μl, HIV-RNA 量 30,000 copy/ml となり，AZT, 3TC に高度耐性，NFV に軽度耐性が生じていたため，2003 年 1 月に d4T＋ddI＋ロピナビル/リトナビル（LPV/RTV）に変更した。この時点でも GOT, GPT は 20～50 IU/l 程度であったが，胆道系酵素（γ-GTP 76～150 IU/l），ビリルビンの軽度上昇（T-Bil 1.1～1.5 mg/dl），ChE の軽度低下（170～229 IU/l）がみられていた。

肝臓に関しては，2003 年 9 月の時点で，CT 上肝脾腫を認め，Child Pugh score では，score7, gradeB（alb 3.0 g/dl：2 点，T-Bil 1.5 mg/dl：1 点，脳症・腹水なし各 1 点，%PT 63.8%：2 点），ICGr15 32.8% と軽度の肝障害を認めたが，完成された肝硬変ではないと診断した。また，肝癌・日和見感染もなく，CD4 陽性リンパ球数も 200/μl 以上あり（9 月 8 日：230/μl），リバビリン＋インターフェロンα（IFNα）療法の開始基準を満たしていたため，患者から同意を取得したのち，HCV に対し，リバビリン＋IFNα 投与開始した[4]。投与スケジュールは，リバビリン 400 mg/day を経口で 24 週間，IFNα600 MIU/day を皮下注で，最初の 2 週間が 6 days/week で後半 22 週間が 3 days/week の計 24 週間投与する予定であったが，投与前は 10 万/μl 前後であった血小板数が 9 月 13 日には 4.8 万/μl と低下したため，リバビリンはそのまま継続したが，IFNα を 3 days/week に減量した。

10 月中旬より全身倦怠感・嘔気が生じ，LA を認めた（血中乳酸 30.8 mg/dl）。その時点では HCV に対する治療よりも HIV に対する治療が優先すると考え，まず 10 月 15 日にリバビリンと IFNα を中止し，外来にて経過観察されていたが，症状悪化がみられたため 10 月 23 日当科入院となった。

入院時身体所見：意識清明。栄養状態不良。結膜貧血（－），黄疸（＋）。胸部異常無し。腹部やや膨隆で波動（＋），肝は右肋骨弓下に 1 横指触れるが，脾は触れず。下肢浮腫なし。皮膚はやや乾燥し，右膝関節には軽度拘縮を認め，右下肢に軽度の知覚低下を認めた。

検査所見（表 1）：入院時の検査所見では，直接型優位のビリルビン，トランスアミラーゼの上昇，アルブミンの低下を認め，乳酸値は 31.2 mg/ml と高値であった。動脈血ガス分析では pH は 7.399 であったが，pO_2 108.3 mmHg と上昇，pCO_2 32.3 mmHg, HCO_3^- 19.6 mmol/l, BE －3.9 mmol/l と低下を認め，代謝性アシドーシスと呼吸性代償の所見がみられた。入院直前の検査では，HIV-1 RNA 量は 50＞copy/ml, HCV RNA 量は 1,370 copy/ml（9 月 8 日のリバビリン開始前は 1,980,000 copy/ml）であり，入院時の CD4 陽性リンパ球数は 521/μl と，HIV に関しては良好なコントロールが得られ，HCV に関しても RNA 量の減少が見られていた。

画像所見：腹部超音波検査・CT では，多量の腹水貯留と肝萎縮，脾腫を認めたが，脂肪肝・門脈塞栓などの所見は認められなかった。

入院後経過：入院時，有症候性の LA がみられており，原因としては NRTI とリバビリンが考えられた。そのためリバビリンは入院 8 日前より中止した。また，それに伴い，

表 1 入院時臨床検査所見

Hematological data				Others	
WBC	7300/μl	BUN	18 mg/dl	urinalysis	
RBC	359×10⁴/μl	S-Cre	1.0 mg/dl	OB (−)	
Hb	13.1 g/dl	UA	6.3 mg/dl	sugar (+)	
Ht	38.6%	Na	131 mEq/l	protein (+)	
Plt	12.1×10⁴/μl	K	4.2 mEq/l	ketone body (−)	
		Cl	99 mEq/l		
TP	6.5 g/dl	Ca	7.5 mg/dl	Stool	
Alb	2.2 g/dl	CRP	0.93 mg/dl	B (+)	
T-Bil	5.4 mg/dl			O (+)	
GOT	111 IU/l	HCV-Ab	(+)	L (−)	
GPT	68 IU/l	HCV-RNA	1.37 k copy		
LDH	477 IU/l	(2003.9 月治療前：1980 k copy)		BGA	
ALP	589 IU/l	HCV-genotype	2b	pH 7.399	
ChE	48 IU/l	HIV-1 RNA		pO_2 108.3 mmHg	
		CD4	521/μl	pCO_2 32.3 mmHg	
		CD4/8	0.91	HCO_3^- 19.6 mmol/l	
		Lactic acid	31.2 mg/dl	BE −3.9 mmol/l	
		(正常値 3.0〜17.0)			

IFNα に関しても単剤で肝障害の副作用が 20% 程度で生じるとされていることと，LA の原因とは考えにくかったものの全身状態を考えるとその時点では HCV に対する治療は一旦中止し，全身状態の改善を最優先とすることとしたため，IFNα に関してもリバビリンと同時に中止とした。また，リバビリン＋IFNα 中止後も症状改善が認められなかったため，入院日には HAART 薬剤（ddI＋d4T と LPV/RTV）も中止した。アシドーシスに対して 7% 炭酸水素ナトリウム，肝機能障害に対して肝庇護剤，経口摂取低下に対し末梢静脈栄養を開始した。入院時より低アルブミン血症があり，入院後も腹水の増加と浮腫認めたため，第 3 病日より 3 日間にわたりアルブミン製剤の投与を行った。第 5 病日には右鎖骨下より中心静脈カテーテルを挿入し，肝障害用アミノ酸輸液を含めた中心静脈栄養を開始した。総ビリルビン値の上昇と血清蛋白の低下が進行し，アルブミン製剤・新鮮凍結血漿の投与，肝庇護剤投与を継続したが，第 18 病日に総ビリルビンは 22.8 mg/dl，第 20 病日に血清総蛋白は 5.4 g/dl に達し，腹水増加を認めた。一方，動脈血ガス分析では pH は 7.400 ± 0.1，HCO_3^- は 20 ± 5 mmol/l の範囲で推移し，極端なアシドーシス進行はみられなかった。血中乳酸値は第 5 病日に 93.8 mg/dl，第 20 病日に 62.2 mg/dl と極端な高値を示したが，入院中は概ね 30〜60 mg/dl の間で推移していた。

2003 年 9 月に施行した上部消化管内視鏡検査では Li F0-1 Cb RC（−）の食道静脈瘤が確認されていたが，第 18 病日に大量吐血がみられ，緊急上部内視鏡施行したところ，食道静脈瘤（Li F2 Cb RC（＋＋））より出血があり，内視鏡的静脈瘤結紮術（EVL）を施行した。胃内には出血源は無く，十二指腸には潰瘍からの出血と，露出血管（出血しておらず）が多数みられ，それらに対しても EVL を施行した。

翌第 19 病日に EVL 施行後の止血確認目的で再び上部消化管内視鏡施行したところ先日 EVL 施行した部位に関しては止血されていたが，十二指腸の肛側に凝血塊を認められ，同部位からの出血が疑われた。

その後，PPF・赤血球輸血・ドパミン投与を行ったが，全身状態の改善は得られず，循環不全により第 21 病日未明に永眠された。第 21 日病日の HIV-1 RNA 定量は 186,400 copy/ml，HCV RNA 定量では＜500 copy/ml と，HAART 中止に伴う HIV-RNA 量の増加は見られたが，抗 HCV 療法に伴う HCV-RNA 量の減少は継続していた。

家族の承諾のもとに施行された剖検では，6,500 ml の腹水と，左右約 200 ml の胸水が認められた。肝臓重量は 1,530 g で，表面は凹凸不整，辺縁は鈍となっていたが，肉眼的な脂肪肝は認められず，肝細胞癌も認められなかった。食道には静脈瘤があり，脾腫と副脾も認めた。肝の病理組織学的所見としては，広範な肝細胞の壊死・脱落像と肝線維化を認め，肝硬変の所見であった。門脈域へのリン

パ球を中心とした炎症細胞浸潤も認められたが，肝小葉内への炎症細胞の浸潤は認められなかった。また，Sudan Ⅲ染色において肝細胞におけるびまん性の小滴性脂肪沈着が確認され，LAによる肝障害の所見として矛盾しなかった。その他には，肝内胆管の胆汁塞栓が認められた。

膵は炎症細胞の浸潤と膵細胞の壊死に加え膵石・出血を認め，膵炎の所見であった。

腎は表面凹凸が著明で，皮質萎縮性の変化が生じ，組織学的には巣状糸球体硬化の所見が見られ，HIV感染者でよく見られるAIDS関連腎症の所見であった。

以上，病理所見からは，肝小葉内へのリンパ球の浸潤が殆ど認められなかったことから，HCVによる肝炎・肝硬変の急激な増悪は，肝不全進行の原因としては可能性が低いと考えられた。一方，LAと胆汁うっ滞型の薬剤性肝障害は，病理所見には矛盾しないと考えられた。膵に関しては，LAで膵炎を生じることがあるとされており，LAの所見として矛盾は無かった。腎は巣状糸球体硬化の所見が強く，腎機能の低下を介しリバビリンなどの腎排泄型薬剤の濃度上昇を引き起こしていた可能性も考えられた。

考　　察

1990年代前半より，HIVに対してHAARTを施行した例において，LAが報告されるようになり，その原因薬剤としてはNRTIのddI, d4Tが多く，頻度としては0.1～0.2%といわれている[5,6]。その機序としては，NRTIのミトコンドリアDNA polymerase γに対する高い親和性によるミトコンドリア障害が考えられている[7]。臨床的には，腹痛，嘔気，嘔吐，呼吸困難，体重減少，肝腫大などの症状を呈し，ミトコンドリア障害による細胞の好気的解糖系の障害に伴う乳酸の増加とアシドーシスがみられ，最終的には肝不全，膵炎，呼吸不全，筋壊死などを生じ，死亡するケースもまれではない（死亡率は48%であったとする報告がある）[5,8]。HAART施行中のLA発症については現在までに多数の報告がある。危険因子については，前述のddI, d4Tの他には，女性の相対危険率が2.5倍であるという報告もあるが，その他の危険因子については明らかになっていない[8]。

LAの治療法としては，原因薬剤の速やかな中止と，炭酸水素ナトリウム投与によるアシドーシスの補正，サイアミン・L-アセチルカルチニン投与によるミトコンドリア毒性の軽減などがあるが，いずれにしても対症療法であり，50%程度という高い死亡率を考えると早期診断が最も重要である。しかし，HAART施行中の患者においては20%程度の症例で無症候性高乳酸血症をきたすことが知られており，臨床症状と動脈血ガス分析によるアシドーシスの有無，そしてLAで特徴的とされる肝脂肪変性を肝生検によって確認することが重要とされているが，肝生検に関しては，HIV感染患者に血友病患者が多く，LA発症時点で血小板数，凝固能などの数値が悪化していることも考えられ，本症例のように施行が困難な例も多いと推測される。

HIV感染患者ではHCVにも重複感染している例が多く，HAARTによりAIDSの発症が抑制され，HIV感染者の生命予後が延長するのに伴い，HCV感染症が生命予後規定因子として重要になってきた。重複感染例においては，HCV-RNA量高値・HCV envelope変異増加によるIFN耐性の増強・治療の副作用が強く出やすいなどの理由により，治療奏功率が低い，肝硬変への進展が早く死亡率が高い，といった問題点がある[9]。治療としては本邦では数年前よりIFNとリバビリンの併用療法が施行されており，奏功率は20～30%（HIV陽性例）で，HCV単独感染例に比較して明らかに低い[9]。

2001年に，ddIとd4Tを含むHAART施行中の15例の患者のHCVに対しIFNとリバビリンを投与したところ，2例にLAを発症したとする報告があり，以降，現在までに36例以上の報告がある[3,9]。その機序としては，リバビリンが5′-ヌクレオチダーゼによるddInoからddIMPへの変換過程におけるリン酸化に関与するIMPレベルの上昇を引き起こし，その結果，活性体への変換を促進することによりミトコンドリア毒性を増強するためと考えられている。IFNに関しては，LA発症に何らかの影響を与えているとする報告は見られない[10]。リバビリン併用投与でのLA発生率は，HAART単独施行時に比し高率の1.9%であるという報告もある[2]。HAARTにIFNとリバビリンを併用した際に生じるLAに関しても，HAART単独施行時と同様に，原因薬剤の速やかな中止と対症療法しか現時点では有効な治療法はない。HAARTとIFN＋リバビリン併用療法施行時のLAの死亡率に関しては，25%という値が報告されており，前述のHAART単独施行時のLAにおける死亡率（50%程度）と比し一見低いように感じるが，HAARTとIFN＋リバビリン併用療法施行時のLA死亡率に関する報告にはLAまで至らない高乳酸血症も死亡率の母数に含まれたものであり，その点を考慮する必要がある[11]。死亡率について直接比較した研究は現在のところないが，一般的には死亡率はHAART単独施行時に比して同等かそれ以上であると考えるべきである[5]。

本症例は，これまで長期の良好なHIVのコントロールが困難な状況であったが，2003年9月の時点でHIV-RNA量のコントロールとCD4陽性リンパ球数の維持が得られていたため，進行しつつあった慢性C型肝炎に対し2003年9月よりIFNαとリバビリンにより治療を開始した。治

療開始時点では完成された肝硬変ではないと診断し，また，上部内視鏡検査では食道静脈瘤を認めたもののRC（－）であった。その後，IFNとリバビリン併用療法開始後1か月で前述の臨床症状と肝機能の急激な悪化を認め，結局は数か月足らずでの食道静脈瘤の悪化・破裂による循環不全と肝不全により死亡した。その原因としては，①HAARTとリバビリン併用によるLA，②プロテアーゼ阻害薬であるLPV/RTV，もしくはNRTIによる直接的な薬剤性肝障害，③慢性C型肝炎の急激な悪化，が考えられた。臨床症状はIFNとリバビリン投与後から生じたものであり，検査所見はLAとして矛盾しなかった。また，病理所見からは，LAに特徴的とされる肝の脂肪変性の所見が認められた[3,5,9]。一方，薬剤性肝障害に特徴的な好酸球の肝小葉への浸潤などの所見は認められなかった。また，慢性C型肝炎における肝小葉へのリンパ球浸潤は認められず，第21病日にはHCV-RNA量が検出限界以下になっていることもあり，肝不全悪化の直接的原因としては否定的であった。以上より，本症例ではNRTIとリバビリンの併用によりLAが生じ，肝障害悪化がみられた可能性が高いと考えられたが，ミトコンドリア障害を直接的には証明できず，薬剤性肝障害を含めた他の原因を完全には否定できなかった。

結　　語

HIV・HCV重複感染例においてHAART＋IFN＋リバビリン併用療法における治療経過中にLAを契機とした致死的肝障害を来した血友病Aの1例を経験し，その原因としては，HAARTとリバビリンの併用療法が最も考えられた。

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Fatal Hepatic Failure in a Hemophilia A Patient with HIV/HCV Co-infection

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Objective : In Japan, most people who have infection with HIV also have infection with HCV caused by administration of coagulation factor products. Fatal lactic acidosis rarely occurs in patients with HIV/HCV co-infection while using both HAART drugs and rivabirin. We experienced a patient with fatal hepatic failure following lactic acidosis after the use of both HAART drugs and rivabirin. We report this rare case with discussion of the results of postmortem examination.

Case Report : A 35-year-old man infected with HIV and HCV after administration of blood products for hemophilia A was started on anti-retroviral therapy for HIV in 1990. He was treated with d4T+ddI+PI (RTV+LPV) from January 2003 after several changes of anti-retroviral drugs. From September 2003, IFNα and ribavirin were also administered for treatment of HCV hepatitis. Symptoms of lactic acidosis initially appeared in October. Although the above-described medication was stopped and treatment for lactic acidosis, including intravenous administration of sodium bicarbonate, was started immediately, hepatic insufficiency rapidly progressed and he died in November 2003.

Conclusion : In this case, rapidly progressing hepatic insufficiency was induced by lactic acidosis. We concluded that hepatic failure was possibly caused by lactic acidosis, side effects of combined therapy with IFNα and ribavirin, and rapid progression to non-compensative liver cirrhosis from HCV hepatitis.

Key words : HIV infection, HCV infection, ribavirin, lactic acidosis, NRTI

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検査値の読み方　連載

B型慢性肝炎の治療に伴うウイルスマーカーの変動

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はじめに

B型慢性肝炎の自然経過は，個人間の差が大きく，病期進展の予想や治療の必要性の判断に関しても画一的でないため，治療の選択やそのタイミングも，検討の必要がある．B型肝炎の治療に関しては，インターフェロン以外に，2000年11月からラミブジンの投与が可能となったが，耐性株の出現と，それに併発する肝炎再燃が問題点としてあげられてきた．しかし，2004年12月からは，ラミブジン耐性株を有する症例にアデホビルの併用も可能となり，治療の選択肢が増えてきた．

本稿では，B型慢性肝炎で種々の治療を行った症例を基に，B型肝炎のデータの読み方と治療の選択に関する検討を示す．

I．症　例

症例は36歳の男性．約10年前からB型慢性肝炎の指摘を受けていた(当時，海外で仕事をしており，ラミブジン治療を勧められたという)．1年前から肝機能障害が増強し，ALTが1,000 IU/*l*以上となり，ウイルス学的にも改善を認めないことから，1999年3月に当科を紹介されて初診した．家族歴は，母親と弟がB型慢性肝炎である．

II．治療経過概略(図1)

1999年3月からラミブジンの投与を開始(図1①)し，治療後にHBV-DNA量の減少，ALTの低下を示し，良好な抗ウイルス効果を認めた(図1②)．しかし，セロコンバージョンが得られないため投与を継続していたところ，17カ月後からHBV-DNA量の再上昇傾向を認め，ALTも24カ月後に110 IU/*l*まで上昇した(図1③)．そのまま経過観察を継続しALTは40～80 IU/*l*程度で推移していたが，33カ月後から急性増悪を認めた(図1④)ため，ラミブジン継続下に強力ネオミノファーゲン

Key words：B型慢性肝炎，ラミブジン，アデホビル，HBVポリメラーゼ領域変異

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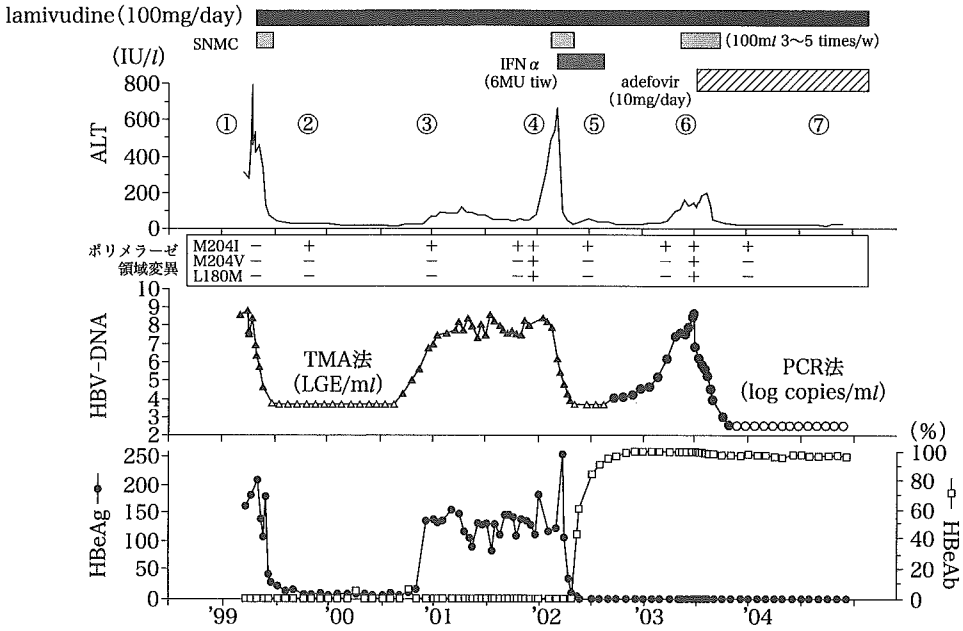

図1 臨床経過

図中の①～⑦は本文を参照．HBV-DNA量の測定法は経過中にTMA法からPCR法に変更している．TMA法，PCR法とも図中白抜き(△，○)は，測定感度以下を示している．

C® の点滴を開始し，さらに，35カ月後からインターフェロンα 600万単位を週3回で24週間投与した(図1⑤)．39カ月後にはセロコンバージョンを認め，ALTも10 IU/l台にまで改善した．しかし，その後，HBe抗体陽性のままでHBV-DNA量の再上昇を示したため，51カ月後からアデホビルの併用を開始した(図1⑥)．その後は，ウイルス学的にも生化学的にも良好な経過で，併用開始18カ月後の現時点まで再燃は認めていない(図1⑦)．

III．考　　案

1．HBe抗原陽性活動性肝炎の治療法の選択

初診時の段階(図1①)では，前医での検査成績で，ALTが高値のまま変動していること，ウイルス量も多めで，HBe抗原陽性/HBe抗体陰性で変化がないことと，さらに患者年齢を考慮すると，経過観察のみでHBe抗原/抗体のセロコンバージョンを期待することは容易ではない可能性が高いと思われたため，本症例では，ラミブジン100 mg/日の連日内服投与を開始した．

わが国の肝臓学会による慢性肝炎の治療ガイド[1]では，HBe抗原陽性活動性肝炎の場合には，年齢・炎症の強さを考慮して治療の必要性を判断し，ウイルス量や肝組織所見を参考にして治療法を選択するよう示している．

2．ラミブジンの治療効果と耐性株の出現

本症例では，ラミブジン投与後，ウイルス量やHBe抗原価は速やかに減少したが，HBe抗原は陰性化せずに低値安定状態となり，HBe抗体も陰性のまま推移し，セロコンバージョンに達することができなかった．

HBe抗原陽性慢性肝炎に対するラミブジン