5 Tassopoulos NC, Volpes R, Pastore G *et al.* Efficacy of lamivudine in patients with hepatitis B e antigen-negative/hepatitis B virus DNA-positive (precore mutant) chronic hepatitis B. Lamivudine Precore Mutant Study Group. *Hepatology* 1999; 29: 889–896.
6 Lau DT, Khokhar MF, Doo E *et al.* Long-term therapy of chronic hepatitis B with lamivudine. *Hepatology* 2000; 32: 828–834.
7 Hadziyannis SJ, Tassopoulos NC, Heathcote EJ *et al.* Adefovir Dipivoxil 438 Study Group. Adefovir dipivoxil for the treatment of hepatitis B e antigen-negative chronic hepatitis B. *N Engl J Med* 2003; 348: 800–807.
8 Marcellin P, Chang TT, Lim SG *et al.* Adefovir Dipivoxil 437 Study Group. Adefovir dipivoxil for the treatment of hepatitis B e antigen-positive chronic hepatitis B. *N Engl J Med* 2003; 348: 808–816.
9 Levine S, Hernandez D, Yamanaka G *et al.* Efficacies of entecavir against lamivudine-resistant hepatitis B virus replication and recombinant polymerases in vitro. *Antimicrob Agents Chemother* 2002; 46: 2525–2532.
10 Knodell RG, Ishak KG, Black WC *et al.* Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. *Hepatology* 1981; 1: 431–435.
11 Chayama K, Suzuki Y, Kobayashi M *et al.* Emergence and takeover of YMDD motif mutant hepatitis B virus during long-term lamivudine therapy and re-takeover by wild type after cessation of therapy. *Hepatology* 1998; 27: 1711–1716.
12 Honkoop P, Niesters HG, de Man RA, Osterhaus AD, Schalm SW. Lamivudine resistance in immunocompetent chronic hepatitis B. Incidence and patterns. *J Hepatol* 1997; 26: 1393–1395.
13 Ling R, Mutimer D, Ahmed M *et al.* Selection of mutations in the hepatitis B virus polymerase during therapy of transplant recipients with lamivudine. *Hepatology* 1996; 24: 711–713.
14 Tipples GA, Ma MM, Fischer KP, Bain VG, Kneteman NM, Tyrrell DL. Mutation in HBV RNA-dependent DNA polymerase confers resistance to lamivudine in vivo. *Hepatology* 1996; 24: 714–717.
15 Gutfreund KS, Williams M, George R *et al.* Genotypic succession of mutations of the hepatitis B virus polymerase associated with lamivudine resistance. *J Hepatol* 2000; 33: 469–475.
16 Ono-Nita SK, Kato N, Shiratori Y *et al.* Susceptibility of lamivudine-resistant hepatitis B virus to other reverse transcriptase inhibitors. *J Clin Invest* 1999; 103: 1635–1640.
17 Papatheodoridis GV, Dimou E, Laras A, Papadimitropoulos V, Hadziyannis SJ. Course of virologic breakthroughs under long-term lamivudine in HBeAg-negative precore mutant HBV liver disease. *Hepatology* 2002; 36: 219–226.
18 Hadziyannis SJ, Papatheodoridis GV, Dimou E, Laras A, Papaioannou C. Efficacy of long-term lamivudine monotherapy in patients with hepatitis B e antigen-negative chronic hepatitis B. *Hepatology* 2000; 32: 847–851.
19 Liaw YF, Chien RN, Yeh CT, Tsai SL, Chu CM. Acute exacerbation and hepatitis B virus clearance after emergence of YMDD motif mutation during lamivudine therapy. *Hepatology* 1999; 30: 567–572.
20 Papatheodoridis GV, Dimou E, Papadimitropoulos V. Nucleoside analogues for chronic hepatitis B: antiviral efficacy and viral resistance. *Am J Gastroenterol* 2002; 97: 1618–1628. Review.
21 Niesters HG, Honkoop P, Haagsma EB, de Man RA, Schalm SW, Osterhaus AD. Identification of more than one mutation in the hepatitis B virus polymerase gene arising during prolonged lamivudine treatment. *J Infect Dis* 1998; 177: 1382–1385.
22 Allen MI, Deslauriers M, Andrews CW *et al.* Identification and characterization of mutations in hepatitis B virus resistant to lamivudine. Lamivudine Clinical Investigation Group. *Hepatology* 1998; 27: 1670–1677.
23 Fu L, Cheng YC. Role of additional mutations outside the YMDD motif of hepatitis B virus polymerase in L(-)SddC (3TC) resistance. *Biochem Pharmacol* 1998; 55: 1567–1572.
24 Ling R, Harrison TJ. Functional analysis of mutations conferring lamivudine resistance on hepatitis B virus. *J Gen Virol* 1999; 80: 601–606.
25 Yeh CT, Chien RN, Chu CM, Liaw YF. Clearance of the original hepatitis B virus YMDD-motif mutants with emergence of distinct lamivudine-resistant mutants during prolonged lamivudine therapy. *Hepatology* 2000; 31: 1318–1326.
26 Ono SK, Kato N, Shiratori Y *et al.* The polymerase L528M mutation cooperates with nucleotide binding-site mutations, increasing hepatitis B virus replication and drug resistance. *J Clin Invest* 2001; 107: 449–455.
27 Das K, Xiong X, Yang H *et al.* Molecular modeling and biochemical characterization reveal the mechanism of hepatitis B virus polymerase resistance to lamivudine (3TC) and emtricitabine. *J Virology* 2001; 75: 4771–4779.
28 Orum H, Nielsen PE, Egholm M, Berg RH, Buchardt O, Stanley C. Single base pair mutation analysis by PNA directed PCR clamping. *Nucleic Acids Res* 1993; 21: 5332–5336.
29 Kirishima T, Okanoue T, Daimon Y *et al.* Detection of YMDD mutant using a novel sensitive method in chronic liver disease type B patients before and during lamivudine treatment. *J Hepatol* 2002; 37: 259–265.
30 Lim SG, Wai CT, Rajnakova A, Kajiji T, Guan R. Fatal hepatitis B reactivation following discontinuation of nucleoside analogues for chronic hepatitis B. *Gut* 2002; 51: 597–599.
31 Lok AS, McMahon BJ. Practice Guidelines Committee, American Association for the Study of Liver Diseases. Chronic hepatitis B. *Hepatology* 2001; 34: 1225–1241.
32 Wang JH, Lu SN, Lee CM, Lee JF, Chou YP. Fatal hepatic failure after emergence of the hepatitis B virus mutant during lamivudine therapy in a patient with liver cirrhosis. *Scand J Gastroenterol* 2002; 37: 366–369.
33 Suzuki F, Tsubota A, Akuta N *et al.* Interferon for treatment of breakthrough infection with hepatitis B virus mutants developing during long-term lamivudine therapy. *J Gastroenterol* 2002; 37: 922–927.

bait5/ztr-tp/ztr-tp/ztr00505/ztr9626-05a | marksj | S=7 | 1/19/05 | 14:17 | Art: 158727

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

Masahiro Onozawa,[1,3] Satoshi Hashino,[1] Koh Izumiyama,[1] Kaoru Kahata,[1] Makoto Chuma,[1] Akio Mori,[1] Takeshi Kondo,[1] Nobuyasu Toyoshima,[1] Shuichi Ota,[1] Sumiko Kobayashi,[1] Shuhei Hige,[1] Tomomi Toubai,[2] Junji Tanaka,[2] Masahiro Imamura,[2] and Masahiro Asaka[1]

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.

(*Transplantation* 2005;79: ●●●–●●●)

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.

[1] Department of Gastroenterology and Hematology, Hokkaido University Graduate School of Medicine, Sapporo, Japan.
[2] Department of Hematology and Oncology, Hokkaido University Graduate School of Medicine, Sapporo, Japan.
[3] Address correspondence to: Dr. Masahiro Onozawa, Department of Gastroenterology and Hematology, Hokkaido University Graduate School of Medicine, Kita-15, Nishi-7, Kita-ku, Sapporo 060–8638, Japan. E-mail address: masahiro.onozawa@nifty.ne.jp.
AQ: 1
Received 23 August 2004. Revision requested 13 September 2004. Accepted 16 October 2004.

ISSN 0041-1337/05/7905-1
DOI: 10.1097/01.TP.0000151661.52601.FB

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. T1
We retrospectively studied hepatitis B surface antigen (HBsAg) (Clinical Laboratory Improvement Amendments [CLIA]), anti-HBs (CLIA), hepatitis B e antigen (HBeAg) (radioimmunoassay [RIA]), anti-hepatitis e antigen antibody AQ: 2
(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) F1
had relapse of hematologic malignancy during the follow-up period.

<ARTICLE DOCTOPIC="Brief Communications" DOCSUBJ="Experimental Transplantation" DATE="March 15, 2005" VID="79" ISS="5" PPF="000" PPL="000" DOI="10.1097/01.TP.0000151661.52601.FB">

C型慢性肝炎　肝組織内HCV-RNAの動態

C型慢性肝炎の肝組織内RNA量の測定
—プラス鎖RNA，マイナス鎖RNA別—

Measurements of strand-specific hepatic HCV-RNA quantities of patients with hepatitis C virus infection

髭　修平

Key words：肝組織内HCV-RNA，プラス鎖HCV-RNA，マイナス鎖HCV-RNA，strand-specific RT-PCR法

はじめに

C型肝炎ウイルス(HCV)の大部分は肝細胞内で増幅し，ダイナミックに肝細胞内外を移動している．HCVを含むフラビウイルス科のRNA複製に関しては，ウイルスの非構造蛋白質や宿主蛋白質の作用も含めて次第に明らかになってきた[1,2]．HCVの複製の過程においては相補的なマイナス鎖RNAを合成し，それを鋳型としてプラス鎖(genomic)RNAを合成する．したがって，マイナス鎖RNAは増幅の中間体と考えられ，その検出はウイルス増殖の直接的な証明となり得る．肝組織内のHCV-RNA量の測定は臨床的に簡便ではないが，更に，プラス/マイナス鎖RNA量の特異的測定には方法論的にも注意が必要である．

本稿では著者の肝組織内のRNA量測定結果を含めて臨床的意義について述べる．

1. 肝組織内HCV-RNAの測定方法

生検あるいは手術による肝組織から核酸を抽出した．検体重量と核酸量には相関を認め，平均すると肝組織1mgあたりの抽出核酸量は1.6μgであった．

肝組織からの核酸抽出にはAGPC法を用いた．抽出したRNA溶液にreverse transcriptaseを用いて逆転写反応を行った．その際，HCV-RNAの検出にはrandom primerを，プラス鎖RNA，マイナス鎖RNAの検出には，各鎖に特異的なanti-sense primer，sense primerを使用した(strand-specific RT-PCR法)．作製されたcDNAにTaq polymeraseを加えてPCRを施行し増幅したが，その定量には標識ヌクレオチドを用いたRT-PCR法を用いた[3]．

2. マイナス鎖特異的HCV-RNA検出の問題点と対策

strand-specific RT-PCRを施行する際，特にマイナス鎖HCV-RNAの検出に関しては，偽陽性の出現についての注意が重要である．その理由として，①virus RNAによるself-priming，②細胞内核酸によるrandom priming，③加えたprimerのfalse primingなどが想定されている[4]．偽陽性出現への対策として，逆転写反応の際に，①ホウ素化合物でRNAの3′末端を修飾する，②tagged primerを用いる，③熱に安定で，逆転写酵素とDNAポリメラーゼ活性を併せもつrTthを使用する，④マイナス鎖が有

Shuhei Hige: Department of Internal Medicine, Gastroenterology and Hematology Section, Hokkaido University Graduate School of Medicine　北海道大学大学院医学研究科消化器内科学

0047-1852/04/¥50/頁/JCLS

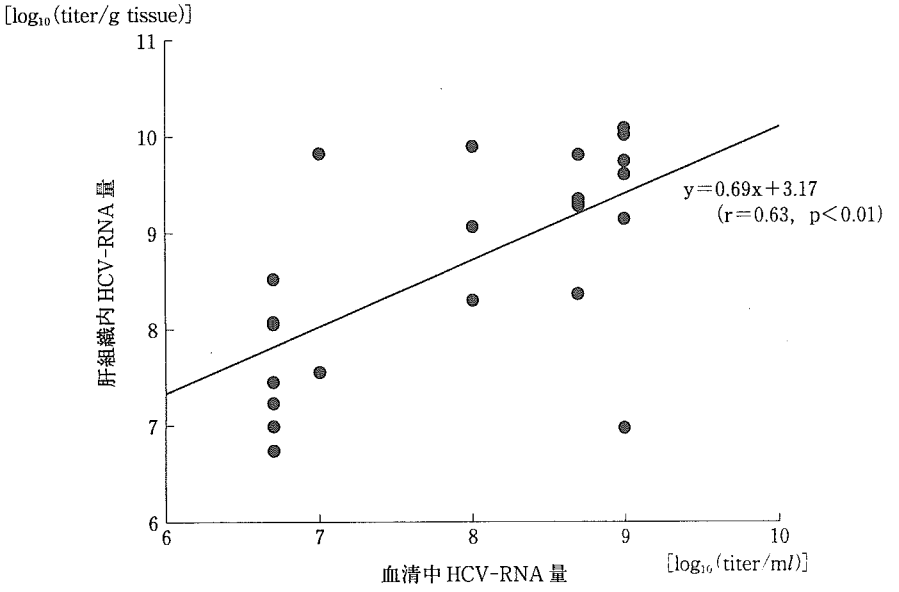


図1 C型慢性肝疾患患者の血清中および肝組織内HCV-RNA量

するpoly A^+領域を指標にRNAを分離後，逆転写を行う，などの方法により検出の特異性が上昇することが報告されている．①では核酸抽出物を$NaIO_4$および$NaBH_4$で処理後に逆転写反応を行う[5]．②ではcDNA合成時に使用するプライマーの5′末端側にHCV-RNAとは異なる塩基配列('tag')をもったものを使用する[6]．③ではrTthを，70℃の高温でMn^{2+}添加下に使用する[6]．この酵素はMn^{2+}キレートで逆転写酵素活性を失活させ，Mg^{2+}添加でDNA依存のポリメラーゼ活性を活性化することができる．通常の逆転写反応は42℃以下で行われることが多いが，反応温度の高い方が感度・特異性ともに上昇する．④は，プラス鎖RNAの3′UTR(非翻訳領域)のpoly(U)配列に対応して有するマイナス鎖5′末端のpoly A^+をビオチン標識oligo (dT) probeでハイブリダイズして分離することでマイナス鎖のみを逆転写反応に進める[7]．

本稿に示す著者の成績は，上記①のGunjiらの方法に準じて以下の化学処理を施した．すなわち，核酸抽出物に0.5M sodium acetate(pH 5.0)，20mM $NaIO_4$を加え，30℃で12時間反応後，10% ethylene glycolを加えてエタノール沈殿した．沈殿物を蒸留水で溶解し，0.1N $NaBH_4$を加えて0℃で1時間反応後に0.1N acetic acidを加えてエタノール沈殿させ，沈殿物を以後の逆転写反応に用いた．

3. 肝組織内のHCV-RNA量

肝組織内のHCV-RNA量と対応する血清中のHCV-RNA量を測定した結果，両者に相関を認めた($r=0.63$, $p<0.01$, **図1**)．したがって，血清中RNA量から肝組織内のRNA量の推測が可能であった．肝組織1gと血清1ml中のHCV-RNA量を比較すると，肝内RNA量が血清中に比べて数十倍多かった．また，肝組織からはプラス鎖，マイナス鎖HCV-RNAの両方が検出された．両者には相関を認め($r=0.63$, $p<0.01$)，マイナス鎖RNA量はプラス鎖RNA量の数%程度であった(**図2**)．

KnodellのHAI(histological activity index)スコアにおける肝の線維化別の肝組織内HCV-RNA量(平均±標準偏差)は，プラス鎖はスコア0群で9.1±1.0，スコア1群で8.6±1.0，スコア3群で8.7±0.8，スコア4群で7.8±1.1[$\log_{10}$(titer/g tissue)]，マイナス鎖はスコア0群で

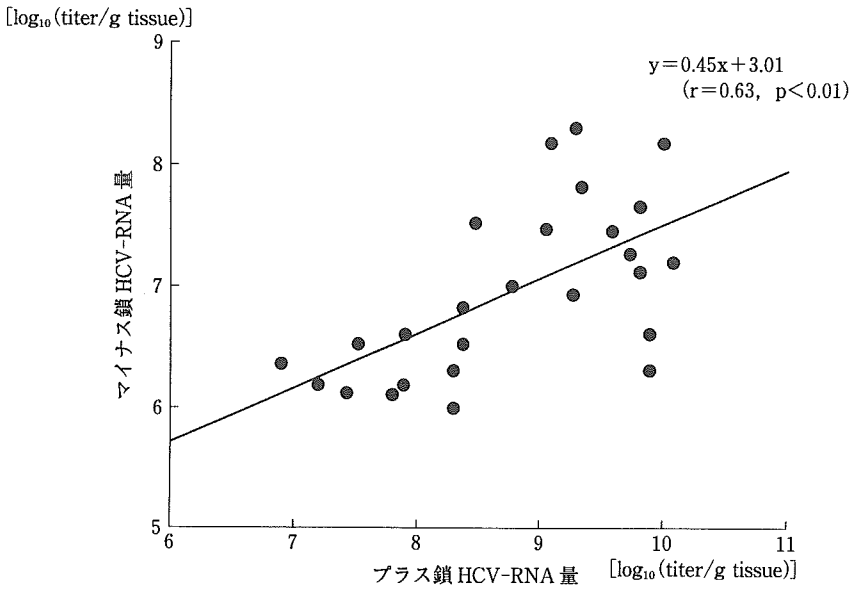


図2 肝組織内のプラス鎖HCV-RNA量とマイナス鎖HCV-RNA量

7.1±0.8，スコア1群で6.9±0.2，スコア3群で7.1±0.7，スコア4群で6.4±0.3[$\log_{10}$(titer/g tissue)]であった(**図3-B**)．プラス鎖HCV-RNA量に関しては，スコア0群とスコア4群の間に有意差($p<0.05$)を示し，肝硬変症例でウイルス量低下を認めた．

一方，肝炎の活動性をHAIのgrade Iからgrade IIIのスコアの合計で示した場合，肝組織内のHCV-RNA量と組織活動性の間に一定の関連は認めなかった(**図3-A**)．更に，血清ALT値と肝組織内HCV-RNA量との間には，プラス鎖，マイナス鎖のいずれとも相関を認めなかった(**図4**)．また，プラス鎖HCV-RNAに対するマイナス鎖HCV-RNAの比率も，肝の組織所見(HAIスコア)や血清ALT値との相関を認めず，HCVはこれらの所見とは無関係に増幅していると考えられた．

4. 肝内HCV-RNA量測定の臨床的意義

前述のとおり，肝組織内のHCV-RNAは血清中のそれに比して単位容積あたりで多量に存在しており，マイナス鎖HCV-RNAが検出される事実と合わせ，肝内でのHCV増殖の裏付けと考えられる．プラス鎖あるいはマイナス鎖RNA量が肝組織内でのHCV増殖の指標と考えた場合，これらは肝組織の活動性や血清ALT値とは相関しなかった．このことは，肝細胞障害が肝組織内HCV増殖に直接的に関連するのではなく，免疫学的反応などの他の要因による結果であることを示している．Negroらも，strand-specific RT-PCR法を用いた肝組織内HCV-RNAの半定量測定成績から，HCVの増幅の程度については血清中HCV-RNA量測定の方が臨床的に有用であると報告している[8]．

おわりに

肝組織内のHCV-RNA量の測定は，肝内HCVの増幅の程度を知るための直接的な検査法である．肝組織内HCV-RNA量は血清中HCV-RNA量とは相関するが，肝組織所見や血清ALT値とは関連せず，C型肝炎がHCVの直接的肝細胞障害以外の機序により起こることを示している．

マイナス鎖HCV-RNAはHCVが組織内で増幅していることの直接的な証明となるが，その検出系の確立には十分な注意が必要である．

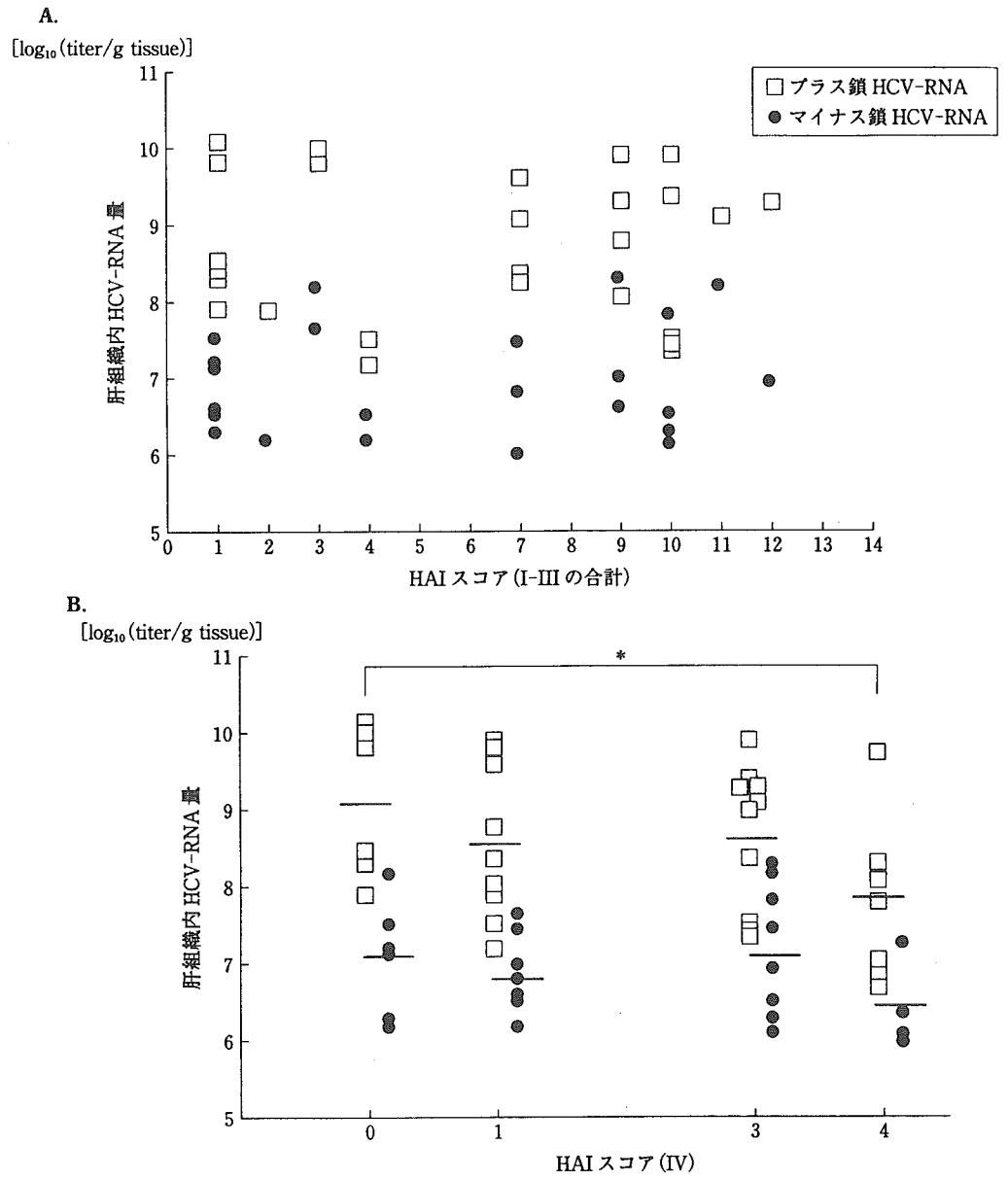


図3 C型慢性肝疾患患者の HAI スコアと肝組織内 HCV-RNA 量 (プラス鎖・マイナス鎖別)

A: HAI grade I-III のスコア合計と肝組織内 HCV-RNA 量

B: HAI grade IV のスコアと肝組織内 HCV-RNA 量

(*$p<0.05$)

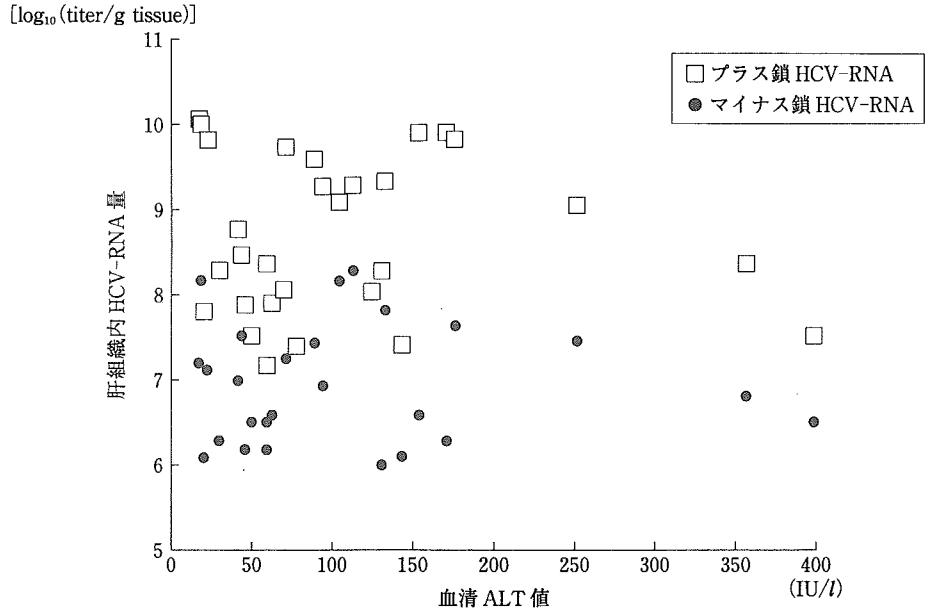


図4 C型慢性肝疾患患者の血清ALT値と肝組織内HCV-RNA量(プラス鎖・マイナス鎖別)

文 献

1) Ahlquist P, et al: Host factors in positive-strand virus genome replication. J Virol **77**: 8181-8186, 2003.
2) Bartholomeusz A, Thompson P: Flaviviridae polymerase and RNA replication. J Viral Hepat **6**: 261-270, 1999.
3) 髭 修平ほか：標識ヌクレオチドを用いたRT-PCR法によるHCV-RNAの定量化の検討. 肝臓 **34**: 52-53, 1993.
4) Sangar DV, Carroll AR: A tale of two strands: reverse-transcriptase polymerase chain reaction detection of hepatitis C virus replication. Hepatology **28**: 1173-1176, 1998.
5) Gunji T, et al: Specific detection of positive and negative stranded hepatitis C viral RNA using chemical RNA modification. Arch Virol **134**: 293-302, 1994.
6) Lanford RE, et al: Demonstration of in vitro infection of chimpanzee hepatocytes with HCV using strand specific RT/PCR. Virology **202**: 606-614, 1994.
7) Takyar ST, et al: Specific detection of minus-strand hepatitis C virus RNA by reverse-transcription polymerase chain reaction on polyA$^+$-purified RNA. Hepatology **32**: 382-387, 2000.
8) Negro F, et al: Detection of genomic- and minus-strand of hepatitis C virus RNA in the liver of chronic hepatitis C patients by strand-specific semiquantitative reverse-transcriptase polymerase chain reaction. Hepatology **29**: 536-542, 1999.

B型慢性肝炎・肝硬変治療症例集

――抗ウイルス薬／ラミブジン・アデホビルピボキシル――

久留米大学名誉教授／米国公益法人国際肝臓研究所理事長　谷川 久一　監修

医薬ジャーナル社

症例 7．ラミブジン投与中止後に肝炎の再燃を起こし，ラミブジン再投与により改善した症例

髭　修平

患者背景

30 歳　男性

診断名	B 型慢性肝炎	罹病期間	10 年	入院 or 外来	入院
既往歴	特記すべき事なし				
家族歴	父：B 型肝硬変症，母および弟：HBV キャリア				
生活歴	飲酒歴，喫煙歴なし				

前治療薬

薬剤名	用量	用法	評価
インターフェロンβ	600 万単位 / 日	静脈内投与，4 週間	無効

病　歴

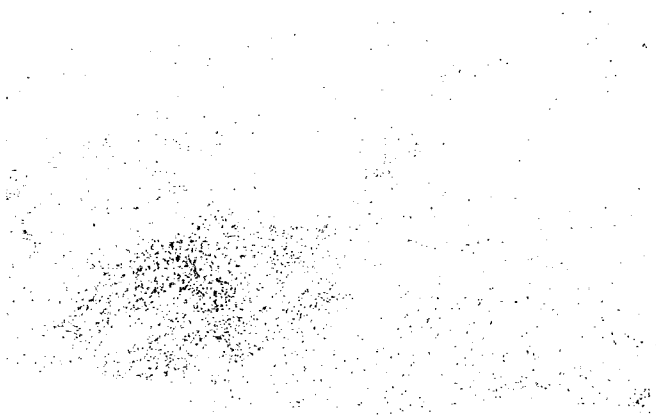
1991 年，眼科処置時に B 型肝炎を発見され，1991 年，1992 年，1997 年の 3 回，インターフェロン治療を受けたが改善せず，HBe 抗原陽性のまま肝炎の再燃を繰り返していた。2000 年 7 月 6 日に再治療を目的として当科に入院した。

経　過

治療開始前の肝生検は F2/A2 の組織像であった（図 1）。2000 年 7 月 10 日からラミブジン 100 mg/ 日を投与開始した。開始初期 4 週間は，インターフェロンを併用した（IFN β 300 万単位 / 日）。治療開始後，HBV-DNA 量の減少と HBe 抗原価の低下を認めたものの，HBe 抗体価の上昇がみられないため，2001 年 3 月から HB ワクチンを併用した。HB ワクチンは 1 回 20 μg を月 1 回の皮下投与とした。投与中に，HBe 抗原価の低下，HBe 抗体価の上昇傾向を認めたが，6 回の投与終了後には治療前のレベルに戻った。2002 年 4 月から再度 HB ワクチン治療を行ったが，同様の反応性で，セロコンバージョンに達しない状態であった。2003 年 3 月からラミブジンの内服を隔日とし，2003 年 7 月には，一旦，内服中止とした。同年 10 月には ALT 値が 100（IU/L）を越し，HBV-DNA 量も 8.22（log copies/mL）にまで上昇した。翌月，HBV-DNA 量が低下傾向になったことを確認した後にラミブジンの投与を再開したところ，2004 年 2 月にはセロコンバージョンを認めた（臨床経過図）。

症例7．ラミブジン投与中止後に肝炎の再燃を起こし，ラミブジン再投与により改善した症例

図1　治療開始前の肝生検組織像



F2/A2 の所見であった。

考　察

本症例の治療経過やポイントを整理すると，

1）20 歳代に 3 回のインターフェロン治療歴があり，さらに今回の治療でもラミブジンに追加してインターフェロンを併用しているにも拘らずセロコンバージョンが得られておらず，インターフェロン治療には抵抗性である。

2）ラミブジン投与後，速やかな抗ウイルス効果を示し，HBV-DNA 量は低値で維持されたが，HBe 抗原は抗原価の低下を認めるものの陽性のままで，HBe 抗体も上昇せず，セロコンバージョンには至らなかった。我々の治療成績では，HBe 抗原陽性例に対するラミブジン投与後のセロコンバージョンは治療後 6 ～ 7 カ月以内に得られるものが多く，このまま治療を継続して著効を得る可能性は低いと予想された。

3）HB ワクチン治療を 2 クール施行した。いずれも，一時的には HBe 抗原 /HBe 抗体価に治療の反応を認めたが，最終的にセロコンバージョンを得る程度には至らなかった。

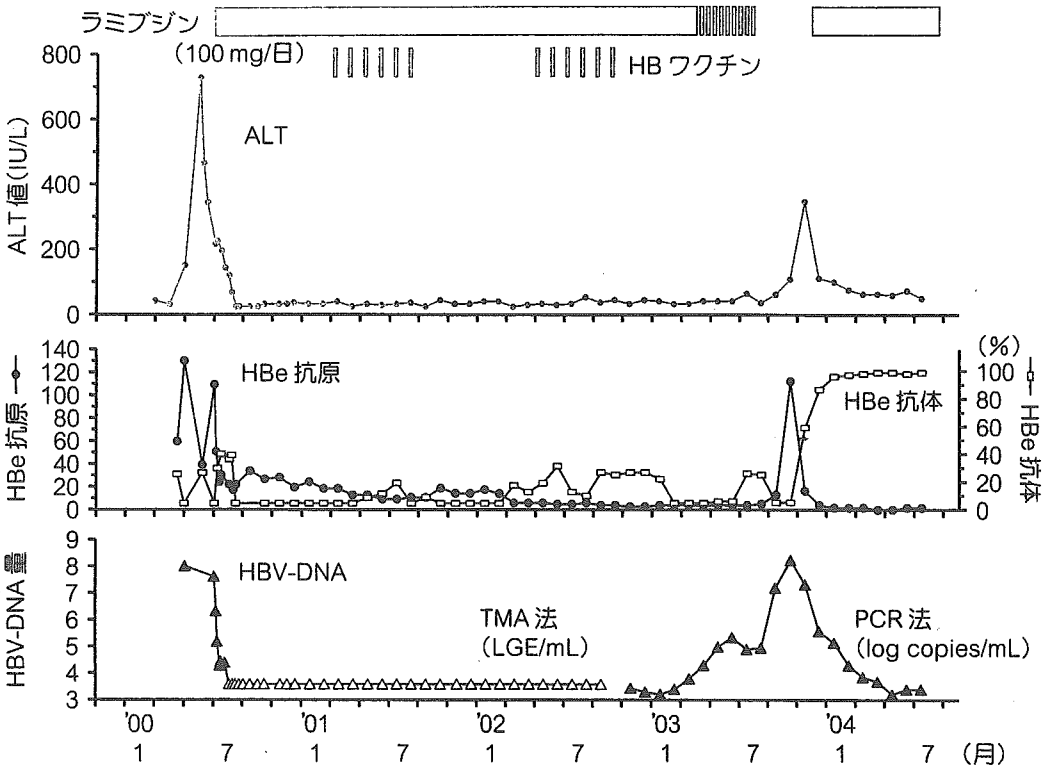
4）ラミブジンを連日投与から隔日投与に減量したところ，HBV-DNA 量は上昇傾向を示したが，4 カ月間の中では ALT 値を上昇させるレベルには至らなかった。

5）ラミブジンを中止したところ，2 カ月後には HBV-DNA 量の著明な上昇を認め，3 カ月後には ALT 値も有意に上昇した。

6）HBV-DNA 量がピーク値を越して減少傾向に入った事を確認後にラミブジン内服を再開したところ，速やかにセロコンバージョンが得られたが，内服再開時には，既に HBe 抗原低

症例 7．ラミブジン投与中止後に肝炎の再燃を起こし，ラミブジン再投与により改善した症例

臨床経過



2003 年 7 月のラミブジン内服中止後に肝炎の再燃を認めたが，同年 11 月から投与を再開し，セロコンバージョンを認めた。

HBV-DNA 量の測定方法は経過中に変更したが，TMA 法での△は測定感度以下を示している。

下，HBe 抗体上昇の傾向を示しており，ラミブジン内服がセロコンバージョンへの流れを加速させたものと考えられた。

インターフェロンやラミブジン投与でセロコンバージョンが得られなかった難治例に対して，ラミブジン休薬による肝障害の再燃を利用してセロコンバージョンを達成させた。本例のように，ラミブジン投与後にウイルス学的には反応を示すもののセロコンバージョンを得られないため長期投与を余儀なくされる症例は少なからず認められ，投与期間の長期化に伴う変異ウイルス出現のリスクを考慮した場合のジレンマとなっている。比較的若年で肝線維化の高度でない症例に対しては，本例のように，意図的にラミブジン投与を中止し，リバウンド後の再投与により改善させる方法も検討可能であると考えられる。

症例 18. ラミブジン投与により肝機能の改善がみられた肝硬変症例（YMDD 変異ウイルス未出現）

髭　修平

患者背景

61 歳　女性

診断名	B型肝硬変	罹病期間	17 年	入院 or 外来	外来
既往歴	21 歳：十二指腸潰瘍，43 歳：尾骨骨折				
家族歴	妹：B型慢性肝炎				
生活歴	ビール 500 mL/ 日，喫煙歴なし				

前治療薬：なし

病　歴

1985 年に尾骨骨折をきたした際に HBs 抗原陽性を指摘され，慢性肝炎の診断を受け，近医で治療を受けていた。1991 年に当科を初診したが，以後，HBe 抗体陽性のまま HBV-DNA 量の変動を認め，慢性肝炎から肝硬変への進展がみられた。さらに，2001 年に肝障害の増強とともに肝予備能の低下傾向を認めたため，2001 年 9 月にラミブジンの投与を開始した。

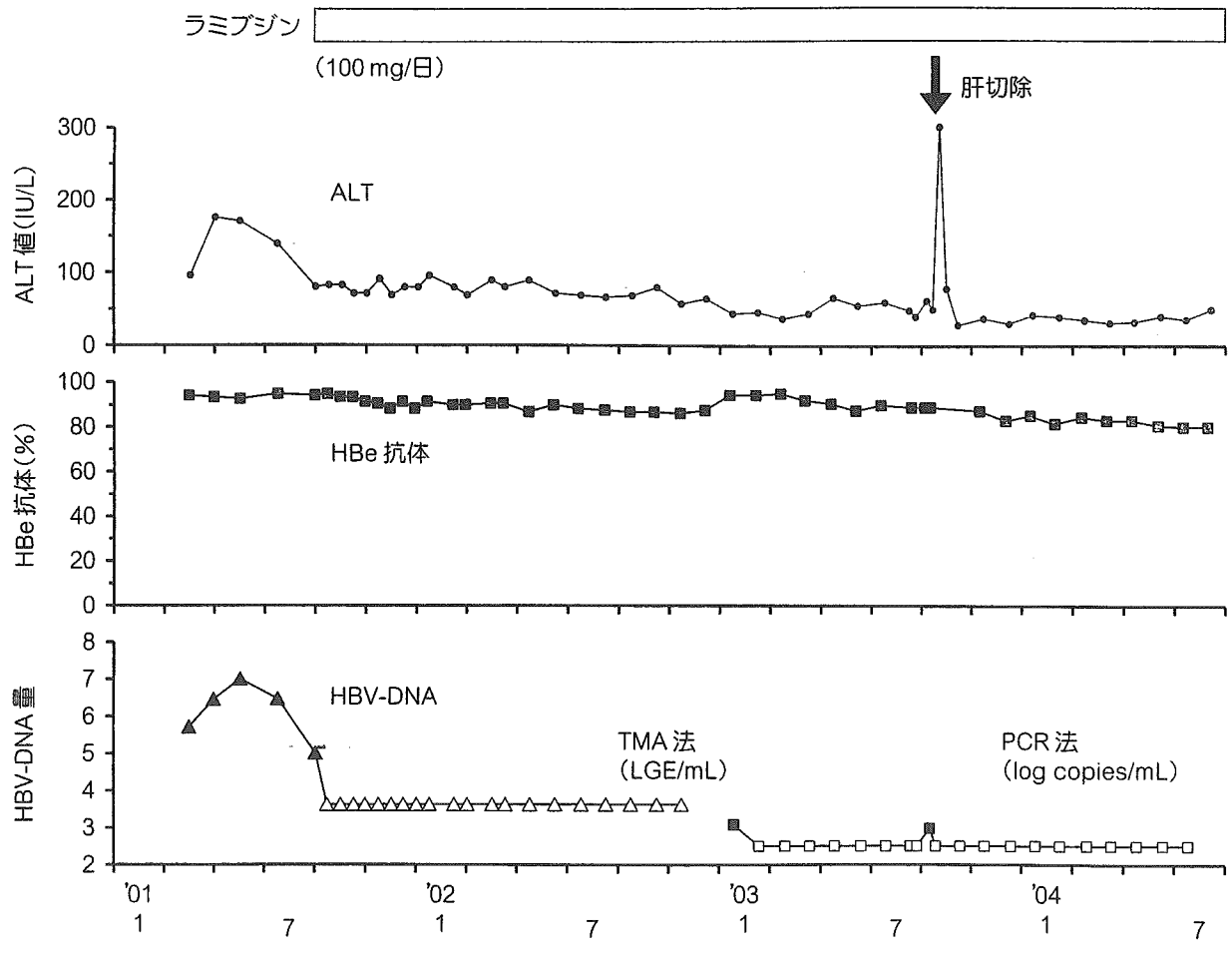
経　過

治療開始時点で，トランスアミナーゼの低下傾向を認め，HBV-DNA 量も減少傾向を示していたが，治療開始後，ウイルス量は急速に減少し，ALT 値も改善した。投与後約 6 カ月間で，アルブミン値は 3.1 から 3.9（g/dL），コリンエステラーゼ値は 130 から 164（IU/L），ヘパプラスチンテストは 33.2 から 49.7（%）へと，肝機能の改善傾向を認めた。現在まで投与開始から 3 年が経過したが，HBe 抗体は陽性を持続し HBV-DNA 量も低値で推移しており，YMDD 変異ウイルスの出現はみられていない（臨床経過図 1，2）。

なお，経過中の 2003 年 7 月に腹部 CT で肝腫瘍を発見され，肝細胞癌の診断にて，同年 9 月 17 日に肝 S4 + S8 の部分切除術を施行した。摘出標本の病理組織学的所見では，背景肝は完成した肝硬変であるが炎症細胞浸潤は乏しく，活動性の低い状態であった（図 1）。手術直後は，一時的に ALT 値の上昇を認めたが，肝予備能の低下は軽度で，順調な回復を示した。

症例 18. ラミブジン投与により肝機能の改善がみられた肝硬変症例（YMDD 変異ウイルス未出現）

臨床経過図 1



2001 年 9 月からラミブジン内服を開始し，肝障害の改善がみられた。2003 年 9 月に肝癌に対する肝切除術を施行され，一時的な肝障害を認めたが，回復は良好であった。HBV-DNA 量は継続して低値を維持している。

HBV-DNA 量の測定方法は経過中に変更したが，TMA 法の△，PCR 法の□は，それぞれの方法での測定感度以下を示している。

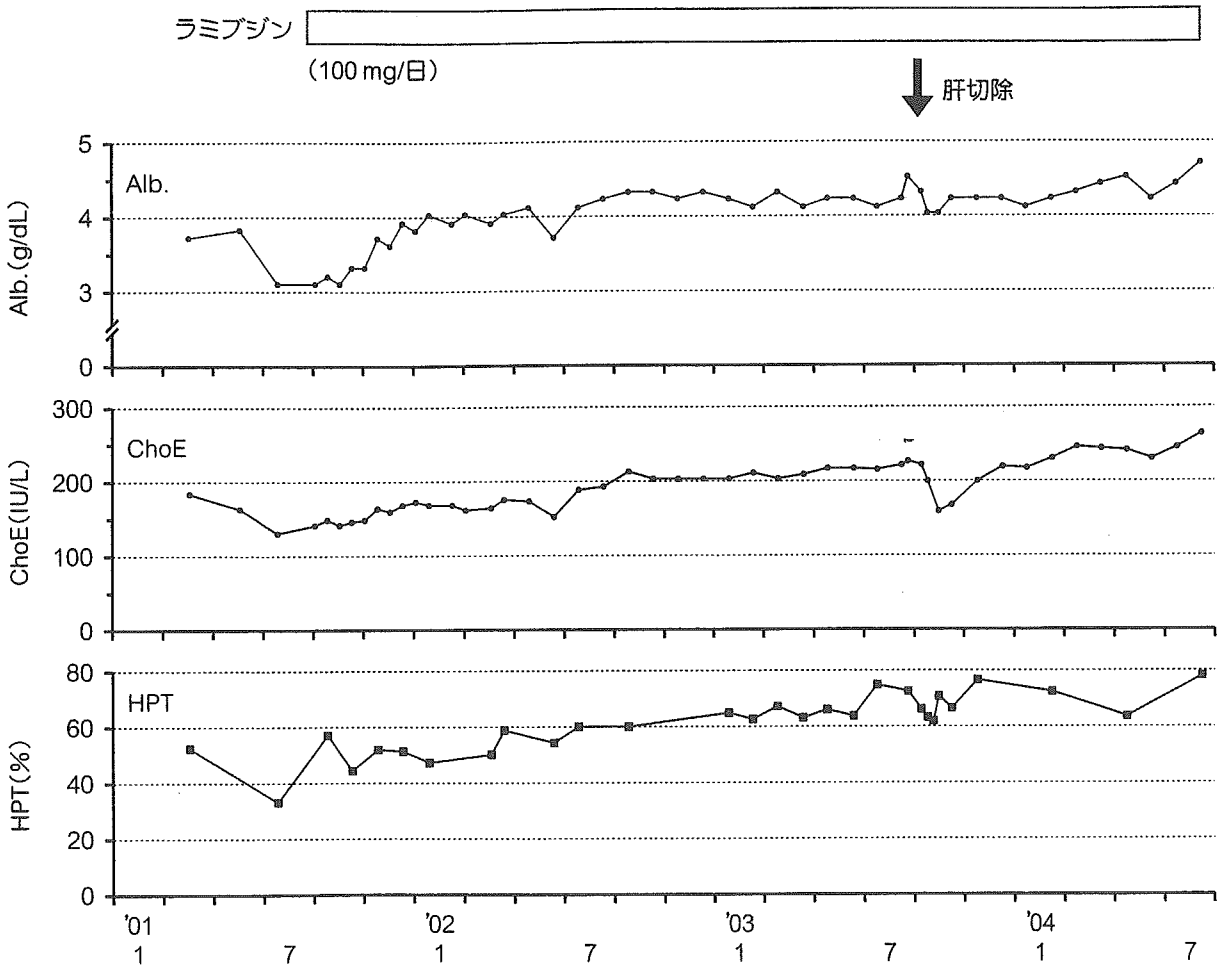
考　察

本症例の治療経過やポイントを整理すると，

1）HBe 抗体陽性で肝炎が持続する症例では，ALT 値は HBV-DNA 量に連動することが多い。したがって，このような症例に対しては，長期的にウイルス量を低値に維持し肝炎の鎮静化を図ることが肝病変の進展抑制の意味で重要である。

症例 18.　ラミブジン投与により肝機能の改善がみられた肝硬変症例
（YMDD 変異ウイルス未出現）

○臨床経過図 2

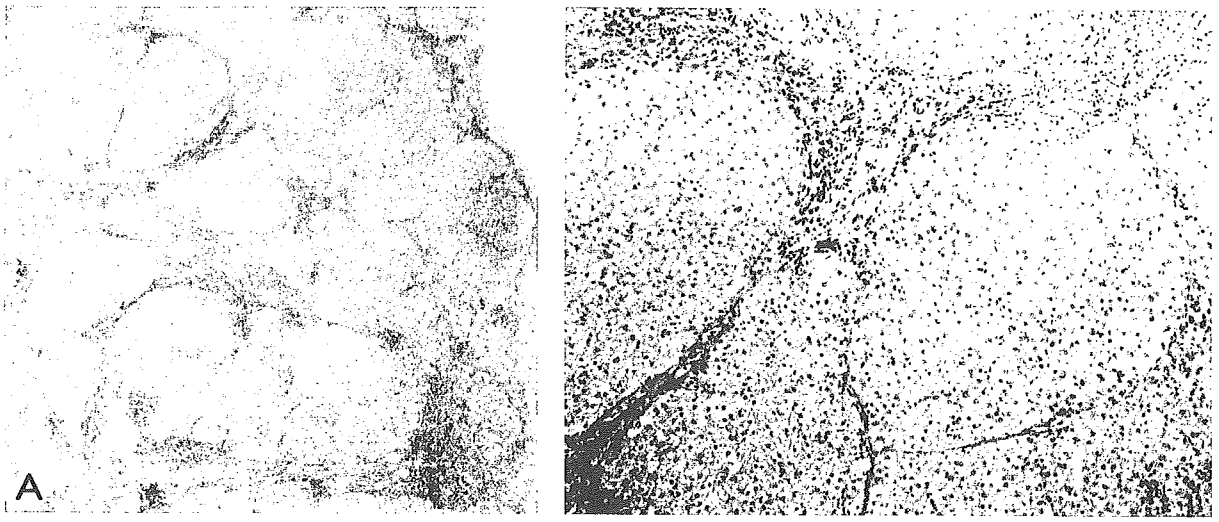


ラミブジン投与後，肝予備能は順調に改善傾向を認めた。肝切除後，一時的に肝予備能の低下を認めたが，その後の回復は良好であった。

2）特に，肝硬変症例では，肝不全への進展を抑止する意味でも有用である。本症例において，投与開始約２年後に肝切除が施行された際の背景肝組織では炎症所見は乏しく，有効性が確認された。

3）３年間のラミブジン継続投与の段階では YMDD 変異ウイルスは出現していない。治療開始時点で e 抗体陽性症例や HBV-DNA 量低値症例では変異ウイルスの出現は比較的低率で，

図 1　2 年後の肝組織像



ラミブジン治療開始の約 2 年後に施行された肝切除時の肝組織像（A：弱拡大，B：強拡大）。完成された肝硬変の所見であるが，肝炎の活動性は軽度であった。

出現時期も，e 抗原陽性や DNA 量高値例に比べて遅い傾向がある。

4）肝癌を合併した場合には，肝癌の進展と肝予備能を総合的に判断して，手術，肝動脈塞栓術，ラジオ波焼灼術，リザーバー動注化学療法などの中から治療法を選択するが，肝予備能の維持は，十分な癌治療の達成のためにも重要となってくる。本症例では肝癌発見以前からのラミブジン内服により肝予備能が改善していたため，安全に手術を施行する事が可能であった。ラミブジン内服開始時の Child-Pugh スコアでは B（7 点）であったが，手術時には A（5 点）に改善していた。

肝炎活動性を持続する肝硬変症例に対しては，肝不全への進展抑制の意味でラミブジン治療は有用である。さらに，肝癌を合併した場合には，最も望ましい肝癌治療法の選択，維持のためにも重要な治療となる。

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

Masahiro Onozawa,[1,3] Satoshi Hashino,[1] Koh Izumiyama,[1] Kaoru Kahata,[1] Makoto Chuma,[1] Akio Mori,[1] Takeshi Kondo,[1] Nobuyasu Toyoshima,[1] Shuichi Ota,[1] Sumiko Kobayashi,[1] Shuhei Hige,[1] Tomomi Toubai,[2] Junji Tanaka,[2] Masahiro Imamura,[2] and Masahiro Asaka[1]

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.

(*Transplantation* 2005;79: 616–619)

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.

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

[1] Department of Gastroenterology and Hematology, Hokkaido University Graduate School of Medicine, Sapporo, Japan.
[2] Department of Hematology and Oncology, Hokkaido University Graduate School of Medicine, Sapporo, Japan.
[3] Address correspondence to: Dr. Masahiro Onozawa, Department of Gastroenterology and Hematology, Hokkaido University Graduate School of Medicine, Kita-15, Nishi-7, Kita-ku, Sapporo 060–8638, Japan. E-mail address: masahiro.onozawa@nifty.ne.jp.
Received 23 August 2004. Revision requested 13 September 2004. Accepted 16 October 2004.

ISSN 0041-1337/05/7905-616
DOI: 10.1097/01.TP.0000151661.52601.FB

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.

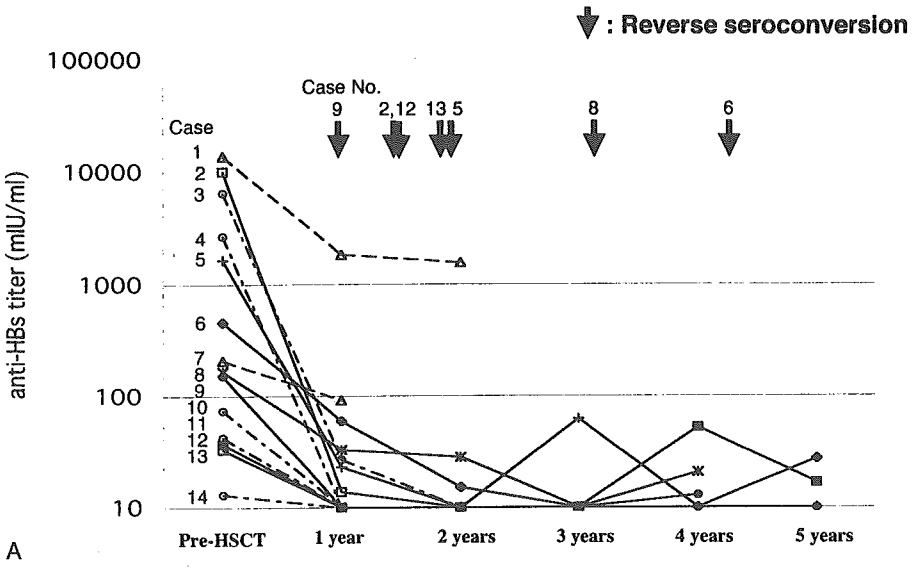
RESULTS

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.

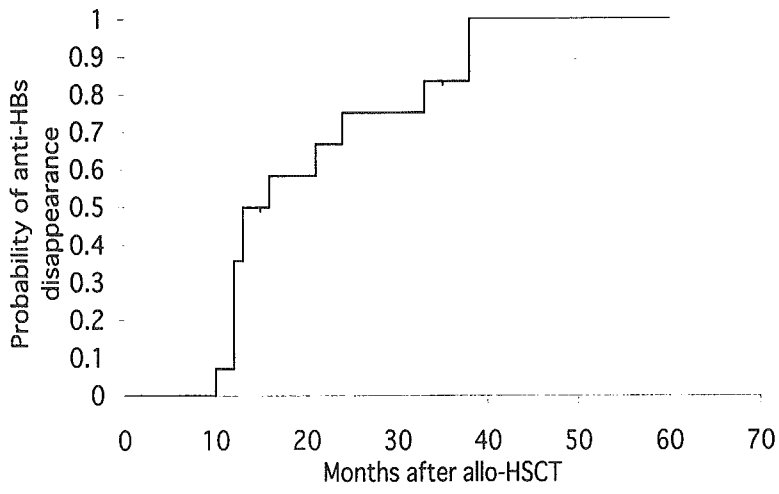
DISCUSSION

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.

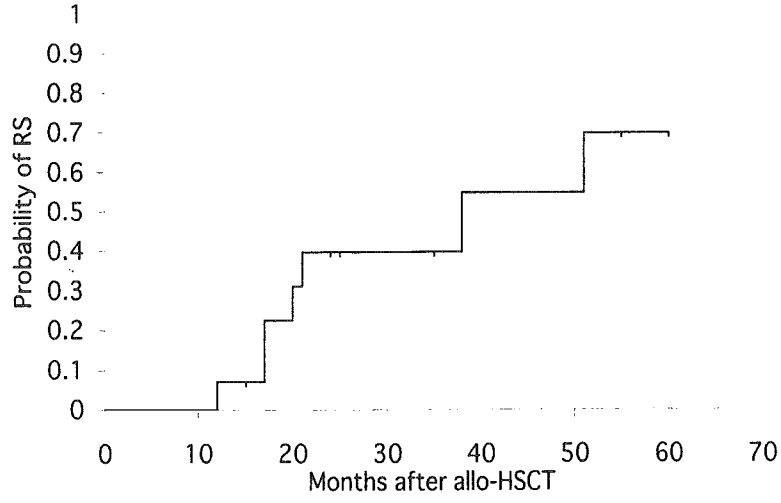
The onset of hepatitis due to RS after HSCT has been reported to be relatively late (6–52 months; median 19 months) (*6–12, 14, 16*) compared with that in cases with HBsAg in pretransplantation. This is probably caused by prolonged existence of recipient-type memory B-cell immunity. Recipient-derived 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



B



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.

ACKNOWLEDGMENTS.

The authors thank all physicians and nursing staff of our HSCT department for providing dedicated care for the patients and Mrs. Tsuda for her help in management of a huge amount of serum samples.

REFERENCES

1. Mason AL, Xu L, Guo L, et al. Molecular basis for persistent hepatitis B virus infection in the liver after clearance of serum hepatitis B surface antigen. *Hepatology* 1998; 27: 1736.
2. Goyama S, Kanda Y, Nannya Y, et al. Reverse seroconversion of hepatitis B virus after hematopoietic stem cell transplantation. *Leuk Lymphoma* 2002; 43: 2159.
3. Degos F, Lugassy C, Degott C et al. Hepatitis B virus and hepatitis B-related viral infection in renal transplant recipients. *Gastroenterology* 1988; 94: 151.
4. Webster A, Brenner MK, Prentice HG, et al. Fatal hepatitis B reactivation after autologous bone marrow transplantation. *Bone Marrow Transplant* 1989; 4: 207.
5. Tsutsumi Y, Kawamura T, Saitoh S, et al. Hepatitis B virus reactivation in a case of non-Hodgkin's lymphoma treated with chemotherapy and rituximab: necessity of prophylaxis for hepatitis B virus reactivation in rituximab therapy. *Leuk Lymphoma* 2004; 45: 627.
6. Chen PM, Fan S, Liu JH, et al. Reactivation of hepatitis B virus in two chronic GVHD patients after transplant. *Int J Hematol* 1993; 58: 183.
7. Kostaridou S, Ladis V, Kattamis A, et al. HBeAg-negative hepatitis B in a previously thalassemic patient during immunosuppressive therapy for chronic GVHD. *Bone Marrow Transplant* 1998; 22: 919.
8. Li Volti S, Pizzarelli G, Galimberti M, et al. Clinical and biochemical reactivation of HBV infection in a thalassemic patient after bone marrow transplantation. *Infection* 1998; 26: 58.
9. Nordbo SA, Skaug K, Holter E, et al. Reactivation of hepatitis B virus infection in an anti-HBc and anti-HBs positive patient after allogeneic bone marrow transplantation. *Eur J Haematol* 2000; 65: 86.
10. Iwai K, Tashima M, Itoh M, et al. Fulminant hepatitis B following bone marrow transplantation in an HBsAg-negative, HBsAb-positive recipient; reactivation of dormant virus during the immunosuppressive period. *Bone Marrow Transplant* 2000; 25: 105.
11. Sakamaki H, Sato Y, Mori SI, et al. Hepatitis B virus reactivation in a patient with chronic GVHD after allogeneic peripheral blood stem cell transplantation. *Int J Hematol* 2001; 74: 342.
12. Hashino S, Nozawa A, Izumiyama K, et al. Lamivudine treatment for reverse seroconversion of hepatitis B 4 years after allogeneic bone marrow transplantation. *Bone Marrow Transplant* 2002; 29: 361.
13. Strasser SI, McDonald GB. Hepatitis viruses and hematopoietic cell transplantation: a guide to patient and donor management. *Blood* 1999; 93: 1127.
14. Seth P, Alrajhi AA, Kagevi I, et al. Hepatitis B virus reactivation with clinical flare in allogeneic stem cell transplants with chronic graft-versus-host disease. *Bone Marrow Transplant* 2002; 30: 189.
15. Dhedin N, Douvin C, Kuentz M, et al. Reverse seroconversion of hepatitis B after allogeneic bone marrow transplantation: a retrospective study of 37 patients with pretransplant anti-HBs and anti-HBc. *Transplantation* 1998; 66: 616.
16. Knoll A, Boehm S, Hahn J, et al. Reactivation of resolved hepatitis B virus infection after allogeneic haematopoietic stem cell transplantation. *Bone Marrow Transplant* 2004; 33: 925.
17. van Tol MJ, Gerritsen EJ, de Lange GG, et al. The origin of IgG production and homogeneous IgG components after allogeneic bone marrow transplantation. *Blood* 1996; 87: 818.
18. Ljungman P, Lewensohn-Fuchs I, Hammarstrom V, et al. Long-term immunity to measles, mumps, and rubella after allogeneic bone marrow transplantation. *Blood* 1994; 84: 657.
19. Parkman R, Weinberg KI. Immunological reconstitution following bone marrow transplantation. *Immunol Rev* 1997; 157: 73.
20. Ilan Y, Nagler A, Adler R, et al. Adoptive transfer of immunity to hepatitis B virus after T cell-depleted allogeneic bone marrow transplantation. *Hepatology* 1993; 18: 246.
21. Lindemann M, Barsegian V, Runde V, et al. Transfer of humoral and cellular hepatitis B immunity by allogeneic hematopoietic cell transplantation. *Transplantation* 2003; 75: 833.

Journal of Viral Hepatitis, 2005, 12, 154–159 doi:10.1111/j.1365-2893.2005.00559.x

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

M. Natsuizaka,[1] S. Hige,[1] Y. Ono,[1] K. Ogawa,[1] M. Nakanishi,[1] M. Chuma,[1] S. Yoshida,[2] and M. Asaka[1] [1]*Gastroenterology & Hematology Section, Department of Internal Medicine, Hokkaido University Graduate School of Medicine, Kita-ku, Sapporo, Japan; and* [2]*Department of Laboratory Medicine, Hokkaido University Hospital, Hokkaido, Japan*

Received November 2003; accepted for publication March 2004

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.

Correspondence: Mitsuteru Natsuizaka, Gastroenterology & Hematology Section, Department of Internal Medicine, Hokkaido University Graduate School of Medicine, North 15, West 7, Kita-ku, Sapporo 060-8638, Japan. E-mail: natsu-m@med.hokudai.ac.jp

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.